Journal of Archaeological Science (1997) **24,** 417–429

The Effects of Sample Treatment and Diagenesis on the Isotopic Integrity of Carbonate in Biogenic Hydroxylapatite

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(*Received 12 October 1995, revised manuscript accepted 15 February 1996*)

The study of the isotopic composition of structural carbonate in fossil vertebrate hydroxylapatite has been hampered by uncertainty concerning sample treatment and arguments about the susceptibility of this material to diagenetic alteration. Our investigation of sample treatment reveals that some methods commonly used to remove organic matter and secondary minerals induce significant isotopic offsets, particularly for oxygen isotopes in bone and dentin. Treatment with dilute sodium hypochlorite, to oxidize organic matter, followed by leaching with very dilute or acetate-buffered acetic acid, to dissolve secondary mineral contaminants, is recommended. Using this protocol, we explore the isotopic fidelity of different carbon- and oxygen-bearing components from individual fossil skeletons of Holocene humans and late Pleistocene mastodons and mammoths. Enamel hydroxylapatite yields reliable results, with low within-group isotopic variability and values that match those expected from well-preserved collagen from the same individual. In contrast, isotopic values from bone and dentin hydroxylapatite are more variable within groups of samples, and many specimens do not produce isotopic values consistent with those expected from collagen or enamel from the same individual. These results suggest that bone and dentin are unreliable source materials for isotopic analysis of structural carbonate, even for relatively young, mid-Holocene to Pleistocene specimens.

Keywords: BONE, TOOTH ENAMEL, TOOTH DENTIN, CARBON ISOTOPES, OXYGEN ISOTOPES, CARBONATE, COLLAGEN, DIAGENESIS, PALAEODIET.

Introduction

Vertebrate hardparts are abundant in the fossil record from the Ordovician to the Recent. The isotopic chemistry of these fossils provides insights into paleobiology and paleoenvironment that are unavailable through traditional morphologic and sedimentologic methods (van der Merwe, 1982; DeNiro, 1987; Koch, Fogel & Tuross, 1994). For these studies to be successful, fossils that have been compromised by post-mortem alteration must be identified and either removed or subjected to rigorous cleaning procedures. Criteria exist for diagnosing when the isotopic composition of skeletal proteins, such as collagen, has been altered (DeNiro & Weiner, 1988; Tuross, Fogel & Hare, 1988; Bocherens *et al*., 1995). Assessing the integrity of the mineral phase of vertebrate skeletons is equally vital.

Bone, tooth dentin, and tooth enamel are composites of mineral, protein, and lipid. The mineral in all three tissues is hydroxylapatite ($Ca_{10}[PO_4]_6[OH]_2$) with a few weight percent carbonate substituting at both hydroxyl and phosphate lattice sites (LeGeros *et al*., 1969; LeGeros, 1981). In addition to lattice-bound, structural carbonate, hydroxylapatite may also contain

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0305-4403/97/050417+13 $25.00/0/as960126

carbonate in hydration layers or in amorphous zones near crystal surfaces (LeGeros *et al.*, 1969; Beshah *et al.*, 1990). Bone and dentin are organic rich (>20%), highly porous, and poorly crystalline, with small crystals (20 × 4 nm) that have numerous defects and high strain. Tooth enamel, in contrast, is nearly anorganic (<2%), non-porous, and more highly crystalline, with larger crystals (130 × 30 nm) that have fewer defects and substitutions(LeGeros, 1981).

Strontium, phosphate, and carbonate in hydroxylapatite have all served as substrates for isotopic analysis, but we will focus on diagenetic alteration of structural carbonate. There has been substantial disagreement concerning the isotopic integrity of structural carbonate in biogenic hydroxylapatite, related both to treatments used to remove diagenetic phases and to the types of tissue analysed (Land, Lundelius & Valastro, 1980; Sullivan & Krueger, 1981, 1983; Schoeninger & DeNiro, 1982, 1983; Nelson *et al.*, 1986). Recently, several authors have argued that while carbonate in bone hydroxylapatite is susceptible to alteration, carbonate in tooth enamel hydroxylapatite is much more retentive of original isotopic signatures (Lee-Thorp & van der Merwe, 1987, 1991; Quade *et al.*, 1992; Wang & Cerling, 1994).

The carbonate recovered from fossils may be contaminated by exogenous sedimentary carbonates, occurring either as pore-filling cements or as bicarbonate adsorbed to crystal surfaces (Krueger, 1991). Carbonate minerals are more soluble than apatite, consequently they can be removed by leaching with dilute acid (Krueger, 1991; Lee-Thorp & van der Merwe, 1991). Post-mortem recrystallization of hydroxylapatite is more difficult to remedy. Because they are composed of small crystals, bone and dentin undergo extensive recrystallization, which has been detected by X-ray diffraction and infrared spectroscopy (Hassan, Termine & Haynes, 1977; Tuross, Behrensmeyer & Eanes, 1989; Bartsiokas & Middleton, 1992; Person *et al.*, 1995). Tooth enamel hydroxylapatite, which is more dense and more crystalline, is less susceptible to recrystallization. If bone recrystallizes to carbonate hydroxylapatite, environmental carbonate may be introduced to the sample, whereas if the product of recrystallization is fluorapatite, the isotopic composition of structural carbonate may be unaffected (Krueger, 1991). In addition to spectroscopic methods, alteration of biologic apatite has been monitored through analysis of carbonate yield (Lee-Thorp & van der Merwe, 1991; Person *et al.*, 1995; Rink & Schwarcz, 1995; Saliège, Person & Paris, 1995). Unusually low carbonate yields may indicate recrystallization to fluorapatite, whereas high values suggest addition of exogenous sedimentary carbonates.

Previous studies have demonstrated that enamel hydroxylapatite is resistant to diagenetic alteration, but that bone and dentin greater than 50,000 years old are often altered (Lee-Thorp & van der Merwe, 1987). The reliability of more recent bone and dentin, particularly material of archaeological interest (1000 to 20,000 years old), has not been established. Saliège *et al.* (1995) demonstrated that bone carbonate from ≈2500 year-old human skeletons from Saharan tombs in Niger yielded reasonable stable carbon isotopes values and ^{14}C dates. However the generality of the results from these burials in a very arid environment is unclear.

A further investigation the isotopic integrity of carbonate in bone hydroxylapatite is warranted for several reasons. First, hydroxylapatite carbonate, including the carbonate in bone and enamel, records the carbon isotope composition of bulk diet (carbohydrates, lipid, and protein), whereas the isotopic composition of collagen is strongly influenced by that of dietary protein (Ambrose & Norr, 1993; Tieszen & Fagre, 1993). Second, because enamel in most mammals forms early in life, it may record a juvenile diet or even prenatal tooth construction. In contrast, bones are remodelled throughout life, and therefore record a weighted average of the lifetime diet (Libby *et al.*, 1964). Consequently, if bone carbonate retains its isotopic composition, it would be an ideal source material for study of changes in the bulk diets of adults. Third, several authors have demonstrated that the difference in carbon isotope value between bone apatite and collagen from the same individual changes between carnivores and herbivores and have suggested that bone apatite-collagen comparisons may be of use in assessing the trophic levels of omnivores (Krueger & Sullivan, 1984; Lee-Thorp, Sealey & van der Merwe, 1989). Finally, with a few exceptions (Quade *et al.*, 1992; Wang & Cerling, 1994), diagenetic effects on oxygen isotope composition of bone and tooth carbonate have not been thoroughly examined.

Our examination of diagenetic alteration of carbonate from biogenic hydroxylapatite has two sections. Because the methods used to remove contaminants from hydroxylapatite have been controversial, we explore treatment artefacts through tests on modern enamel and bone. Tooth enamel from living animals contains no secondary carbonate, little organic matter, and large crystals, which offer a small surface area for bicarbonate adsorption. Thus untreated enamel should give "correct" isotope values against which to assess isotopic effects of different treatments. Although modern bone lacks secondary carbonates, it is organic rich, and its extremely small crystals offer a large surface area for bicarbonate adsorption. Thus untreated bone may not yield "correct" isotope values for structural carbonate.

Second, we examine isotopic fidelity in Holocene humans and Pleistocene proboscideans by comparing the carbon and oxygen isotopic compositions of bone or dentin hydroxylapatite to those of collagen and enamel hydroxylapatite from the same individual. If the bone or dentin have not been altered, we expect carbonate in their hydroxylapatite to be similar to that from diagenetically-resistant enamel in the same

individual. Also, because there are consistent differences in carbon isotope value between hydroxylapatite and collagen within an individual (Krueger & Sullivan, 1984; Lee-Thorp *et al.*, 1989; Bocherens & Mariotti, 1992), and because we analysed only specimens with well-preserved collagen (Tuross *et al.*, 1988), we can use the apatite-collagen carbon isotope difference as a measure of hydroxylapatite alteration. Finally, having identified specimens with altered hydroxylapatite, we examine X-ray diffraction patterns from a subset of samples to search for crystallographic changes that could be used to diagnose isotopic alteration.

Materials and Methods

Samples

Treatment experiments were performed on tooth enamel from a modern elephant and on bone from a modern impala. X-ray diffraction patterns were analysed from a suite of modern bone and dentin samples. Long term preservation was examined in four groups of fossils: Holocene humans from Sully, SD ($\approx$500 BP), Chemochechobee, GA ($\approx$1000 BP), and the Tennessee Valley (Eva, Cherry, Ledbetter) (3000–5000 BP), and late Pleistocene mastodons and mammoths from the Great Lakes area (radiocarbon dates on four specimens span from $\approx$10,400 to 11,850 BP) (Koch, 1989). All samples, except the Holocene human bones, were collected as fine powders by drilling. For analysis of structural carbonate, pieces of Holocene human bones (femoral diaphyses) were powdered under liquid nitrogen in a Spex mill. To obtain enamel that formed after weaning, samples were collected from as far back in the tooth row as possible (M2 or M3 for humans; M1, M2, or M3 for proboscideans). Dentin from fossil proboscideans was collected adjacent to the pulp cavity; this material formed in the last few years of life.

Treatment experiments

We tested the effects of 2% NaOCl and 30% H_2O_2, two solutions used to oxidize organic matter, and NaOCl followed by either 0·1 M acetic acid (pH$\approx$2·9), 1·0 M acetic acid (pH$\approx$2·4), or 1 M acetic acid-calcium acetate buffer (pH$\approx$4·5), which are used to remove diagenetic carbonates. Enamel powders were soaked for 1 day and bone powders for 3 days in 2% NaOCl, using 0·04 ml solution/mg sample, rinsed five times with excess distilled/deionized water, then lyophilized. Enamel powders were soaked for 1 day in 30% H_2O_2, then rinsed and lyophilized. Samples receiving acid or buffer treatments were first treated with NaOCl, rinsed five times with water, then soaked in either 0·1 M acetic acid for 3 days or 1 M acetic acid or 1 M acetate buffer for 24 h, again using 0·04 ml solution/mg sample. Following acid or acetate buffer treatment, samples were rinsed five times, then lyophilized. To establish that treatment removed all non-structural carbonate, we tested acidic treatments on mixtures of enamel and calcite. A 9:1 enamel:calcite mixture was treated with 0·1 M acetic acid and 1 M acetate buffer, whereas 1 M acetic acid was tested on a 1:1 enamel:calcite mixture.

The potential for isotopic exchange in treatment was examined by performing a set of experiments using ^{18}O-enriched water ($\delta^{18}O_{SMOW} = +40‰$). An enriched 2% NaOCl solution was prepared by 50:50 dilution of enriched water and reagent-grade 4% NaOCl stock. Likewise, we prepared dilutions of 0·1 M and 1 M acetic acid solutions by mixing 100% acetic acid with +40‰ water. Therefore acid solutions had $\delta^{18}O$ values of 40‰, whereas the $\delta^{18}O$ value of the NaOCl solution, while not measured, was probably $\geq +20‰$. Samples were rinsed with unenriched distilled/deionized water, which had a $\delta^{18}O$ of $\approx -6‰$.

Preparation of fossil samples

Fossil bone and dentin were reacted for 3 days in 2% NaOCl, rinsed five times with water, soaked for 3 days in 0·1 M acetic acid, rinsed five times with water, then lyophilized. A subset of human bones was then treated with 1 M acetic acid, rinsed, and lyophilized. Treatment of fossil enamel hydroxylapatite was similar, except samples were reacted in 2% NaOCl for 1 rather than 3 days. Holocene human collagen was extracted from bone chunks by demineralization in 0·5 M EDTA, pH 7·2, at 4°C for $\approx$7 days (Tuross *et al.*, 1988). After demineralization a pale yellow collagen replica was obtained, which was washed 15 times in distilled/deionized water, then lyophilized. To obtain collagen from proboscidean dentin, powders were decalcified using 0·5 M HCl, rinsed five times with water, then lyophilized.

Stable isotope and weight percent carbonate analysis

To obtain CO_2 from collagen for carbon isotope analysis, $\approx$3 mg samples were sealed in preheated, evacuated quartz tubes with CuO and Cu metal, combusted at 900°C for 1 h, then cooled at a controlled rate; the CO_2 was isolated cryogenically (Tuross *et al.*, 1988). To release structural carbonate as CO_2, hydroxylapatite was dissolved in 100% phosphoric acid. Hydroxylapatite from all the fossils and the modern bones was reacted for 5 h at 50°C in evacuated vessels, then the CO_2 was isolated cryogenically (Koch *et al.*, 1990). The isotope compositions of CO_2 from collagen and hydroxylapatite from all fossils and the modern bones were measures on a Nuclide 6-60 RMS or a Finnigan MAT 252 mass spectrometer at the Geophysical Lab. Isotopic analysis of modern tooth enamel was conducted using an ISOCARB automated carbonate system attached to a VG OPTIMA isotope ratio monitoring mass spectrometer in the stable isotope geochemistry laboratory at Princeton University. Briefly, $\approx$10 mg of hydroxylapatite was dissolved in an

evacuated vessel containing constantly stirred 100% phosphoric acid at 90°C. Samples were reacted for 10 min, with continuous trapping of evolved CO_2 and H_2O. Following reaction, the CO_2 was admitted to the source of the mass spectrometer and analysed while the reaction vessel was constantly stirred under vacuum for 5 min prior reaction of the next sample.

Oxygen and carbon isotopes are reported as $\delta = [(R_{sample}/R_{standard}) - 1] \times 1000$ where $R = {}^{13}C/{}^{12}C$ or ${}^{18}O/{}^{16}O$, and the standards are PDB for carbon and SMOW for oxygen. To calculate a $\delta^{18}O$ value for structural carbonate from the $\delta^{18}O$ value of CO_2 gas produced by acid dissolution, we used the oxygen isotope fractionations characteristic for calcite dissolution at 50° and 90°C (Koch *et al.*, 1990). Standard deviations (1σ) for replicate analyses of organic standards were ± 0·1‰ for $\delta^{13}C$. No standards exist for carbonate in hydroxylapatite, but deviations for modern elephant enamel were <0·1‰ for $\delta^{13}C$ and $\delta^{18}O$ for samples analysed with the ISOCARB system. S.D.s were ± 0·15‰ for carbon and oxygen isotopes in calcite reacted in evacuated vessels at 50°C.

To estimate weight percent carbonate for modern enamel hydroxylapatite [(weight CO_3/weight sample) × 100], we monitored the amount of CO_2 generated from samples with a pressure transducer on the VG OPTIMA. The S.D. for weight percent carbonate from elephant enamel was ± 0·05%.

X-ray diffraction analysis

The purity and crystallinity of fossil bone and dentin hydroxylapatite were monitored through X-ray diffraction analysis of 10 human bones and 12 proboscidean dentin samples. Patterns from fossils were compared to bone and dentin patterns from recent mammals. Modern comparison samples received the same treatment as fossils (NaOCl followed by 0·1 M acetic acid). Samples were ground with an agate mortar and pestle to a fine powder, then transferred to a quartz plate with acetone. Diffraction patterns were measured on a Philips PW-1720 diffractometer at the Conservation Analytical Laboratory using graphite-monochromatized Cu Kα radiation. Samples were scanned from 2° to 90° 2θ with a step size of 0·02 2θ and a counting time of 1 s.

Isotopic Effects of Sample Treatment

Results of treatment experiments

Differences in isotope value among replicate analyses of untreated enamel and bone were low (Table 1). To visualize the effects of treatment on enamel or bone, we plot isotope difference values ($\Delta^{18}O_{ptr\text{-}utr}$ versus $\Delta^{13}C_{ptr\text{-}utr}$, defined in Table 1) in Figures 1(a) & (b). NaOCl and H_2O_2 treatment did not significantly affect the isotope values of enamel (Figure 1(a)), but in bone, NaOCl produced substantial variability and shifted mean isotope values relative to untreated samples (Table 1; Figure 1(b)). NaOCl treatment followed by either 0·1 M or 1 M acetic acid or 1 M acetate buffer increased $\delta^{18}O$ values and decreased $\delta^{13}C$ values for both enamel and bone (Table 1; Figure 1(a) & (b)). The magnitude of these effects was greatest for 1 M acetic acid and least for acetate buffer, and was greater for bone than enamel (Table 1; Figure 1(a) & (b)). Finally, bone treated with ${}^{18}O$-enriched NaOCl followed by 0·1 M acetic acid had a $\delta^{18}O$ value lower than samples receiving non-enriched 0·1 M acetic acid treatment, whereas bone treated with ${}^{18}O$-enriched NaOCl followed by 1 M acetic acid had a $\delta^{18}O$ value 1‰ higher than any other sample (Figure 1(b)).

The calcite mixed with the enamel had $\delta^{13}C$ and $\delta^{18}O$ values of − 25·6‰ and 14·9‰, respectively (Table 1). Enamel/calcite mixtures treated with NaOCl followed by 0·1 M or 1 M acetic acid or acetate buffer had $\delta^{18}O$ values intermediate between those for untreated enamel and values for enamel receiving these treatments. For example, enamel treated with NaOCl followed by 1 M acetic acid had a mean $\delta^{18}O$ value of 28·1‰, whereas the enamel/calcite mixture receiving this treatment had a value of 27·5‰, while untreated enamel averaged 26·5‰. Carbon isotope values did not differ significantly between pure enamel and enamel/calcite mixtures for these treatments.

A possible explanation of the lower $\delta^{18}O$ values of enamel/calcite mixtures is incomplete removal of calcite. If we assume, for the moment, that the lower $\delta^{18}O$ values of enamel/calcite mixtures were due to end member mixing between pure treated enamel and calcite, we would estimate that 5% or less of the oxygen in the treated mixtures was derived from calcite (5% for 1 M acetic acid, 4% for 0·1 M acetic acid, and 2% for 1 M acetate buffer). However, because the calcite had a $\delta^{13}C$ value ≈14‰ lower than that for enamel, incomplete removal of calcite should impact carbon isotope values as strongly as it does oxygen isotope values. The fact that the $\delta^{13}C$ values of treated enamel/calcite mixtures were not lower than $\delta^{13}C$ values for pure treated enamel indicates that incomplete removal of calcite **was not** the cause of lower $\delta^{18}O$ values. We consider other potential sources for lower $\delta^{18}O$ values below.

Weight percent carbonate for untreated enamel was 3·8 ± 0·1% (Table 1), in the range expected for structural carbonate in biologic hydroxylapatite (LeGeros & LeGeros, 1984). The percent carbonate values for different treatments with acetic acid or buffer ranged from 3·6 to 3·7% (Table 1). In general, percent carbonate values overlapped among all NaOCl, H_2O_2, and acid-treated samples, including the enamel/calcite mixtures, and there was no association between carbonate yield and the magnitude of isotopic offset for samples.

Samples used in treatment tests were sacrificed to isotopic analysis, so examination of these samples by X-ray diffraction was precluded. We subjected a set

Table 1. Comparison of pretreatments effects on enamel, enamel/calcite mixtures, and bone

Treatment	$\delta^{13}C$	$\delta^{18}O$	$\Delta^{13}C$ ptr-utr	$\Delta^{18}O$ ptr-utr	% CO_3
Enamel					
Untreated	−11·80	26·46			3·86
Untreated	−11·71	26·46			3·88
Untreated	−11·62	26·51			3·61
Untreated	−11·58	26·58			3·97
Average ± 1σ	−11·68 ± 0·09	26·50 ± 0·05			3·83 ± 0·13
2% NaOCl	−11·47	26·45	0·21	−0·05	3·88
2% NaOCl	−11·40	26·57	0·28	0·07	4·04
2% NaOCl	−11·68	26·53	−0·00	0·03	3·91
2% NaOCl	−11·63	26·54	0·05	0·04	3·98
Average ± 1σ	−11·55 ± 0·11	26·52 ± 0·04	0·13	0·02	3·95 ± 0·06
30% H_2O_2	−11·65	26·78	0·02	0·28	4·03
30% H_2O_2	−11·67	26·61	0·01	0·11	3·82
Average	−11·66	26·70	0·02	0·19	3·93
NaOCl→1 M Buffer	−11·79	26·95	−0·12	0·45	3·77
NaOCl→1 M Buffer	−11·83	26·96	−0·15	0·46	3·83
NaOCl→1 M Buffer	−11·81	27·02	−0·13	0·52	3·84
NaOCl→1 M Buffer	−11·77	27·01	−0·09	0·51	3·82
NaOCl→1 M Buffer	−11·79	26·87	−0·11	0·37	3·69
NaOCl→1 M Buffer	−11·76	27·00	−0·08	0·50	3·58
NaOCl→1 M Buffer	−11·76	27·18	−0·09	0·68	3·43
NaOCl→1 M Buffer	−11·77	27·27	−0·09	0·77	3·51
Average ± 1σ	−11·79 ± 0·02	27·03 ± 0·12	−0·11	0·53	3·68 ± 0·15
NaOCl→0·1 M Acetic	−11·87	27·38	−0·19	0·87	3·71
NaOCl→0·1 M Acetic	−11·79	27·52	−0·12	1·02	3·74
NaOCl→0·1 M Acetic	−11·91	27·39	−0·24	0·89	3·54
NaOCl→0·1 M Acetic	−11·88	27·40	−0·20	0·90	3·43
Average ± 1σ	−11·86 ± 0·04	27·42 ± 0·06	−0·19	0·92	3·61 ± 0·13
NaOCl→1 M Acetic	−11·95	28·06	−0·28	1·56	3·68
NaOCl→1 M Acetic	−11·94	28·01	−0·27	1·51	3·72
NaOCl→1 M Acetic	−11·93	28·04	−0·25	1·53	3·64
NaOCl→1 M Acetic	−11·96	28·16	−0·28	1·66	3·72
Average ± 1σ	−11·95 ± 0·01	28·07 ± 0·06	−0·27	1·57	3·69 ± 0·03
Enamel/Calcite Mixtures					
NaOCl→1 M Buffer	−11·81	26·68	−0·13	0·18	3·77
NaOCl→1 M Buffer	−11·82	29·69	−0·14	0·18	3·81
Average	−11·82	26·69	−0·14	0·18	3·79
NaOCl→0·1 M Acetic	−11·87	27·00	−0·19	0·49	3·54
NaOCl→0·1 M Acetic	−11·85	26·93	−0·17	0·42	3·73
Average	−11·86	26·97	−0·18	0·46	3·64
NaOCl→1 M Acetic	−12·01	27·49	−0·34	0·99	3·82
Calcite: average	−25·61	14·94			
Bone					
Untreated	−7·71	29·82			nd
Untreated	−7·71	30·42			nd
Untreated	−7·70	30·24			nd
Average ± 1σ	−7·71 ± 0·01	30·16 ± 0·25			
2% NaOCl	−7·98	31·62	−0·27	1·46	nd
2% NAOCl	−8·00	30·60	−0·29	0·44	nd
2% NaOCl	−7·61	32·73	0·10	2·57	nd
Average ± 1σ	−7·86 ± 0·18	31·65 ± 0·87	−0·16	1·49	
NaOCl→1 M Acetate/Acetic Buffer	−8·00	31·78	−0·29	1·62	nd
NaOCl→1 M Acetate/Acetic Buffer	−7·92	32·08	−0·21	1·92	nd
Average	−7·96	31·93	−0·25	1·77	
NaOCl→0·1 M Acetic	−8·43	31·92	−0·72	1·76	nd
NaOCl→0·1 M Acetic	−8·00	32·62	−0·29	2·46	nd
Average	−8·22	32·27	−0·51	2·11	
NaOCl→1 M Acetic	−8·57	31·70	−0·86	1·54	nd
NaOCl→1 M Acetic	−8·51	32·54	−0·80	2·38	nd
NaOCl→1 M Acetic	−8·49	32·76	−0·78	2·60	nd
Average ± 1σ	−8·52	32·33	−0·82	2·17	
NAOCl→0·1 M Acetic-^{18}O Spike	−7·96	31·42	−0·25	1·26	nd
NaOCl→1 M Acetic-^{18}O Spike	−7·93	33·66	−0·22	3·50	nd

$\Delta^{18}O_{ptr\text{-}utr} = \delta^{18}O_{pretreated\ sample} - \delta^{18}O_{untreated\ sample}$.
$\Delta^{13}C_{ptr\text{-}utr}$ is calculated similarly.
nd = not determined.

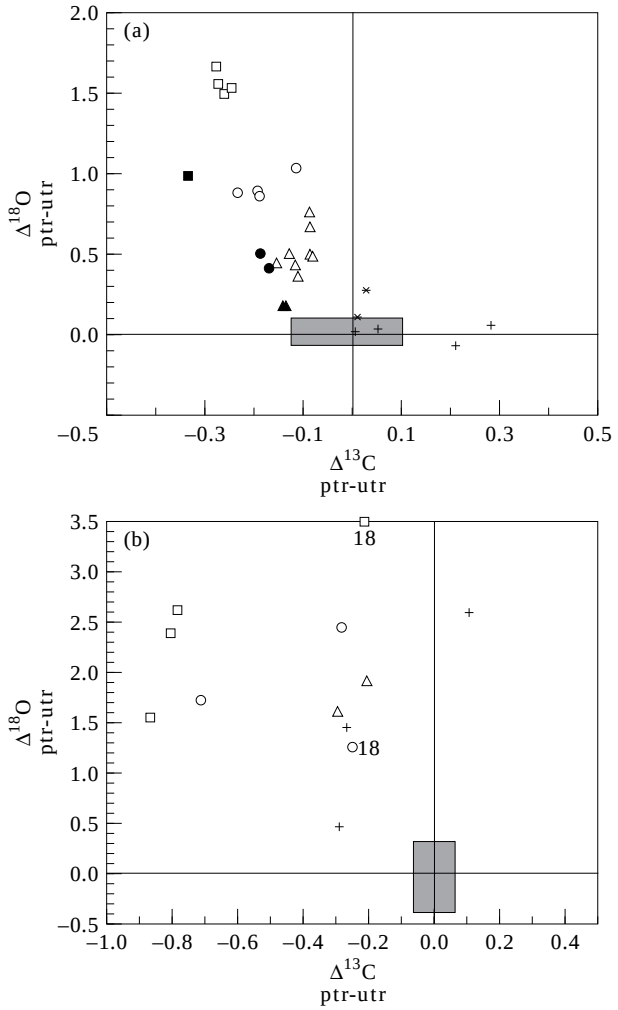

Figure 1. (a) Plot of isotope difference values between untreated modern elephant tooth enamel and enamel receiving different treatments. (b) Plot of isotope difference values between untreated modern impala bone and bone receiving different treatments. Stippled boxes represent range of values for untreated samples. +: NaOCl; × : H_2O_2; ○: NaOCl/0·1 M acetic acid; □: NaOCl/1 M acetic acid; △: NaOCl/1 M acetic acid/calcium acetate buffer; ●: NaOCl/0·1 M acetic acid on 9:1 enamel/calcite mixture; ■: NaOCl/1 M acetic acid on 9:1 enamel/calcite mixture; ▲: NaOCl/1 M acetic acid/calcium acetate buffer on 1:1 enamel/calcite mixture; ○: NaOCl/0·1 M acetic acid with ^{18}O-enriched solutions; □: NaOCl/1 M acetic acid with ^{18}O-enriched solutions.

of modern samples (five dentin, five bone) to treatment with NaOCl followed by 0·1 M acetic acid for diffraction analysis of structural changes (Figure 2 (a–c)). Table 2 presents parameters derived from diffraction patterns. The diffraction patterns had narrower peaks and greater peak resolution then expected for typical bone hydroxylapatite (e.g. Figure 2(a–b)) (LeGeros & LeGeros, 1984; Sillen, 1989), perhaps due to minor recrystallization or loss of poorly crystalline fractions during treatment or post-mortem exposure and weathering (Tuross *et al.*, 1989). Several modern dentin samples had substantial amounts of brushite ($CaHPO_4{\cdot}2H_20$) (Figure 2(c); Table 2). No modern bone sample had detectable brushite.

The source of isotopic artefacts during sample treatment

Treatment with NaOCl and H_2O_2 did not affect the isotope values of enamel, whereas treatment with NaOCl did alter the isotope values of bone. However bone is approximately one-third organic, thus the shifts induced by NaOCl may reflect removal of actual contaminants. Once collagen was removed from bone, acetic acid or acetate buffer treatment caused $\delta^{18}O$ values to increase and $\delta^{13}C$ values to decrease by values similar to those observed for tooth enamel receiving these treatments. Lee-Thorp & van der Merwe (1991) also observed decreases in $\delta^{13}C$ value when anorganic bone was treated with 1 M acetic acid, and Quade *et al.* (1992) demonstrated that fossil enamel treated with 1 M acetic acid had higher $\delta^{18}O$ values than enamel treated with 0·1 M acetic acid. Thus the pattern of increasing oxygen isotope values and decreasing carbon isotope values following treatment with solutions of lower pH is a general phenomenon.

There are three non-exclusive explanations for the trends induced by pretreatment. Perhaps isotopic trends are caused by recrystallization in pretreatment solutions. Prolonged immersion in 1 M acetic acid, or more brief immersion in more concentrated acids, promotes recrystallization of biological hydroxylapatite to brushite (Lee-Thorp & van der Merwe, 1991; Sillen & Sealy, 1995). Because brushite contains water derived from treatment solutions, recrystallization provides an isotope exchange mechanism that might explain the offsets in $\delta^{18}O$ value, particularly in bones treated with ^{18}O-enriched solutions. Yet brushite contains no carbon, so its formation cannot alter $\delta^{13}C$ values. Furthermore, none of the modern or fossil bones contained brushite (Table 2), suggesting it was not a major factor in treatment alteration.

A second possibility is that biological hydroxylapatites are mixtures of fractions that differ in solubility, and that these different solubility fractions are isolated by treatment with acids of different molarity (Sillen, 1989). For example, hydroxylapatite that is rich in carbonate is poorly crystalline and more soluble in acidic solutions than carbonate-poor hydroxylapatite or fluorapatite (LeGeros & Tung, 1983; Nelson *et al.*, 1983). Associating isotopic differences with different solubility fractions is difficult, however. Such differences might occur if an animal shifted its diet or habitat during tooth or bone formation. Yet similar isotopic trends have been found in a wide range of modern and fossil taxa from different geographic regions (Lee-Thorp & van der Merwe, 1991; Quade *et al.*, 1992; this study), indicating that the explanation must be independent of animal ecology.

Finally, biological hydroxylapatites contain structural carbonate in phosphate and hydroxyl lattice sites

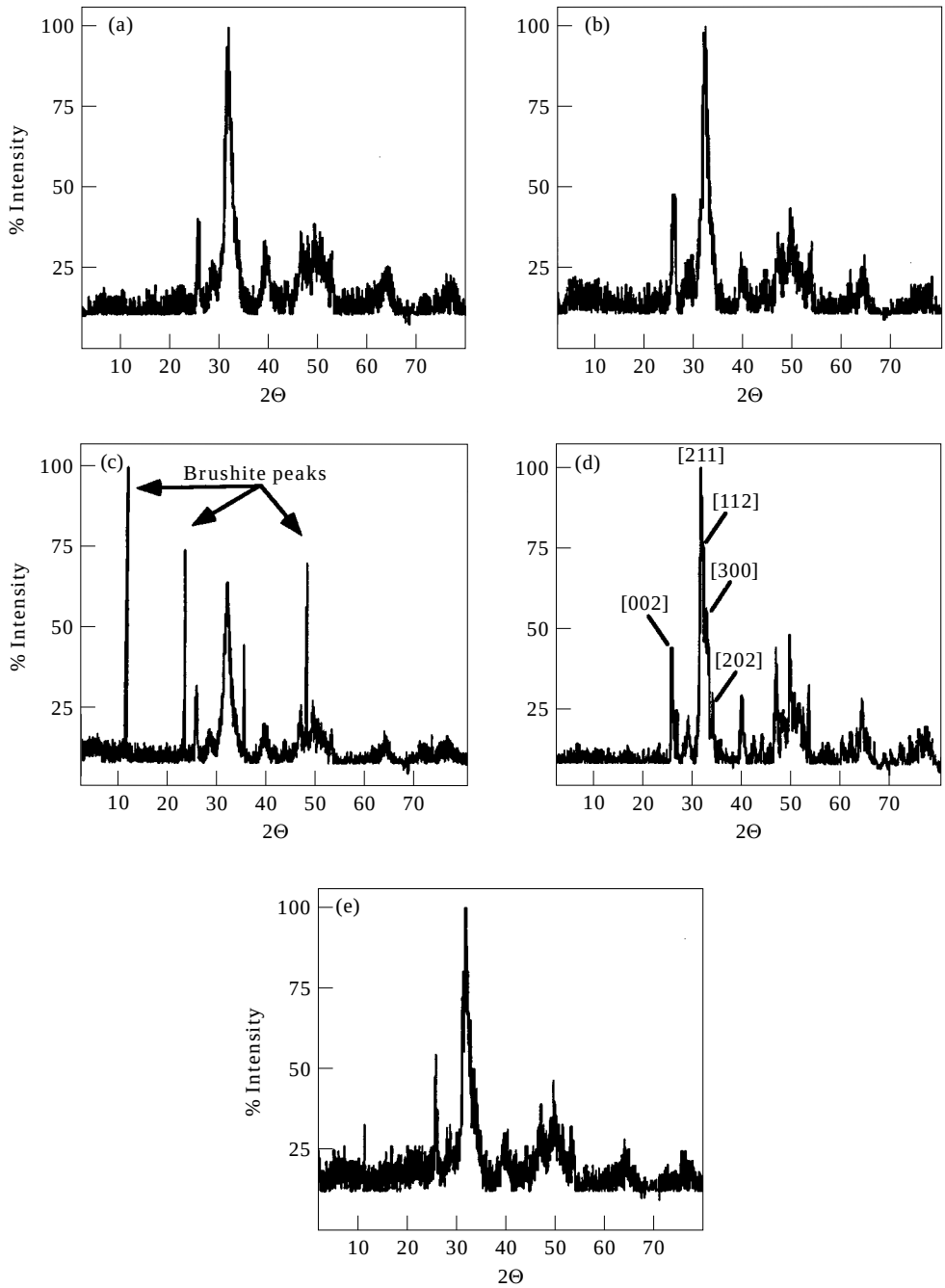

Figure 2. X-ray diffraction patterns for a suite of modern and fossil bones and dentin all of which had received NaOCl/0·1 M acetic acid treatment. (a) Modern bone from juvenile wildebeeste, (b) modern African elephant molar dentin, (c) modern hippopotamus molar dentin with substantial brushite contamination, (d) bone form Archaic human at Eva site (12/54), note increased peak separation relative to modern bone, (e) dentin from Late Pleistocene Chittinango mammoth.

(LeGeros *et al.*, 1969; LeGeros, 1981; Beshah *et al.*, 1990). Carbonate moieties in these sites are involved in different chemical bonds. As isotope fractionations in minerals are ultimately related to mass dependent differences in bond energy, it is plausible to assume that these sites have different isotope fractionations. If partial dissolution by acidic solutions changes the proportions of carbonate in each site it could induce isotope effects that are consistent across taxa, regardless of geography or ecology. For example, during dissolution, the more weakly bonded site may be more susceptible to removal, leading to a disproportionate representation of either hydroxyl or phosphate site carbonate on the mineral surface. Because the crystallites of bone and dentin are so small (e.g. only a few unit cells wide), they present a large, potentially

Table 2. X-ray diffraction parameters for modern and fossil mammals compared to a monitor of isotopic alteration

Specimen	Sample type	Brushite	Peak sep.	[002] FWHM	[002] Height	$\Delta^{13}C$ bn or dn-en
Modern mammals						
Wildebeeste	B	A	L	0·26	37	
Impala	B	A	M	0·25	46	
Zebra	B	A	L	0·27	41	
Elephant	B	A	L	0·27	32	
Elephant	B	A	L	0·28	41	
Elephant	TD	P	L	0·22	73	
Elephant	TD	P	L	0·13	64	
Hippopotamus	MD	P	L	0·29	48	
Elephant	MD	A	L	0·38	46	
Buffalo	MD	A	L	0·33	48	
Archaic humans						
LD 25/85	B	A	M	0·27	42	− 1*
CH 74/8	B	A	H	0·24	47	+3*
CH 74/36	B	A	H	0·26	44	+4*
EV 12/44	B	A	H	0·24	52	+2
EV 12/51	B	A	H	0·26	45	− 5*
EV 12/54	B	A	H	0·25	46	+2
EV 12/66	B	A	H	0·22	44	0
EV 12/74	B	A	H	0·24	44	+2*
EV 12/118	B	A	H	0·30	42	+2*
EV 12/184	B	A	M	0·26	44	− 3*
Pleistocene proboscideans						
UMMP 14404	MD	A	L	0·28	46	+1
UMMP 22798	TD	A	L	0·28	58	+14
UMMP 24240	MD	A	L	0·29	47	+3
UMMP 54910	MD	A	L	0·24	58	+1*
UMMP 57648	TD	A	L	0·28	55	+7
UMMP 57705	TD	A	L	0·34	48	+5
UMMP 59936	MD	A	L	0·19	66	− 1
UMMP Heisler	TD	A	L	0·26	54	+11
MSU Sheathelm	TD	A	L	0·24	65	+7
MSU Sheathelm	MD	A	L	0·33	48	+10
NYSM V45	TD	A	L	0·22	49	+4*
WMU Powers	TD	A	L	0·28	63	0

Specimens—see Tables 3 and 4. Sample type—B, bone; TD, tusk dentin; MD, molar dentin. Brushite—A, absent; P, present. Peak sep., a qualitative measure of distinctness of [211, [112], [300], and [202] reflections—L, low; M, moderate; H, high. [002] FWHM and [002] Height, measures of peak width at half maximum height (in °2θ) and relative peak height (intensity [002]/intensity of highest peak, [112]). $\Delta^{13}C_{\text{bn or dn-en}}$, a measure of carbon isotope alteration in specimen, calculated as $\delta\,^{13}C_{\text{bone or dentin}} - \delta^{13}C_{\text{enamel}}$ for each individual. *Indicates individuals for which enamel values were not determined. For these specimens, carbon deviation was claculated using the mean enamel value for the population (see Tables 3 & 4).

altered, surface area relative to their unaffected crystal cores. If correct, this mechanism predicts an association between the isotope value of apatite and lattice site occupancy that changes consistently during acid attack. These predictions are testable through coupled stable isotope analysis and structural analysis via Fourier-transformed infrared spectroscopy.

Our experiments indicate that treatment with either dilute NaOCl or H_2O_2, followed by either 1 M acetic acid-calcium acetate buffer of 0·1 M acetic acid is recommended. These treatments remove organic matter and secondary and adsorbed carbonates without substantial partial dissolution of hydroxylapatite. Treatment with 1 M acetate buffer induces the smallest isotopic offsets in both bone and enamel. The production of brushite during treatment of some dentin samples with 0·1 M acetic acid is troubling. These modern dentin samples were collected from well-preserved specimens (a zoo elephant and a lightly weathered hippopotamus tooth from Kenya), and brushite was not present in untreated dentin from these specimens. Thus even dilute solutions of acetic acid have a pH low enough to promote recrystallization. For this reason 1 M acetic acid-calcium acetate buffer may be preferred.

Diagenetic Trends in Holocene and Pleistocene Fossils

Results of within individual isotope comparisons

Diagenetic effects on the isotopic composition of dentin hydroxylapatite from proboscideans were profound. Assuming that these animals consumed C_3 plants with a $\delta^{13}C$ value of ≈ -25‰ (O'Leary, 1988), that the diet-apatite carbon isotope fractionation was $\approx +12$‰, and that the diet-collagen carbon isotope fractionation was $\approx +5$‰ (Lee-Thorp *et al.*, 1989),

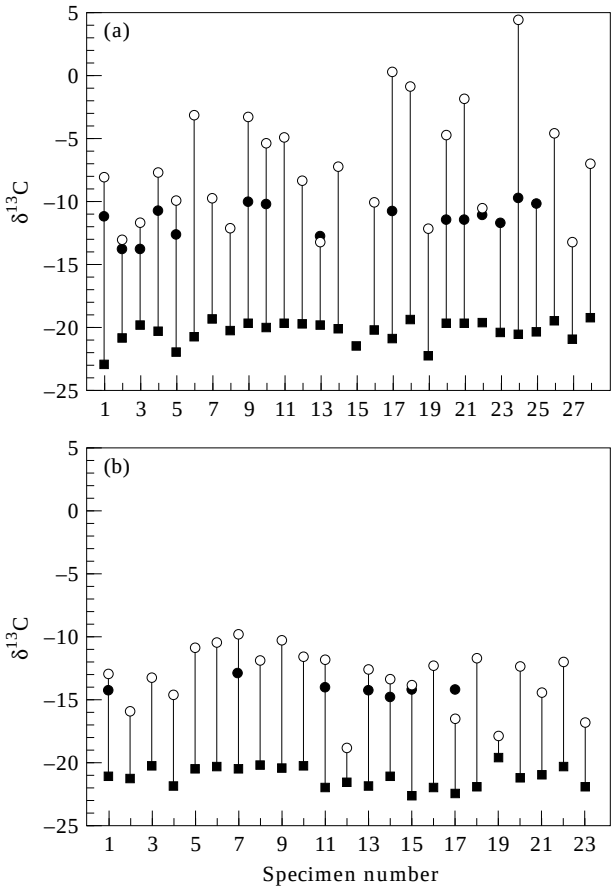

Figure 3. Isotopic values for different carbon-bearing fractions in different individual specimens of (a) dentin from late Pleistocene probosideans (11 Ka) and bone from Archaic humans (~5 Ka). Lines connect different samples from the same specimen. ■: Collagen; ●: enamel hydroxylapatite; ○: dentin or bone hydroxylapatite.

then hydroxylapatite from these animals should average −13‰ and collagen should average −20‰. While collagen and enamel had invariant $\delta^{13}C$ values in this range, values for dentin hydroxylapatite were highly variable and many specimens had excessively high $\delta^{13}C$ values (Figure 3(a); Table 3). Dentin and enamel hydroxylapatite form at approximately the same time in ontogeny, and therefore should have similar isotope values, yet in Pleistocene proboscideans they differed in $\delta^{13}C$ value by 4·5‰, on average, though the $\delta^{18}O$ difference was small (≈0·4‰).

Diagenetic alteration of proboscidean dentin was also indicated by apatite–collagen $\delta^{13}C$ difference values ($\Delta^{13}C_{ap-col}$). In modern herbivores, collagen and hydroxylapatite differ in $\delta^{13}C$ value by 7–9‰ (Lee-Thorp *et al.*, 1989; Bocherens & Mariotti, 1992). Recent experiments indicate that animals consuming lipids, carbohydrates, and proteins that are isotopically distinct can exhibit a wide range of $\Delta^{13}C_{ap-col}$ values (Ambrose & Norr, 1993; Tieszen & Fagre, 1993), but there is no reason to expect such diversity in the diets of late Pleistocene proboscideans. Dentin $\Delta^{13}C_{ap-col}$ values averaged 13·2 ± 4·6‰ (range 6·6–25·0‰), whereas for enamel hydroxylapatite, the mean value of 6·1 ± 1·4‰ (range 6·3–11·8‰) fell in the expected range. The extreme $\Delta^{13}C_{ap-col}$ values for dentin are well outside the range of typical outliers seen in studies of modern faunas. Because collagen $\delta^{13}C$ values were invariant, ranging only ≈2‰, variations in dentin $\Delta^{13}C_{ap-col}$ values must result from altered hydroxylapatite.

Diagenetic effects on human bones were more subtle. On average, Archaic human collagen and enamel $\delta^{13}C$ values were invariant and in the range expected for populations consuming plants and meat from a 100% C_3 foodweb (Figure 3(b); Table 4). Several Archaic human bones had unreasonably low hydroxylapatite $\delta^{13}C$ values (e.g. 25/23, 12/51, 12/114, 12/184) and many specimens had higher $\delta^{13}C$ values for bone than the average for diagenetically resistant enamel (≈ −14‰) (Table 4). On average, $\delta^{13}C$ and $\delta^{18}O$ values for bone hydroxylapatite were only ≈1‰ higher than those for enamel, but the data from bones were more variable (Table 4). Is it possible that the high variability in bone hydroxylapatite from Archaic humans is a true reflection of diet, perhaps due to changes in diet during life or differences among individuals, and is not a product of diagenesis? Enamel mineralization occurs during a relatively short period early in an animal's life, whereas bone is deposited and remodelled throughout life. If diet is changing during life or is variable among individuals, it should be most obvious in enamel. Such differences would be partially erased and averaged in bone by remodelling throughout life. The fact that different Archaic individuals had the same enamel $\delta^{13}C$ value, but differed widely for bone hydroxylapatite $\delta^{13}C$ is evidence of bone diagenesis, not differences in diet.

The Woodland populations (Chemochechobee, GA; Sully, SD) had $\delta^{13}C$ values for collagen and bone hydroxylapatite in the range expected for humans that had C_4 plants (mean $\delta^{13}C$ ≈ −12‰; O'Leary, 1988), such as corn, in their diets (Table 4). The variability of $\delta^{13}C$ values from Woodland collagen and bone hydroxylapatite were similar and somewhat higher than observed at the Archaic sites. In the Woodland sites, high variability in $\delta^{13}C$ values most likely reflects true isotopic heterogeneity in the diet. Because $\Delta^{13}C_{ap-col}$ values are affected by isotopic differences in different biochemical fractions of diet and by trophic level (Krueger & Sullivan, 1984; Lee-Thorp *et al.*, 1989; Ambrose & Norr, 1993; Tieszen & Fagre, 1993), they cannot be used to diagnose alteration in omnivorous species, such as humans, with complex diets containing a mix of C_3 and C_4 foods.

To exclude the possibility that the high isotopic variability and unusual values observed in bone and dentin reflect incomplete removal of secondary carbonates, a subset of human bones was treated with 1 M acetic acid. Seven of eight samples exhibited isotopic trends identical to those seen in treatment experiments

Table 3. C and O isotope values for collagen, dentin and enamel from Late Pleistocene mastodons and mammoths from the southern Great Lakes

Specimen number	Sample type	$\delta^{13}C$ collagen	$\delta^{13}C$ dentin	$\delta^{18}O$ dentin	$\delta^{13}C$ enamel	$\delta^{18}O$ enamel
Mastodons						
UMMP 8394	M	−22·8	−7·9	24·1	−11·0	23·0
UMMP 14404	M	−20·8	−12·9	24·7	−13·6	23·9
UMMP 11733	M	−19·8	−11·6	23·0	−13·5	22·6
UMMP 24240	M	−20·1	−7·5	21·9	−10·7	24·3
UMMP 24836	M	−21·8	−9·7	22·2	−12·4	22·6
UMMP 34302	T	−20·8	−2·8	23·5	nd	nd
UMMP 37811	T	−19·2	−9·7	22·8	nd	nd
UMMP 54910	M	−20·1	−12·2	22·3	nd	nd
UMMP 57648	T&M	−19·6	−3·2	24·5	−9·9	23·5
UMMP 57705	T	−19·9	−5·3	21·6	−10·1	24·1
UMMP 58028	T	−19·4	−4·8	22·8	nd	nd
UMMP 58075	M	−19·6	−8·1	21·1	nd	nd
UMMP 59936	M	−19·7	−13·1	22·4	−12·4	22·9
UMMP 61246	T	−19·9	−7·0	23·7	nd	nd
UMMP Burning Tree	T	−21·3	nd	nd	nd	nd
UMMP Cole	M	−20·1	−9·9	22·1	nd	nd
UMMP Heisler	T	−20·7	0·4	25·2	−10·6	23·4
ALMA Parker	T	−19·3	−0·7	24·2	nd	nd
BMS E26274	T	−22·1	−12·0	23·7	nd	nd
MSU Sheathelm	M	−19·6	−4·6	23·3	−11·2	22·6
MSU Sheathelm	T	−19·6	−1·7	24·9	nd	nd
WMU Powers	T	−19·4	−10·3	21·7	−10·6	22·0
Mastodons		−20·2	−7·4	23·1	−11·4	23·2
Mean ± 1σ		±1·0	±4·2	±1·2	±1·3	±0·7
Mammoths						
UMMP 11732	M	−20·2	nd	nd	−11·6	25·4
UMMP 22798	T&B	−20·3	4·6	25·8	−9·6	21·0
UMMP 44381	M	−20·2	nd	nd	−10·0	22·7
MOTT CC	T	−19·5	−4·4	20·8	nd	nd
NYSM V26	T	−20·6	−13·0	20·3	nd	nd
NYSM V45	T	−19·0	−6·7	21·2	nd	nd
Mammoths		−20·0	−4·9	22·0	−10·4	23·0
Mean ± 1σ		±0·6	±7·3	±2·5	±1·1	±2·2
All Specimens		−20·2	−7·0	22·9	−11·2	23·1
Mean ± 1σ		±0·9	±4·7	±1·5	±1·3	±1·1

ALMA, Alma College; BMS, Buffalo Museum of Science; MOTT, Mott Community College; MSU, Michigan State University; NYSM, New York State Museum; UMMP, University of Michigan Museum of Paleontology; WMU, Western Michigan University. B, bone; T, tusk; M, molar.

on modern bone; $\delta^{13}C$ values dropped by ≈1·5‰, while $\delta^{18}O$ values increased by ≈2·5‰ (Table 4). In several cases, treatment shifted the $\delta^{13}C$ value of bone to match that expected from enamel or collagen from the same specimen (e.g. 25/12, 74/14, 12/57). In an equal number of cases, 1 M acetic acid treatment did not shift bone $\delta^{13}C$ values toward predicted values (e.g. 25/23, 12/92, 12/114). When $\delta^{18}O$ values from 0·1 M and 1M acetic acid treated bone were compared to those from enamel (e.g. 25/12, 74/14, 12/57, 12/92), 1 M treated bones were consistently shifted away from "correct" enamel values. Overall, treatment with 1 M acetic acid did not improve the isotopic results for bone or dentin.

Qualitatively, X-ray diffraction patterns from fossils and modern bone or dentin were similar, however none of the fossils contained brushite (Table 2; Figure 2(d) & (e)). Changes in the relative height or width of reflections (e.g. [002] or [310]) have been used to monitor hydroxylapatite crystallinity (Bonar *et al.*, 1983; Tuross *et al.*, 1989; Hedges, Millard & Pike, 1995). We discovered no clear differences in [002] height or width, either between modern and fossil specimens, or between isotopically altered and unaltered fossils (Table 2). One qualitative difference did emerge between modern and Archaic human bones; the [211], [112], [300], and [202] reflection were distinct in fossils, whereas in modern bones, the peaks merged into one broad peak (Figure 2(a) & (d)). However this peak sharpening, which denotes an increase in crystallinity (Bartsiokas & Middleton, 1992; Person *et al.*, 1995), occurred in all fossil human bones, not just in bones with altered isotope values.

The source of diagenetic trends in fossil bone and dentin

We discovered diagenetic effects on the isotopic composition of structural carbonate from Holocene and Pleistocene samples that were similar in direction to those found in earlier studies (Lee-Thorp & van der

Table 4. C and O isotope values for human collagen, enamel, and bone

Specimen numbers	$\delta^{13}C$ collagen	$\delta^{13}C$ bone 0·1 M acetic	$\delta^{18}O$ bone 0·1 M acetic	$\delta^{13}C$ bone 1 M acetic	$\delta^{18}O$ bone 1 M acetic	$\delta^{13}C$ enamel	$\delta^{18}O$ enamel
Archaic sites—no corn in diet							
Ledbetter							
25/12	−21·2	−13·0	26·0	−14·0	27·6	−14·3	24·2
25/23	−21·2	−16·1	23·9	−16·0	25·7	nd	nd
25/61	−20·4	−13·5	26·3	nd	nd	nd	nd
25/85	−21·7	−14·6	23·8	nd	nd	nd	nd
Cherry							
74/08	−20·5	−10·9	26·4	nd	nd	nd	nd
74/10	−20·3	−10·5	25·6	nd	nd	nd	nd
74/14	−20·5	−9·8	25·4	−12·6	27·1	−13·0	25·2
74/16	−20·2	−11·8	26·5	nd	nd	nd	nd
74/36	−20·3	−10·4	26·7	nd	nd	nd	nd
Eva							
12/42	−20·2	−11·5	24·4	nd	nd	nd	nd
12/44	−21·9	−11·8	26·9	nd	nd	−14·0	24·6
12/51	−21·5	−18·8	21·4	nd	nd	nd	nd
12/54	−21·7	−12·6	26·0	nd	nd	−14·1	24·6
12/57	−21·2	−13·4	25·1	−14·7	25·7	−14·8	24·3
12/66	−22·5	−13·9	25·6	nd	nd	−14·1	24·2
12/74	−21·7	−12·5	27·0	nd	nd	nd	nd
12/92	−22·3	−16·5	24·5	nd	nd	−14·3	24·8
12/101	−21·9	−11·7	25·7	nd	nd	nd	nd
12/114	−19·6	−17·9	22·6	−19·7	24·8	nd	nd
12/118	−21·1	−12·4	26·3	nd	nd	nd	nd
12/136	−20·9	−14·5	25·9	nd	nd	nd	nd
12/170	−20·3	−12·0	23·2	nd	nd	nd	nd
12/184	−21·5	−16·7	28·7	nd	nd	nd	nd
Archaic	−21·1	−13·3	25·4	−15·4	26·2	−14·1	24·6
Mean ± 1σ	±0·8	±2·5	±1·6	±2·4	±1·0	±0·6	±0·3
Woodland sites—corn in diet							
Sully, SD							
381345	−11·8	−8·2	19·4	nd	nd	nd	nd
381352	−13·8	−6·0	18·2	nd	nd	nd	nd
381357	−14·2	−7·8	18·4	nd	nd	nd	nd
381381	−11·2	−4·7	20·3	nd	nd	nd	nd
381408	−11·0	−4·7	20·9	nd	nd	nd	nd
381466	−11·2	−4·3	20·7	nd	nd	nd	nd
Sully	−12·2	−6·0	19·6				
Mean ± 1σ	±1·4	±1·7	±1·2				
Chemochechobee, GA							
31/B20	−9·9	−4·7	25·9	nd	nd	nd	nd
32/B21	−9·8	−5·4	26·1	−5·9	29·2	nd	nd
36/B27	−9·1	−4·3	26·4	nd	nd	nd	nd
45/B14	−13·1	−5·7	26·0	−6·5	27·8	nd	nd
46/B16	−11·5	−5·0	26·0	nd	nd	nd	nd
Chemoche	−10·7	−5·0	26·1				
Mean ± 1σ	±1·6	±0·5	±0·2				

Merwe, 1987; Quade *et al.*, 1992). Fossil bone and dentin hydroxylapatite typically had $\delta^{13}C$ values higher than those predicted by dietary models, and more variable than the $\delta^{13}C$ values of tooth enamel from the same populations. However, Lee-Thorp & van der Merwe (1987) detected only minor differences in isotope composition (<2·5‰) between bone and enamel hydroxylapatite for samples ranging from 65,000 to 100,000 years in age. Similarly, Saliège *et al.* (1995) were able to recover life-time stable carbon isotope and consistent radiocarbon dates from fossil bone. We observed diagenetic isotope shifts in bone and dentin of much greater magnitude on a Pleistocene/Holocene time scale. Clearly, the extent of alteration may vary with depositional environment, depending on the isotope composition of reservoirs that might exchange with the fossil and locally controlled variations in the rate of alteration. Yet the immediate controls on the rate of hydroxylapatite alteration, as well as a complete explanation for the differences in extent of diagenesis between bone and enamel, are unknown.

The cause of isotopic alteration in fossil bone and dentin can be constrained by diffraction data. Fossils lack diffraction peaks indicating contamination by non-apatitic carbonate minerals or recrystallization to brushite (Table 2; Figure 2). Although the crystallinity of some fossil human bones had increased, this recrystallization was not uniformly associated with isotopic alteration. Given these observations, one possible mechanism for isotopic alteration is deposition of carbonate hydroxylapatite after burial. Krueger (1991) has argued that more highly crystalline fluorapatite is the dominant mineral formed in geological settings, and therefore that formation of carbonate hydroxylapatite is not a viable diagenetic mechanism. Yet, carbonate hydroxylapatite does precipitate inorganically *in vitro* (Nancollas *et al.*, 1989), and marine phosphate nodules are composed of relatively poorly crystalline carbonate-rich hydroxylapatite (Shemesh, 1990). Also, hydroxylapatite precipitation in decaying organisms has been inferred in fossils (Martill, 1988) and demonstrated in experiments (Briggs & Kear, 1993). Finally, Hedges *et al.* (1995) argued that "nodular" structures in bones with poor histological preservation were recrystallized hydroxylapatite. Thus the deposition of carbonate hydroxylapatite in fossil bones cannot be ruled out as a diagenetic mechanism.

Conclusions

Our experiments indicate that care must be taken to minimize, or at least render consistent, isotopic offsets produced during pretreatment of samples. These offsets are greatest for oxygen isotopes in bone, and least for carbon isotopes in tooth enamel. These offsets are large for treatment with acids of high molarity (e.g. 1 M acetic acid or higher). Until the source of these large offset is better understood, we suggest using 0·1 M acetic acid or 1 M acetic acid-calcium acetate buffer. If these offsets are a product of the separation of hydroxylapatite with different proportions of carbonate in the available lattice sites, then no treatment gives an unambiguous "right" answer. The key to obtaining consistent results will be extreme consistency in the concentration of solutions used, in the proportion of solution to sample, and in the time for each treatment step.

Comparisons of different carbon- and oxygen-bearing materials from the same individual provide a method for assessing diagenetic alteration in Pleistocene and Holocene specimens. Carbon isotope values from enamel hydroxylapatite and collagen were invariant and gave mutually consistent results. Bone and dentin hydroxylapatite from the same individuals were more variable. In many cases, dentin from fossil proboscideans and bones from Archaic humans had carbon isotope values that were altered, rendering these materials useless for paleodietary analysis. The diagenetic effects on oxygen isotope composition were less profound. Finally, while our results agree with previous studies demonstrating that bone and dentin are relatively unreliable (Lee-Thorp & van der Merwe, 1987, 1991), we discovered that they alter much more rapidly than previously recognized, precluding reliable isotopic determinations even on these relatively young, mid-Holocene materials.

Acknowledgements

We thank H. Bocherens, B. J. Johnson, A. Longinelli, D. P. Schrag and two anonymous reviewers for comments on the manuscript, D. C. Fisher and A. K. Behrensmeyer for access to samples, and C. S. Campell for technical assistance in the X-ray diffraction analysis. This project profited greatly from discussions with A. K. Behrensmeyer, J. A. Lee-Thorp, and A. Sillen. This research was supported by NSF EAR 91-05160, the Carnegie Institution of Washington, the Smithsonian Institution, and Princeton University.

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