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Altered states: Effects of diagenesis on fossil tooth chemistry

MATTHEW J. KOHN,[1,*] MARGARET J. SCHOENINGER,[2] and WILLIAM W. BARKER[3]

[1]Earth and Environmental Sciences and Institute for Geophysics and Planetary Physics, Lawrence Livermore National Laboratory, L-202 Livermore, CA 94550, USA
[2]Department of Anthropology, University of Wisconsin, Madison, WI 53706, USA
[3]Department of Geology and Geophysics, University of Wisconsin, Madison, WI 53706, USA

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Abstract—Investigation of modern and fossil teeth from northern and central Kenya, using the ion microprobe, electron microprobe, and transmission electron microscope, confirms that fossil tooth chemistry is controlled not only by the diagenetic precipitation of secondary minerals but also by the chemical alteration of the biogenic apatite. Increases in the concentrations of Fe, Mn, Si, Al, Ba, and possibly Cu in fossil vs. modern teeth reflect mixtures of apatite and secondary minerals. These secondary minerals occur in concentrations ranging from ~0.3% in enamel to ~5% in dentine and include sub-μm, interstitial Fe-bearing manganite [$(Fe^{3+}, Mn^{3+})O(OH)$], and smectite. The pervasive distribution and fine grain size of the secondary minerals indicate that mixed analyses of primary and secondary material are unavoidable in *in situ* methods, even in ion microprobe spots only 10 μm in diameter, and that bulk chemical analyses are severely biased. Increases in other elements, including the rare earth elements, U, F, and possibly Sr apparently reflect additional alteration of apatite in both dentine and enamel. Extreme care will be required to separate secondary minerals from original biogenic apatite for paleobiological or paleoclimate studies, and nonetheless bulk analyses of purified apatite may be suspect. Although the PO_4 component of teeth seems resistant to chemical alteration, the OH component is extensively altered. This OH alteration implies that bulk analyses of fossil tooth enamel for oxygen isotope composition may be systematically biased by ±1‰, and seasonal records of oxygen isotope composition may be spuriously shifted, enhanced, or diminished. *Copyright © 1999 Elsevier Science Ltd*

1. INTRODUCTION

Teeth and other biogenic phosphatic tissues form the sample basis of many dietary and climate studies. Analysis of modern teeth and bones has shown links between oxygen isotopes and climate (e.g., Longinelli, 1984; Luz et al., 1984, 1990; Ayliffe and Chivas, 1990), carbon isotopes and diet (e.g., Tauber, 1981), trace element content and trophic level (e.g., Toots and Voorhies, 1965; Brown, 1973; Elias et al., 1982), Ca isotopes and trophic level (Skulan et al., 1997), and Sr or Pb isotopes and migration patterns (e.g., Ericson, 1985; Sealy et al., 1991; Price et al., 1994; Koch et al., 1995; Ezzo et al., 1997). Interpretations regarding diagenetic effects on biogenic phosphates are quite contradictory, in part because of the methods employed. In many trace element studies, bulk analysis is applied to a few elements in both modern and fossil materials. Any modern vs. fossil changes are ascribed to diagenesis, and any similarities to diagenetic resistance. Not surprisingly, this approach has led to conflicting conclusions. For example, Lambert et al. (1985) and Byrne and Parris (1987) found no difference in Zn concentrations between modern and fossil bones, and concluded that Zn is resistant to diagenesis, whereas Bocherens et al. (1994) concluded that although enamel may be resistant to Zn alteration, dentine and presumably other porous phosphatic tissues are not. As a further complication, Grupe and Piepenbrink (1989) found that it is nearly impossible to remove fungal material from bones, which can bias Zn analyses and lead to erroneous conclusions. The fidelity of Sr and phosphate oxygen in bone are also strenuously debated (e.g., Nelson et al., 1986 and Tuross et al., 1989 vs. Sillen, 1986 and Sealy et al., 1991; Kolodny et al., 1996 vs. Barrick and Showers, 1994).

A major disadvantage of many studies is that they focus on chemical trends of bulk materials and rarely investigate the mechanisms by which compositions and mineralogy change. The small number of elements commonly analyzed provides little leverage in distinguishing different diagenetic processes, and the use of bulk analyses makes it difficult to eliminate the influence of secondary materials on trace element budgets. Furthermore, although bone has been frequently investigated, little attempt has been made to combine textural, mineralogical, and chemical information for modern and fossil teeth, despite the widespread assumption that tooth enamel is more resistant to diagenetic alteration and is therefore the material of choice for biological or climatological studies.

The purpose of this study is to characterize the textural, mineralogical, and chemical features of fossils from northern Kenya in comparison with modern samples from nearby areas. The main instruments employed were the ion and electron microprobes and the transmission electron microscope. In the investigation of fossil teeth, the ion probe has been used in only one study of marine fish (Grandjean and Albarède, 1989), the electron probe has only rarely been used for imaging and textural interpretations (e.g., Bell et al., 1991), and we know of no TEM studies. The new data highlight the importance of μm to sub-μm scale, intercrystalline, secondary oxyhydroxides in controlling the bulk chemical composition of fossil teeth, yet

Address reprint requests to *Matthew J. Kohn, Department of Geological Sciences, University of South Carolina, Columbia, South Carolina 29208 USA (mjk@geol.sc.edu).

Table 1. Elements in teeth and their uses.

Element	Site	Concentration (ppm)	Uses (C, I)[a]
Ca	Ca	~370,000	Trophic level (I)
Na	Ca	1000's	Diet (C)
Mg	Ca	1000's	Diet (C)
Zn	Ca	100's–1000's	Diet (C)
Sr	Ca	100's–~1000	Diet (C), Migration (I)
Ba	Ca	10's–~100	Diet (C)
REEs	Ca	<1	Diagenesis (C)
U/Pb	Ca	<1	Chronology (I)
O	PO_4, OH, CO_3	~450,000	Climate (I)
C	P, OH	~6000	Diet (I), Climate (I)
H	OH	~2000	Diet (I), Climate (I)
K	Ca	100's–~1000	
P	P	~180,000	
Si	P	10's–100's	
Al	P	<10–~100	
B	P	<10–10's	
Cl	OH	1000's	
F	OH	10's–100's	Diagenesis

[a] C vs. I indicates whether concentration or isotope composition are used. Data from this study: Schoeninger (1979), Lambert et al. (1985), Byrne and Parris (1987), Driessens and Verbeeck (1990), Price et al. (1992), and Ezzo et al. (1997).

demonstrate that chemical alteration of biogenic apatite is also significant. The data have additional implications on the use of fossil teeth in future trace element and isotopic studies.

2. TOOTH STRUCTURE AND CHEMISTRY

Teeth consist of a tough inner core ("dentine" or "dentin"), and an outer brittle carapace ("enamel"). Enamel contains ~96% calcium phosphate (by weight), ~3% water, and ~1% organic matter; dentine is 70% to 75% calcium phosphate, ~20% organic matter, and 5% to 10% water (Driessens and Verbeeck, 1990; Hillson, 1986). Structurally, enamel is extremely compact, with little pore space, large phosphate crystallites (>1000 nm long), and a decussate texture; in contrast, dentine is porous with 1-μm-diameter tubules, and much smaller crystallites (<100 nm long; Hillson, 1986). The greater organic content and smaller crystal size of dentine may make it more susceptible to alteration than enamel.

Enamel phosphate has a composition of $\sim Ca_{4.5}[(PO_4)_{2.7}(HPO_4)_{0.2}(CO_3)_{0.3}](OH)_{0.5}$ (e.g., Driessens and Verbeeck, 1990, p. 107 and 128) where CO_3 and HPO_4 substitution for PO_4 is charge balanced by vacancies in the Ca and OH sites. Other important substitutions include additional CO_3 and Cl in the OH site, and Na and Mg in the Ca site. Dentine phosphate has less PO_4 and Ca, and higher Mg and CO_3 (Driessens and Verbeeck, 1990, p. 165). The range of elemental substitutions and occurrence of several light stable isotopes in teeth leads to many applications in biogeochemistry and paleoclimatology (Table 1). In general, stable isotope investigations focus on the dependence of oxygen and carbon isotope compositions on food and water intake, coupled with the dependence of food and water compositions on climate (for oxygen) or photosynthetic mechanism (for carbon). Trace element studies principally rely on differential discrimination of a specific element relative to Ca in the body. For example, Sr is discriminated against relative to Ca in the body, and so herbivores have a lower Sr/Ca compared to plants, while carnivores have a still lower Sr/Ca than herbivores (e.g., Comar et al., 1957; Schoeninger, 1979; Sillen, 1981). Similar or inverse behaviors for other trace elements lead to analogous approaches in studying food chains and trophic levels. Studies of migration patterns (e.g., Sealy et al., 1991; Koch et al., 1995) use the fact that the radiogenic isotope composition (Sr and/or Pb) of an animal reflects that of the underlying rock where it lives. If an animal migrates, it will either have an isotope composition out of equilibrium with its new location (assuming isotope compositions are different), or early- vs. later-formed phosphate will have distinct compositions. Clearly, all these approaches require fidelity of trace element compositions or isotope ratios to diagenetic assault. Thus, the results we describe here have implications for a wide range of paleobiology and paleoclimate studies.

3. SAMPLES

Modern samples of Burchell's zebra (*Equus burchelli*) and dikdik (*Rhynchotragus guentheri*) were obtained from surface-exposed skeletons from the Sibiloi National Park, in northern Kenya, and samples of Grant's gazelle (*Gazella granti*) were obtained from a game ranch near Nairobi. These samples were used in two previous studies investigating modern climate and oxygen isotope compositions (Kohn et al., 1996, 1998). The Nairobi samples were fresh. Weathering of the Sibiloi samples ranged up to stage 3, implying 0 to 7 yr of exposure (Behrensmeyer, 1978). The modern teeth we analyzed typically showed a factor of 2 to 5 variation in the concentration of any one element. This mostly reflects systematic compositional differences between animals (e.g., dikdik vs. zebra), which in turn may reflect diet and drinking water composition, minor variations in soil type, and possibly also weathering stage (not all modern samples are from exactly the same location or were exposed for the same length of time). However, the concentrations of many elements in fossil teeth are 2 to 4 *orders of magnitude* higher than in modern teeth, and so any small differences in preburial concentrations of trace elements were likely swamped by postburial effects. Fossil samples of hippo-

potamus, giraffe, elephant, pig, and bovid (unspecified) were obtained courtesy of Dr. Meave Leakey and the National Museums of Kenya, and were collected from the ~3.9 Ma hominid site at Allia Bay, Kenya (Leakey et al., 1995). Unlike the modern samples, which are uniformly white or slightly yellowish, the fossil samples are all strongly colored. Fossil tooth interiors are dark brown to black, whereas surfaces and cracks are white, pale blue, brown, black, or mottled.

4. TECHNIQUES

Three analytical approaches were used: (a) ion microprobe spot analysis, (b) scanning electron microprobe imaging, spot analysis and X-ray mapping, and (c) TEM imaging and analysis. These techniques combine differing spatial and compositional resolutions. The ion microprobe resolves concentrations at the ppm- to sub-ppm level on 8 to 10-μm-diameter spots, the scanning electron microprobe has spatial and analytical resolutions of ~1 μm and ~250 ppm, respectively, whereas the TEM images at the subnanometer level but in this study is analytically qualitative. Prior to any analysis, all samples were bleached overnight in a 3% aqueous NaOCl solution, then rinsed and ultrasonicated repeatedly in distilled water. One fossil sample (hippopotamus) and all modern teeth described here were mounted together in epoxy, sectioned, polished, and analyzed using both the ion and electron microprobes. A matching section of the hippo tooth was analyzed with the transmission electron microscope. All other fossil samples were subsequently mounted together and analyzed only with the electron microprobe. All quantitative analyses were collected at least 50 μm from the physical edge of the teeth (i.e., away the surface exposed to weathering and soils) to avoid potential problems with local surface leaching and alteration.

Ion Microprobe. Spot analyses were collected on carbon-coated samples, using the Cameca IMS 3f instrument maintained at the Lawrence Livermore National Laboratory. A primary O^{2-} ion beam was used with an accelerating voltage of 15 keV and a beam current of ~1 nA. Analytical spots were ~8 to 10 μm in diameter. Voltage switching focused specific isotopes of the elements of interest (e.g., ^{44}Ca), and interfering masses were discriminated with a mass resolving power of ~1100. Counts were measured on a Faraday cup for Ca and P, and on an electron multiplier for the other elements, and 10 to 20 blocks of data were collected on each spot and averaged for each analysis. For modern teeth, these settings limit analytical resolution to ~0.1 ppm for most isotopes except for ^{55}Mn, ^{54}Fe, and ^{65}Cu. There were clear molecular interferences at these three masses, so that minimum detection limits for these elements were ~10 to 20 ppm. A 50 V offset was applied to the accelerating voltage of the secondary ion beam to help eliminate molecular interferences, and this offset was determined automatically every fifth block using the ^{44}Ca peak. The magnitude of the offset depends on the degree of electrical charging of the sample. For modern enamel, the offset shifted dramatically as the primary ion beam eroded through the carbon coat and caused sample charging, whereas no such change for fossil enamel and dentine was observed. This indicates that the fossil material is much more electrically conductive than modern enamel, a point that is discussed below. Because the main purpose of this study was to compare modern vs. fossil materials, accuracy is of less importance than precision. Nonetheless, analyses were standardized using the main standard NBS 610 glass, which nominally contains 500 ppm of each element of interest. This standardization was checked for major and minor elements (i.e., except for Ce, U, Nd, B, Al, and Cu) based on subsequent electron microprobe analysis of adjacent spots. Excepting halogens, the apparent compositions based on NBS 610 glass were found to be within ~±25% of the electron microprobe analyses. Given the extreme differences in matrix compositions (Ca–phosphate vs. high-Si glass), the differences in composition as determined by the ion and electron microprobes are not surprising. To account for the greater accuracy of the compositions determined by electron microprobe, the NBS standardization was adjusted for the elements Mg, F, Cl, Fe, and Mn by the following factors: 0.80, 0.50, 0.38, 1.28, and 1.05.

SEM. Electron microprobe analyses were collected with the fully automated JEOL 733 Superprobe at the Lawrence Livermore National Laboratory. Spot analyses were standardized using natural and synthetic oxides, silicates, and phosphates, and were collected using an accelerating voltage of 15 keV, a beam current of 20 nA (measured on brass), an expanded beam of 5 μm, four wavelength-dispersive spectrometers, and count times of 20 s for major elements (Ca and P on teeth, Fe and Mn on oxyhydroxides) and 120 s for minor and trace elements (F, Na, Mg, Al, Si, Cl, Mn, Fe, Sr, Ba, Ce, Nd, and U). These conditions yield typical minimum detection limits of ~250 ppm for minor and trace elements. X-ray maps were collected with a 3–10 μm pixel resolution, count times of 30 to 100 ms, and a current of 200 nA.

TEM. Tooth samples were impregnated with epoxy, and 30-μm-thick petrographic thin sections were prepared using manual grinding techniques and an acetone-soluble adhesive. Thin sections were reimpregnated with M-bond before polishing to <30 μm thickness in order to prevent disaggregation (Barker and Banfield, 1996). Two-mm-diameter slices were removed from thin sections and Ar ion milled to electron transparency. These samples were coated with carbon and were examined either in a Philips CM20UT HRTEM operated at 200 kV or in a LEO 912 energy-filtered transmission electron microscope (EFTEM) operated at 120 kV in zero loss energy filtered mode. Energy dispersive X-ray microanalysis was performed using a NORAN germanium detector with a light element-capable Norvar window and associated Voyager analytical hardware and software.

5. RESULTS

5.1. Effect of Diagenesis on Chemical Compositions

Fossil teeth are unquestionably altered chemically compared to their modern counterparts (Fig. 1; Tables 2 and 3). Trace and minor element concentrations in modern enamel (ME) vs. fossil dentine (FD) compare as follows: (a) REEs and U are sub-ppm in ME vs. ~100 ppm in FD, (b) transition metals, Al, Si, and Ba are <10 to ~100 ppm in ME vs. >100 to 10000 ppm in FD, and (c) F is 30 to 300 ppm in ME vs. several wt.% in FD. Fossil enamel compositions are generally intermediate between modern enamel and fossil dentine, and thus fossil enamel has also been altered. However, Si and Al in fossil enamel show much less alteration than Mn, Fe, or Ba. The source of these chemical

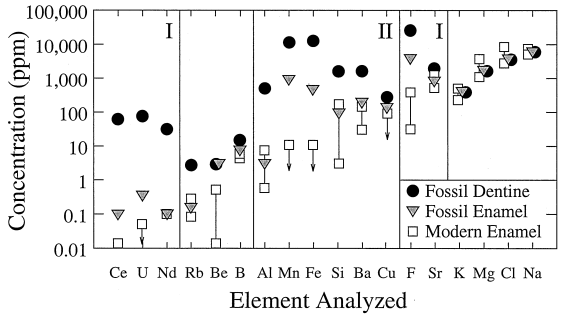

Fig. 1. Ion microprobe analyses of composition vs. element for modern enamel (squares), fossil enamel (triangles), and fossil dentine (circles), showing strong increases in the concentrations of many trace and minor elements associated with fossilization. Regions denoted "I" correspond to elements likely associated with chemical alteration of apatite. The increased concentration of region "II" elements likely reflects mixed apatite and oxyhydroxide analyses. Although all fossil materials are altered, alteration is greater in dentine than in enamel. Vertical lines connecting squares show range of compositions measured for modern samples, and mostly reflect compositional differences between species.

signatures could reflect chemical alteration of the apatite crystallites, the occurrence of secondary minerals smaller than the ~10 μm ion probe spot size, or both.

Several factors point towards contamination by fine-grained secondary Fe– and Mn–oxides or oxyhydroxides. First, such minerals are common stains and the fossil teeth are quite dark. Second, Fe– and Mn–oxides are semiconductors, and their presence would explain why the fossils did not charge under the primary O^{2-} beam of the ion probe. Third, two elements whose concentrations showed the greatest increases in fossil materials are Fe and Mn. Finally, Fe+Mn+Ba–oxyhydroxides have been occasionally reported in association with fossil bone (e.g., Parker and Toots, 1972; Shahack–Gross et al., 1997). Whereas the ion probe analyses are spatially and analytically among the most detailed measurements made on fossil teeth to date, even a 10-μm spot size is unable to resolve the possible occurrence of secondary minerals, and other more spatially precise analytical and imaging techniques are required.

5.2. Secondary Mineral Occurrence

Backscattered electron imaging via the scanning electron microscope shows significant differences in modern vs. fossil teeth. Modern dentine has a low average atomic number (low Z) compared to enamel (Fig. 2a), reflecting a lower concentration of Ca-phosphate and a higher concentration of interstitial low-Z organic material in dentine. However, backscattered electron intensities are reversed in fossil materials: average Z for fossil dentine is noticeably higher than for fossil enamel. Inasmuch as the Ca and P concentrations are certainly not higher in fossil dentine vs. modern or fossil enamel (Tables 2 and 3), the higher Z for fossil dentine must instead reflect a greater concentration of $Z > 20$ elements, such as Fe, Mn, Ba, and possibly REEs + U. Close inspection of fossil dentine (Figs. 2b and 2c) further reveals that the brighter BSE signal is not uniformly developed, but rather preferentially associated with dentine tubules. These tubules are heterogeneously infilled

Table 2. Compositions of modern and fossil teeth as measured by ion probe microanalysis.

	Fossil Hippo Dentine (4)		Fossil Hippo Enamel (6)		Gazelle M1 Enamel (3)		Gazelle M3 Enamel (8)		Dikdik Enamel (4)		Zebra Enamel (3)	
Elem.	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
Be	2.0	1.2–2.7	2.2	0.00–6.0	0.2	0.00–0.6	0.1	0.00–0.3	0.3	0.03–0.7	0.1	0.07–0.15
B	14	13–18	8.2	6.0–9.7	3.9	3.3–4.6	4.7	3.9–6.3	5.4	5.2–5.9	4.6	4.4–4.8
F	21,000	18000–29,000	4100	2900–5800	29	26–32	31	17–45	34	31–49	300	150–480
Na	6500	5000–7500	6800	5300–7900	6800	6600–6900	5900	5400–6500	7800	7500–8400	6500	6450–6650
Mg	1500	1300–1600	1300	1100–1400	1800	1800–1900	1400	1000–1900	2100	1800–2300	2400	1900–2900
Al	530	330–790	2.7	1.7–3.6	7.3	4.3–10.2	2.4	0.3–6.7	1.1	0.2–2.8	0.80	0.33–1.50
Si	1700	1300–2600	110	50–150	120	80–140	43	7–98	25	3–76	12	7–19
P	14%	11–18	19	15–22	17	17–18	17	16–19	19	17–19	19	18–21
Cl	2300	1900–3200	5800	3300–8800	4200	3800–4700	4300	3300–5000	4700	3300–5500	6300	6000–6550
K	270	120–410	240	150–300	240	220–260	280	260–300	360	300–420	270	240–290
Ca	35.7%	35.6–35.8	35.7	35.6–35.9	35.7	35.6–35.7	35.8	35.7–36.1	35.7	35.7–35.8	36	36–36
Fe	16,500	9300–23,000	670	390–1100	≤26	24–29	≤22	20–23	≤17	12–28	≤14	12–15
Mn	11,900	10,000–13,300	1150	960–1500	≤19	5–47	≤12	7–16	≤4	2–5	≤2.0	1.7–2.3
Cu	200	150–240	19	11–23	≤24	23–24	≤18	13–22	≤15	12–24	≤14	12–16
Rb	2.1	0.95–3.4	0.30	0.08–0.81	0.23	0.22–0.25	0.22	0.16–0.28	0.16	0.11–0.18	0.10	0.7–0.13
Sr	1900	1700–2000	690	540–750	650	630–670	710	630–780	630	460–700	1200	1100–1250
Ba	1700	800–3000	210	150–280	120	100–150	98	85–114	32	29–34	52	45–59
Ce	64	0.15–174	0.09	0.00–0.33	0.00	0.00	0.00	0.00–0.02	0.00	0.00	0.01	0.00–0.01
Nd	47	0.28–95	0.11	0.10–0.13	0.09	0.09–0.10	0.10	0.06–0.24	0.05	0.03–0.08	0.04	0.01–0.06
Th	0.02	0.01–0.04	0.04	0.00–0.15	0.00	0.00	0.00	0.00	0.00	0.00–0.02	0.00	0.00
U	79	57–124	0.38	0.07–1.73	0.04	0.00–0.06	0.00	0.00	0.03	0.02–0.07	0.03	0.02–0.04

Note: Numbers in parentheses indicate number of analyses collected of that material. All concentrations reflect that of the entire element (not the specific isotope measured), and are in ppm except for P and Ca, which are in wt.%. Compositions were corrected for measured concentrations in NBS 610 glass and electron microprobe analysis of adjacent spots. Modern gazelle samples (two teeth) come from near Nairobi, dikdik (two teeth), and zebra (one tooth) are from the Sibiloi National Park, adjacent to northeastern Lake Turkana, northern Kenya, and the fossil Hippo sample is from Alia Bay, eastern Lake Turkana.

Table 3. Compositions of modern and fossil teeth as measured by electron probe microanalysis.

	Fossil Dentine (9)		Fossil Enamel (9)		Modern Enamel (7)		Oxyhydroxide (6)	
Oxide	Average	Range	Average	Range	Average	Range	Average	Range
P_2O_5	34.47	30.55–36.49	38.34	35.95–39.47	39.59	38.75–40.14	0.00	0.00–0.00
SiO_2	0.12	0.00–0.66	0.08	0.00–0.74	0.01	0.00–0.05	12.06	10.31–16.28
Al_2O_3	0.03	0.00–0.10	0.01	0.00–0.03	0.01	0.00–0.05	0.96	0.51–1.52
MgO	0.28	0.13–0.55	0.27	0.20–0.34	0.34	0.24–0.53	0.82	0.45–1.07
FeO	1.23	0.32–2.55	0.34	0.05–2.13	0.01	0.00–0.03	54.67	26.12–62.21
MnO	1.13	0.50–4.57	0.23	0.12–0.69	0.01	0.00–0.03	9.56	3.49–35.54
CaO	49.92	46.05–51.82	50.48	48.99–51.32	49.88	49.52–50.66	0.67	0.00–3.47
BaO (ppm)	1000	0–5600	200	0–700	150	0–450	13200	1800–59,900
SrO (ppm)	1500	500–2000	600	0–1100	750	0–2000	800	0–2500
Nd_2O_3 (ppm)	650	0–2200	250	0–1150	400	0–1050	250	0–950
Ce_2O_3 (ppm)	1400	0–4400	600	0–1900	550	0–1300	1200	250–2450
UO_2 (ppm)	200	0–450	N.D.		N.D.		N.D.	
Cl	0.32	0.22–0.37	0.40	0.33–0.47	0.43	0.34–0.52	0.35	0.20–0.55
F	2.29	1.95–2.73	0.45	0.00–1.74	0.10	0.00–0.19	0.47	0.00–1.12
Wt% Total	90.25		90.80		90.55		81.11	

Note: Numbers in parentheses indicate number of analyses collected of that material. For enamel and dentine, four fossil teeth were analyzed. For modern teeth, five samples were analyzed. For oxyhydroxide analyses in sample 4879, the Ca content was adjusted by subtracting 1.3 times the measured wt.% P_2O_5, P_2O_5 was then set to zero, and the wt%s renormalized to the original wt% total.

with a high-*Z* alteration mineral (bright spots, Fig. 2c), and chemical or secondary mineral alteration within the fossil dentine is indicated by light patches surrounding individual tubules (Fig. 2c). Modern dentine lacks infilling minerals and light patches associated with tubules. Electron microprobe analysis shows relatively high concentrations of Fe and Mn for fossil dentine and enamel, and high concentration of Ba, Al, and Si in fossil dentine (Table 3). Texturally and chemically, every fossil sample from Allia Bay that we examined with the electron microprobe shows these alteration patterns.

In one sample (4879; pig), coarse high-*Z* minerals have precipitated at the intersection of a crack and the dentine–enamel interface (Figs. 2d and 2e). The coarse grain size of these particles allows collection of energy dispersive spectra unaffected by mixture with apatite (Fig. 3), and this indicates that the minerals are Fe+Mn−oxyhydroxides, with some Si enrichment. Because a focused electron beam damages this mineral within a few minutes, quantitative analyses were collected with a defocused beam, and this caused mixing of analyses with adjacent apatite. Consequently, the Ca content of each analysis was corrected based on the measured P content and an average wt.% ratio of CaO/P_2O_5 of 1.3 in enamel, and the results were renormalized to the original measured wt.% total (Table 3). These analyses further indicate substantial enrichment in Ba and Al. X-ray maps of the spatial distribution of Ba, Fe, and Mn (Fig. 4) show correlations among these elements as well as patchy zoning, indicating that alteration is heterogeneously distributed.

At the most detailed level, TEM imaging of fossil dentine (Figs. 5a and 5b) reveals submicron size clays in the dentine tubules, coprecipitated with Fe+Mn−oxyhydroxides that are compositionally similar to those found via electron microprobe in sample 4879 (Fig. 3). Complete identification of the oxyhydroxides has not yet been possible. Birnessite $[(Na,Ca,K)Mn_7O_{14}\cdot 3H_2O]$ and hollandite $[BaMn_8O_{16}]$ have been reported as secondary minerals in fossil bone (Parker and Toots, 1972; Shahack–Gross et al., 1997). However, in situ TEM diffraction patterns on the submicron particles resolved only manganite [MnO(OH)], smectite, and apatite.

6. DISCUSSION

6.1. Secondary Minerals and Apatite Alteration

One explanation of the chemical signatures in fossil dentine and enamel is that the apparent alteration is the result of mixed analyses that include submicron sized, interstitial Fe+Mn-oxyhydroxides ± silicates. As clays contain high concentrations of Si ± Al, and the coarse oxyhydroxides contain abundant Fe, Mn, and Ba, this mechanism explains the increase in five elements in dentine most strongly affected by diagenesis. For enamel, the strong increases in Fe, Mn, and Ba between modern and fossil materials, but not for Al and Si, indicates that oxyhydroxides alone, and not clays or other silicates, may cause the mineralogical and bulk chemical alteration.

Simple mass balance shows that even small amounts of oxyhydroxide can readily explain most of the measured compositions of the fossil samples. In this calculation, it is assumed that the measured composition in the altered material reflects a mixture of unaltered apatite, whose composition can be estimated from the modern sample, and oxyhydroxide, whose composition is known from measurements on coarse crystals from sample 4879. This leads to the following equation for any element *i*:

$$[\mathrm{i}]_{\mathrm{Fossil}} = (1 - X)\cdot[\mathrm{i}]_{\mathrm{Modern\ Enamel}} + X\cdot[\mathrm{i}]_{\mathrm{Oxyhydroxide}}, \quad (1)$$

where [i]is the concentration of *i*, and *X* is the proportion of oxyhydroxide responsible for the measured concentration in the fossil. In practice, such calculations are complicated by the wide variation in composition for these elements in the oxyhydroxide, and the possible additional occurrence of clays with high concentrations of Si ± Al. However, measured Fe+Mn concentrations are nearly constant in the oxyhydroxide despite variations in Fe/Mn, and so a sum of both elements provides a

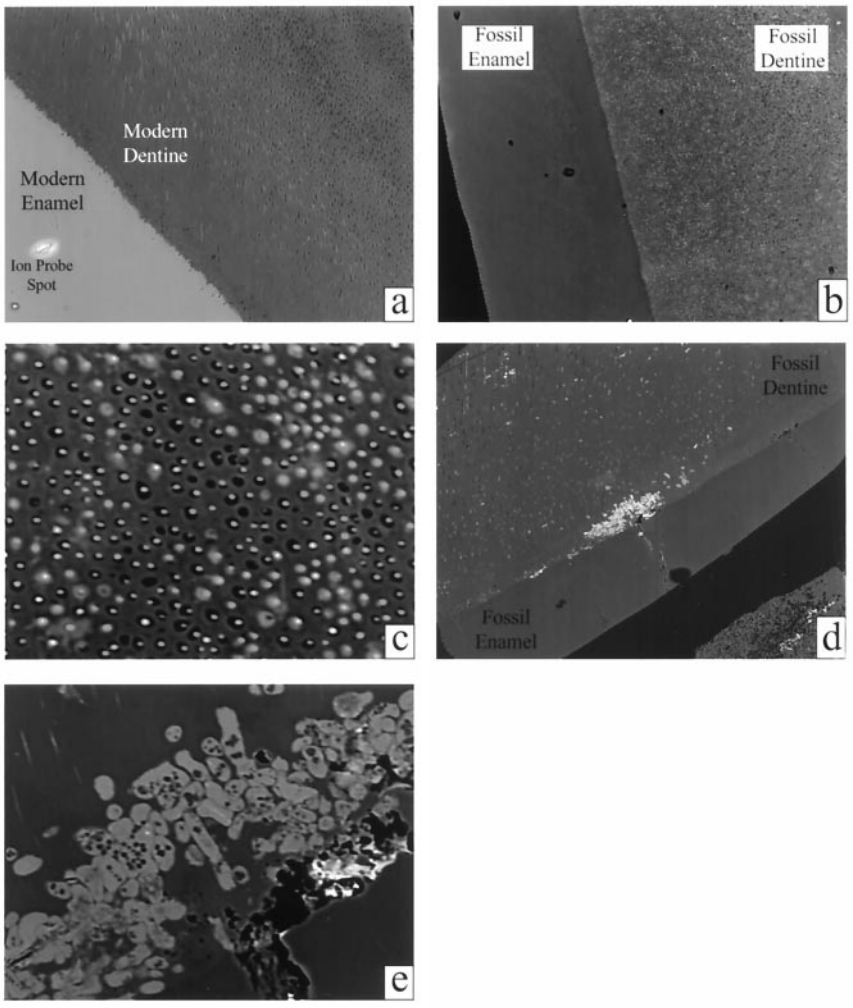

Fig. 2. Backscattered electron images of modern and fossil enamel and dentine. (a) In modern teeth, enamel has a brighter image compared to dentine because dentine has a higher concentration of low-atomic-number (low *Z*) organic materials. Note that modern dentine also contains numerous unfilled tubules. Sample 2306 (gazelle). (b) In fossil teeth, dentine contains more high-*Z* elements, and so has a brighter image compared to enamel. The patchiness in the dentine image is caused by a heterogeneous distribution of secondary alteration minerals and attendant apatite alteration. Sample 4864 (bovid). (c) Closeup of fossil dentine shows that alteration is spatially associated with the tubules, which are filled with a high-*Z* material. Sample 4864 (bovid). (d) In fossil sample 4879 (pig), coarse oxyhydroxides have precipitated at the intersection of a crack and the dentine–enamel interface. Box shows area of Fig. 2e. (e) Closeup of oxyhydroxides in fossil sample 4879.

more robust estimate. For Si and Al, the measured oxyhydroxide composition provides a maximum estimate of the amount of contaminating material required to explain [Si] and [Al] (because a mixture of oxyhydroxide with clay would have a higher concentration of Si and Al than oxyhydroxide alone), whereas the highest measured [Ba] in the oxyhydroxide (~4 wt.%) provides a minimum limit. These calculations (Table 4) indicate that admixtures of ~5% and ~0.3% oxyhydroxide ± clay can explain the measured Fe+Mn, Si, Al, and Ba compositions of fossil dentine and enamel, respectively. The chemical similarity of Cu to Fe and Mn suggests that its increase in concentration during fossilization can be analogously explained.

Mixed analyses of apatite and secondary minerals do not, however, explain all the measured chemical differences between modern and fossil teeth. Assuming that the composition of fossil dentine is only the result of admixtures of ~5% oxyhydroxide, the expected concentration of the rare earth elements (REEs), U, F, and Sr in the contaminating oxyhydroxide phase can be backcalculated using Eqn. 1 to solve for $[i]_{Oxyhydroxide}$ and compared to the measured concentrations. These calculations (Table 5) show that with the possible exception of Ce, none of these elements are explained well by oxyhydroxide contamination alone. Although some of the measured compositions could be the result of mixtures with clay, the strong chemical affinity of these elements for the Ca and OH sites in apatite suggests they instead reflect chemical changes in the apatite crystallites themselves. Several previous studies support this conclusion by showing REE and U concentrations of 10's to 10,000's of ppm in fossil dentine and enamel (Grandjean et al., 1987; Grandjean and Albarède, 1989;

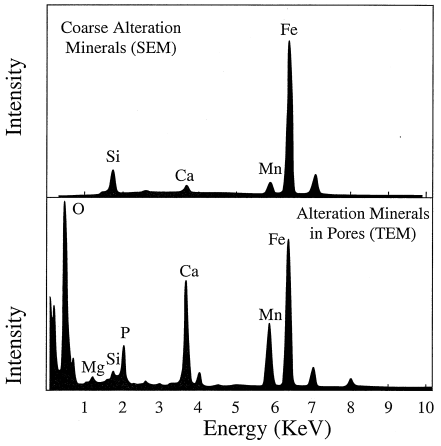

Fig. 3. Energy dispersive spectra for oxyhydroxide minerals. (a) Coarse-grained oxyhydroxide in sample 4879 (pig), shows large Mn, Fe, Ca, and Si peaks, smaller Cl and Al, and no P. (b) Sub-μm sized grains in fossil hippopotamus sample are mixed with surrounding apatite (Ca and P peaks), but also show large Fe, Mn, and Si peaks.

Toyoda and Tokonami, 1990; Denys et al., 1996; Stuart-Williams et al., 1996). It is unlikely that such high concentrations could result from small proportions of interstitial secondary minerals, and we conclude that increases in these elements reflect chemical alteration of the original apatite crystals. Most importantly, chemical alteration of apatite was not limited to dentine, as indicated by the increased concentration of REEs and U in fossil vs. modern enamel. Thus, apatite crystals in enamel are not immune to chemical alteration, despite enamel's lower original organic content and porosity.

6.2. Implications for Trace Element Studies

The recognition of mixed analyses at the micron scale weakens considerably previous conclusions regarding the importance of diagenesis on the chemistry of biogenic apatite. For example, Bocherens et al. (1994) and Denys et al. (1996) used principal component analysis to show that some elements, such as Mn, Fe, and Ba, are good indicators of diagenetic alteration. Although they did not specify whether the alteration reflected secondary minerals or chemical changes to the apatite, the

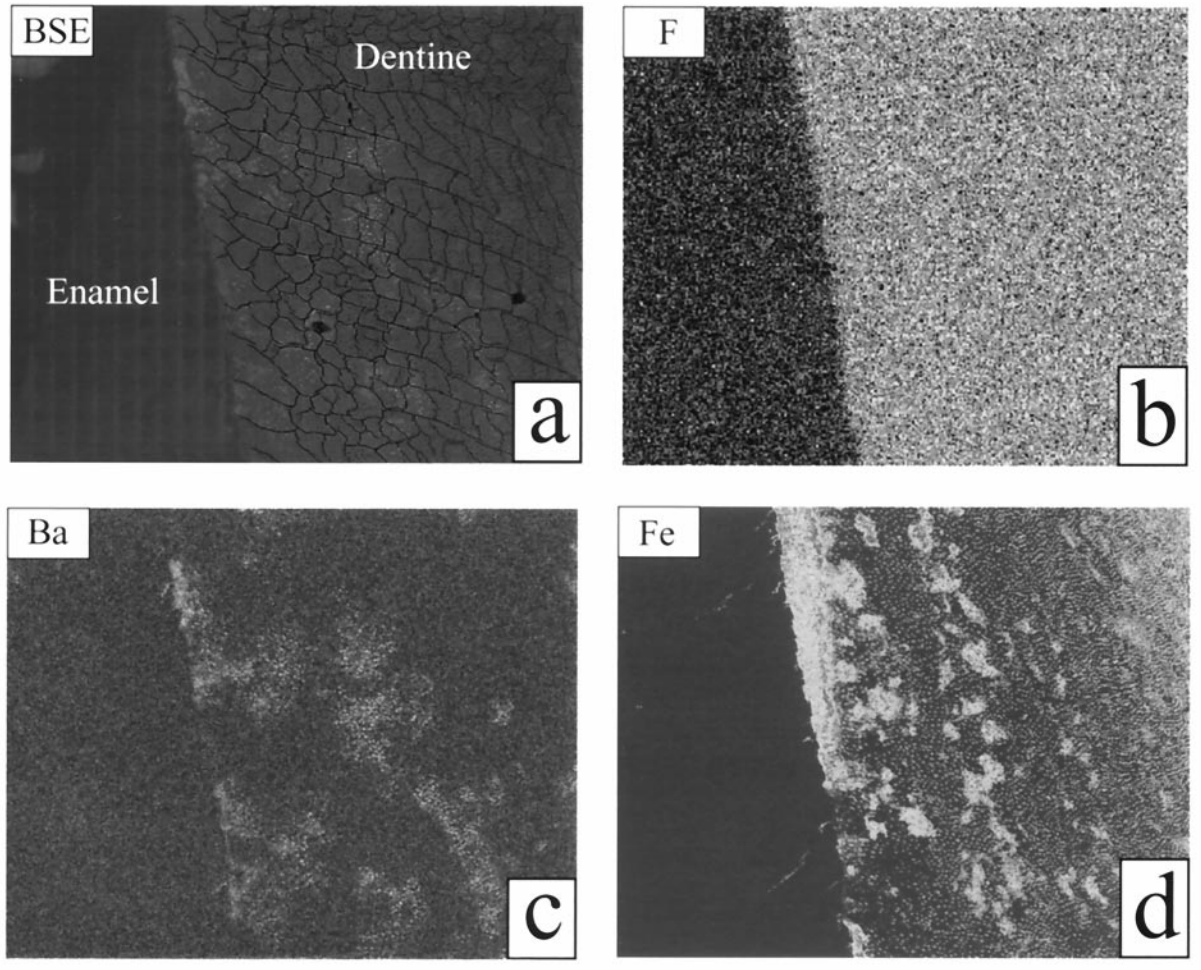

Fig. 4. Images of the dentine–enamel interface in sample 4864 (bovid). (a) Backscattered electron image. (b) F X-ray map. (c) Ba X-ray map. (d) Fe X-ray map. Ba, Fe, and high-*Z* (brightness on BSE image) show spatial correlations that are unrelated to F, which is uniformly distributed in dentine.

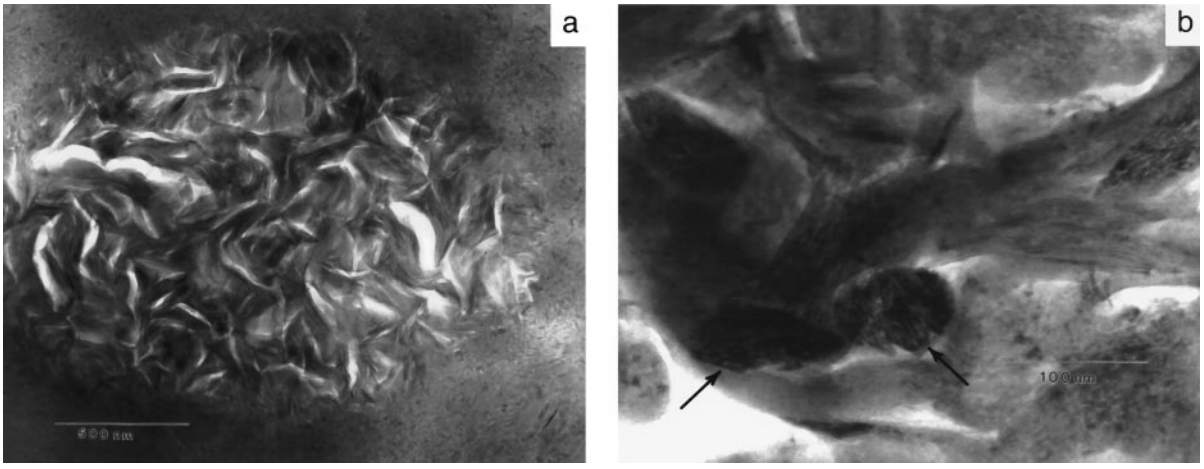

Fig. 5. Transmission electron microscope images of tubules in fossil hippopotamus sample. (a) A single tubule is filled with a mixture of clay particles and an oxyhydroxide mineral (manganite?). (b) Closeup, with arrows pointing to two oxyhydroxide grains.

detailed ion and electron beam data for fossils vs. modern samples presented here clearly implicate secondary minerals as the principal cause of such variations. This result, that some of the most readily recognized elemental changes in fossil teeth do not reflect alteration of the biogenic apatite, partially supports studies seeking to use original tooth chemistry to infer paleobiological and paleoclimatic features.

However, many elements in apatite *are* affected by diagenesis, including REEs, U, F, and possibly Sr, and extreme care must be taken in future studies of trace elements and isotopes that employ elements in the Ca and OH sites of apatite for retrieving primary biologic signals. Ca, Sr, and Pb isotopes are useful for inferring modern trophic level and migration patterns (Ericson, 1985; Sealy et al., 1991; Price et al., 1994; Koch et al., 1995; Ezzo et al., 1997; Skulan et al., 1997). However, in the Allia Bay teeth, it is likely that the U + REE increases in enamel apatite, and U + REE + Sr increases in dentine apatite herald alteration of other Ca-site elements and their isotope compositions. Thus, rather than assuming that teeth are unaltered unless proven otherwise, it should be assumed that all fossil teeth are altered, and that methods will have to be developed to differentiate diagenetic from paleobiological signals. For example, acid leaching of bone affects the retrieved Sr isotope composition, presumably because of differential solubilities of diagenetically altered vs. unaltered biogenic apatite (e.g., Sillen, 1986). A conclusive demonstration that such approaches indeed cleanly separate apatites of different composition and origin would greatly advance the reliability of paleodietary and other trace element studies of fossil materials. Probably the most diagnostic elements for tracing chemical alteration of apatite are U and the REEs. They are strongly partitioned into apatite, readily analyzed, yet occur in extremely low abundance in modern teeth (e.g., Driessens and Verbeeck, 1990, p. 111). We suggest that future trace element and isotope studies bolster assertions of Ca-site compositional fidelity through U or REE analysis, as material with little or no U+REEs will more likely contain the primary biological signal.

6.3. Oxygen Isotopes and Paleoclimate

Our trace element data and textural observations have additional implications for the recovery of oxygen isotope compositions, which is of special interest in the analysis of fossil teeth for paleoclimatic studies. Three analytical approaches have been used for modern teeth: (1) preferential analysis of only the PO_4 component for its oxygen isotope composition (e.g., Longinelli, 1984; Luz et al., 1984); (2) acid dissolution to recover the CO_3 component (e.g., Kolodny and Kaplan, 1970; Koch et al., 1989, 1997); and (3) bulk analysis using laser fluorination or thermal decomposition (Cerling and Sharp, 1996; Kohn et al., 1996). At issue is the robustness of each of these sampling approaches for potentially altered materials. In this discussion we do not consider stable isotopes of H and C. Given the extensive exchange between F and OH, it is unlikely that primary biologic D/H ratios are preserved in fossil teeth.

Table 4. Estimates of the amount of contaminating oxyhydroxide required to explain compositions of fossil materials.

Element (i)	$[i]_{OH}$ (wt %)	$[i]_{FD}$ (ppm)	$[i]_{FE}$ (ppm)	$[i]_{ME}$ (ppm)	Amount (FD)	Amount (FE)
Fe+Mn	50	24,000	1600	<10	5%	0.3%
Si	5	1650	100	50	≤3%	≤0.1%
Al	0.5	525	3	5	≤10%	0
Ba	≤4.0	1700	200	80	≥4%	≥0.3%

Note: Weight percents of the oxyhydroxide phase are elemental concentrations, rather than the oxide weight percents reported in Table 3.

Table 5. Predicted and measured Sr, REE, U, and F concentrations (in ppm) of oxyhydroxide.

Element	Predicted concentration	Measured concentration
Sr	20,000	600 ± 600
Ce	1300	900 ± 500
Nd	600	200 ± 250
U	1600	300 ± 300
F	54,0000	3400 ± 3500

Note: Calculations based on measured fossil dentine and modern enamel compositions (Tables 2 and 3), using Eqn 1 and assuming 5% interstitial oxyhydroxide.

Carbon isotope alteration has been extensively discussed by Wang and Cerling (1994) and Koch et al. (1997).

PO_4. Because the elements found to be diagenetically altered in biogenic apatite exclusively occupy the Ca and OH sites of the apatite, but not the P site, no definitive conclusion can be reached regarding analysis of PO_4 for $^{18}O/^{16}O$. The only elements analyzed that likely substitute for P are Si, Al, and B, and they either do not change composition significantly (B), or their compositions are obscured by mixed analyses (Si and Al). Nonetheless, it is generally recognized that abiotic dissolution–reprecipitation is negligible in near-surface environments, and does not change oxygen isotope compositions (Kolodny et al., 1983). Consequently, microbial catalysis is the one likely mechanism for altering PO_4 $\delta^{18}O$ (Kolodny et al., 1983; Kastner et al., 1990). Our textural observations do have implications on the likelihood of the involvement of microbes in diagenesis of enamel and dentine.

Although bacteria are well-known producers of Mn+Fe–oxyhydroxides, it is unlikely that bacteria directly precipitated the oxyhydroxides observed in our samples. Secondary minerals are dispersed throughout the dentine, and are concentrated within and near the tubules. Most bacteria have minimum diameters of ~1 μm. Although they could possibly move along the larger cracks, the tubules within the dentine are only ~1 μm across, and it is difficult to envision microbes forcing their way into such small channels, precipitating secondary minerals. It is even less likely that bacteria could infiltrate the compact portions of the dentine or the enamel, which have much smaller intercrystalline pore space, yet also contain interstitial secondary oxyhydroxides. If bacteria or other microorganisms are involved in this secondary mineral precipitation, then it is more likely through the release of extracellular catalytic enzymes (e.g., Tebo et al., 1997). Such enzymes could, in principle, permeate the teeth and facilitate secondary mineral precipitation.

Even if extracellular bacterial enzymes or exopolymers do facilitate secondary mineral precipitation and cause some dissolution or transport of phosphate, we do not believe this will affect the oxygen isotope composition of residual materials. Although PO_4 may be removed from the interior of teeth, it should not undergo isotopic exchange until it is within the cell itself (Blake et al., 1997, 1998); and once it is biologically processed, external to the tooth, it is unlikely to return to the same site from which it was derived. Furthermore, reprecipitation of previously dissolved phosphate often produces distinctive apatite balls, several microns in diameter (Hirschler et al., 1990; Blake et al., 1998). Insofar as the exquisite ultrastructure of teeth is preserved in the minutest detail at the micron level, reprecipitation of either biotically or abiotically dissolved PO_4 is unsupported, and we conclude that PO_4 oxygen in the residual enamel in our fossil teeth is probably unaltered. However, we do recommend that future studies employ micron-scale imaging techniques to investigate whether bacteria have precipitated secondary apatite.

CO_3 and Bulk O Analyses. Because ~90% of the carbonate component of teeth resides in the P site of apatite (Elliott et al., 1985), and because this site seems resistant to diagenetic alteration, it is likely that most of the CO_3 in teeth is also resistant. However, the remaining 10% resides in the OH site and may not be so immune to exchange or alteration. Similarly, bulk analyses of teeth must account for the 5% to 6% total oxygen residing in the OH site, which is obviously affected by diagenesis. Although the differences in internal fractionations are unmeasured for the two carbonate components in the OH and P sites, and for OH vs. PO_4, some simple calculations provide estimates of the potential error introduced to CO_3 or bulk analyses of altered teeth.

First, the difference in $\delta^{18}O$ between CO_3 and PO_4 in teeth [$\Delta^{18}O(CO_3 - PO_4)$] is ~8‰ at 38C (Longinelli and Nuti, 1973; Iacumin et al., 1996; Bryant et al., 1996c). Although $\Delta^{18}O(PO_4 - OH)$ is unknown, limits can be placed based on empirical and theoretical studies. Bulk analyses of teeth give isotope compositions similar to PO_4 alone (Kohn et al., 1996), and as the ratio of oxygen in the CO_3 and OH components is ~1.2, a rough estimate of $\Delta^{18}O(PO_4 - OH)$ is ~+10‰. Analysis of the different components of low-temperature kaolinite (Hamza and Epstein, 1980) show an internal fractionation between Al- or Si-bound O^{2-} and hydroxyl of ~+20‰. In contrast, theoretical estimates of the intracrystalline isotope fractionation for apatite give a reversed fractionation value at 38°C of $\Delta^{18}O(PO_4 - OH)$ ~−30‰ (Smyth, 1989; Smyth and Clayton, 1988; Zheng, 1996). So, we assume that $\Delta^{18}O(PO_4$–OH) is between −30 and +20‰. Because 5% to 6% of total tooth oxygen resides in this component, it affects a bulk analysis by −1.5‰ to +1‰. If the different carbonate components have a smaller internal fractionation, say −10‰ to +10‰, then the CO_3 in the OH site affects a bulk carbonate analysis by ±1‰.

During diagenesis, OH is replaced by F, and the obvious rapidity of F–OH exchange implies that OH-site oxygen must additionally undergo isotopic exchange with sediment pore waters. Simple F–OH exchange in enamel ranges from negligible to extensive (>50% exchange; Table 2). Removal of the OH component will likely change a bulk tooth analysis by −1.5‰ to +1‰ (if all OH is removed), and removal of CO_3 in the OH site would affect a bulk carbonate analysis by ±1‰. Fortunately, spectroscopic and electron microprobe techniques can readily identify whether these components have been lost, and should be applied routinely in isotope studies of fossil teeth.

The effect of isotope exchange of OH-site oxygen (either OH or CO_3 components) on bulk tooth or bulk CO_3 $\delta^{18}O$ is harder to identify and depends on the actual pore water composition and temperature. In general, animals have a higher body water $\delta^{18}O$ and higher body temperature than soil and groundwater, and the fractionation between water vs. phosphates, carbonates, or silicates increases with decreasing temperature with a typical proportionality of ~0.25‰/°C at 0 to 40°C (e.g., Friedman and O'Neil, 1977). As the tooth CO_3 component is enriched in ^{18}O relative to water, exchange with lower $\delta^{18}O$ water will be offset

by the larger fractionation occurring at lower temperature. For example, if pore water $\delta^{18}O$ is 5‰ lower than the original body water in which the tooth was precipitated, and T is ~20°C lower, then partial to complete exchange of CO_3 in the OH site will cause no shift to bulk CO_3 $\delta^{18}O$. In contrast, because OH–oxygen could be enriched or depleted in ^{18}O, partial to complete exchange with the same pore water at the same temperature could cause shifts in OH–oxygen ranging from 0‰ to −10‰, resulting in a 0‰ to −0.5‰ shift in bulk $\delta^{18}O$.

In some instances, secondary minerals could also bias bulk isotope analyses. If there is a large isotopic difference between secondary oxyhydroxide and original biogenic apatite, say ±20‰, then the high concentration of oxyhydroxides in dentine (~5%) could cause analyses to be biased by as much as ±1‰. However, the low abundance of secondary minerals in enamel (0.3%) implies that inclusion of oxyhydroxides should not affect analyses by more than ~0.1‰. Oxyhydroxides can also be dissolved preferentially, leaving apatite intact (Price et al., 1992), and allowing collection of unbiased analyses.

In sum, bulk analyses of altered teeth for total oxygen isotope composition may differ from original compositions by ~±1‰ (F–OH exchange ± isotope exchange of OH components), leading to significant interpretational uncertainties. Depending on how diagenesis proceeds, this could be manifested either as a constant shift in measured values, or as systematic variations along the length of a tooth. Modern teeth show seasonal isotopic variations of the order 1‰ to 6‰ (Bryant et al., 1996a, 1996b; Kohn et al., 1996, 1998; Fricke and O'Neil, 1996, Fricke et al. 1998a, 1998b; Sharp and Cerling, 1998; Kohn, unpubl. data), and clearly incautious bulk analysis of fossil teeth could spuriously increase or degrade an original seasonal signal substantially.

7. CONCLUSIONS

In contrast to previous studies of diagenesis, which have emphasized either chemical alteration of apatite or physical contamination by secondary materials, our combined ion probe, electron probe, and TEM investigations implicate both processes. Increases in U, REEs, F, and possibly Sr are best explained by chemical alteration of the Ca and OH sites of original biogenic apatite within both dentine and enamel, whereas increases in Al, Si, Mn, Fe, Ba, and probably Cu are the result of contamination by sub-μm-sized diagenetic oxyhydroxides ± clay. The ubiquitous alteration of the Ca and OH sites implies that future studies of paleodiet and migration patterns will require considerable effort to distinguish original biological signals from diagenetic obfuscation. The textures of the teeth and secondary minerals imply that alteration is not directly mediated by microorganisms, although catalysis of the formation of secondary minerals by extracellular enzymes is possible. The absence of direct bacterial involvement in alteration supports the use of O– and C–isotope analyses of the PO_4 − site of tooth enamel. However, the clear alteration of the OH site mitigates the usefulness of bulk analyses for O, as these can be potentially biased by ±1‰. Future trace element studies should include analysis for U and/or REEs, as these may be the best indicators of chemical alteration of primary apatite.

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