Pergamon

Geochimica et Cosmochimica Acta, Vol. 68, No. 1, pp. 1–12, 2004

0016-7037/04 $30.00 + .00

doi:10.1016/S0016-7037(03)00278-3

Experimentally-controlled carbon and oxygen isotope exchange between bioapatites and water under inorganic and microbially-mediated conditions

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(*Received September* 26, 2002; *accepted in revised form April* 17, 2003)

Abstract—Modern bone and enamel powders have reacted at 301 K with ^{13}C- and ^{18}O-labelled waters under inorganic and microbial conditions. The aim of the study is to investigate the resistance of stable isotope compositions of bioapatite carbonate ($\delta^{13}C$, $\delta^{18}Oc$) and phosphate ($\delta^{18}Op$) to isotopic alteration during early diagenesis. Rapid and significant carbon and oxygen isotope changes were observed in the carbonate and phosphate fractions of bone apatite before any detectable change occurred in the crystallinity or organic matter content. These observations indicate that chemical alterations of bone apatite are likely to start within days of death. Enamel crystallites are much more resistant than bone crystallites, but are not exempt of alteration. Non removable carbon and oxygen isotope enrichments were measured in the carbonate phase of bone (50–90%) and enamel (40%) after the acetic acid treatment. This result indicates that a significant part of ^{13}C and ^{18}O-labelled coming from the aqueous fluid has been durably incorporated into the apatite structure, probably through isotopic exchange or secondary carbonate apatite precipitation. As a result, acetic acid pre-treatments that are currently used to remove exogenous material by selective dissolution, are not adequate to restore pristine $\delta^{13}C$ and $\delta^{18}Oc$ values of fossil apatites. Under inorganic conditions, kinetics of oxygen isotope exchange are 10 times faster in carbonate than in phosphate. On the opposite, during biologically-mediated reactions, the kinetics of oxygen isotope exchange between phosphate and water is, at least, from 2 to 15 times faster than between carbonate and water. Enamel is a more suitable material than bone for paleoenvironmental or paleoclimatical reconstructions, but interpretations of $\delta^{18}Op$ or $\delta^{13}C$ values must be restricted to specimens for which no or very limited trace of microbial activity can be detected.

1. INTRODUCTION

Since the pioneering studies of Sullivan and Krueger (1981), Luz et al. (1984) and Longinelli (1984), carbon and oxygen isotope analyses of carbonate and phosphate compounds of biogenic apatite (i.e., bioapatite), which forms the mineral phase of teeth and bones, have been widely recognized as powerful paleoenvironmental proxies. The carbon isotope composition ($\delta^{13}C$) of carbonate from bioapatite reflects that of diet and is commonly used to reconstruct dietary preferences in areas where feeding resources of distinct isotopic compositions are available (Koch et al., 1994). The oxygen isotope compositions of carbonate ($\delta^{18}Oc$) or phosphate ($\delta^{18}Op$) from biogenic apatites are used as a climatic proxy because they are closely related to that of ingested water which, in turn, is a function of mean annual temperature and humidity (Dansgaard, 1964; Rozanski et al., 1993). The validity of extending such proxies back to the past are dependent on the absence of significant postdepositional interactions between fossil material and the burial environment.

Twenty years after the first applications, the survival of carbon and oxygen isotope signatures in carbonate and phosphate from fossil bioapatite is still a matter of debate. Controversies stem mostly from a lack of knowledge concerning (1) the diagenetic mechanism involved, (2) the agent (inorganically or biologically-mediated reactions), and (3) a quantitative estimate of the role played by the apatite structure in bone and enamel diagenesis.

At the Earth surface, several mechanisms can be responsible for the modification of $\delta^{13}C$ and $\delta^{18}O$ values of biogenic apatites:

1. precipitation of secondary minerals within and at the surface bioapatite crystals. Of greatest concern are (1a) carbonate minerals (calcite, siderite, etc.) and (1b) phosphates (apatite, brushite, etc.);
2. adsorption of ions at sites on the surface of crystals;
3. isotopic exchange during dissolution/reprecipitation processes.

These mechanisms can be reduced to two basic types: the addition of new material (mechanisms 1 and 2) and the isotopic exchange between the apatite and surrounding aqueous fluids (3). The identification of the diagenetic process is an important issue because it determines the ability to remove diagenetic contaminants and in turn, the reliability of fossil data. Acetic acid leaching protocols were designed to remove alteration resulting from type 1a and 2 mechanisms (Krueger, 1991); they are of limited help in case of secondary apatite precipitation, or

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isotopic exchange during recrystallization (Schoeninger and DeNiro, 1982; Wright and Schwarcz, 1996).

Until the mid-1990s the role of micro-organisms in the diagenetic alteration of bioapatites was more or less ignored. Under inorganic conditions and at low temperature, phosphate oxygen is considered to remain largely unaffected by isotopic exchange, whereas carbonate oxygen is more sensitive (Tudge, 1960; Lécuyer et al., 1999). Therefore, δ^{18}Op values of bone and enamel apatite were prefered to δ^{18}Oc values for reconstructing past climates (Huertas et al., 1997; Fricke et al., 1998) or deciphering dinosaur physiology (Barrick and Showers, 1994, 1995; Barrick et al., 1996). However, in several studies, fossil bone or dentine, were found to exhibit anomalous δ^{18}Op values when compared to enamel. These data were attributed to oxygen isotope exchange between phosphate and water catalysed by bacteria or microbial enzymes (Ayliffe et al., 1994; Kolodny et al., 1996; Sharp et al., 2000). As a result, the use of δ^{18}Op values is now restricted to fossil enamel.

Bioapatite structures vary widely between enamel, dentine and bone. These differences have a considerable influence on the isotopic behavior of bioapatites during diagenesis. Because bone and dentine are highly porous and composed of small crystals that have numerous defects (Brudefold and Soremark, 1967; Trautz, 1967), they can either be contaminated by exogenous minerals or undergo extensive recrystallization more easily than enamel. Quantifying the preservation of the isotopic signal in fossil enamel is necessary because this highly-mineralized tissue constitutes the best material for paleoenvironmental investigations. However, the resistance of enamel to isotopic alteration relative to other tissues has not been experimentally investigated so far.

This paper presents the results of the first experiments designed to examine the effects of both inorganically and microbially-mediated reactions on carbon and oxygen isotope compositions of modern bone and enamel apatite during early diagenesis. Un-like previous published experimental protocols that involved heating (Person et al., 1996) or acid etching (Nielsen-Marsch and Hedges, 1997) to promote a rapid but very aggressive alteration of the apatites, we chose to simulate natural conditions by measuring labelled isotopic exchange between apatites and water-saturated natural soils. Experimental results will be discussed regarding the current opinions on diagenetic alteration of biogenic apatites, with emphasis on

- the efficiency of pretreatments for removing contaminants such as organic matter and secondary carbonate,
- the kinetics of oxygen and carbon isotope exchange between apatite and aqueous fluids under inorganic conditions and in the presence of micro-organisms,
- the reliability of enamel isotopic compositions as proxies of past environments.

Implication for paleoenvironmental studies based on fossil bioapatites will be discussed in light of our experimental results.

2. EXPERIMENTAL METHODS

Two laboratory "incubation" experiments were designed to study the effects of inorganic vs. microbially-mediated reactions of carbon and oxygen isotope exchange between bioapatite and water (Fig. 1). The objective was to simulate diagenetic processes occurring in the field. To observe such isotopic exchanges over 1 yr of laboratory experiments, the bone and enamel samples were powdered and reacted with ^{18}O- and ^{13}C-labelled water and soil organic matter. Rates of isotopic exchange between mineral and fluids are dependent on several physico-chemical parameters like temperature, pH and grain size (Cole and Ohmoto, 1986). During microbially-mediated, rates of phosphate-water oxygen isotope exchange may also be strongly dependant on the abundance and type of micro-organisms involved in the reaction. However, because our experiments were designed to document first order patterns of isotopic modifications and because natural systems are complex, no attempt was made to quantify the abundance and type of micro-organisms, as well as speciation and concentration of dissolved species.

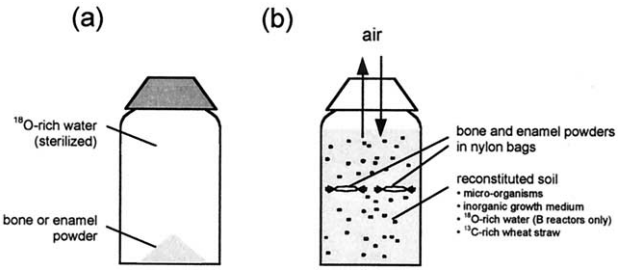

Fig. 1. Reaction vessels for the inorganic (a) and microbial (b) experiments.

2.1. Material

Enamel material was sampled from three teeth of modern *Hippopotamus africanus* found in several locations in Africa. Around 5 g of enamel were ground using a mortal and a pestle until obtaining a fine powder ($<$100 μm). Around 10 g of cortical bone was removed from the mandible of a modern cow that was raised in Vendee (western France). Because modern bone contains organic matter (including lipids), this tissue offers a greater resistance to grinding and it was not feasible to grind bone as thin as for enamel ($<$700 μm). Each powder was carefully homogenized.

2.2. Experimental Designs

2.2.1. Microbial Experiment

This experiment was designed to examine the effects of microbial mediation on C and O isotopic exchanges. Sixteen aliquots of enamel and bone powders were weighed and placed in small nylon bags (porosity 10 μm), each bag containing $\sim$150 mg of enamel or 250 to 400 mg of bone powder (Table 1). Enamel and bone are placed at half height in sixteen 134-mL flasks, each of them containing 120 g of reconstituted soil (95% sand, 5% kaolinite). Experimental soils were inoculated with the supernatant of a natural soil solution that was sampled near the INRA station of Grignon, France. The natural mixture of micro-organisms is grown on a mixed organic-inorganic medium containing 4.8 g of crushed wheat straw (50 wt.% C) labelled at 5% of ^{13}C. Inorganic growth medium is made of 238 mg of NH_4NO_3, 32.5 mg of $NH_4H_2PO_4$,16.4 mg of $CaCl_2$, 21.1 mg of $MgSO_4$, and 271.1 mg of KNO_3. The soil in each flask was saturated with 15 to 20 wt.% of water with a low δ^{18}O value of -16‰. Each sample was buffered at a pH of 7 and closed with a permeable film allowing gaseous exchange with the atmosphere. During the first weeks, soil weight loss indicated that some evaporation occurred, leading to a water ^{18}O-enrichment of $\sim$10‰ after 12 weeks. The film was replaced, and evaporation and ^{18}O-enrichment stopped. During the 9th week, following a partial dehydration of the soil, enriched water (δ^{18}O = $+170$‰) was added to half of the remaining reactors. The input of such isotopically-labelled water was decided to favor the identification of isotopic exchange between apatite and water. The experiments were carried out at 28°C in the dark during a period of 1 yr, two samples were withdrawn for isotopic analysis after 14, 28, 42, 84, 112, 143, 220 and 365 d of reaction. At these desired times, nylon bags were opened, the powders were dried at

Table 1. Weight of enamel and bone powder before and after the microbial experiment. Differences (Δm) are expressed in mg and %.

N° sample	Time of reaction (d)	Mass before reaction (mg)	Mass after reaction (mg)	Δm (mg)	Δm (%)	N° sample	Time of reaction (d)	Mass before reaction (mg)	Mass after reaction (mg)	Δm (mg)	Δm (%)
Enamel						Bone					
E1-A	14	151.0	143.8	–7.2	–4.8	O1-A	14	274.6	280.9	6.3	2.3
E1-B	14	158.9	153.5	–5.4	–3.4	O1-B	14	251.9	258.2	4.3	1.7
E2-A	28	149.1	135.0	–14.1	–9.5	O2-A	28	267.7	276.3	8.6	3.2
E2-B	28	149.8	141.1	–8.7	–5.8	O2-B	28	256.4	266.8	10.4	4.1
E3-A	42	148.4	140.1	–8.3	–5.6	O3-A	42	302.3	313.1	10.8	3.6
E3-B	42	148.8	136.6	–12.2	–8.2	O3-B	42	300.9	309.7	8.8	2.9
E4-A	84	146.3	136.3	–10.0	–6.8	O4-A	84	317.0	330.5	13.5	4.3
E4-B	84	151.1	135.3	–15.8	–10.5	O4-B	84	293.6	304.5	10.9	3.7
E5-A	112	150.7	150.5	–0.2	–0.1	O5-A	112	334.2	347.7	13.5	4.0
E5-B	112	150.4	148.8	–1.6	–1.1	O5-B	112	356.9	372.8	15.9	4.5
E6-A	143	152.7	149.1	–3.6	–2.4	O6-A	143	334.2	353.6	19.4	5.8
E6-B	143	154.4	150.7	–3.7	–2.4	O6-B	143	356.9	359.6	2.7	0.8
E7-A	220	147.3	141.7	–5.6	–3.8	O7-A	220	393.5	401.1	7.6	1.9
E7-B	220	155.8	154.4	–1.4	–0.9	O7-B	220	392.0	402.8	10.8	2.8
E8-A	365	151.2	147.6	–3.6	–2.4	O8-A	365	400.1	428.1	28.0	7.0
E8-B	365	156.1	145.7	–10.4	–6.7	O8-B	365	400.3	417.5	17.2	4.3

50°C before examination by the stereomicroscope to check that no pollution coming from the soil has entered the bag.

2.2.2. Inorganic Experiment

This control experiment is an inorganic analog of the microbial experiment for oxygen isotope exchange. This experiment is very similar to that designed by Krueger (1991) for bones. Three aliquots of bone and three aliquots of enamel, each of them containing 200 mg of powder were placed in six glass tubes. Four tubes were fulfilled with 10 mL of ^{18}O-enriched deionized water, whereas the last two tubes contained deionized water at a natural abundance level. Waters were poisoned with 50 μL of 3% mercuric chloride Hg_2Cl_2 to maintain sterile conditions. Tubes were sealed then placed at 28°C in the dark, and agitated periodically during the time of experiment. Exchange experiments were carried out until 60 and 105 d, respectively. At these desired times of sampling, aliquots of water were sampled whereas bone and enamel powders were removed, rinsed and dried at 50°C.

2.3. Analytical Methods

2.3.1. Growth Medium of Microbial Experiment

Oxygen isotope ratios of soil water, and carbon isotope ratios of dissolved inorganic carbon (DIC) were measured after their extractions. Water was extracted from soil after distillation under vacuum at 90°C and oxygen isotope ratios were determined following the CO_2-H_2O equilibration method with analytical precision better than 0.1‰ using an VG-Optima mass spectrometer. Contents of DIC were measured by centrifugation on a Shimazu total organic analyzer with analytical precision better than 3%. Carbon dioxide was extracted from DIC samples by reaction with phosphoric acid under vacuum at 90°C. Carbon isotope ratios were measured with a VG-Optima mass spectrometer. Analytical precision was better than 1‰ in the case of high δ^{13}C values. Results are reported using the delta notation in per mil (‰) relative to SMOW for oxygen and PDB for carbon.

The evolution of C and N contents of the solid phase of the soil, as well as δ^{13}C values, were monitored by using a Carlo-Erba CHN analyser coupled to a VG-Sira 10 mass spectrometer. Standard deviation was 0.1% for C and N contents. Precision for high δ^{13}C values is probably less good than that specified for natural abundances (0.1‰). All elemental and isotopic data represent averages of six analyses.

2.3.2. Enamel and Bone Samples

Bone and enamel powders used for the microbial experiment were examined before and after incubation in reactors. A few milligrams of samples were fixed using a solution of sodium cacodylate and glutaraldehyde at 2% for 2 h, then dried in five volumes of ethanol with increasing concentration, from 60 to 100% by volume. Each specimen was gold-coated under vacuum and was mounted on an aluminium stub. Observations were made using a Leica S 440 scanning electron microscope (SEM), at 20 kV.

Organic matter was removed from all enamel and bone samples before isotopic analyses of their mineral phases. The proteinous fraction of bone and enamel was oxidized in 10 mL of 2 to 3% NaOCl solution. Enamel and bone powders were soaked for 1 and 3 d respectively. The solutions were changed every 12 h, then rinsed 10 times with distilled water and dried overnight. Since bone also contains lipids, bone powders were defatted before the NaOCl treatment. Bone lipids were solubilized in a dichloromethanol 2:1 solution (ultrasounded three times for 10 min), then dried overnight. Samples treated with acid were first soaked with NaOCl, rinsed 10 times, then soaked in 10 mL of 1 mol/L acetate buffer for 24 h, rinsed 10 times again, then dried overnight. Carbon (δ^{13}C) and oxygen (δ^{18}Oc) isotope ratios of carbonate from apatite were measured before and after acid treatment. CO_2 was produced by reaction with 100% H_3PO_4 under vacuum at 50°C. Isotopic analyses were performed on a VG-Sira 9 mass spectrometer with a precision of 0.1 and 0.2‰ for carbon and oxygen, respectively. We used the CO_2 pressure read on the ion gauge of the mass spectrometer to calculate weight percentage carbonate of the sample after calibration with carbonate standards. Oxygen isotope ratios of phosphate (δ^{18}Op) were measured after the NaOCl treatment. Apatite was converted to Ag_3PO_4 and reduced to CO_2 in the presence of a stoichiometric amount of graphite at a temperature of 1100°C, following the method of O'Neil et al. (1994) modified by Lécuyer et al. (1998). Oxygen isotope ratios were measured with a VG Prism and a Delta-E mass spectrometer and were corrected from the offset that was observed due to low yields of O'Neil et al.'s (1994) method compared to fluorination (Lecuyer et al., 1998; Venneman et al., 2002).

The total amounts of carbon and nitrogen in bone were measured using a Carlo-Erba CHN analyser coupled to a VG-Sira 10 mass spectrometer.

Fourier transform infra-red (FTIR) spectroscopic analyses were performed on bone and enamel powders to examine changes in crystallinity during incubation experiments. Infra-red analyses were performed using a Magna-IR 560 Nicolet FTIR spectrometer. Crystallinity

Table 2. Humidity, C and N contents, C and O isotope ratios of organic matter and water in the reactors from the inorganic and microbial experiments.

N° sample	Time of reaction (d)	Position in reactor	Water				Organic matter[a]		
			Humidity (wt. %)	DIC (ppm)	$\delta^{13}C_w$ (‰)	$\delta^{18}O_w$ (‰)	$[N]_{org}$ (%)	$[C]_{org}$ (%)	$\delta^{13}C_{org}$ (‰)
Inorganic experiment									
O0	105		100			–6.0			
E0	105		100			–6.8			
O1	60		100			817.9			
E1	60		100			758.3			
O2	105		100			913.8			
E2	105		100			917.8			
Microbial experiment									
1A	14						1.7 ± 0.3	16.5 ± 3.0	1803 ± 11
1B	14						1.5 ± 0.3	13.8 ± 3.7	1818 ± 12
3A	42						1.3 ± 0.2	13.6 ± 1.9	1817 ± 12
3B	42						1.0 ± 0.1	13.0 ± 1.7	1820 ± 4
4A	84	Higher	14.4			–5.3			
		Medium	14.7			–5.3			
		Lower	14.2			–5.3			
4B	84	Higher	13.5			167.5			
		Medium	14.4			168.6			
		Lower	15.0			168.7			
5A	112	Higher	15.3			–6.1	1.1 ± 0.2	9.7 ± 2.0	1809 ± 19
		Medium	17.2			–6.2			
		Lower	15.3			–6.3			
5B	112	Higher	14.2			175.8	1.3 ± 0.2	10.1 ± 1.3	1826 ± 4
		Medium	15.1			174.0			
		Lower	16.0			177.2			
7A	220	Higher	16.1		1743	–5.7	0.4 ± 0.1	9.1 ± 2.0	1800 ± 6
		Medium	16.5	1734	1783	–5.8			
		Lower	20.3	1955	1773	–5.8			
7B	220	Higher	16.5	25		164.7	1.2 ± 0.1	7.6 ± 0.8	1792 ± 7
		Medium	17.8			167.0			
		Lower	20.2	19		167.5			
8A	365	Higher		12	1909		0.7 ± 0.1	7.8 ± 1.4	1822 ± 3
		Medium		20					
		Lower		16	1681				
8B	365	Higher		705			0.3 ± 0.0	6.2 ± 1.2	1780 ± 12
		Medium		742					
		Lower		727					

[a] Six measurements were made after homogeneization of the soil.

is quantified by the splitting factor (SF) as defined by Weiner and Bar-Yosef (1990). Precision for SF values is better than 0.1 for a given sample.

3. RESULTS

3.1. Inorganic Experiment

Oxygen isotope compositions of waters are given in Table 2 while δ^{18}Oc, δ^{18}Op values, and carbonate contents of bone and enamel are presented in Table 3.

Changes in δ^{18}O values of both carbonate and phosphate oxygen appeared rapidly (Fig. 2) during the exchange experiments and were 10 times more important in the carbonate than in the phosphate compound. Enamel samples show less marked δ^{18}O variations in the carbonate phase, whereas phosphate oxygen isotope ratios did not show any evolution. A small variability (0.4%) in the carbonate content of bone was also detected. Since deionized water did not contain dissolved carbon, and glass tubes were sealed, this variability cannot be explained by adsorption of secondary carbonate. Because carbonate estimates are deduced from the mass of the mineral phase, this variability could be explained by an incomplete removal of bone water or organic matter during the preliminary treatments of some samples.

The acetic acid treatment of reacted samples induced a 30% decrease in carbonate content, and a small isotopic shift in the carbon (~1‰) and oxygen (~1–6‰) isotope compositions of bone apatite. We note that acid-leached apatites do not retain their original oxygen isotope ratios, and this treatment was less efficient in bone for which only 12 to 32% of the isotopic shift was removed vs. ~60% in enamel.

3.2. Microbial Experiment

3.2.1. Soil Measurements

The measurements are given in Table 2. The evolution of carbon and nitrogen contents of the soil shows that ~70% of the organic matter was mineralized by micro-organisms after 1 yr of experiment. No significant shifts in δ^{13}C values of organic

Table 3. Elementary (C, N), isotopic (C, Oc, Op) and spectroscopic (FTIR) analyses and carbonate content of bone and enamel reacted under inorganic and microbial conditions.

		Before the acid treatment							After the acid treatment				
		Before NaOCl			After NaOCl								
N° sample	Time of reaction (d)	C (%)	N (%)	C/N	$[CO_3^{2-}]$ (%)	$\delta^{13}C$ (‰)	$\delta^{18}Oc$ (‰)	SF	$[CO_3^{2}]$ (‰)	$\delta^{13}C$ (‰)	$\delta^{18}Oc$ (‰)	$\delta^{18}Op$ (‰)	SF
Inorganic experiment													
Bone													
O0	0				6.0	–5.5	28.5			–4.2	26.9	17.4	
O1	60				6.4	–4.9	39.1		4.3	–3.8	36.2	18.4	
O2	105				6.4	–4.9	48.9		4.4	–4.1	41.4	20.0	
Enamel													
E0	0					–2.6	27.4		3.7	–2.5	27.5	17.9	
E1	60					–2.5	30.3		3.8	–2.5	28.6	17.9	
E2	105					–2.5	32.3		3.5	–2.4	29.4	17.9	
Microbial experiment													
Bone													
First set of water $\delta^{18}Ow = –6‰$													
O0	0	13.7	4.2	3.3	6.1	–5.0	28.5	3.51	4.5	–3.8	26.1	17.0	4.33
O1-B	14	12.2	4.2	2.9					4.3	–0.9	26.3		
O2-A	28	11.6	3.9	3.0					4.5	0.1	26.2		
O2-B	28	12.1	4.1	2.9					4.8	1.0	25.6		
O3-A	42	11.8	4.0	2.9					4.3	0.3	26.0		
O3-B	42	11.9	3.9	3.1					4.6	3.5	25.8		
O4-A	84	12.4	4.4	2.8					4.5	10.1	26.3	17.0	
O5-A	112	12.6	4.4	2.9	6.1	45.1	27.7	3.85	4.3	24.4	26.3		4.28
O5-A (48h in acetic acid)									4.4	22.3	25.5		
O5-A (72h in acetic acid)									4.0	22.1	25.8		
O5-A (96h in acetic acid)									3.9	21.5	26.4		
O6-A	143	12.2	3.7	3.3	6.1	96.0	26.6		4.2	46.5	25.7		
O7-A	220	13.0	4.2	3.1	6.5	96.7	28.4		4.4	65.8	26.3	17.4	
O8-A	365				6.7	25.9	26.7		5.0	19.0	26.3	16.8	
Second set of water $\delta^{18}Ow = +170‰$													
O4-B	84	12.4	4.2	2.9					4.5	13.1	26.7	18.6	
O5-B	112	12.6	4.3	2.9	6.1	25.1	30.3		4.5	12.5	27.0	25.5	
O6-B	143	12.7	4.2	3.1	6.0	80.0	30.4		4.0	35.4	27.4	20.1	
O7-B	220	12.5	4.2	3.0	5.8	19.9	29.0	3.59	3.9	17.9	27.5	36.5	
O8-B	365				7.4	99.5	32.9	3.50	5.8	69.3	29.6	22.6	
Enamel													
First set of water $\delta^{18}Ow = –6‰$													
E0	0								3.8	–2.4	27.4	17.7	5.07
E1-A	14								3.8	–2.4	27.4		
E1-B	14								3.8	–2.2	27.4		
E2-A	28								3.7	–2.0	27.7		
E2-B	28								3.9	–2.2	27.5		
E3-A	42								3.9	–2.0	27.0		
E3-B	42								3.5	–1.4	27.4		
E4-A	84								3.8	0.9	27.3	17.6	
E5-A	112				4.0	29.7	26.7		3.9	2.7	27.2		
E6-A	143								3.8	4.8	27.3		4.95
E7-A	220				4.2	112.9	27.4	4.05	3.9	18.4	27.3	17.8	
E8-A	365				3.8	10.7	28.1		3.5	1.2	27.8		
Second set of water $\delta^{18}Ow = +170‰$													
E4-B	84								3.8	2.7	27.3	17.9	
E5-B	112				3.9	12.2	28.4		3.7	0.6	27.5	17.8	
E6-B	143								3.7	2.5	27.5	17.3	
E7-B	220				3.9	8.6	28.4		3.9	1.1	27.6	20.0	
E8-B	365				3.8	63.0	30.8	4.35	3.7	3.2	28.0	17.5	

matter accompanied microbial degradation, and average $\delta^{13}C$ values of soil organic carbon range from 1780 to 1825‰. The concentration and carbon isotope ratios of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) were measured in the higher, medium and lower parts of the reactors after 220 and 365 d. DIC concentrations were homogeneous within a reactor but very variable among them with values ranging from 20 to 1950 ppm. Measured $\delta^{13}C_{DIC}$ values are close to that of coexisting organic matter, ranging from 1681 to 1909‰. Therefore, we assume that the dissolved inorganic carbon available to react with bioapatites during the experiment has a $\delta^{13}C$ value of about +1800‰, similar to that of organic matter. No significant changes in soil humidity and $\delta^{18}O_w$ were observed during the experiment, suggesting that the second film was successful in

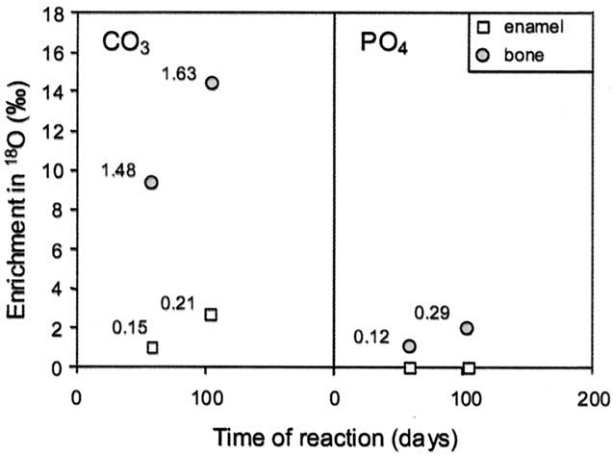

Fig. 2. ^{18}O-enrichments (after acid treatment) in carbonate and phosphate from bone and enamel powders that reacted under inorganic conditions. Percent of oxygen isotope exchange are indicated for each bone and enamel sample.

preventing evaporation. Between days 84 and 220, ^{18}O-enriched reactors had δ^{18}O values between +166 and +175‰ with no noticeable trend, whilst the other series had a narrower range of δ^{18}O values, between −6 and −5‰.

3.2.2. Bone and Enamel Measurements

Bone samples become brownish soon after the beginning of the experiment. This color change is reminiscent of that observed for archaeological skeletal remains in natural subaqueous environments (Bocherens et al., 1997), and could be related to extraneous organic matter, such as humic acids, or to the decomposition of bone organic matter. Extensive colonization by various micro-organisms was observed on the bone surface after seven months of incubation. At least two forms of microorganisms were found very close to bone surface; bacteria and fungi. Hundreds of bacteria were observed on each bone fragment, either dispersed on the bone surface or concentrated around the collagenous fibrils (Figs. 3A and 3B). Fungi are represented by spores and mycelium (Figs. 3B and 3C). We observed a weight increase of bone powders after the incubation process (Table 1), thus indicating that a component from the soil (organic or mineral phase, water) had been incorporated into the bone structure during incubation. Soil water could be partly responsible for this weight increase since no significant change in the mineral or the organic phase was detected in bone samples throughout the experiment. During exchange experiments, total carbon contents of bones slightly decreased (11.8–13.0% against 13.7% in fresh bone) but N contents remained unchanged (3.7–4.4% against 4.2% in fresh bone), thus pro-

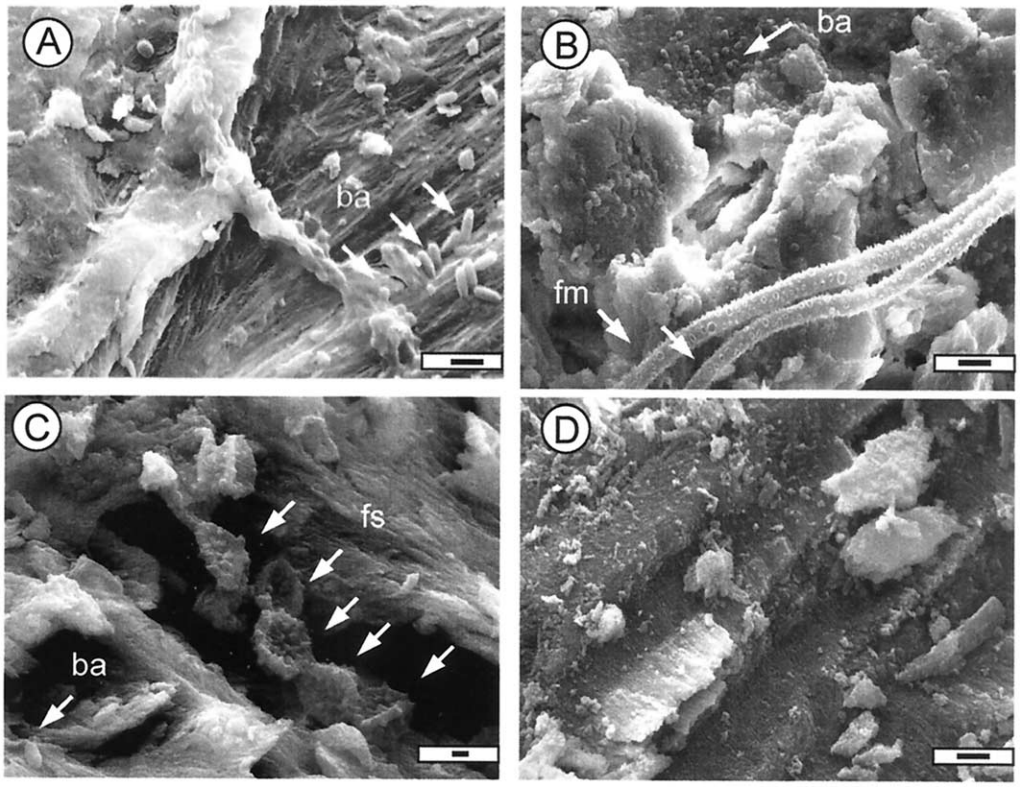

Fig. 3. Scanning Electron Micrographs of bone (A–C) and enamel (D) fragments after reaction in presence of soil micro-organisms. (A) Bacterially-infested bone fragment after 16 weeks of reaction. Note that bacteria (ba) are closely packed along fibrillae of bone organic matter. Bar = 1 μm. (B) Bone fragment invaded by bacteria and fungal mycelium (fm) after 1 yr of reaction. Bar = 3 μm. (C) Bone fragment with bacteria and fungal sporangia (fs) after 1 yr of reaction. Bar = 1 μm. (D) Enamel fragment after 20 weeks of reaction. No microorganism detected on the enamel surface. Bar = 1 μm.

viding C/N ratios between 2.8 and 3.3 typically in the known range for modern bones (Bocherens et al., 1997). Some samples were characterized by a slight increase in their carbonate content or crystallinity (Table 3) but no evolution was observed. These minute changes are far less significant than those resulting from the acetic acid treatment. Contrasting with this pattern, bone crystals suffered both a sharp increase in crystallinity and a decrease in carbonate content during the acetic acid treatment. From 25 to 40 wt.% carbonate were removed after 1 to 4 d of immersion in the acid solution.

After reaction, enamel was less tainted than incubated bone and was not affected by visible postmortem scavenging. No trace of bacteria or fungi could be detected by SEM microscopy even after 1 yr of experiment (Fig. 3D). Slightly lower masses were measured after incubation (Table 1), possibly resulting from an incomplete removal of the powder from the nylon bags. Fresh enamel is better crystallized than bone (SF = 4.5 against 3.5) and contains less carbonate (~4 vs. 6 wt.%). No significant increase in carbonate content or crystallinity was detected during the experiment. Acetic acid is less aggressive on enamel than bone, and <10% of carbonate was leached after 24 h of treatment.

Stable isotope compositions of phosphate and carbonate from bone and enamel apatite are presented in Table 3 and Figure 4. Carbon and oxygen isotope evolutions of both carbonate and phosphate phases indicate interactions with soil water. Isotopic changes are much more important in bone than in enamel and were not totally eliminated by the acetic acid even after 4 d of treatment (Table 3, sample O5A). In the carbonate phase, the most important changes are observed for carbon with a ^{13}C-enrichment that can be >100‰ in bone or enamel before the acetic acid treatment. The carbonate oxygen appears to be much more resistant with an observed ^{18}O-increase which does not exceed 5‰ in ^{18}O-enriched (B) reactors. Unlike carbonate oxygen, phosphate oxygen isotope compositions can be shifted by as much as 20‰ in bone. Phosphate oxygen in enamel is more stable, and only one sample (E7-B) was significantly ^{18}O-enriched by ~2‰. The absence of significant shift in δ^{18}Op values in A reactors suggests that the δ^{18}O value of dissolved phosphate from the inorganic growth medium must be very close to that of bone and enamel apatite and that soil water from the B reactors is the only ^{18}O-enriched source available for oxygen isotope exchange. Progressive ^{13}C- and ^{18}O-enrichments are documented during incubation, a pattern that could be linked to the progressive mineralization of organic carbon due to bacterial activity.

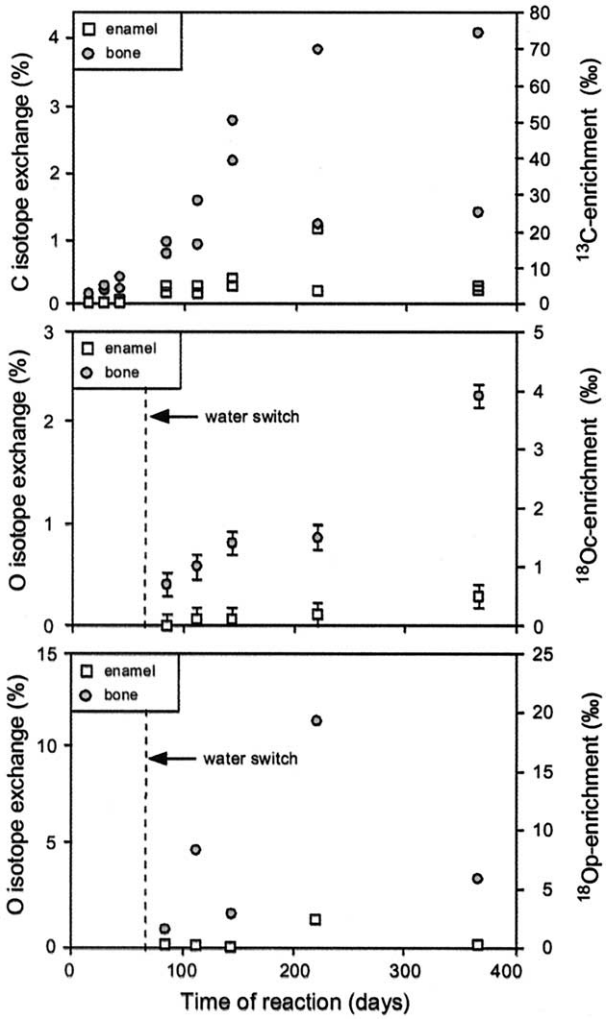

Fig. 4. ^{13}C- and ^{18}O-enrichments in carbonate and phosphate measured after the acetic acid treatment of bone and enamel powders that reacted under microbial conditions. Percent of C and O exchange are calculated assuming average carbon and oxygen isotope compositions of waters of +1800 and +166‰, respectively. In the C panel, enamel and bone data from the A and B reactors are plotted for each day. In the O panels, only data from ^{18}O-enriched (4B–8B) reactors are plotted.

4. DISCUSSION

Despite the increased surface area provided by the grinding and the rapid colonization of bone apatite by soil micro-organisms, limited changes in organic content of incubated bones were observed after 1 yr of experiment. Experimental conditions (humidity, protection from light) were probably favorable to bone and enamel apatite survival. This is in agreement with the conclusions given by Tuross et al. (1989) who found that molecular and crystallographic changes of wildebeest bones where much less advanced for skeletons that were buried in a soil or protected from open sunlight compared to exposed ones. Because apatite is closely linked to the organic fraction, mainly collagen, its fate is highly dependent on the resistance of the organic fraction to chemical hydrolysis, and the degradation of both phases are expected to proceed simultaneously (Tuross et al., 1989; Hedges et al., 1995) As the organic matrix decomposes, the crystallites are released and can interact with the burial environment, the smallest being leached. Although bone organic matter has not reached an advanced stage of degradation, significant chemical changes were measured in the mineral phase of bone, and to a lesser extent, enamel. This result indicates that changes in the carbon and oxygen isotope compositions of bioapatite were rapidly initiated, within days of burial, and before any documented significant reorganization of bone or enamel apatite.

4.1. Characterization and Elimination of the Diagenetic Contaminants From the Carbonate Phase

The carbon and oxygen isotope ratios of bone and enamel samples have significantly shifted from initial values during the two experiments (Figs. 2 and 4). In the inorganic experiment, precipitation of secondary carbonate on the apatite surface is not expected to occur, since deionised water from the inorganic experiment is free of dissolved carbonate or bicarbonate ion, and sealed glass tubes prevent water to equilibrate with atmospheric CO_2. However, deionised water has a pH <7 and could partly dissolve HCO_3^- and CO_3^{2-} ions that are adsorbed on the surface of bone and to a lesser extent, enamel. This will provide some bicarbonate ions to exchange oxygen with enriched water. These ions, in turn, could be incorporated to the apatite structure via adsorption, and/or isotopic exchange and would not be removed by NaOCl treatment. However, δ^{18}Oc and δ^{18}Op values remained high after the acid treatment, thus indicating unambiguously that isotopic exchange between carbonate apatite and water is involved in the diagenetic process.

The situation is different when considering the microbial experiment because various amounts of dissolved inorganic carbon were present in the system and may combine at pH of 6 to 7 with water to produce bicarbonate ions. However, we emphasize that the experimental conditions as well as the accuracy of carbonate content measurements were not initially optimized for deciphering the source of C and O isotope enrichments in the carbonate fraction. But it is noteworthy that, considering the high δ^{13}C values of the dissolved inorganic carbon and the lack of significant ($>$0.4 wt.%) increase in the carbonate content of apatites during the experiments, even minute amounts of secondary carbonate could accommodate shifts up to +100‰ in δ^{13}C values as measured in some samples.

In this framework, the response of bone and enamel to the acid treatment can be used as an indirect information. The acetic acid treatment was not sufficient to completely remove C and O isotope enrichments in either bone or enamel. In the microbial experiment, the soluble fraction from enamel was responsible for 61 to 92% of the observed isotopic shifts, whereas only 12 to 54% of the contamination was removed from the bone (Fig. 5). Both the ^{13}C enrichment measured before the acid treatment and the efficiency of this treatment to remove the diagenetic fraction are highly dependent on DIC concentrations. For two contemporaneous reactors, ^{13}C enrichments and acid efficiency are higher when DIC concentrations are high, suggesting that adsorbed carbonate or the precipitation of secondary carbonate minerals could have contributed to the measured isotopic enrichment in these samples (Tables 2 and 3). In reactors 7B and 8A, DIC concentrations are low (15–25 ppm), and acetic acid eliminates only 10 to 30% of the isotopic shift in bone and 60% in enamel. This limited efficiency is very similar to that calculated for bone and enamel reacted under inorganic conditions with no DIC present in water. This result suggests that in reactors 7B and 8A, the major fraction of the ^{13}C enrichment was durably incorporated into the structure of bone and enamel apatite, either via isotopic exchange or precipitation of secondary phosphate minerals.

Low efficiency for the acetic acid treatment in bone challenges the conclusions of Krueger (1991) who reacted bone

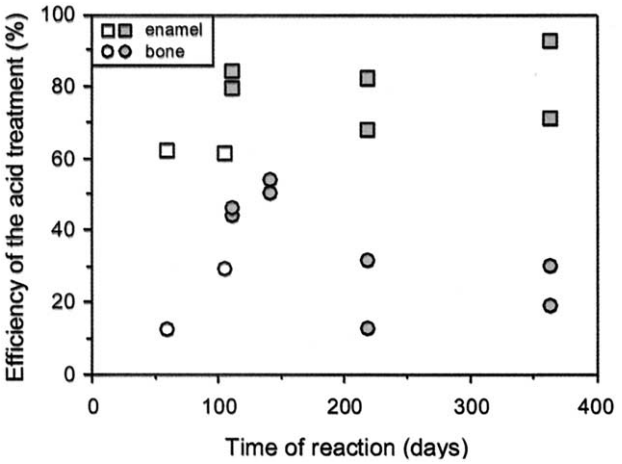

Fig. 5. Efficiency of the acid treatment of bone and enamel samples that reacted under inorganic (open symbols) and microbial (closed symbols) conditions. Acid treatment efficiency (in %) is expressed as the difference between the isotopic enrichment measured before and after the acid treatment, divided by the isotopic enrichment measured before the treatment. Calculations are based on bone and enamel δ^{18}Oc values in the case of the inorganic experiment, and bone and enamel δ^{13}C values in the case of the microbial experiment.

apatite during 550 d in ^{13}C-enriched sterilized water and observed that at least 99% of the contaminating carbonate phase had been removed by the acetic acid pretreatment. Two differences with Krueger's (1991) experiment may account for such a discrepancy. Firstly, in Krueger's (1991) experiment the solution had a bicarbonate concentration of 1000 ppm $CaCO_3$ equivalent and a significant part of the isotopic composition of the soluble phase could be explained by the presence of secondary carbonate, which should had been easily dissolved during the acid treatment. Secondly, Krueger (1991) used archaeological bone instead of modern bone because he thought that the organic matter in bone would have limited the surface of reaction between the solution and the apatite micro-crystals. Extensive literature has shown that the degradation of bone organic matter is accompanied by a crystal size increase, and that fossil bone crystal sizes become very similar to that of modern enamel (Tuross et al., 1989; Ayliffe et al., 1994; Person et al., 1995). In Krueger's (1991) experiment, the soluble fraction of archaeological bone is 14 to 122 times more isotopically-enriched than the non soluble one, a range which compares well with the ranges of 17 to 63 or 41 to 209 calculated for enamel in our inorganic and bacterial experiments, respectively. With respect to these arguments, Krueger's (1991) conclusions are more likely valid for enamel rather than bone diagenesis.

Thus, examination of the effects of acetic acid on reacted bioapatites sheds some light on the processes operating during fossil bone and enamel diagenesis, respectively. This cleaning procedure was recognized by many authors as both necessary and efficient to remove adsorbed carbonate ions or calcite (Sullivan and Krueger, 1981; Lee-Thorp and van der Merwe, 1987; Krueger, 1991; Bocherens et al., 1996; Koch et al., 1997) but remains of limited help if isotopic exchange between carbonate apatite and dissolved inorganic carbon operates as the main diagenetic process (Schoeninger and DeNiro, 1982). High

efficiency of the acetic acid treatment in enamel suggests that alteration in this tissue happens early, probably via recrystallization of a small fraction of poorly crystallized enamel and that subsequent exchange is minimal compared to bone crystallites. This experimental work is in agreement with past studies on fossil enamel (Lee-Thorp and van der Merwe, 1987; Wang and Cerling, 1994; Koch et al., 1997).

4.2. Inorganic vs. Microbial Diagenesis

4.2.1. Carbon Isotopes

During the microbial experiment, shifts in enamel and bone carbon isotope ratios are rapid and significant and can account for up to 4% of the apatite $\delta^{13}C$ values after the acid pretreatment (Fig. 4). In comparison, ^{18}O-enrichments of apatite carbonate are very small, and suggest preferential incorporation of labelled carbon vs. oxygen. This result is very surprising because C and O from DIC are incorporated together in apatite carbonate, and preferential enrichment to one element vs. another relative to solution concentrations is not expected. Simulations of carbon isotope diagenesis based on water-rock interaction models indicate that under inorganic conditions and at low temperature, isotopic exchange between carbon apatite and dissolved inorganic carbon is very limited when compared to carbonate oxygen (Wang and Cerling, 1994). Given the small DIC contents (<0.1%) measured in the solutions, the shifts in $\delta^{13}C$ measured in bone and enamel samples would require partial to total oxygen isotope equilibrium between apatite carbonate and water to fit the predictions of the model which is, by far, not the case. This trend may be an indirect consequence of the presence of micro-organisms in the vicinity of reacted apatites. Grupe and Pipenbrick (1989) have demonstrated experimentally that microbial (fungal) activity was responsible for the concentration of various trace elements in bones. Similarly, microbial activity could be responsible for increased DIC concentration within the bone or enamel crystallites compared to that measured in ambient fluids. Our result could be explained by a rapid incorporation of CO_2 into lattice sites following hydration. Computing Wang and Cerling (1994)'s model for bone and enamel from reactor 8B, DIC concentrations of ~70,000 and 20,000 ppm within the bone and enamel crystallites, respectively, are required to obtain the observed the results. Unfortunately we did not measure in situ the DIC concentration during the experiment, thus prevailing to test this hypothesis. Additional inorganic and microbial experiments are currently underway to elucidate the preferential enrichment of apatites in ^{13}C observed during our microbial experiment.

4.2.2. Oxygen Isotopes

In the apatite of living vertebrates, oxygen from phosphate and carbonate is in isotopic equilibrium with body water, and coexisting $\delta^{18}Oc$ and $\delta^{18}Op$ values are linearly related (Bryant et al., 1996; Iacumin et al., 1996). This property was used by Iacumin et al. (1996) as a test for identifying diagenetic alteration in fossil teeth and bones. Because different rates of oxygen isotope exchange in the phosphate-water and carbonate-water systems are expected in the case of inorganic or microbially-mediated interactions, the $\delta^{18}O$ values of altered fossils should deviate from equilibrium values. Results of our two experiments revealed that the presence or absence of micro-organisms during early diagenesis controls the rates of oxygen isotope exchange between phosphate in apatite and soil water. Reacted apatites are characterized by two very distinct patterns of diagenesis, each of them leading to a substantial deviation from their initial equilibrium isotopic compositions (Fig. 6).

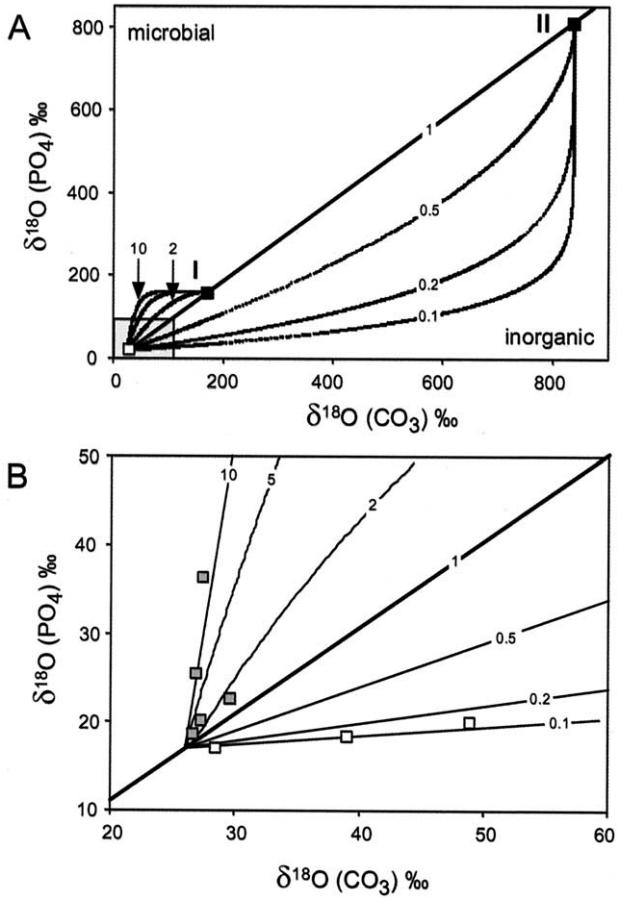

Fig. 6. (A) Numerical simulation of exchange trajectories in a $\delta^{18}Op$-$\delta^{18}Oc$ space for various k_p/k_c ratios according to Gregory and Criss (1986). Along a single-trajectory exchange curve, samples that plot the farthest from the initial point have suffered the highest fluid-rock ratio. Carbonate-water and phosphate water fractionation equations are used to estimate the oxygen isotope composition of end-members I and II according to average oxygen isotope compositions of reacting waters in the microbial ($\delta^{18}O_w = +166‰$) and inorganic ($\delta^{18}O_w = +818‰$) experiments, respectively. (B) Figure 6B focuses on the fit of bone data with the model (located on the lower left part of Fig. 6a.). Under inorganic conditions (open squares) $k_p/k_c = 0.1$ whereas in the presence of micro-organisms (closed squares) k_p/k_c is comprised between 2 and 15.

Under inorganic conditions, kinetics of isotopic exchange between carbonate oxygen and water are higher than those between phosphate oxygen and water. This result confirms that the phosphate component of bioapatites is very resistant to inorganic oxygen exchange with water at low temperature (Blake et al., 1997; Lécuyer et al., 1999). Under microbially-mediated conditions, the rate of oxygen isotope exchange between phosphate and water is much faster than that observed

for carbonate oxygen. In bone apatite, >12% of the phosphate oxygen could had experienced isotopic exchange. Partial oxygen isotope exchange occurred because microbes are able to break inorganic P-O bounds (Blake et al., 1997).

Using the formulation of Gregory and Criss (1986), the kinetics of isotopic exchange between phosphate and water or carbonate and water is formulated as

$$f = 1-\exp^{-(kt)} = \frac{\delta^{18}O_{(i)}-\delta^{18}O_{(t)}}{\delta^{18}O_{(i)}-\delta^{18}O_{(e)}} \quad (1)$$

where f is the fraction of isotopic exchange between mineral and fluid, $\delta^{18}O_{(i)}$ is the initial oxygen isotope composition at $t = 0$, $\delta^{18}O_{(t)}$ is the composition measured at any time t, $\delta^{18}O_{(e)}$ is the composition at equilibrium, and k is the rate constant of the reaction. However, the rates constants k_p and k_c—defined for the PO_4-H_2O and CO_3-H_2O systems, respectively—are unknown. According to the experimental conditions, in a buffered open system, the kinetically-controlled $\delta^{18}O$ values of coexisting carbonate and phosphate in apatite may be directly compared by rearranging Eqn. 1 to eliminate t, and exponentiating the results (Gregory and Criss, 1986):

$$1 - f_c = (1-f_p)^{k_c/k_p} \quad (2)$$

where f_c and f_p are the fractions of isotopic exchange for the carbonate-water and the phosphate-water systems, respectively.

In a $\delta^{18}Oc$-$\delta^{18}Op$ space, a set of curves was computed to describe the rates of oxygen isotope exchange for various k_p/k_c ratios (Fig. 6). The two end-members of the isotopic reaction are the compositions of reactants at the initial state ($f = 0$) and at equilibrium ($f = 1$). The $\delta^{18}O$ values of both carbonate and phosphate at equilibrium are calculated using the composition and temperature of reacting waters according to the oxygen isotope fractionation factors known for the phosphate-water (Longinelli and Nuti, 1973) and carbonate-water (Friedmann and O'Neil, 1977) systems. Actually, the choice for using the Friedmann and O'Neil's (1977) equation is not totally appropriate because this equation was defined for calcium carbonate, not apatite carbonate. Solving the PO_4-H_2O and the CO_3-H_2O equations at $T = 37°C$ gives a difference between $\delta^{18}Oc$ and $\delta^{18}Op$ which is ~1.8‰ higher than the difference predicted by empirical equations determined by using modern mammals (Bryant et al., 1996; Iacumin et al., 1996). However, because there is no fractionation equation available for the apatite carbonate-water system, and because the isotopic changes produced by labelled waters are one order of magnitude higher, the relative small uncertainties in the knowledge of the fractionation relationships are not relevant here.

More the k_p/k_c ratio differs from 1 more the convex ($k_p/k_c > 1$) or concave ($k_p/k_c < 1$) curvature is pronounced (Fig. 6A). Because of very limited isotopic exchange during the experiments, the curves that fit the data look like straight lines (Fig. 6B). Under inorganic conditions, kinetics of oxygen isotope exchange are 10 times faster in carbonate than in phosphate. On the opposite, during biologically-mediated reactions, the kinetics of oxygen isotope exchange between phosphate and water is from 1.5 to 15 times faster than between carbonate and water. Our diagenetic end-member for the phosphate compound is calculated assuming equilibrium PO_4-H_2O exchange catalyzed by microbes and does not take into account the possibility of incomplete isotopic exchange. Partial (~50%) exchange was observed by Blake et al. (1997) during microbially-mediated oxygen isotope exchange between dissolved phosphate and water and may occur under conditions of excess PO_4. If such partial exchange occurred during our microbial experiment, our model would underestimate k_p values and therefore k_p/k_c ratios. For example, a k_p/k_c ratio of 30 is calculated for O7B assuming a 50% exchange according to Blake et al. (1997).

4.3. Implications for Paleoclimatic Reconstructions

Our experimental data confirm that enamel is the most reliable tissue for paleoenvironmental studies. A quantitative treatment of data in terms of relative resistance to isotopic exchange of enamel compared to bone may be difficult to achieve for several reasons. Since the surface area of a mineral is critical in determining rates of isotopic exchange (Cole and Ohmoto, 1986), applying our results obtained on powders to tridimensional objects is certainly not straightforward. Moreover, bone and enamel apatites differ in many ways: crystal size, degree of mineralization, porosity and chemical composition. These parameters, which are likely to evolve during apatite diagenesis, control the kinetics of isotopic exchange as well. Further experiments will be necessary to explore their importance during early diagenesis.

Kinetics of oxygen and carbon isotope exchange between fossil apatites and surrounding aqueous fluids are strongly controlled by the inorganic or microbially-mediated mechanism of alteration. Given the wide range of k_p/k_c ratios calculated during our experiments, it is very likely that the relative degrees of interaction between apatites and the local environment will not be predictable when micro-organisms are involved in the process of isotopic exchange. Series of $\delta^{18}O$ values measured in hypsodont enamel are frequently used as a proxy for short term (i.e., seasonal) climate change because in this tissue, mineralization occurs basically from apex (upper part) to cervix (lower part) of the crown as it erupts and is not remodeled once formed. In modern enamel, the carbonate-phosphate equation (Bryant et al., 1996; Iacumin et al., 1996) predicts similar range of $\delta^{18}Op$ and $\delta^{18}Oc$ measured in one hypsodont tooth. Fricke et al. (1998) and Fox and Fisher (2001) measured low range of $\delta^{18}Op$ values compared to $\delta^{18}Oc$ in tooth enamel from several Paleocene-Eocene pantodonts and Pleistocene proboscideans, respectively, indicating preferential alteration of one fraction relative to another. High k_p/k_c ratios during tooth enamel diagenesis could have produced these large (up to 1.5‰) and unexpected differences measured in fossil hypsodont enamel. This result raises the possibility that, at least in some cases, phosphate oxygen in enamel may experience bacterially-mediated reactions leading to partial reequilibration with the oxygen isotope composition of the diagenetic fluids.

Bacteria and fungi represent a large part of the soil biomass. Microbiological activity is frequently invoked to explain early diagenetic changes that are expressed by a decrease in protein contents, and also increases in crystallinity and porosity of apatites (Hedges et al., 1995). A microbial control of the isotopic interactions between soil water and apatites are most likely very common at the Earth surface.

5. CONCLUDING REMARKS

Our experiments indicate that a significant part of ^{13}C and ^{18}O that derived from the aqueous fluid have been rapidly incorporated into the apatite structure, probably through isotopic exchange or secondary carbonate apatite precipitation. In most cases, usual chemical treatments are not efficient for restoring the original $\delta^{13}C$ and $\delta^{18}Oc$ values of fossil apatites since only part of the alterations are removed by acetic acid. Enamel was found to be more resistant than bone to isotopic alteration. This result is in agreement with previous studies based on fossil material, and confirms experimentally that enamel is the more suitable material for paleoenvironmental reconstructions.

Under inorganic conditions, the k_p/k_c is estimated close to 0.1, thus confirming that phosphate oxygen is more resistant than carbonate to isotopic exchange with water. On the opposite, during biologically-mediated reactions, the kinetics of oxygen isotope exchange between phosphate and water is, at least, from 2 to 15 times faster than between carbonate and water. Soil micro-organisms are responsible for large variations in $\delta^{18}Op$ and $\delta^{13}C$ values measured in bones but also in enamel in a lesser extent. Depending on the inorganic or biologically-mediated mode of apatite-water interactions, we can conclude that it is not possible a priori to know whether the oxygen isotope composition of phosphate has been more preserved than carbonate in fossil apatites. If fossil enamel can be selected with minimal or no trace of microbial activity, its $\delta^{18}Op$ and $\delta^{13}C$ values can be safely used for the reconstitution of paleoenvironments.

Acknowledgments—This work could not have been obtained without the generous help and support of several colleagues and friends. Advice and remarks from Simon Sheppard were particularly appreciated and helped us to improve this manuscript. We acknowledge with thanks the valuable help of Cyril Girardin and Josiane Guillemot in preparing the growth medium, and Stephan Borenstajn and Graziella Midelet in obtaining SEM photographs. ^{13}C-enriched straw was provided by Sylvie Recous and Olivier Delfosse, whereas Thierry Bariac gave us the ^{18}O-enriched water. We are indebted to Patricia Richard, Micheline Grably, François Martineau and Jean-François Saliège for their technical assistance. We also wish to thank Guillaume Morin for allowing us extensive use of the FTIR spectrometer. Paul Koch and anonymous reviewer are thanked for helpful reviews of this manuscript.

Associate editor: J. Horita

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