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Intra-tooth study of modern rhinoceros enamel $\delta^{18}O$: Is the difference between phosphate and carbonate $\delta^{18}O$ a sound diagenetic test?

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ABSTRACT

The carbonate and phosphate oxygen isotopic difference ($\Delta^{18}O_{C-P}$) has been suggested as a test for diagenetic alteration of isotope values in fossil apatite samples. The generally accepted $\Delta^{18}O_{C-P}$ value for well-preserved enamel is ~9‰. Only a few studies have focussed on the potential natural variability of this difference in modern and fossil mammal teeth. The aim of this study is to document the value of the carbonate-phosphate oxygen isotopic fractionation factor ($\alpha_{CO3-PO4}$) at an intra-tooth level. We measured the enamel phosphate and carbonate oxygen isotopic compositions of two upper molars (M1 and M2) of *Rhinoceros unicornis* following a sequence from the apex to the cervix of the crown. The average $\alpha_{CO3-PO4}$ is 1.0082±0.0007. The mean value of the $\Delta^{18}O_{C-P}$ difference is 8.4±0.7‰, close to the literature values given for other species. However, we also show that the values of the $\Delta^{18}O_{C-P}$ difference for *R. unicornis* upper molars can vary on a ~2‰ range in the same tooth. We develop a physiological model to explain the origin of inter-species and intra-tooth $\Delta^{18}O_{C-P}$ difference and to understand their implications on the status of $\Delta^{18}O_{C-P}$ as a tool for identifying diagenesis. The results imply that, by increasing the dataset of phosphate and carbonate oxygen isotopic composition for modern mammal species, we will be able to define a maximal and precise envelope corresponding to the well-preserved samples and to check a possible species dependence on the $\delta^{18}O_C/\delta^{18}O_P$ relationship.

1. Introduction

The oxygen isotope compositions (noted $\delta^{18}O$)[1] of mammalian tooth bioapatite are related to $\delta^{18}O$ of local environmental water and hence can reflect climatic conditions at the time of growth. The $\delta^{18}O$ of phosphate and carbonate of the biogenic apatite precipitate in isotopic equilibrium with body water at constant temperature (Luz et al., 1984; Bryant and Froelich, 1995). For most large mammals, $\delta^{18}O$ of body water is directly related to that of their ingested (drinking and dietary) water (Luz et al., 1984; Luz and Kolodny, 1985; D'Angella and Longinelli, 1990; Bryant and Froelich, 1995; Kohn et al., 1996). This relationship varies among species; for instance some animals obtain large amounts of their water supply from the food that they eat (e.g. giraffe) — these are often non-obligate drinkers. Very small animals with rapid metabolism (e.g. micro-mammals) may have larger non-water ^{18}O inputs (Luz et al., 1984). In large, obligate drinking mammals, the $\delta^{18}O$ of phosphate and carbonate of the biogenic apatite provides an indirect measure of the local precipitation $\delta^{18}O$ compositions that are preserved in local environmental water. According to Dansgaard (1964) and Rozanski et al. (1993), the rainwater $\delta^{18}O$ is locally related to climatic variables (surface air temperature and amount of precipitation). The interest of this relationship is well established in paleobiology, paleocology and paleoenvironmental studies. However, one of the main limits for systematic successful studies is the preservation of the isotopic signal of the biogenic apatite.

In biogenic apatite (carbonate-hydroxyapatites described as $[Ca_9((PO_4)_{4.5}(CO_3)_{1.5})OH_{1.5}]$, Mc Connell, 1952; LeGeros et al., 1996), oxygen is present in three different locations in the crystal lattice: in the phosphate, carbonate and hydroxyl groups. It is generally accepted that phosphate oxygen isotope ratio ($\delta^{18}O_P$) is less susceptible to diagenetic processes than carbonate oxygen isotope ratio ($\delta^{18}O_C$) because P–O chemical bonds in apatite are stronger than the C–O bonds. However different studies demonstrated that microbial activity affected $\delta^{18}O$ values from phosphate (oxygen isotope equilibrium between dissolved phosphate ions and water is rapidly promoted by

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[1] Oxygen isotopic results are expressed in relative delta (δ) per mil (‰) units which are defined as follows:

$$\delta^{18}O = \left[\left(\frac{18_O}{16_O}\text{sample}/\frac{18_O}{16_O}\text{standard}\right)-1\right]*1000,$$

with respect to an international standard (V-PDB_Vienna_Pee Dee Belemnita or V-SMOW_Vienna_Standard Mean Ocean Water).

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microbial activity; e.g. Blake et al., 1997; Sharp et al., 2000; Blake et al., 2001). Zazzo and coworkers (2004a,b) presented evidence from laboratory experiments, that paleoclimate reconstructions based on $\delta^{18}O_P$ analyses are invalid if they are performed on mammalian teeth affected by bacterial or microbial alteration. Iacumin et al. (1996b) examined the isotopic variations on both $\delta^{18}O_C$ and $\delta^{18}O_P$ of modern mammal bones and teeth and showed a constant difference of ~9‰ between carbonate and phosphate oxygen isotopes ratios ($\Delta^{18}O_{C-P}$). This value has been confirmed by the data of several authors (Bryant et al., 1996; Zazzo, 2001). This standard difference subsequently became regarded as a test of the isotopic integrity of fossil apatite material (e.g. Iacumin et al., 1996a; Fricke et al., 1998; Genoni et al., 1998; Lecuyer et al., 1998; Shahack-Gross et al., 1999; Fox and Fisher, 2001; Lecuyer et al., 2003; Palombo et al., 2005; Arppe and Karhu, 2006). The carbonate-phosphate oxygen isotopic tooth enamel fractionation factors ($\alpha_{CO3-PO4}$) have been measured for about 14 mammal species (Bryant et al., 1996; Iacumin et al., 1996b; Zazzo, 2001). Values concerning the intra-individual (Bryant et al., 1996), inter-species (Iacumin et al., 1996b) and intra-species (Zazzo, 2001) variations of the $\alpha_{CO3-PO4}$ are available in the literature. They are all interpreted with respect to the linear relationship between $\delta^{18}O_C$ and $\delta^{18}O_P$ values ($\delta^{18}O_C$ versus $\delta^{18}O_P$) and show similar slopes.

In this study $\delta^{18}O_C$ and $\delta^{18}O_P$ measurements are conducted on the enamel of modern M1 and M2 teeth from a *Rhinoceros unicornis* individual from Thailand. Rhinoceros are water-dependant mammals; 82% of their ingested water comes from drinking water (Clauss et al., 2005). Thus their teeth are potentially good indicators of the $\delta^{18}O$ values of local waters and precipitation. Our aims are to firstly study the intra-tooth variability of the phosphate and carbonate isotopic composition and of the $\Delta^{18}O_{C-P}$ differences from one individual, and secondly, to check whether Iacumin's linear correlation is valid for *Rhinocerotidae*, by comparing our intra-tooth $\Delta^{18}O_{C-P}$ results with existing literature data on enamel tooth (Bryant et al., 1996; Iacumin et al., 1996b; Shahack-Gross et al., 1999; Fox and Fisher, 2001; Zazzo, 2001). Such information on *Rhinocerotidae* intra-tooth carbonate–phosphate isotopic signals and on their difference can be used to test the possible presence of diagenetic alteration in fossil remains of the same or related species. *Rhinocerotidae* remains are widely preserved and abundant in terrestrial sediments in Pakistan and Thailand (Tassy et al., 1992; Welcomme and Ginsburg, 1997; Welcomme et al., 1997, 2001; Antoine et al., 2003a,b). Finally, we discuss on the basis of our rhinoceros intra-tooth results, how the $\Delta^{18}O_{C-P}$ difference could be used as a diagnostic tool for identifying diagenesis.

2. Materials and methods

During fieldwork in Thailand in 2002, teeth from a modern rhinoceros skull (*Rhinoceros unicornis*, Linnaeus, 1758) were extracted by the veterinary office of the Chiang Mai zoo (North Thailand, 18°48'22.4"N, 98°56'48"E, 355 m alt.). This rhinoceros died in its second year, in late summer of 1989, and since it died in the zoo, we can reasonably assume that the skull was not subsequently exposed to weathering or heat. Observations on tooth eruption and wear patterns of the lower and upper jaws show that both eruption order and teeth

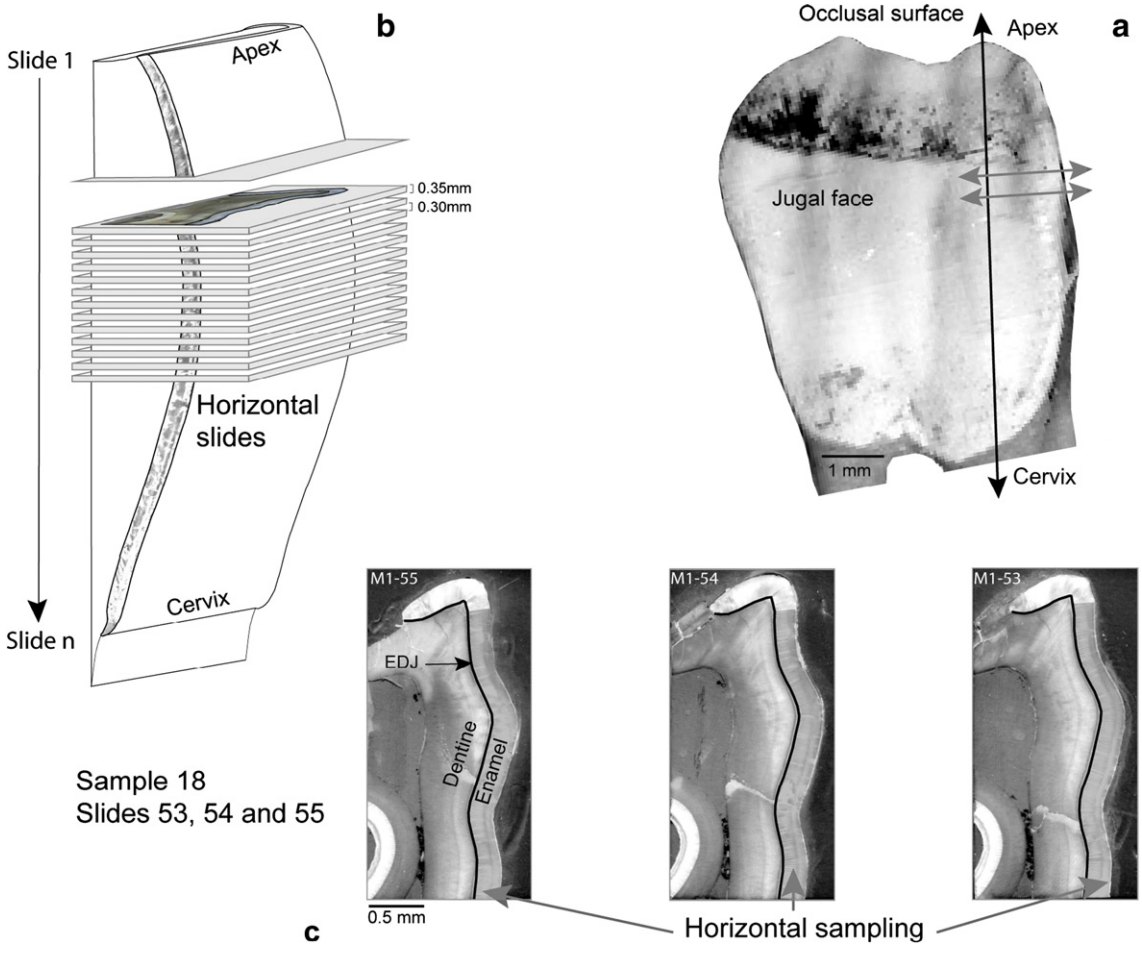

Fig. 1. Schematic representation of the enamel microsampling procedure. a — Jugal view of the rhinoceros M1, and representation of the horizontal slide orientation. b — Three-dimensional representation of tooth slides cut from the apex to the cervix perpendicular to the growth axis. c — All the enamel thickness is sampling between the enamel-dentine junction and the tooth surface (example: sample 18 consisted of three mixed adjacent slides (53–55)).

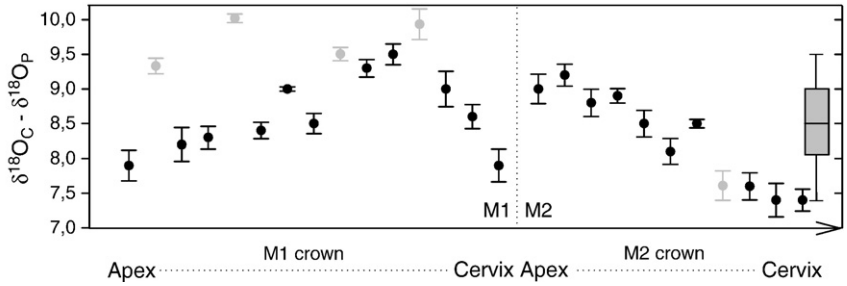

Fig. 2. Intra-tooth oxygen isotope variations of enamel bioapatite obtained for carbonate and phosphate. Profiles are plotted from apex to cervix. Each point highlighted in grey corresponds to the values which we do not take into account for discussion purposes.

formation and mineralization of rhinoceroses is M1-P2-P3-M2-P4-M3 (Groves, 1967; Guérin, 1980). In this study, we used the first two upper molars (M1 and M2). The M2 is incompletely mineralized in the last third of its crown. As the enamel formation of the M1 and M2 teeth started during the intra-uterine life, we expected that weaning/nursing signal would be present in their enamel isotopic records. Nursing will results in a modification of the drinking water $\delta^{18}O$ signal.

To obtain precise high resolution measurements, microsampling was done on horizontal slides (0.3 mm thickness) cut parallel to the occlusal surface using a diamond wire-saw (Fig. 1a). The sampling sequence was made from the occlusal surface to the root, perpendicular to the growth axis (Fig. 1b). Sampling was performed through the whole enamel thickness (~0.22 cm) between the enamel-dentine junction and the tooth surface (Fig. 1c), using a Micromill™ sampler (Dettman and Lohmann, 1995). We use 50 mg and 0.2 mg of powder from the same sample, for isotopic analyses of phosphate and carbonate groups, respectively. For the purposes of $\Delta^{18}O_{C-P}$ comparison presented in this paper, the necessary mass for simultaneous oxygen isotope analyses of both the phosphate and carbonate was obtained by mixing 3–5 adjacent slides.

The $\delta^{18}O_C$ analyses were performed on a Finnigan Mat 252 mass spectrometer equipped with an automated (Bremen-type) carbonate preparation line at the Vrije Universiteit of Amsterdam. The reproducibility of oxygen measurements of structural carbonate in apatite, determined by replicate of NBS19 standards as well as replicate sample analysis, is better than ±0.09‰ (1σ). Isotopic compositions are given in the conventional δ-notation relative to V-PDB and converted to V-SMOW with the Coplen et al. (1983) equation: $\delta^{18}O_{V-SMOW} = 1.03091\ \delta^{18}O_{PDB} + 30.91$.

The PO_4^{3-} radical was isolated for isotopic analysis of oxygen phosphate, via Ag_3PO_4 precipitate. The isolation of PO_4^{3-} from enamel samples follows the techniques described by Stephan (2000) and Vennemann et al. (2002) adapted from Crowson et al. (1991) and O'Neil et al. (1994). The oxygen isotope composition was measured on CO generated by reducing Ag_3PO_4 using a high-temperature conversion-elemental analyser (TC-EA) connected online to a ThermoFinnigan Delta^{Plus} XP mass spectrometer. The overall reproducibility determined by replicate of trisilverphosphate standards as well as replicate sample analysis was better than ±0.2‰ (1σ). As before, isotopic composition is expressed in the conventional δ-notation relative to V-SMOW. All samples were run in duplicate and the reported values are the mean of at least two consistent measurements.

3. Results

3.1. Rhinoceros unicornis

Horizontal milling of M1 and M2 gave 30 and 21 samples respectively of enamel powder for isotopic analysis. Half the number was used for phosphate analysis compared to carbonate group analyses. Oxygen isotope ratios of the carbonate group ($\delta^{18}O_C$) and phosphate group ($\delta^{18}O_P$) of the M1 and M2 are presented in Figs. 2 and 3 (and in the electronic Table 1).

Oxygen isotope ratios from the carbonate group fluctuate in a narrow range between 25.8 and 27.3‰ for M1 and between 25 and 26‰ for the M2 (electronic Table 1). There is a difference of 1.1‰ between the averaged values of M1 (26.8 ± 0.3‰) and M2 (25.6 ± 0.3‰). The M1 $\delta^{18}O_C$ values increase from 26.4 to 27.3‰, and then decrease to 25.8‰. The M2 record shows the highest values at the beginning (26‰), and then exhibits a plateau (25.8‰) followed by a decrease until 25.2‰ corresponding to the less mineralized part of the enamel.

Oxygen isotope ratios of the M1 phosphate fluctuate between 16.9 and 18.6‰. The M2 $\delta^{18}O_P$ record increases from 16.7 to 18.4‰. Average isotopic values are 18 ± 0.5‰ and 17.4 ± 0.5‰ for M1 and M2 respectively (electronic Table 1). The horizontal sampling method used in this study results in collection of sample from adjacent sections of the same subvertical growth lines. These samples should have a similar isotopic value as they contain a large amount of material mineralized at the same time (ie. any natural fluctuations should be smoothed); and yet several phosphate measurements deviate from 0.7 up to 1.6‰ compared to the values displayed by surrounding samples (e.g., samples 3, 10, 18, and sample 24 for the M1 tooth, and sample 13 for the M2 tooth). Such abrupt variations may be analytical artefacts. Alteration or fractionation is possible during chemical treatment to isolate the PO_4^{3-} radical. During isolation of phosphate, it is important to eliminate any organic matter present in the apatite.

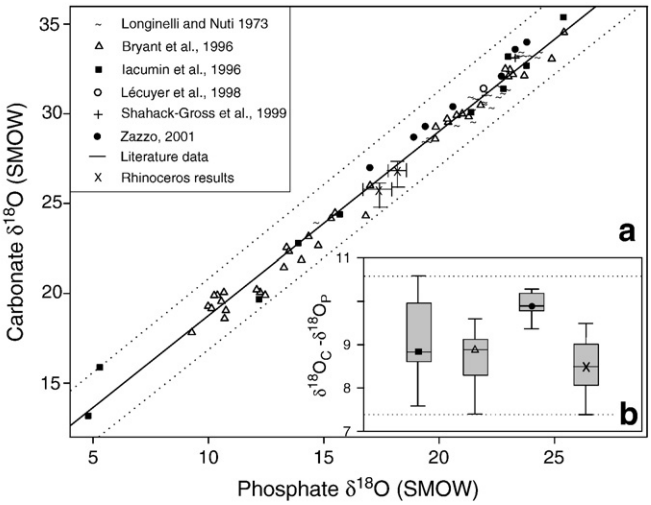

Fig. 3. a — Regression of $\delta^{18}O_C$ on $\delta^{18}O_P$ for modern biogenic apatite of samples reported in the literature: modern carbonate shells (Longinelli and Nuti, 1973; Lecuyer et al., 1998), modern *Eqqus* (Bryant et al., 1996), *Gazella gazella* (Shahack-Gross et al., 1999), *Hippopotamus amphibius* (Zazzo, 2001) and several modern mammals (Iacumin et al., 1996b), together with the average rhinoceros values with their intra-tooth variability. The straight line refers to the least squares regression calculated for modern literature samples. Dotted lines refer to the envelope of the total values. b — Box plot for each mammal literature study and rhinoceros molars. Boxes represent the 50% of the variability, horizontal bars inside the boxes represent the median and vertical bars the maximum $\delta^{18}O_C - \delta^{18}O_P$ values.

Table 1
Relationship between $\delta^{18}O_C$ and $\delta^{18}O_P$ for each sample group quoted in literature and our rhinoceros data

Study	Equation $\delta^{18}O_C/\delta^{18}O_P$	n	r	p	t	p
Longinelli and Nuti, 1973	$\delta^{18}O_C = 1.014\ (\pm 0.053)\ \delta^{18}O_P + 8.68\ (\pm 1.11)$	26	0.971	<0.001	$t_{24} = -1.47$	0.150
Bryant et al., 1996	$\delta^{18}O_C = 1.022\ (\pm 0.019)\ \delta^{18}O_P + 8.41\ (\pm 0.33)$	37	0.994	<0.001	$t_{35} = -1.82$	0.077
	$\delta^{18}O_C = 1.026\ (\pm 0.015)\ \delta^{18}O_P + 8.35\ (\pm 029)$ *	8	0.999			
Iacumin et al., 1996b	$\delta^{18}O_C = 1.015\ (\pm 0.043)\ \delta^{18}O_P + 8.79\ (\pm 0.79)$	10	0.992	<0.001	$t_8 = -0.69$	0.505
Lécuyer et al., 1998		1				
Shahack-Gross et al., 1999		1				
Zazzo, 2001	$\delta^{18}O_C = 1.020\ (\pm 0.051)\ \delta^{18}O_P + 9.50\ (\pm 1.07)$	7	0.994	<0.001	$t_5 = -0.65$	0.542
Rhinoceros M1 (this study)		15	−0.223	0.4323		
Rhinoceros M2 (this study)		11	−0.584	0.587		
All mammal literature	$\delta^{18}O_C = 1.037\ (\pm 0.026)\ \delta^{18}O_P + 8.57\ (\pm 0.504)$	27	0.985	<0.001	$t_{25} = 1.46$	0.07
Fox and Fisher, 2001	$\delta^{18}O_C = 1.106\ (\pm 0.171)\ \delta^{18}O_P + 5.23\ (\pm 3.60)$	5	0.962	0.0042	$t_3 = -0.99$	0.392
F:AM 38257		14	0.521	0.0553		
F:AM 38258		15	0.084	0.7662		
F:AM 38259		8	0.454	0.2742		
F:AM 38269		13	0.078	0.799		
F:AM 38270		6	0.230	0.6853		

The relationship is expressed by a last square regression line equation and simple correlation (r) coefficient (and p values) and results of Student test on regression slope compared to 1 (t and p values). For Bryant et al. (1996) dataset we calculated two last square regression line equations, the first with all dataset and the second indicated with the symbol * with an average value per specimen analysed.

Improper silver orthophosphate precipitation can produce errors of 2–3‰ (Stuart-Williams and Schwarcz, 1995). Four samples out of 26 have values inconsistent with surrounding samples and were discarded. Oxygen isotope values of phosphate group range from 17.8 to 18.6‰ and 16.7 to 17.9‰ for M1 and M2 respectively.

The M1 $\Delta^{18}O_{C-P}$ values exhibit a general increase from 7.9 to 9.5‰ followed by a decrease to 7.9‰. The M2 $\Delta^{18}O_{C-P}$ values decrease rather regularly all along the record from 9.2 to 7.4‰. The corresponding averaged values are 8.5‰ (±0.6‰) for M1 and 8.2‰ (±0.7‰) for M2 (Fig. 2). The average of the apparent isotopic fractionation factor between carbonate and phosphate groups ($\alpha_{CO3-PO4}$) of the M1 and M2 teeth is 1.0082±0.0007 ($\Delta^{18}O_{C-P}$=8.4±0.7‰).

3.2. Comparison with literature datasets

Mean $\delta^{18}O_P$ versus mean $\delta^{18}O_C$ values of the rhinoceros M1 and M2 (n=12 and n=10 for M1 and M2 respectively) are plotted in Fig. 3a

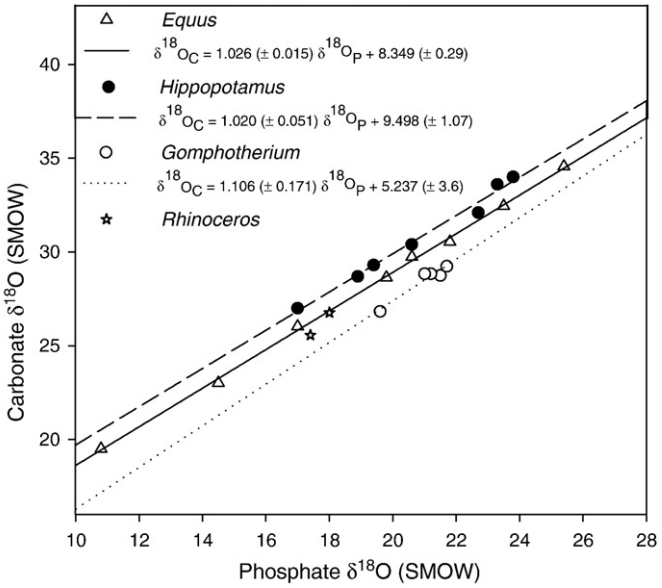

Fig. 4. Species regression of $\delta^{18}O_C$ on $\delta^{18}O_P$ for modern equus (Bryant et al., 1996), modern hippopotamus (Zazzo, 2001) and fossil gomphothere (Fox and Fisher, 2001) species. When intra-tooth and intra-specimen values are available, we have used an average value per specimen to calculate each species regression line. Each regression line shows statistically similar slope but different intercepts.

and compared with tooth oxygen isotopic composition values of different modern mammal and non-mammal species described in the literature (Longinelli and Nuti, 1973; Bryant et al., 1996; Iacumin et al., 1996b; Lecuyer et al., 1998; Shahack-Gross et al., 1999; Zazzo, 2001; electronic Table 2). Strong correlations between $\delta^{18}O_C$ and $\delta^{18}O_P$ exist for each dataset (r>0.97, p<0.001; Longinelli and Nuti, 1973; Bryant et al., 1996; Iacumin et al., 1996b; Zazzo, 2001). The slopes of these regression lines do not differ significantly from 1 (student test on the slope, p>0.05; Table 1). The new $\delta^{18}O_C/\delta^{18}O_P$ relationship using the modern mammal dataset (literature data plus the rhino data) is $\delta^{18}O_C$=1.037 (±0.026) $\delta^{18}O_P$+8.57 (±0.504), with r=0.98 (p<0.0001). When intra-specimen or intra-tooth values are available we used specimen mean values to calculate this new correlation. Our $\Delta^{18}O_{C-P}$ values are consistent with the literature data (Fig. 3b; Brown–Forsythe test shows that the variance homogeneity hypothesis is accepted: p=0.094). However, Fig. 3b also points out the importance of the inter-species $\Delta^{18}O_{C-P}$ variability, which reaches 3.1‰ (Iacumin et al., 1996b). This inter-species variability is higher than that at the intra-tooth level described earlier for our rhinoceros teeth 1.6 and 1.8‰ for M1 and M2 respectively and those of *Equus* teeth (2.1‰, Bryant et al., 1996) or *Hippopotamus* teeth (0.9‰, Zazzo, 2001).

We then performed statistical analyses between $\delta^{18}O_P/\delta^{18}O_C$ relationships available in the literature for several mammal species, *Equus* (Bryant et al., 1996), *Hippopotamus* (Zazzo, 2001) and *Gomphotherium* (an extinct elephant, Fox and Fisher, 2001). When intra-tooth and intra-specimen data are available, we used specimen average values to calculate the three species regression lines between $\delta^{18}O_P$ and $\delta^{18}O_C$ (Fig. 4). ANCOVA analyses with effect tests show that 1) the species regression slopes are statistically identical (p=0.83) and do not differ significantly from 1 (p>0.1, student test on the slope) and 2) the intercepts of the three species regression lines are significantly different (p=0.0004). Our rhino data are aligned on the *Equus* regression line. There is no significant correlation (r<0.58, p>0.05) between $\delta^{18}O_C$ and $\delta^{18}O_P$ at intra-tooth level (for the *Gomphotherium* and the rhinoceros; Table 1) while at intra-species level the correlation is significant (when using the whole *Gomphotherium* dataset, r=0.96, Table 1).

4. Discussion

The mean $\Delta^{18}O_{C-P}$ found for the M1 and M2 rhinoceros teeth (8.4±0.7‰) is consistent with the one commonly described in the literature (~9±0.8‰), that has been proposed as a threshold for testing diagenetic effects on fossil apatite material (e.g. Iacumin et al., 1996a; Fricke et al., 1998; Genoni et al., 1998; Lecuyer et al., 1998;

Shahack-Gross et al., 1999; Fox and Fisher, 2001;Lecuyer et al., 2003; Palombo et al., 2005; Arppe and Karhu, 2006). The constant difference between $\delta^{18}O_C$ and $\delta^{18}O_P$ of enamel may be explained implicitly by an isotopic fractionation (molecular mass dependence) between the oxygen of body water and that of the PO_4^{3-} and CO_3^{2-} enamel lattice ions, at constant temperature. The presence of water molecules in the enamel matrix results from diffusion processes from blood and enzymatic activity (Nagy, 1989). The enamel crystallises in thermodynamic equilibrium with body water (Longinelli, 1984; Luz et al., 1984; Iacumin et al., 1996b). Interestingly, the relationship $\delta^{18}O_C$ *versus* $\delta^{18}O_P$ described for modern shells (Longinelli and Nuti, 1973) is similar to that obtained for enamel (Fig. 3, Table 1), strongly suggesting that physicochemical processes involved in biomineralization are dominant (Westbroek and Marin, 1998; Marin et al., 2000).

Nevertheless, the $\Delta^{18}O_{C-P}$ inter-species variability from the existing literature may reach 3.4‰. The comparison of the intercept values of the different species $\delta^{18}O_P/\delta^{18}O_C$ linear regressions shows significant differences ($p=0.0004$). So far there has been no quantitative analysis of the standard deviation values around the generally accepted mean $\Delta^{18}O_{C-P}$ difference and consequently no discussion on the origins of these changes. Why might they exist?

Part of the reason may be analytical since the literature and our data were obtained in different laboratories using different analysis methods (fluorination or high-temperature reduction). However, as shown in the study of Iacumin et al. (1996b), for a same analytical dataset the inter-species variability can reach 3.1‰; thus, the analytical procedure cannot be the only reason.[2]

A biological reason might be put forward to explain this variability. It is accepted that enamel crystallises from body water in thermodynamic (or near-thermodynamic) equilibrium. However, body temperature of warm blooded organisms ranges between ~35 to 42 °C (Ivanov, 2006). Body temperatures of horse, elephant, hippopotamus and white rhinoceros species are respectively 38.4 °C (Refinetti and Piccione, 2005), 36.5 °C or between 32.5 and 37.5 °C according to Rees, 2002, 35.5 °C (Luck and Wright, 1959) and from 34.5 to 37.5 °C (Allbrook et al., 1958). This wide range might partly cause the inter-species variations of the $\Delta^{18}O_{C-P}$ values. The differences between the intercepts ($p=0.0004$) of the $\delta^{18}O_P/\delta^{18}O_C$ species regression lines show that there is a significant effect of the species on $\delta^{18}O_P$ and/or $\delta^{18}O_C$ values.

Even though correlations between $\delta^{18}O_P$ and $\delta^{18}O_C$ look good for a group of individuals ($p<0.001$), within a single tooth there is often no correlation between the two ($p>0.05$) as it is the case for our rhinoceros results but also for the Gomphothere data (Table 1; Fox and Fisher, 2001). Some processes internal to the individual can also have small impacts on the $\delta^{18}O_P$ or $\delta^{18}O_C$ during enamel formation without affecting the correlation between $\delta^{18}O_P$ and $\delta^{18}O_C$ for an entire group of specimens of a same species. Development and mineralization of teeth have been the subjects of descriptive or analytic investigations (*in vivo* and *in vitro*). Despite that the biological or physicochemical processes are not yet fully understood, enamel formation is considered to be a progressive process divided into two stages: matrix formation and maturation (Weinmann et al., 1942). Biomineralization is strongly controlled by micro-environmental parameters (matrix physicochemical conditions) such as pH, temperature, ionic concentration and ionic strength (Simmer and Fincham, 1995). For example, at the physiological temperature, amelogenin molecular self-assembly is extremely sensitive to local pH changes (Robinson et al., 1995; Moradian-Oldak et al., 1998). Smith et al. (1996) showed that in weakly acidic conditions (pH=6.28) there is an activation of new caseinase enzymes at the expense of permanent enzymes inhibiting proteinase activities. They also demonstrated pH fluctuations at focal areas (6.2 to 7.2) during the maturation phase. The effect of this pH modulation on the chemistry of the enamel crystal is not known but it does alter protein/mineral interactions (Robinson et al., 2005). For homoeothermic organisms, pH variations could potentially affect intra-tooth $\Delta^{18}O_{C-P}$ as pH has a significant effect on $\delta^{18}O$ fractionation in carbonates (Adkins et al., 2003). Because mammalian body temperature is subject to circadian variations of 1 to 4 °C (Refinetti, 1999; Brown et al., 2002; Reffinetti and Piccione, 2005) the intra-tooth $\Delta^{18}O_{C-P}$ values could be affected in the same way as body temperature difference between mammalian species affect the inter-species $\delta^{18}O_P/\delta^{18}O_C$ relationship.

The enamel at the M2 crown base was softer, as indicated by easier drilling, and suggests an incomplete mineralization of the sampled enamel close to the cervix. The unmineralized enamel could affect the $\Delta^{18}O_{C-P}$ values and explain the lower values obtained for the cervix M2 samples (Fig. 2). Thus, enamel maturation process could potentially contribute to the rhinoceros intra-tooth $\Delta^{18}O_{C-P}$ variability. During the maturation process the mineral content of the enamel increases considerably from 10–20% to 80–90% (Weinmann et al., 1942; Robinson et al., 1981; Robinson et al., 1995). This increase occurs to the detriment of the enamel matrix concentration. It is possible that phosphate and carbonate are not precipitating from the same fluid at the same time. The resorption of the protein enamel matrix could also potentially modify the local ionic microenvironment and thus PO_4^{3-} and CO_3^{2-} availability for enamel maturation along the tooth crown.

What are the implications of variability in these intra-tooth and inter-species $\delta^{18}O_P/\delta^{18}O_C$, to identify diagenesis? When one measures intra-tooth $\delta^{18}O_C$ and $\delta^{18}O_P$, it is preferable to use a mean value to test diagenesis, as at this time intra-tooth variability origin is not well-understood and especially because this intra-tooth variability did not modify the general relationship between carbonate and phosphate. On the other hand, the inter-species variability increases significantly robustness of the test. If, as we suggest, body temperature influences the $\delta^{18}O_C/\delta^{18}O_P$ relationship, it seems important to clarify this effect.

We recommend quantifying the $\Delta^{18}O_{C-P}$ difference with respect to the relationship in the literature. The described modern dataset (including 15 species) gives a linear relationship $\delta^{18}O_C$ versus $\delta^{18}O_P$ characterized by a mean $\Delta^{18}O_{C-P}$ difference of 8.9±0.7‰. The envelope of the total values shows that the preserved enamel is ranged between 7.2 and 10.6‰. The number of studies at the species level is required in order to improve the robustness of such a diagenetical test with an exhaustive statistical study and to determine a maximal envelope corresponding to the well-preserved samples. It is important to measure the temperature effect on the linear relationship $\delta^{18}O_C$ versus $\delta^{18}O_P$, and hence the species dependence of this relationship.

5. Conclusion

Our data on *Rhinocerotidae* enamel are broadly consistent with existing literature data for other species correlating $\delta^{18}O_C$ and $\delta^{18}O_P$ values with a slope statistically equal to one. Physicochemical processes (e.g. temperature, pH) during enamel formation and hydroxyapatite crystallization may well explain divergences from the ideal $\Delta^{18}O_{C-P}$ value. Jointly performed intra-tooth analyses on phosphate and carbonate groups from modern mammal tooth enamel show significant differences in $\Delta^{18}O_{C-P}$; while at intra-species level (with average value by specimen), no divergence is observed. The $\Delta^{18}O_{C-P}$ difference, calibrated using modern specimens may therefore be used as a benchmark for evaluating diagenetic alteration of the isotopic integrity of fossil enamel. Our results show that by increasing the datasets of phosphate and carbonate oxygen isotopic composition for modern mammal species, it is possible to define a maximal and precise envelope corresponding to the well-preserved samples. Moreover, by studying the species dependence and/or body temperature dependence on the

[2] Nevertheless, the need for common preparation and analytical techniques for apatites and a new standard or standards for apatite isotope analysis are pre-requisites for the international scientific community as discussed during the Paris "2005 Bioapatite" meeting.

$\delta^{18}O_C/\delta^{18}O_P$ relation, we will be in a position to make this diagenetical test more robust and quantitative.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.palaeo.2008.03.039.

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