DIRECT DATING OF HUMAN REMAINS

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June 2010

A thesis submitted for the degree of Doctor of Philosophy of the Australian National University
The contents of this thesis are the results of original research and have not been submitted for a higher degree to any other university or institution.

Renaud Joannes-Boyau
2010
Acknowledgments

Working towards my doctorate in the Research School of Earth Sciences at the Australian National University was an outstanding and challenging experience. It would not have been possible for me to achieve my goals without the valuable support of many people. Here is a small tribute to most of those people.

I owe my deepest and sincere gratitude to my principal supervisor, Professor Rainer Grün, for offering me the opportunity to undertake this PhD, for all his valuable advice and for sharing his extensive knowledge. Thank you Rainer for your continued support as well as the time you spent supervising my work and always guiding me in the right direction. I am very grateful for having been able to interact with you on a daily basis, which allowed me to gain so much knowledge.

I am deeply grateful to my other supervisor, Dr Steve Eggins, for his valuable advice regarding both professional and personal matters. His kind support and guidance have proven of great value to this study, and I hope to have the pleasure to collaborating further with Steve in the future.

I warmly thank Thomas Bodin, my very good friend and colleague, who sat with me for several hours, helping me with the Simulation Annealing program; it is not possible nor sufficient to express my gratitude with only a few words. I hope we will have the pleasure to collaborate and meet at conferences in the future.

I would like to thank in particular Professor Ian Jackson and Dr John Fitzgerald for numerous valuable discussions and advice. I am also grateful to Dr Les Kinsley and Harri Kokkonen for their help in sample preparation and other technical assistance.

I would like to express my appreciation to Professor Freddy Callens and Dr Henk Vrielinck for welcoming me in their magnetic resonance EPR/ENDOR Group in the Department of Solid State Sciences at the University of Ghent, Belgium. I am grateful
for their helpful advice and support, which proved particularly valuable during the early stages of my research.

This thesis would not have been possible without the endorsement of Professor Ross Griffiths who kindly signed my PhD scholarship offer. I also would like to show my gratitude to Professor Brian Kennett, who greatly enriched the experiences of student life in the Research School of Earth Sciences, by helping create a fruitful, friendly and interactive research environment.

I owe my deepest gratitude to my partner and best friend Elisabeth, who stood by me all those years, giving me comfort, love and attention when I needed it the most. I will cherish always those years of happiness and joy, even during times of high pressure, and I promise to try to be as supportive during your PhD as you were during mine. Je t’aime de tout mon coeur; je promets de devouer ma vie à te rendre heureuse.

This thesis would not have been possible without the love of my parents, who supported me during the dark ages of my teenage-hood, which lasted for nearly 10 years. Avec tout mon coeur et ma reconnaissance pour tout ce que vous avez fait pour moi, pour m’avoir toujours aîné et pour m’avoir toujours encourage, merci.

To my brother Olivier, who consistently believed in me: thank you for supporting me without the slightest hesitation; thank you for always answering when I needed help; thank you for your unconditional love. I wish you all the best for your life. Take good care of your self and your family.

I would like to thank Dr Alice Gorman and Dr Lynley Wallis for editing this thesis, for there advice and friendship. I particularly want to emphasise my gratitude to Lynley for the opportunity that she gave me with the ECR funding which turned out to be a magnificent experience, but on top of that a valuable addition to my CV. Thank you both for your valuable help.

I am indebted to my many friends, collaborators and colleagues. In particular, to Dr Maxime Aubert for all the great times we had together down the coast since the beginning of my time in Australia, for “lowering” your French so I could understand,
as well as your valuable professional help, I wish you, Maxime, all the best for the future. To Ian Moffat for his kindness, friendship and considerable advice, sitting on the bench outside OHB-B, thank you. I am also grateful for your valuable assistance preparing my ARC application; I wish you the best of luck for your PhD and your career. I would like to thank Tegan Kelly for sharing her office with me and enduring my awful singing, as well as my terrible French accent during all these years. I am also very grateful to her for correcting many pages of poor English syntax. Tegan, I wish you a wonderful wedding, a happy ever after marriage and a fruitful career, best of luck to you and Cluan. I would also like to express my gratitude to Mathieu Duval, for the valuable talks and the challenging badminton games we had when he visited RSES. I hope I will have the pleasure to collaborate with you soon, and offer my congratulations for your recent position in Spain.

I would like to thank Thomas and Shinta for their friendship and all the great time we have had together. I will cherish in my memory all the week-ends at the coast and the road trip in Tasmania that we did, I wish you a wonderful life and congratulation on your magnificent little baby boy, Jaures. Take good care you three.

I would like to show my gratitude to the administrative staff of RSES, in particular Mike Avent (School Manager) and Eric Ward (Building Manager), for their friendship and great senses of humor.

The financial support of the Australian National University, the Research School of Earth Sciences and the Earth Environment Group is gratefully acknowledged.

Lastly, I offer my regards to all of those who supported me in any respect during the completion of this project.
ABSTRACT

To understand human evolution, archaeologists require precise chronologies to compare and contrast the fossil collection. While indirect dating techniques of human occupation sites allow quantitatively precise than direct dating techniques, the accuracy of indirect dating is frequently poor. Direct dating of human fossils older than 50 to 60,000 radiocarbon years limits is limited to OSL and ESR.

“...We are somewhat in the position of the drunken man who has lost his keys on a dark stretch of road but who is searching for them a few yards away, under a lamp post, because the light is better there. Thus, we must sometimes content ourselves with dating sites or strata in sites which are far removed from critical hominid loci, but which are better suited for dating and can, hopefully, be correlated to hominid sites.”

H. SCHWARCZ, 1992
ABSTRACT

To understand human evolution, archaeologists require precise chronologies to compare and contrast the fossil collection. While indirect dating techniques of human occupation sites usually applied on sediments are somehow more precise than direct dating techniques, the accuracy of indirect dating is frequently poor. Direct dating of human remains older than 50 to 60 ka (radiocarbon limit) is limited to U-series and ESR techniques. To minimize the impact of direct dating on valuable archaeological samples, non-destructive U-series and ESR analysis have to be carried out using specifically designed protocols.

Both methods are seriously compromised by the fact that teeth accumulate large amounts of uranium following their deposition in sediments. During the three years of this PhD, a 2D mapping protocol has been developed on the isotope distributions and elemental concentrations of uranium and thorium in fossil teeth using laser ablation ICP-MS. Isotopic maps of enamel and dentine show complex patterns that imply that systematic mapping of fragments would provide accurate insight for U-series and ESR internal dose assessment. A fossil Neanderthal tooth from Payre (France) showed negligible U-migration through the external enamel surface compared to the internal migration from the dentine, with great implications for ESR dating.

Non-destructive ESR analyses are carried out on enamel fragments instead of powders to minimize the impact of analysis on samples. Nevertheless, the ESR spectra of fragments have a high angular dependency which complicates their study and the establishment of experimental protocols. During this PhD, new measuring protocols and analytical decomposition of ESR spectra have allowed to gain new insight on the composite nature of the signal. The development of comprehensive model describing the influence of several oriented and non-oriented CO$_2$ radicals in the spectra with complex kinetics and transfer processes has shown that major age underestimation can be expected for most fossil tooth enamel. The new model suggests that fossils such as the Irhoud specimen or Broken Hill had there age underestimated by around 30%, propelling the Irhoud specimen amongst the oldest anatomically modern humans in Africa.
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LIST OF SYMBOLES AND ABBREVIATIONS

$^{14}C$ Radioisotope of carbon, usually refers to radiocarbon dating

$^{238}U, ^{234}U$ Radioisotopes of uranium

$^{230}Th$ Radioisotope of thorium, (daughter isotope of $^{234}U$)

$\alpha$ Alpha particle

$\beta$ Beta particle

$\gamma$ Gamma rays

AAR Amino-Acid Racemisation

AICOR Anisotropic $CO_2^-$ radicals, also called oriented $CO_2^-$ radicals

BEB Buccal-Enamel Boundary

BH Broken Hill fragments

D-A Diffusion-Adsorption model

DEJ Dentine-Enamel Junction

ESR Electron Spin Resonance

HAp Hydroxyapatite

H # Holon fragments (# indicates the fragment position in the lamella)

IR Jebel Irhoud fragments

LA-ICP-MS Laser Ablation Inductively Coupled Plasma Mass Spectrometer
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>MH</td>
<td>Modern Human fragments</td>
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<tr>
<td>NOCOR</td>
<td>non-oriented CO$_2^-$ radicals</td>
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<tr>
<td>OSL</td>
<td>Optically Stimulated Luminescence</td>
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<tr>
<td>SA</td>
<td>Simulation Annealing procedure</td>
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<tr>
<td>TL</td>
<td>Thermoluminescence</td>
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<tr>
<td>TIMS</td>
<td>Thermal Ionisation Mass Spectrometer</td>
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<tr>
<td>TT</td>
<td>Thermal Treatment</td>
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<tr>
<td>U-series</td>
<td>Uranium-series</td>
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<tr>
<td>U-Th</td>
<td>Uranium and thorium, either ratio or dating</td>
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<tr>
<td>UV</td>
<td>Ultraviolet</td>
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INTRODUCTION

"Man is by nature a social animal," wrote Aristotle, "and the affluent society is the proper habitat of the individual" (Aristotle 1925, p. 341). While the notion of home is vital to most members of the animal kingdom, the comprehension of the concept of home is one of the fundamental characteristics that differentiate humans from the rest of the animal kingdom (Dewsbury and Ploger 1967). In the first observations of the movements of stars by the celestial navigator, and the noting of the passing of seasons and days, through in the development of the atomic clock, have made it possible to estimate, comprehend and measure the time that passes and the time that has past. Attempting to understand the past, especially human evolution, without a precise chronology can be likened to a two-dimensional picture, a momentary glimpse that has no meaning. In order to better comprehend the past and refer to specific time periods, scientists divide the past according to distinct criteria including geological, archaeological, biological or historical divisions.

Quaternary Geochronology

One of these divisions, the Quaternary era, is a singular period of the geological time scale of particular interest, not only because it is the most recent era within which we live, but because the Quaternary was the theatre for the evolution of hominids. The Quaternary was characterized by successive interglacial and glacial phases, which are major transformations for hominids and arguably drove evolutionary processes.
Measuring time seems such a simple task in the contemporary world where most humans possess watches and mobile phones. Yet Saint Augustine, in his confessions, said “What then is time? If no one asks me, I know what it is. If I wish to explain it to him who asks, I do not know anymore” (St Augustine, c.397). While the notion of time is shared amongst members of the animal kingdom, the comprehension of the concept of time is one of the fundamental characteristics that differentiate humans from the rest of the animal kingdom (Fraisse and Piaget, 1967; Ivry and Schlerf, 2008). From the first observations of the movements of stars in the celestial sky, and the noting of the passing of seasons and days, through to the development of the atomic clock, humans have tried to estimate, comprehend and measure the time that passes and the time that has past. Attempting to understand the past, especially human evolution, without a precise chronology can be likened to a two-dimensional picture, a palimpsest of objects that has no meaning. In order to better comprehend the past and refer to specific time periods, scientists divide the past according to distinct criteria including geological, archaeological, biological or historical divisions.

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research has been undertaken on materials and sediments from the Quaternary period in order to create a chronological frame for archaeological, palaeo-anthropological and palaeoenvironmental research. To obtain absolute age estimates for selected materials, scientists can utilise a range of dating techniques, each with their own limitations with respect to the material able to be dated and the time range within each is applicable (Figure A and Table A).

Figure A: Simplified Quaternary chronology linked with climate changes recorded with the variation of $^{18}$O, and with the approximate dating range of dating techniques (from Shackleton and Opdyke, 1976).
As shown in Table A, a few dating methods can be successfully applied directly to hominid remains. Those that are suitable include radiocarbon dating ($^{14}$C), uranium series (U-series), electron spin resonance (ESR) and amino acid racemization (AAR). Because the dating range of $^{14}$C is negligible (∼50 ka maximum) compared to the time periods involved in hominid evolution (∼6 Ma; Stringer et al., 1994), U-series dating (∼500ka) and ESR dating (∼2 Ma) are extremely valuable as a means of generating the chronological framework for human evolution. Unfortunately, however, these techniques have several limitations including complex mechanisms and frequently materials available for possible dating turn out to be unsuitable.

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Table A: Most in used dating techniques applied for archaeological and geological materials. Precision of dating techniques according to the sample (★ applicable, but regularly not reliable; ★★ applicable and reliable, after Grün, 2008)

Despite these problems, the direct dating of hominid fossils remains preferable to other techniques where the age of the sample is interpreted based on its association with the
surrounding dated sediments as it avoids any inaccuracies generated from the misinterpretation of taphonomic processes within the site. Schwarcz (1992) highlighted this problem, warning that indirect dating is potentially extremely hazardous owing to the possibility of misinterpretation of archaeological strata. Therefore, improving direct dating techniques such as ESR and U-series is seen as a priority by the archaeological scientific world in order to validate proposed chronologies and hypotheses about human evolution.

PART 1: Material and Methods

Introduction to ESR and U-series dating of fossil tooth

In combination, the techniques of U-series and ESR dating offer a powerful tool for accurate age estimations, however, each remains constrained by complex mechanisms operating within the materials being dated. Bone, which presents complexities for U-series (Pike, 2000) is not suitable for ESR dating (Grün and Schwarcz, 1987). Hence, in order to apply the two dating techniques to a single sample, fossil tooth is the only suitable material. The U-series technique is based on the disequilibrium of the decay chain between two radiogenic isotopes uranium and thorium. The ratio between the parent isotopes $^{234}$U and the daughter isotopes $^{230}$Th are measured to determine the age of the sample. In contrast to uranium-lead dating, where the product of the decay accumulates through time, thorium itself will decay and therefore constrains the dating range (half life ~75ka).

Chapter 1, 2 and 3 present respectively the background information relating to the principles underpinning the ESR dating technique, uranium and U-series geochemistry, and to tooth structure. Understanding equally ESR signals and U-uptake mechanism (Millard, 1993; Millard and Hedges, 1996) along with tooth enamel crystal
organisation is the key to apply successfully U-series and ESR dating. While Chapter I and II deals with the theory of the two dating techniques, Chapter 4 and 5 reviews previous and new dating protocols including mass spectrometer mapping analysis and ESR measurement experiments on powdered tooth and tooth fragments in both fossil and modern tooth enamel.

Archaeological sites

Chapter 6 briefly describes the archaeological sites from which the tooth samples studied in the thesis were excavated:

- Payre in southern France near the Rhône River (Combier, 1967; Moncel, 2003). Payre represents a very interesting site in regards of hominin evolution as it produced the remains of 14 *Neanderthalensis* tooth fragments in different levels of the site (Valladas et al., 2008);

- Broken Hill in Zambia containing the Kabwe skull (Woodward, 1921; Pycraft et al., 1928) and Jebel Irhoud in Morocco (Smith et al., 2007). Hominid fossils from these sites are considered critical for understanding hominin evolution, especially in the context of the out-of-Africa debate [references].

- The open air, Lower Paleolithic site of Holon in Israel (Porat et al., 1999). This site provides a unique perspective on hominin behaviour, technology and subsidence during a key evolutionary period (Chazan and Horwitz 2008). A bovid fossil tooth from Holon played a central part in this thesis since most of the ESR theoretical models were developed based on studies of this fragment.

- The archaeological cave site of Jebel Irhoud (Morocco) is well-known for several hominin fossil remains discovered since 1991, including portions of two adult skulls (Irhoud 1 and Irhoud 2), a child’s mandible (Irhoud 3), and a child’s humerus (Irhoud 4). Until recently the site age had been based on the ESR dating of horse teeth from Level 17, estimated to be ca 130 ka using an early uptake model and ca 190 ka assuming a linear uptake (Smith et al., 2007). The human remains from Irhoud are potentially amongst the oldest anatomically modern humans in Africa.
PART 2: Results and Discussion

2D mapping of fossil tooth: new approach for U-uptake and internal dose assessment

To be able accurately assess internal dose for ESR dating, the incorporation patterns and distribution of U-isotopes are critical. Chapter 7 presents a new approach to understanding U-uptake through the use of a 3-dimensional, high resolution protocol for mapping uranium and thorium concentration and distribution within teeth. This protocol allows previously poorly understood incorporation patterns to be overcome by providing data at a far higher spatial resolution than was formerly possible. The development of a mapping system resolves the often heterogeneous distribution of uranium and thorium isotopes which in the past frequently lead to dose estimation miscalculation and U-series age inaccuracy. The use of Laser Ablation Inductively Coupled Plasma Mass Spectrometer (LA-ICPMS) with a specifically designed ablation chamber allows a rapid investigation of large samples providing the unique opportunity to fully visualize complex U-uptake patterns.

Improving the understanding of ESR spectra of fossil tooth enamel fragments

Previously most direct dating protocols for fossil teeth involved the use of powder samples, causing irreversible damage to samples. Hominid remains are exceptionally rare and valuable, and therefore scientists are understandably eager to minimise the use of destructive analytical techniques. Consequently, it was considered necessary to develop new direct dating protocols that minimized damage caused through analysis. Chapter 8, Two types of CO₂ radicals threaten the fundamentals of ESR dating of tooth enamel (Grün et al., 2008b), describes a preliminary study on a new protocol for the ESR measurements of tooth fragments. The new protocol allows a solid tooth fragment to be analysed without the need for converting it to powder; thus it can be easily restored to the tooth following analysis. The study shows that while the response of the enamel powder
is qualitatively similar to environmental and laboratory dosing, preliminary results on laboratory irradiated fragments show significantly different ESR responses. The emergence in the fragment of two different \( \text{CO}_2^- \) radicals, one strongly anisotropic (AICOR) and one with no preferential orientation (NOCOR), invisible in the powder spectrum, induces concerns about dating accuracy. The AICOR:NOCOR ratio of naturally irradiated samples compared to laboratory \( \gamma \)-irradiation of 90:10 to 60:40, respectively, demonstrates that the qualitative response is significantly different between the two irradiations. Until further investigation of the response of tooth enamel fragments, the specific measuring protocol described in this paper creates a powder-like spectrum obtained by measuring the sample that is rotated around three principal axes over 360° then merged into one single spectrum. This powder-like spectrum is potentially the same as a powder spectrum, and dose estimation could have been carried out identically to previous studies. Unfortunately, the presence of at least two distinct \( \text{CO}_2^- \) radicals in the spectrum suggests that this approach is not valid and thus the relationship between the two radicals has to be fully understood before such a non-destructive ESR dating method can be developed. Brik et al. (1998) showed that the two radicals have significantly different kinetic behaviours. The AICOR is described as being more stable than the NOCOR, with disappearance of the second species after one hour of thermal treatment at 200°C, while the AICOR can be observed after one hour at 300°C. Brik et al. (1998) also advanced the hypothesis of a possible mass transfer process that occur between the two radicals, with the NOCOR transferring into the AICOR form. The complex process leading to the transformation of one species in to the other form called “mass transfer process” is poorly described with insufficient evidence. Further study (Chapter 9) *Thermal behavior of orientated and non-orientated CO\(_2\) radicals in tooth enamel* (Joannes-Boyau and Grün, 2009) was required to accurately observe and characterise the effect of thermal treatment on the radicals involve in the ESR response of fossil tooth enamel. This study described modification in the spectra with an attempt to separate the two radicals supposedly involved in the signal. After thermal treatment, the spectra show a shift in the angular response of the fragment unrelated to the disappearance of the NOCORs. Other heating-induced processes may provoke a significant change of preferential orientation.
Because heating resulted in qualitative differences, an objective decomposition system had to be investigated to understand processes that may occur between radicals. The study *Decomposition of the angular ESR spectra of fossil tooth enamel fragments* (Joannes-Boyau et al., 2010b) *(Chapter 10)* introduces for the first time the simulating annealing procedure (SA) that allows the separation of overlapping signals in the spectra. The study shows that the ESR spectrum of fossil tooth enamel is a composite signal created by a mix of three categories of radicals: isotropic components also called non-CO$_2^-$ radicals, NOCORs and two distinct AICORs. The identification of two radicals R$_1$ and R$_2$ tentatively related to orthorhombic and axial radicals respectively, introduces a new perspective on the explanation of anisotropy variations induced by thermal treatment. The new SA procedure successfully applied to thermal behaviour could allow the observation of processes leading to differences between natural and laboratory irradiation. The study *Decomposition of the laboratory irradiation component of angular ESR spectra of fossil tooth enamel fragments* (Joannes-Boyau et al., 2010b) *(Chapter 11)* describes the decomposition of the laboratory irradiation-induced spectra. The comparison of natural and laboratory $\gamma$-irradiation shows that the two AICORs present dissimilar radiation sensitivity, with only the orthorhombic species being created by $\gamma$-exposure, while the natural is a mix of both radicals. Understanding the formation and transfer processes that lead to the observed mix of CO$_2^-$ radicals in fossil tooth enamel spectra is essential for the reliable application of ESR dating. On that basis, radiation sources that can influence the AICOR ratio, including UV and $\beta$-irradiation had to be investigated using the SA annealing procedure.

Studies on retrospective dosimetry suggested that UV may contribute significantly to the overall ESR intensity (El-Faramawy, 2005; Liidja et al., 1996; Nilsson, 2001). Brik et al. (1998) and Vorona et al. (2007) showed that UV induced spectra in modern teeth contained significantly less NOCORs than those by $\gamma$-irradiation. *Chapter 12* presents the article *Decomposition of UV induced ESR spectra in enamel fragments of a modern and a fossil tooth* (Joannes-Boyau et al., 2010a). The study explores, using the SA procedure, the composition of the ESR spectrum induced by UV
lights. It appears that the influence between UV and $\gamma$-irradiation on the ESR response is qualitatively different, by inducing a different ratio of anisotropic radicals. The study also shows not only that the AICORs are presenting strong differences between UV and $\gamma$ exposure, but that the amount of NOCORs in the total spectra is clearly different.

**Chapter 13, Decomposition of beta-ray induced ESR spectra of fossil tooth enamel** (Joannes-Boyau and Grün, 2010b) compares the influence of $\beta$-irradiation on the composition of AICORs and NOCORs in the total spectra. Because there are structural differences between the buccal-enamel boundary (BEB) (Hillson 1986) and the volume close to the dentine-enamel junction (DEJ), one may expect different radiation responses in these domains. However, the energy of gamma irradiation will be more or less evenly distributed in the enamel (provided that charged particle equilibrium is assured) while beta irradiation will mainly affect domains close to the irradiated surface owing to a strong attenuation of beta particles (Brennan et al., 1997; Marsh et al., 2002). This chapter explores difference ESR response to beta-irradiation between the two domains DEJ and BEB.

Because Chapter 13 shows that different volumes of enamel can give rise to very different responses to irradiation, the study **A comprehensive model for CO$_2$ radicals in fossil tooth enamel: implication for ESR dating of human remains** (Joannes-Boyau and Grün, 2010c) presented in **Chapter 14** focuses on the influence of laboratory $\gamma$-irradiation on two fragments of tooth physically separated from the same piece. The bottom fragment, containing the enamel near the DEJ, and the top fragment, containing the BEB, show different radical orientation and different amount of NOCORs. Furthermore, quantitative differences between $\gamma$ and $\beta$-irradiation induced spectra were observed. These differences were initially attributed to a strong attenuation of beta rays in the enamel, suggesting the differences observed were induced by the enamel domains rather than by the irradiation type.

In combination the two aforementioned studies by Joannes-Boyau and Grün, (2010a, b) shed light on the possible transfer between the different types of CO$_2$ radicals
through preliminary annealing experiments. At the same time, the angle formed by the two radicals $R_1$ and $R_2$ (around 25°) is similar in all measurements including in the natural, after thermal treatment, as well as after UV and $\beta$-irradiation. The constant offset between the two radicals suggests they are most likely located in the same enamel domains. The CO$_2$ radical model developed in the latest study investigates potential transfer processes between the two AICORs (orthorhombic to axial) and between the AICORs and NOCORs. In light of these complex transfer mechanisms, the amount of NOCORs could influence the accuracy of dating by underestimating the equivalent dose.

When first measured the Jebel Irhoud fossil enamel fragment gave rise to unusual spectra with high angular dependency. In Chapter 15, Dose assessment of the fossil tooth fragments from Jebel Irhoud (Morocco) using the SA decomposition procedure, we have applied the new decomposition procedure to investigate the influence of gamma irradiation on a different tooth fragment. This study unravels the differences between the Holon and Irhoud fragments with a distinctive response to irradiation. The two fragments show different induced AICORs ratios, with an unexpected growth of $R_2$ to irradiation. These results allow speculation about the heterogeneity of the behaviour of enamel fragments with irradiation.

**PART 3: Summary and Conclusions**

The concluding Chapters summarize the advances made in the understanding of the complex composite ESR signal of fossil tooth enamel fragments, and justify the new approach developed for their study. Furthermore, I outline a potential protocol that can be used to more appropriately date hominin remains utilising a combined U-series and ESR dating approach.
PART 1
CHAPTER 1
1. ELECTRON SPIN RESONANCE METHOD

The use of Electron Spin Resonance (ESR) or Electron Paramagnetic Resonance (EPR) spectroscopy allows the identification and quantification of paramagnetic materials. The Electron Spin Resonance (ESR) phenomenon was first discovered by Zavoisky (1944, 1945); it is now a common method for analysing the structure of solids and liquids in physics, chemistry, biology and crystallography.

1.1 Principles

Most materials are not magnetic, however, will demonstrate magnetic properties when they are exposed to an external magnetic field. Three main categories of magnetic materials can be distinguished:

- **Ferromagnetic** materials have a strong induced magnetisation which can be retained even in the absence of an externally applied magnetic field, contrary to paramagnetic properties.

- **Diamagnetic** materials have a magnetic field in opposition to an externally applied magnetic field, causing a repulsive effect. The external magnetic field alters the orbital velocity of the electron around the nucleus, changing the magnetic dipole moment in the opposite direction of the external field. In this material all electrons are paired inducing the magnetic moment to be null.

- **Paramagnetic** materials have unpaired electrons or an uneven number of electrons, therefore the magnetic moment will be different than zero. Paramagnetism can only be measured when an external magnetic field is applied. Thus, the total magnetisation drops to zero when the external field is removed. Even in the presence of an external field, the magnetisation in paramagnetic materials is comparatively small owing to a small number of spins that are orientated by the field. In the case of classic atomic physics, we consider the electron as a negatively charged sphere rotating around the nucleus (orbital motion) and around itself (spin). The intrinsic property of spin defines the magnetic moment of an electron, which can be
considered then a magnetic dipole behaving as a bar magnet. The magnetic dipole \( \vec{\mu} \) can be obtained with the angular momentum \( \vec{L} \) by:

\[
\vec{\mu} = \frac{-e}{2m_e} \vec{L}
\]  
(1.1)

where \(-e\) is the elementary charge, \(m_e\) the electron mass.

### 1.1.1 Zeeman Effect

When paramagnetic materials are placed in an external magnetic field, the electron's magnetic moment changes accordingly to the orientation of the external field. This allows electrons to be separated into two energy levels depending on their spin orientation, either opposite to or in the same direction of the field; this is known as the Zeeman Effect. The energy difference (\(\Delta E\)) between the two levels is directly proportional to the external magnetic field \(H\), as described by the relation:

\[
\Delta E = g\beta_e H_0
\]  
(1.2)

where \(g\) is the g-factor, \(\beta_e\) Bohr magneton (\(\beta_e=9.274.10^{-24} \text{ J T}^{-1}\)) and \(H_0\) is the magnetic field at resonance.

Because the electron appears twice as effective in producing a magnetic moment \(g \sim 2\), the electron spin is one-half of a particle \(M_s = \frac{n}{2}\), with \(n\) = the number of unpaired electrons (Figure 1.1). The Zeeman Effect was first observed by Peter Zeeman (1897) on the hydrogen transition, with simple spin of \(M_s=1/2\), whereby two energy levels can be distinguished:
\[ E_1 = -\frac{1}{2} g \beta H \quad \text{and} \quad E_2 = +\frac{1}{2} g \beta H \]  

(1.3)

The resonance is obtained when the magnetic moment flips into the opposite energy induced by the electromagnetic frequency \( \nu \) and can be written:

\[ \Delta E = g \beta_e H_0 = h \nu \]  

(1.4)

with \( h \) the Planck constant \( (h = 6.626 \times 10^{-34} \text{ J s}) \).

![Diagram](image.png)

Figure 1.1: Orientation of the magnetic moment of an electron in an external magnetic field. \( M_s \), spin quantum number; \( \mu_s \), magnetic moment of the spin; \( \omega \), precession frequency; \( \nu \), microwave frequency that is required for inducing a flip of the magnetic moment into the opposite direction. (after Grün, 2006)

The orientation of the magnetic moment of the electron can go either way. The Maxwell-Boltzmann equation allows the quantification of electrons in either energy level:

\[ \frac{N_1}{N_2} = e^{\left( \frac{\Delta E}{kT} \right)} = e^{\left( \frac{h \nu}{kT} \right)} \]  

(1.5)
with \( N_1 \) and \( N_2 \) representing the number of electrons in the \( E_1 \) and \( E_2 \) energy level of the Zeeman Effect, respectively; \( k \) is the Boltzmann constant (\( k = 1.38 \times 10^{-23} \text{ J K}^{-1} \)); and \( T \) is the temperature.

At thermodynamic equilibrium \( N_1 > N_2 \) a transfer between the two populations is observable. Nevertheless, according to the Maxwell-Boltzmann equation, for a certain \( v \) value at a certain temperature, resonance can be observed, with energy transfer from both levels. If electrons from the \( E_1 \) level are transferred to the \( E_2 \) level, the system is gaining energy, defined as absorption phenomenon detected by ESR spectroscopy.

Alternatively, electrons transferring from the \( E_2 \) to \( E_1 \) energy level will release energy (Figure 1.2).

As previously mentioned, when the magnetic field is turned off, the system returns to the equilibrium state, called relaxation. The relaxation time varies depending on the system, and can be separated into two processes: the initial relaxation where the lattice returns to equilibrium referred to as the spin-lattice relaxation time (\( T_1 \)). The second process (\( T_2 \)) is completed when the other spins reaches equilibrium and is known as the spin-spin relaxation time.

### 1.2 ESR Spectroscopy

Electron Spin Resonance spectroscopy is based on the interaction between an electromagnetic radiation and the paramagnetic materials. It allows the study of chemical
species that have one or more unpaired electrons, including organic and inorganic free radicals (Calas Hawthornw, 1988; Geiger, 2004).

![Figure 1.3: Spectroscopy and the application domain (Frequency GHz)](image)

1.2.1 Spectrometer

The magnetic field used to obtain the resonance depends on the microwave frequency (Figure 1.3 see above). Several bands (frequency value) can be used; one of the most common is the X band, with a frequency close to 9.5 GHz (Table 1.1). The spectrometer allows the measurement of electromagnetic radiation absorption by the paramagnetic material. The instrumentation consists of three main components: an electro-magnet, a microwave generator, and a detection device. The ESR cavity is located between the two electromagnets, which generates a magnetic field. A microwave bridge, situated above the cavity, is generated though a klystron or gun diode fixed microwaves at a constant power. The microwaves penetrate the cavity though the wave guide.
<table>
<thead>
<tr>
<th>Band</th>
<th>$B_{\text{res}}(T)$</th>
<th>Frequency (GHz)</th>
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</thead>
<tbody>
<tr>
<td>L</td>
<td>$3.92 \times 10^6$</td>
<td>1.1</td>
</tr>
<tr>
<td>S</td>
<td>$1.07 \times 10^7$</td>
<td>3.0</td>
</tr>
<tr>
<td>X</td>
<td>$3.48 \times 10^7$</td>
<td>9.75</td>
</tr>
<tr>
<td>Q</td>
<td>$1.2 \times 10^8$</td>
<td>34.0</td>
</tr>
<tr>
<td>W</td>
<td>$3.4 \times 10^8$</td>
<td>94.0</td>
</tr>
</tbody>
</table>

Table 1.1: Related Magnetic field, $B_{\text{res}}$ for a $g = 2$ signal at selected microwave frequencies (Bruker ®)

The microwave then travels to the detection component where it is compared to the incident wave in order to identify any absorption that may have occurred. An iris controls the amount of microwave into the cavity, by changing its impedance.

Figure 1.4: Schematic representation of the Bruker E500 Elexys spectrometer used at the Research School of Earth Sciences at the Australian National University. The cavity is located between two electromagnets generating a magnetic field, while microwave produced by the microwave bridge are directed though the wave guide directly inside the cavity where the sample is. The modulation and detection device is linked to the spectrometer and to a computer that records the adsorption. The spectrometer was used in X band with a standard rectangular cavity topped by an ER 218PG1 programmable goniometer that allows to rotate the sample in the cavity.
1.2.2 Spectra

At resonance, the sample partially absorbs the microwaves, inducing a variation in the electric current that goes through the crystal in the cavity. As absorption occurs the current is modified which the detector will record. Because it is electronically advantageous to amplify an AC signal, a low intensity field $H_m$ superposes the field $H$, inducing a modulation in the absorption signal. The modulated signal will be the derivative of the actual signal (figure 1.5).

![Resonance Diagram](image)

*Figure 1.5: Integral or absorption curve ($A$) and the derivative ESR spectra ($dA/dH$). $H$ is the external magnetic field (Marfunin, 1979).*

The signal is characterised by several parameters including the shape, the width, the intensity and the $g$-factor. The latter, also referred to as the $g$-value or dimensionless magnetic moment, characterizes the magnetic moment and gyromagnetic ratio of a particle or nucleus.
The g-value can be calculated using (1.4) equation:

\[ g = \frac{h \nu}{\beta e H_0} \] (1.6)

where \( h \nu \) the energy difference, \( \beta e \) the Bohr magneton and \( H_0 \) the magnetic field at resonance.

The g-factor of a free electron corresponds to \( g = 2.0023 \), however, the electron spin g-value is always influenced by surrounding interactions with other unpaired electrons, precursors, and the nucleus of atoms. The measured g-value will be offset from the free electron g-value which allows us to identify and characterise surrounding interactions (Marfunin, 1979).

1.2.3 Anisotropy of the g-factor

The crystal matrix of the enamel is an assemblage of ions and atoms which creates an electrostatic field affecting paramagnetic centres. Atoms and ions surrounding the paramagnetic electron have a direct influence on the disturbance that occurs. In the case of a cubic symmetry, the disturbance will be the same on the three axes of the crystal and the g-factor will not change. In the case of a non-cubic symmetry, the interruption will depend on the orientation of the site relative to the magnetic field and there will be two or three different g-values depending on the symmetry of the crystal. In the case of powder, each microcrystal is oriented randomly to the external magnetic field; therefore all possible orientations occur at the same time in the same ratio. The ESR spectrum will be the superposition of all signals and all g-values. A paramagnetic element in a cubic symmetry will have only one g-value, which gives a symmetrical or isotropic peak (Figure 1.6a). In a lattice where two axes are of equal value and the third is different (rhombic system), two values of g are possible which gives an anisotropic spectrum (Figure 1.6b). If the lattice has three unequal axes (orthorhombic system) there will be
three possible g-values resulting in an anisotropic spectrum with three peaks (Figure 1.6c).

In addition to interactions that occur with the crystal lattice, three other interactions can be described: the fine, hyperfine and super-hyperfine structure. The fine structure, also described as spin-orbit interaction, results from the interaction between the magnetic moment associated with the electron spin and the electron orbital angular momentum. The hyperfine structure corresponds to the interaction of the nuclear spin with the electron spin, inducing the formation of a splitting of the signal equally spaced and of same intensity. The super-hyperfine structure, which corresponds to an interaction between the hyperfine structure with the nuclear spin, induces the formation of a splitting of the signal with distinct intensities. In theory, the signal width should not change, as it is inversely proportional to the relaxation time. Nevertheless, it appears that strong anisotropy, superposition of radicals spectra and thermal treatment can influence signal width measurements. Further, the intensity is directly proportional to the number of unpaired electrons. According to the Maxwell-Boltzmann equation (1.5), the greater the number of unpaired electron, the greater the absorption and therefore the intensity of the derivative. Nevertheless, when the microwave energy is too substantive to be entirely absorbed by the electron population, then the intensity reaches a maximum which no longer reflects the unpaired electron population; this phenomenon is commonly referred to as microwave saturation.
1.3 ESR dating

ESR was applied to geochronology for the first time by Zeller et al. (1967). However, the systematic practical application of ESR dating began with the work of Ikeya (1975) involving the dating of a calcite sample from Akiyoshi Cave in Japan. Since these first studies, the method has been widely applied by several laboratories and researchers, on different materials, including carbonates (Ikeya, 1978; Yokoyama et al., 1981; Grün, 1989; Bahain et al., 1994), quartz (Yokohama et al., 1985; Toyoda and Ikeya, 1994), bones (Ikeya, 1978; Yokoyama et al., 1981, 1982) and teeth (Schwarz, 1985; Grün et al., 1988, 2007; Grün and McDermott, 1994; Falguères et al., 1997, Grün, 2006). ESR dating is a palaeodosimetric technique which consists of measuring the effect of natural radioactivity record by the sample. Techniques such as Optically Stimulated Luminescence (OSL) or Thermoluminescence (TL) are based on the same principle whereby the energy measured is directly proportional to the absorbed energy over time by the material.

1.3.1 Electron trapping: Band Model

In solid state physics, trapped electrons and holes can be represented with the band model, where electrons are localized by pair of opposite spins on the bands, and define the possible energy level they can reach (Figure 1.7). The difference between the valence and the conduction band is the energy needed to break a bond in the crystal. When a bond is broken, the electron has absorbed enough energy to leave the valence band and to be transferred into the conduction band. Near the valence band remain positively charge holes; most of the time electrons will recombine with the holes, returning the crystal back to an electrically
neutral state. Crystals, such as hydroxyapatite, contain defect sites (vacancies, impurities, interstitial atoms) where electrons and holes can be trapped (e.g. Grün, 2006).

Figure 1.7 shows that the amount of trapped electrons and holes are linked to the amount of radiation received by the crystal. The activation energy $E_a$ characterises the traps and its stability, and represent the required energy to free the electron from the trap. At the same time, ionizing radiation can split molecular bonds, leading to the formation of free radicals such as $CO_2^-$ for the hydroxyapatite crystal (Callens et al., 1989).

The stability of traps or radicals is proportional to the value of the activation energy $E_a$, the thermal stability increases with the $E_a$. The life expectancy $\tau_e$ of an electron trapped or of an unpaired electron associated with an organic radical can be estimated using the Arrhenius equation:

$$\frac{1}{\tau_e} = \nu_0 e^{-\frac{E_a}{kT}}$$

where $k$ the Boltzmann constant, $T$ the temperature, $\nu_0$ the frequency and $E_a$ the activation energy.

The life expectancy of an electron trapped in a crystal can vary greatly from a few seconds to a few million years, depending on the material, the trap and environmental conditions. ESR dating consists of measuring the amount of paramagnetic centres created by natural or laboratory irradiation. The intensity measured is proportional to the amount of radioactivity received by the material over time (the archaeological or geological dose), the amount of traps or radicals available (sensitivity) and the exposition time (age of the material, Grün, 1993).
1.3.2 Equivalent dose

ESR dating is only possible since the materials being dated behave as a natural dosimeter, meaning that the sample records radiation over time. The palaeodose is the dose received by the sample with time, related to the age of the sample. The usual method for determining this is to add doses, and then the intensity is plotted versus the irradiation dose. The additive dose response curve, by extrapolation with an exponential function, will indicate the dose that the sample has received through time (Figure 1.8). The dose is described as equivalent since it is obtained with laboratory gamma irradiation, while the actual dose received in nature is the sum of radiation from multi-energetic emissions $\alpha$, $\beta$, $\gamma$ and cosmic rays.

![Diagram](image)

*Figure 1.8: Determination of the equivalent dose $D_e$ (example of the 2122A Fossil tooth enamel sample). Left: Dose response curve of the ESR intensity (a.u) signal against the dose received. The $D_e$ is obtained by extrapolation of the curve to cross the x-axis. Right: ESR spectra of fossil tooth enamel increasing with irradiation.*

To obtain a dose response curve, intensities measured are plotted against a laboratory irradiation dose. Grün (1996) estimated that the dose response curve can be best fitted with an exponential curve. The trapping is constrained by the limited number of traps available in the crystal, as well as an ability to trap more electrons inversely proportional to the number of electron already trapped. Exponential fitting is more accurate than a linear fitting; however, it may only be an approximation of the curve that actually occurs. Grün (1989) defined the properties (Table 1.2) that the ESR measurement must have in order to provide reliable results for a $D_e$ estimation. These are basic conditions that must be satisfied, otherwise results may contain large systematic errors.
The dose that has been absorbed by the sample during the burial time is the $D_e$, and can be written as:

$$D_e = \int_{t=0}^{t=T} D(t) \, dt$$  \hspace{1cm} (1.8)$$

where $D$ is the dose rate, and $T$ the time of exposure, which correspond to the age of the sample.

The dose rate $D(t)$ corresponds to the amount of radiation received by the sample. If we assume that the dose rate is constant, the age of the sample will be

$$T = \frac{D_e}{D}.$$  \hspace{1cm} (1.9)$$

- The initial signal (at $t_0$) is either zero or can be experimentally determined.
- The signal intensity grows proportionally to the dose received.
- The signals must have a thermal stability, which is at least one order of magnitude higher than the age of the sample.
- The number of traps is constant. Recrystalisation, crystal growth or phase transitions must not have occurred.
- The signals should not show anomalous fading.
- The signals are not influenced by sample preparation (grinding, exposure to laboratory light, etc.).

*Table 1.2: Properties that ESR measurement must satisfy in order to give reliable dose. (Grün, 2006)*

### 1.3.3 Internal and external dose

The external dose is derived from the environment surrounding the sample, including from cosmic rays. Radioelements taken into account in the calculation of the external dose rate are thorium, uranium and potassium. Different techniques can be used to
identify the concentration of these elements, either by counting the number of particles emitted (e.g. spectrometry α), or by measuring directly the isotopic concentration (e.g. mass spectrometry).

The external dose is expressed by the following equation:

\[ d_{\text{(external)}} = (kd\alpha_{\text{ext}} + d\beta_{\text{ext}} + d\gamma_{\text{ext}} + d_{\cos})t \] (1.10)

where \(kd\alpha_{\text{ext}}\) alpha dose rate and \(k\) the \(\alpha\)-efficiency, \(d\beta_{\text{ext}}\) beta dose rate, \(d\gamma_{\text{ext}}\) gamma dose rate and \(d_{\cos}\) the cosmic ray dose rate.

The internal dose is expressed by the following equation:

\[ d_{\text{(internal)}} = (kd\alpha_{\text{int}} + d\beta_{\text{int}})t \] (1.11)

where \(kd\alpha_{\text{int}}\) alpha dose rate and \(k\) the \(\alpha\)-efficiency, \(d\beta_{\text{int}}\) beta dose rate.

The internal dose rate is calculated from the radioelement emission of \(\alpha\) and \(\beta\) particles within the sample (1.11). Tooth enamel is usually free of Th and K, therefore only U-isotopes have to be considered. The internal dose rate adds further complexity, as the amount of U-isotopes in the tooth may vary through time. Therefore the uranium uptake history plays an important role in the calculation of the average dose rate (Grün, 2006). In the past, uranium uptake was often assumed, and conventionally two models were used: the early uptake and the linear uptake. In the first model, uranium was modelled as being incorporated within the tooth after a short time; in the second model uranium uptake was modelled as being continuously diffused during burial at a linear rate. The differences in these models may cause extremely large uncertainties.

The alpha efficiency corresponds to the ability of alpha particles to create an ESR signal. Because alpha particles have an important ionisation energy a significant local saturation can be observed, inducing fewer paramagnetic centres to be created. The \(k\) factor in front
of $d_{\alpha_{ext}}$ in the equation (2.0) is calculated in order to correct for the attenuation of alpha source within the material. The factor varies depending on the material. For example, the alpha efficiency was estimated at 0.06 ±0.01 in corals by Radke and Grün, (1988) while Yokoyama et al., (1982b) estimated at 0.26 ±0.1, 0.2 ±0.1 in quartz (Yokoyama et al., 1985), 0.07 ±0.01 in shells and 0.13 ±0.2 in fossil tooth enamel (Grün and Katzenberger-Apel, 1994).

The annual dose, which corresponds to the dose that the sample has received over a year, can be written:

$$D_{\text{annual}} = d_{\text{(external)}} + d_{\text{(internal)}}$$

Grün (1987) has shown that the external $\alpha$ contribution in the total dose rate is only a few percent, and can often be negligible. In contrast to $\alpha$ particles, the influence of $\beta$ and $\gamma$ rays in the electron trapping process is significant. The external $\beta$ dose rate must be calculated from sediments directly surrounding the sample being dated (Grün 1986, 2006). The external dose is derived from the chemical analysis of U, Th and K of the sediment. The water surrounding the sample has to also be included in the calculation of the $\beta$ and $\gamma$ dose rates. $\gamma$-rays are usually measured in situ, when possible; otherwise the external $\gamma$ dose rate is derived from the chemical analysis of sediments. The $d_{cos}$ corresponds to the amount of cosmic rays received by the sample, which will depend on the altitude, latitude and the depth of the sample within the sediment, as well as the nature of sediment (Prescott et al., 1988, 1994).

### 1.3.4 Errors in ESR dating

The ESR dose estimation error is estimated with the equation described by Yokoyama (Yokoyama et al., 1985):
\[ \Delta T = T \sqrt{\left( \frac{\Delta D_e}{D_e} \right)^2 + \left( \frac{\Delta D}{D} \right)^2}. \] (1.13)

Random error is considered to be in the range of 5-7%. In ESR dating, systematic errors are usually unknown and therefore cannot be corrected. The systematic calibration errors can be sometimes as high as 5% (Grün, 2007). The largest error in ESR dating of tooth enamel is usually associated with the unknown U-uptake. When using parametric models, such as early or late uptake, errors in the 50% range are often found.

Previous studies have defined two main calculation techniques: *Jack-knifing* (Grün and Macdonald, 1989; Lyons et al., 1992) and Monte-Carlo simulation (Chumak et al., 1999 and Grün, 2002). The *Jack-knifing* technique consists of suppressing one random data point each time the \( D_e \) is calculated. By doing so each data point is weighted on its influence on the \( D_e \). The error represents the range of values the \( D_e \) can take. In contrast, the Monte-Carlo technique (frequently used in similar problems), consist of generating random data when changing parameters for the \( D_e \) estimation. The study by Grün and Brumby (1994) has shown that the *Jack-knifing* technique is constrained by experimental data quality and frequently gives superior error values in comparison to the Monte-Carlo technique. A common mistake by non-scientists or even by geochronologist themselves, is the negligence of error, especially in the estimation of the \( D_e \). The error calculation is considered, with good reason, as important as the \( D_e \) calculation itself.
CHAPTER 2
2. THE URANIUM SERIES METHOD

The $^{230}$Th/$^{234}$U dating technique was applied for the first time on corals in the mid-1950s (Potratz et al., 1955; Barnes et al., 1956). These studies demonstrated the relationship between coral age and the disequilibrium in the uranium decay chain. This method since has been applied on marine shells (e.g. Stearns et al., 1965; Kaufman et al., 1971), calcite (e.g. Schwarcz, 1981), bones (e.g. Rae and Ivanovich, 1986; Pike et al., 2002; Eggin et al., 2003, 2005) and on teeth (Bischoff et al., 1988). Uranium-series dating uses three different radioactive decay chains, $^{238}$U, $^{235}$U and $^{232}$Th, which by disintegration give rise to three stable isotopes $^{206}$Pb, $^{207}$Pb and $^{208}$Pb, respectively.

![Diagram of Uranium-series decay chains](image)

Figure 2.1: Main isotopes of the two uranium and the thorium decay chains. The alpha and beta decays are indicated by the $\alpha$ and $\beta$ symbols. The red bold boxes indicate the isotopes used for U-series dating; Boxes in green are the stable isotopes ending the chain, the remaining isotopes are the main $\alpha$ emitters.
The U-series dating technique offers the possibility of dating past events and objects up to 500,000 years in age. However, the exact range can differ from one sample to another, depending on the uranium migration and concentration.

2.1 Uranium/Thorium geochemistry

Both uranium and thorium belong to the actinides family, or actinoid series, composed of 15 chemical elements that lie between actinium and lawrencium on the periodic table, with atomic numbers between 89 and 103. The actinide series derives its name from the first element in the series, actinium (Ac). Only thorium and uranium occur naturally in the earth's crust in trace quantities. Neptunium and plutonium have been known to show up naturally in trace amounts in uranium ores as a result of bombardment (Essien, 1990). The remaining actinides were discovered in nuclear fallout, or were synthesized. All actinides are radioactive elements. Uranium, thorium, and protactinium are highly electronegative elements (U=1.22 and Th=1.11). They can be found in a wide range of oxidation states, for thorium: $3^+$ and $4^+$, and for uranium: $3^+$, $4^+$, $5^+$ and $6^+$.

2.1.1 Oxidation state $4^+$ of Uranium

When both uranium and thorium are in the $4^+$ oxidation state, they are insoluble at low temperatures, however, they can be transported, if they are trapped to inorganic (silicates, alumino-silicates, iron oxyhydroxyde) or organic colloids. Uranium (IV) is the reduced tetrapositive cation of uranium, called uranous.

2.1.2 Oxidation state $6^+$ of Uranium

Uranium (VI) is soluble in water, through the reaction that transforms $U^{4+}$ into a complex uranyl of valence $U^{6+}$.

$$U^{4+} + 2H_2O = UO_2^{2+} + 4H^+ + 2e^- \quad (2.1)$$

The uranyl ion is the dipositive cation $UO_2^{2+}$, which can form several uranyl complexes. In this configuration, uranium is in $+6$ oxidation state ($U^{VI}$). The dominant uranyl complex will depend on several factors such as pH, temperature and the surrounding
elements. Three dominant species of uranyl can be produced in pure water: \( \text{UO}_2^{2+} \), \( \text{UO}_2\text{OH}^+ \) and \( (\text{UO}_3)_3\text{(OH)}_2^{4+} \); however, if the pH is greater than 5, other complexes will form (Langmuir, 1978). Concentrations of uranyl in water are usually extremely low (<1 ppb), due to the high insolubility of uraninite, except when the pH is below 3 or above 7. However, uranium transportation generally occurs in oxidizing surface and ground waters, usually \( \text{UO}_2^{2+} \) or uranyl fluorides, phosphates or carbonate complexes (Langmuir, 1978).

2.1.3 Uranium mobility

The dating technique of U-series is based on the different solubility of \( ^{230}\text{Th}, ^{234}\text{U} \) and \( ^{238}\text{U} \) in natural water, which leads to a disequilibrium of the isotopic ratio. Due to this disequilibrium between these nuclides, we are able to date samples including bones, teeth, speleothems, corals and shells. After the death of the organism (in the case of bones, teeth, corals or shells), the remains may end up buried in the ground, either through deliberate burial (human sepulchre) or through natural ground aggradation. During the initial period after death, the decomposing organic tissues create local reducing conditions (Rae and Ivanovitch, 1986), which induce the reduction of the uranyl ion (U\(^{VI}\)) by ground water. Uranium precipitates into U\(^{IV}\), through the reaction:

\[
\text{UO}_2^{2+} + 4\text{H}^+ + 2\text{e}^- = \text{U}^{4+} + 2\text{H}_2\text{O} \quad (2.2)
\]

The migration/incorporation pattern of uranium into fossil bones and teeth is a complex matter that needs to be fully understood, especially for ESR/U-series combined dating.

2.2 U-series dating of human remains

Most models, such as the D-A model (Millard and Hedges 1995, 1996; Millard and Pike, 1999; Pike et al., 2002, see Section 2.2.3) was developed on bones; however, the present study focuses primarily on fossil teeth, which show different incorporation patterns of uranium.
2.2.1 Principles

If the fossil (i.e. tooth) is considered to be a closed system, after the first uranium uptake at \( t=0 \), we assume that all dental tissues (dentine, enamel and cement) will be thorium free and that no other uranium uptake will occur. As only one uranium uptake event took place, the amount of thorium \((^{230}\text{Th})\) measured today \((t=\text{T})\) will be the result of the disintegration of \(^{238}\text{U}\) decay chain (Figure 2.2). The isotopic ratio \(^{230}\text{Th}/^{234}\text{U}\) at \( t=\text{T} \) is directly related to the time since the uranium uptake, and can be determined with the half-life of \(^{230}\text{Th}\) of about 75,200 years (Cheng et al., 1998).

![Graph showing the accumulation of \(^{230}\text{Th}\) and its relation to \(^{234}\text{U}\) over time.]

**Figure 2.2:** The \(^{230}\text{Th}\) accumulation comes from the decay of \(^{234}\text{U}\), which is the daughter isotope of \(^{238}\text{U}\). The \(^{230}\text{Th}\) concentration increases until the number of isotopes reaches equilibrium (from Grün et al. 2003).

The age calculation can be done with a few simple equations, after complex measurement and model analysis. The \(^{230}\text{Th}\) is the daughter of \(^{234}\text{U}\) in the \(^{238}\text{U}\) decay chain (see above Figure 2.1). The decay rate of uranium \(^{234}\text{U}\), is described by

\[
\frac{-d^{234}\text{U}}{dt} = \lambda_{^{234}\text{U}},
\]

(2.3)

where \(^{234}\text{U}\) is the number of atoms involved, and \(\lambda_{^{234}\text{U}}\) is the decay constant of \(^{234}\text{U}\). The general formula can be written:
\[ A_r = A_p \left(1 - e^{-\lambda_d t}\right) \]  \hspace{1cm} (2.4)

where \( A_p \) is the activity of the parent isotope and \( \lambda_d \) is the decay constant of the daughter isotope.

Therefore, the \(^{230}\text{Th}\) activity can be expressed as:

\[ ^{230}\text{Th} = ^{234}\text{U} \left(1 - e^{-\lambda_{230} t}\right) = ^{238}\text{U} \left(1 - e^{-\lambda_{230} t}\right) \]  \hspace{1cm} (2.5)

only if \( \frac{^{234}\text{U}}{^{238}\text{U}} = 1 \), with \( \lambda_{230} \) is the decay constant of \(^{230}\text{Th}\).

The equation requires that \(^{234}\text{U}\) and \(^{238}\text{U}\) are in equilibrium at \( t=0 \). However, in ground water the ratio \( ^{234}\text{U}/^{238}\text{U} \) can be as high as 10 (the average value is around 1.5) due to an excess of \(^{234}\text{U}\) (Gascoyne, 1992). The excess is present in continental water mainly due to the \( \alpha \)-recoil phenomenon; the disintegration of \(^{238}\text{U}\) triggers the emission of an \( \alpha \) particle and induces the recoil of \(^{234}\text{U}\). The atom traverses the crystal lattice inducing damages and sometimes ejection directly into the solution (Cherdynstev et al., 1955; Kigoshi 1971; Fleischer 1980, 1988).

Because the half-life of \(^{234}\text{U}\) is 244 ka, which remains within the dating range of the U-Th method, the variation of the \( ^{238}\text{U} / ^{234}\text{U} \) ratio has to be considered, until restoration of equilibrium (Ivanovitch and Harmon, 1992) (Figure 2.3).

\[ \frac{^{230}\text{Th}}{^{234}\text{U}} = \frac{^{238}\text{U}}{^{234}\text{U}} \left(1 - e^{-\lambda_{230} t}\right) + \left(1 - \frac{^{238}\text{U}}{^{234}\text{U}} \right) \frac{\lambda_{230}}{\lambda_{230} - \lambda_{234}} \left(1 - e^{-\left(\lambda_{230} - \lambda_{234}\right) t}\right) \]  \hspace{1cm} (2.6)
2.2.2 U-series dating of open systems

The theory discussed in the previous section, is based on the principle that the dated material (in our case tooth) behaves as a closed system with respect to uranium. Unfortunately, teeth appear to be an open rather than closed system, meaning that the material does potentially interact with the surrounding environment after death. Uranium uptake into bones and teeth is complex and difficult to describe and the migration of U and Th isotopes has a direct impact on the calculated ages of teeth: if uranium migrates late into the bone or if thorium is leached away, the age calculation will be too young; alternatively, if the uranium is leached or if some thorium migrates into the remains, then the age calculation will be too old (Figure 2.4). One has to keep in mind that thorium leaching from bones or teeth has not been observed in nature so far.
Figure 2.4: The effect of uranium and thorium mobilisation on measured U-series ages

Despite the fact that bones and teeth do not appear to behave as a closed system, the ESR and U-series dating techniques remains extremely valuable. This is principally because the radiocarbon dating range of 50 ka is negligible compared to length of hominid evolution of a few million years; thus, even though ESR and U-series age calculations are mostly unreliable, palaeoanthropologists have few other options available to them through which they can establish chronology.

2.2.3 Diffusion-Absorption model

The concentration of uranium and thorium in modern bones and teeth is virtually zero (<1 to 50 ppb - Tandon et al., 1998), while in archaeological samples the concentration can be up to hundreds of ppm. Uranium is incorporated through the diffusion of uranyl (UO$_2^{2+}$) complex, which is adsorbed on the surface of hydroxyapatite crystals (Millard, 1996; Millard and Hedges 1995, 1996; Millard and Pike, 1999; Pike et al., 2002). The Diffusion-Adsorption model (D-A) attempts to describe the spatial allocation of the isotopes from the uranium decay chain in bones and teeth. The slow diffusion of uranium from the sediments to within the sample creates a uranium distribution close to a $\cup$-shape across the bone. Through time the concentration of uranium tends to equilibrate itself, therefore the $\cup$-profile will flatten (Figure 2.5).
Figure 2.5: D-A model of Millard & Hedges (1996) and Pike et al. (2002), the upper graph shows the distribution of the concentration of uranium in the bone according to the D-A model. Lower graph shows the U-series age measured on the bone; ages are modelled with different Diffusion-Adsorption parameters.

The D-A model allows the identification of uranium leaching from the system. When this situation occurs the U-concentration will not be constant and therefore it will not present a \( \cup \)-shape; if the uranium profile in the bone does not suit the D-A model, then the bone is rejected and will not be dated. At the same time, this model allows the identification of bones with complex uranium uptake histories. While the D-A model is a powerful tool, in some cases it cannot be applied, as some samples may have spent a long period in the ground without accumulating uranium, until local hydrology changes. The calculated age will thus end up being an underestimate, but with a \( \cup \)-profile (Figure 2.6). Figure 2.6 shows frequent diffusion patterns, with rapid, continuous or recent U-uptake and at the bottom of the figure the diffusion shape with uranium being leach at the surface. Alas, the D-A model is not applicable to tooth enamel material, as the U-uptake generally take place through the root canal and the dentine. The uranium diffusion through the dentine only take place in one direction, and is constrained by the dentine-enamel junction (DEJ) inducing a complex 3D diffusion pattern.
Figure 2.6: Diffusion prediction using the D-A model for various geochemical scenarios. (Pike et al., 2002)
CHAPTER 3
3. DATING FOSSIL TOOTH ENAMEL

Tooth enamel behaves as a natural dosimeter. For the past 40 years ESR spectroscopy has been widely applied in the assessment of past radiation doses in archaeological and geological dating applications (Rink, 1997; Grün, 2000a), as well as accident dosimetry usually named retrospective dosimetry (i.e. Tchernobyl employers IAEA, 2002; Survivors of the Hiroshima and Nagasaki nuclear bombs, Tatsumi-Miyajima, 1978). The enamel is able to record doses from a few mGy to several thousand Gy (Sholom et al., 2006; Schwarcz et al., 1994).

The dating process of tooth enamel is identical to that applied to retrospective dosimetry protocols, with the only difference being that in order to estimate the age of the sample an annual dose rate must be calculated. Direct dating of hominid remains using ESR spectrometry was first applied to fossil bones in early 1980 (Ikeya and Miki, 1980; Yokoyama et al., 1981). Nevertheless, the intrinsic constitution of bones does not allow for reliable dating when compared to fossil tooth, the latter of which is better preserved over long periods (Grün and Invernati, 1985; Schwarcz and Zymela, 1985).

3.1 Dental Anatomy

The evolution of teeth through time has lead to various morphologies, from the simple lines of conical sharks’ teeth to mammals possessing very complex tooth structures. Mammalian teeth, including those of humans, are encased in an osseous cavity of the jawbone and are maintained by a ligament. The majority of mammals, excepting the rodents, have two series of teeth: deciduous and permanent. The organisation of the tooth itself, as well as the jaw, is closely linked to the diet. Hominids, carnivores and herbivores all show a very similar arrangement, composed by three dental tissues: enamel, dentine and cement.
Bones and teeth have the advantage of being constructed in very hard materials, thereby increasing their chance of survival in the archaeological and sedimentary record. Indeed, tooth is the hardest organ of human tissues, generally composed of a free crown and one or more roots established in the oral cavity (jawbone and mandible; Cate, 1998).

Teeth are heterogeneous organs comprised of with organic and inorganic structures (Figure 3.1). The histology of the structure explains the definitive organisation of the different components. Teeth growth depends on the species of animal involved. For example, humans grow their teeth during childhood, yet the teeth of some herbivores grow continuously throughout their life. Therefore, looking at teeth and jaws can be a powerful tool for identifying their species of origin, diet, possible migration patterns, palaeoenvironments and dating. The most commonly used tissues for dating purposes are enamel (ESR and U-series) and dentine (U-series).

![Figure 3.1: Cross section of a human molar showing the principal tissues found in the organ.](image)

The root is attached to the bone in the alveolar bone socket, held together with a “joint” made by complex fibres, called the periodontal ligament. Teeth are enclosed in heavily built bony jaw structures. The root is constituted by cement and dentine, built with living tissues (Cate, 1998). Established on the alveolar edge of the jawbone (upper jaw), the mandible (lower jaw) forms the dental arcade (parabolic curve made by tooth on each jaw). The centre of the crown contains vessels and nerves, known as the pulp cavity (Ross et al., 2003). The tooth is covered with dentine, with cement to the level of the root and enamel on the crown level. Teeth arranged into rows, are called dentition.

The organisation of dentition differs between mammals, depending on their diet as mentioned above. The organisation of enamel, dentine, cement and also the periodontal ligament can also be very different from one species to another. Further, even within the same individual, teeth differ from one another, depending on their functions.
3.1.1 Cement

The consistency of cement is very similar to that of bones, however, the main difference is that the cement is not vascularised. Jaws of modern humans are built with a cement covered by the gum while, for example, in herbivores the cement encloses the entire tooth, even the inside between dentine and enamel. As opposed to enamel or dentine, cement is constituted by a succession of cells creating a friable tissue. The cement does not contain a large percentage of mineral-bearing zones when compared to dentine and enamel, only 45%; 33% are organic components and 22% water (Ross et al., 2003). Cement usually presents a layered structure composed by collagen fibres and cementocyte cells (Hillson, 2005).

3.1.2 Dentine

![Figure 3.2: SEM picture of the dentine from a Neanderthal tooth from Payre (France).](image)

The dentine is the internal part of the tooth from the roots to the dentine-enamel junction (DEJ) and enrobing the pulp cavity (Figure 3.1, above). Its composition is similar to bones with a mix of collagen fibres (~20%) and hydroxyapatite crystals approximately 20 to 100 nm in length (Hillson, 2005). Collagen is a glycoprotein fibre, which plays the role of a frame in bone or dental tissues (DiLulloDagger et al., 2002). It is exuded by conjunctival tissue and has the particularity to be a non extendible fibre, conferring to the dentine a high resistance to mechanical pressure.

Like enamel or cement, dentine is not a vascular tissue, however, because tubules or canaliculi are found throughout the dentine, some odontoblasts (HAp secreting cells) can travel across the structure. Boyde and Lester (1967) described two different structures
within the dentine: the intertubular and peritubular domains. These can be macroscopically differentiated by obvious distinct fibrillar phases that have a significant implication for U-series dating.

3.1.3 Enamel

Dental enamel is the most highly mineralized structure in the human body and is formed within a unique, extracellular matrix derived through the synthesis secretion of proteins by ameloblast cells (Johnson, 1998). The mature enamel has a complex structure, providing a tissue which is very well adapted to resist the mechanical stresses of masticating. It constitutes an interface layer between organic and pseudo-organic tissue within the oral environment. The enamel does not present any cells or vestige of cells; it is an inorganic mineral structure, composed of 96 to 97% of hydroxyapatite (Ca_{10} (PO_{4})_6 (OH)_{2}) crystal, <1% percent of organic impurities (0.4-0.9%), and 2 to 3% of interstitial water (Hillson, 1986; Driessens, 1980).

![Enamel structure](image)

*Figure 3.4: Simplified Enamel prism organisation in beams*

HAp crystals are hexagonal elongated prisms (~4 to 8 μm diameter and ~16 μm long), grouped in beams, parallel in the direction of the length, extending from the DEJ to the
BEB, and agglomerate to each other by a semi-amorphous interprismatic phase (Figure 3.4) (Driessens, 1980; Lester and Koenigswald, 1989).

The interprismatic crystals do not present any preferential orientation, with an anarchistic growth. They provide a pathway for substances such as fluorine, calcium or uranium, to penetrate the enamel by adsorption (Martin et al., 1988; Lester and Koenigswald, 1989). Lakes (1993) defined enamel using a hierarchic structure, of six distinct levels from n=0 to n=5. At the first level, n=0, the enamel tissue is described as a compact and solid structure, highly mineralised. The second level, n=1, distinguishes three different phases: mineral, organic and aqueous. The next four levels are distinctions within the mineral phase, defined as n=2 prismatic and interprismatic phases, n=3 prisms beams, n=4 nanocrystals, and n=5 unit cell.

![Figure 3.5: Detail of enamel hydroxyapatite nano-crystal agglomerated, parallel in the direction of the length. Behind the first layer, a second layer with a different orientation can be observed.](image)

**Mineral Phase.** The unit cell (n=5) described by Lakes (1993) corresponds to the bulk arrangement of atoms within a given type of crystal, which repeat periodically in 3-dimension corresponding to the crystal lattice. The unit cell dimension in the 3-dimension axis (a*b*c) are 9.418*9.418*6.884 Å (Simmer and Fincham, 1995). The nano-crystal lattice corresponds to a combination of unit cell (n=4), forming hexagonal prisms (n=3) grouped in beam separated by interprismatic phases or interrow sheet (n=2). The interprismatic domain remains poorly understood, with a possible amorphous constitution.
(Sander, 1997; Roveri et al., 2009; Sato et al., 2002) or a mixture of misaligned nanocrystal ranging from 40° to 65° orientation offset from the direction of the growth (Simmer and Fincham, 1995) (Figure 3.5).

**Organic Phase.** The organic phase represent a very small amount of the total composition of the enamel (<1%). Two organic phases can be distinguished, one thermally stable fills gaps in the interprismatic domain (annealed between 300° and 500°C), while the second, less stable phase envelopes the nano-crystal (annealed at 150° to 250°C; Brik et al., 2000b).

**Aqueous phase.** Water (2 to 3 % of the enamel composition) is found in two different forms in the crystal: one at the surface of the crystal commonly named adsorbed water, and the other trapped within the crystal lattice known as absorbed water (LeGeros et al., 1978). The main difference between the two types of water is the reversibility of evaporation after thermal treatment. The absorbed water is unstable at temperatures between 150° and 200°C, while the adsorbed water can be evaporated as low as 100°C, however, can be recovered by rehydration of the enamel.

The enamel is formed through a process called *amelogenesis* directly occurring after the dentinogenesis leading to the dentine formation. This process is considered to have two stages. The first stage is known as the secretory phase and the second stage as the maturation phase (Simmer and Fincham, 1995; Smith, 1998; Fincham et al., 1999). The secretory phase starts with the differentiation of ameloblast that becomes polarized and elongated in the DEJ domain. The ameloblast start secreting enamel proteins that become partially mineralised by the enzyme alkaline phosphatase. Following the first depository layer known as *Tomes* processes, the ameloblast move away from the dentine and start secereting the second layer of enamel. The *Tomes* processes lead to the creation of a distinct layer, frequently assimilated with the DEJ, that shows a particular orientation and organisation different to the rest of the enamel matrix (Simmer and Fincham, 1995). The maturation stage finalises the mineralization of pre-existing crystal that elongates in the
direction of the length induced by the transformation of proteins such as ameloblastins, amelogenins and enamelines (Smith, 1998; Hilson, 2005; Fincham et al., 1999).

3.1.4 Schmelzmuster, HSB and Enamel Prisms

Mammalian enamel corresponds to the most evolved and complex tissues of the animal kingdom (Koenigswald and Clemens, 1992). It can be characterized in three hierarchic levels of complexity, the Schmelzmuster which corresponds to the spatial distribution of enamel, the Hunter-Schreger bands (HSB) which describe the decussation characteristic and the enamel prisms that can be sub-divided in modules, types, rods or pattern (1 to 3). While, the three hierarchic levels are found in the mammalian enamel, strong differences occur within the arrangements between species. In the case of human enamel the Schmelzmuster describes a complex spatial organization of the enamel type from the DEJ to the buccal-enamel boundary. Enamel types can be categorise in two groups the prismatic and non-prismatic (or interprismatic) crystal (see above, 3.1.3).

While the enamel is constructed with different modules, the prismatic crystal shows a complex pattern on it self with strong variation between the DEJ and the BEB. The later description corresponds to decussation of the crystal prisms, and can formed a particular pattern in human and bovid enamel the Hunter-Schreger bands (HSB). HSB thickness and organization varies between species, individuals, teeth and within the same enamel fragment (Korvenkontio, 1934; Walhert, 1989; Koenigswald and Clemens, 1992). In the particular the bovid tooth of Holon (later used in this thesis) the HSB formed a serpentine shape from the DEJ to the BEB defined by two parameters: the inclination (vertical view) and the orientation (tangential view) with prisms crossing one another in a complex pattern. One has to keep in mind that the prisms direction does not refer to the actual orientation of the crystallites within the prisms but to the direction of the prisms sheath. Finally the enamel prism varies in length width and shape within the enamel crystal. Prisms are composed by bundles of diverging crystallites that agglomerate together with rather small angular offset in there average orientation (Koenigswald and Clemens, 1992; Sander, 1996).
3.2 Hydroxyapatite (HAp)

Enamel is composed of specific apatite crystal, mainly hydroxyapatite with the formula (Ca$_5$ (PO$_2$)$_3$OH), however, usually written (Ca$_{10}$ (PO$_4$)$_6$ OH$_2$) because of the two unit cell entities. It crystallises in a hexagonal (dipyramidal) crystal system, with a specific gravity of 3.08, a density of 3.156 g/cm$^3$ and 5 on the Mohs hardness scale. Nevertheless, the structural organisation of enamel still divides scientists, as experiments present HAp as hexagonal or monoclinic system (Kimiya su et al., 2002; Bystrov et al., 2006).

3.2.1 Substitution and Interaction

The HAp crystal is, because of its biological origin, filled with impurities within the crystal lattice. The hydroxyl ion (HO$^-$) can be replaced within the crystal, known as substitution $A$ by fluoride, chloride or carbonate. The substitution $B$ consists of replacing the phosphorous group PO$_4^{3-}$ by another radical, such as CO$_2^-$ radical (Arends et al., 1987; Battistone et al., 1967; Bakhos et al., 1977; Tochon-Danguy, 1980; Callens, 1987). Further information about this essential-for ESR dating of tooth enamel-substitution will be presented later in this chapter. The incorporation within the crystal of ions or radicals can induce significant modifications of the crystal lattice leading to changes in the physical and chemical properties of the HAp (Young, 1974; Narasaraju and Phebe, 1996; Driessens, 1980).

Frank et al. (1960) and Poole and Silverstone (1973) confirmed that an exchange is possible between the apatite ion and the surrounding environment. One third of the ions from the crystal grid may be substituted by ions which have diffused from the environment, whether it is a heteroionic or an isoionic substitution. With respect to substitution it should be noted that there is a greater chance for it on the crystal surface than within the crystal (Kimiya su et al., 2002). Also, experiments on the migration of heavy ions such as uranium shows that the migration in enamel is relatively slow, and concentrations are extremely low.
3.2.2 Incorporation of Carbonates

The incorporation of carbonates within the crystal lattice is critical for ESR dating, as carbonate ions play the role of precursor for the creation of the paramagnetic \( CO_2^- \) radical. Carbonates are known to substitute the hydroxyl ion (10%) and the phosphorous group (90%) in site A and B, respectively (LeGeros, 1981; Elliott et al., 1985) (3.1). The general formula for the carbonated hydroxyapatite is (Suetsugu et al., 2000):

\[
\text{Ca}_{10-x/2} \left[ (\text{PO}_4)_{6-x} (\text{CO}_3)_x \right] \left[ (\text{OH})_{2-2y} (\text{CO}_3)_y \right]
\]  \hspace{1cm} (3.1)

The incorporation of carbonate groups occurs in two occasions during amelogenesis and diagenesis. Carbonate ions are exuded by the ameloblastes to increase the pH to avoid demineralisation (Smith, 1998). The quantity of carbonates varies within the enamel layers, with a mass percentage decreasing from the DEJ to the BEB, of 3.9% to 2.25%, respectively (Simmer and Fincham, 1995; Smith, 1998). Further, studies have speculated that diagenetic processes occurring during burial could lead to the incorporation of new carbonates within the lattice (Michel et al., 1995). The study by Michel et al., (1995) showed that the carbonate concentration found in cervidae fossil enamel is higher compared to modern enamel, especially at the B site (substituting the phosphorous group). The hypothesis propound relates to complex diagenetic process, however, one has to keep in mind that the carbonate concentration could have been different in the past.

3.2.3 Diagenetic processes

Several studies have proven that differences in burial time and environment resulted in diagenetic alterations of fossil tooth and bones (e.g. Collins et al., 1995; Reich et al., 2002a,b; Lebon, 2008). The intrinsic organisation of bones and dentine is in many aspects very similar, and physical and chemical alteration seems to happen in both tissues. Dauphin and Williams (2004) have shown that dentine can be affected by an increase of the canaliculi width through dissolution processes, precipitation processes and organic
deterioration. These mechanisms induce the dentine to increase its porosity increasing the possibility of water (including ions in solution) penetrating the fossil. Berna et al. (2004) have shown that recrystallisation can occur within the bone or the dentine structure after dissolution induced by acid water. Nevertheless, the process of dissolution and recrystallisation had thus far never been observed on enamel. In fact, no diagenetic processes with the exception of creation of a crystal defect or modification of electronic state has been observed on tooth enamel. Several studies have shown that crystal defects appear during burial (Henderson et al., 1983; Pate et al., 1989; Michel et al., 1995; Kohn et al., 1999). However, hypotheses relating to the processes of incorporation and substitution within the crystal are still under debate. Nevertheless, electronic modification of ions already incorporated within the crystal is clearly observable. Ionising radiation emitted mainly from uranium, thorium and potassium transform the electronic states of elements, groups and radicals. One has to keep in mind that uranium is known to migrate in low concentration in to the enamel, most certainly by adsorption. The α recoil from the disintegration, is also acknowledged to greatly damage the crystal lattice.

3.3 Dating fossil tooth enamel

3.3.1 ESR signal of fossil tooth enamel

The complex ESR signal of fossil enamel is composed by a multitude of radicals located in different domains of the enamel that forms the composite spectra by superposition. The asymmetrical spectrum around g=2 is composed by radicals with different radiation and temperature sensitivity.
Figure 3.6: Shape of the ESR spectra of fossil tooth enamel powder representing the three main peaks T1, B1 and B2 with g-values gx, gz and gy for T1, zero passing and B2 respectively.

The spectrum is shaped by three main peaks T1, B1 and B2, with g-value at around g=2.0030, g=2.0015 and g=1.9975, for T1, zero passing and B2 respectively (Figure III.6), attributed to several CO2- radicals used for dose reconstruction. Nevertheless, other radicals are known to interfere with the main signal including CO3-, CO33-, methyl, SO2-, O-, O3-, CO- and several wide lines (Callens et al., 1995). The CO33- (Vanhaelewyn et al., 2000a), O- (Vanhaelewyn et al., 2000b), along with SO2- (Barabas, 1992), methyl lines (Bouchez et al., 1988) and CO3- (Callens et al., 1989) are temperature dependent interfering with the T1-B1 region, however, no intensity increase induced by irradiation has been observed. One damper has to be put on the methyl radical, as the radiosensitivity is still under debate, with contradictory results (Vanhaelewyn et al., 2000a) describing the signal as increasing with irradiation under 5000 Gy, while in this research the methyl lines did not increase. The study by Vanhaelewyn et al. (2000a) used a multivariable spectral decomposition method MCFLA (Maximum Likelihood Common Factor Analysis) which has given six main components. In this study, we have designed a different approach using a Simulating Annealing (SA) Monte-Carlo decomposition methods, that has shown different results which will be discussed in the chapters to follow.
<table>
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<th>$g_y$</th>
<th>$g_z$</th>
<th>$g_{iso}$</th>
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<th>$A_y$</th>
<th>$A_z$</th>
<th>$A_{iso}$</th>
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<td>422</td>
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<td>2.0016</td>
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Table 3.1: Spin Hamiltonian parameters of carbonate-derived radicals in several materials ($^{13}$C hyperfine parameters are in MHz). Hap$^-$ = hydroxyapatite; sc$^-$ = single crystal; po$^-$=powder. (from Callens, personal communication)

In the existing literature, most dose estimations are based on peak to peak measurements of $T_1$-$B_1$ or $T_1$-$B_2$ (e.g. Falguères et al., 1997; Chen et al., 2001) However, the $T_1$ peak is very sensitive to interfering radicals, as demonstrated by the simulation presented in
Figure 3.7. The B2 position does not experience any modification, and has been proposed for dating purposes by Callens et al. (1998). It is important to remember that a non-orientated $CO_2^-$ is presumably interfering on the entire spectra (Grüner et al., 2008a).

Vanhaelewyn et al. (2000) has simulated interfered spectra, obtained by admixing 20% of abovementioned radicals that are known to interfere with the $CO_2^-$ main signal. (Figure 3.7).

![Figure 3.7: Effect of 20% of interfering radicals ($O^-, O_3^-, CO^-, CO_3^-, CO_3^{2-}$) on the $CO_2^-$ radical ESR signal, the resulting spectrum is represented below respectively to each simulation. The dotted line designates the undisturbed $CO_2^-$ signal. The small spectra of $O^-, O_3^-$ and $CO_3^-$, represent the full ESR signals of the corresponding defects over wider magnetic field ranges (Vanhaelewyn, 2000a, c)](image)

To be able to produce accurate dose estimations, the contribution of each radical has to be investigated, separated, quantified and kinetically understood.
3.3.2 Two types of CO$_2$- radicals

The number and types of CO$_2$- radicals contributing to the total ESR signal of tooth enamel is still under debate. Brik et al. (2000a) identified seven different types of CO$_2$- radicals with very similar g-values, while other studies (Callens et al., 1995; Brik et al., 1998; Ishchenko et al., 2002; Scherbina and Brik, 2000a) are separating two main types of CO$_2$- radicals with significantly different kinetic properties. The first radical, non-oriented CO$_2$- radical (NOCOR), is formed by radicals with no-preferential orientation giving rise to a powder spectrum, identical at every angle. The second radical type, commonly named oriented radicals (AICOR), is strongly anisotropic and is responsible for the angular variation observed in fragments. It is not possible to differentiate the two species in the powder spectrum due to the nature of the NOCORs. Unlike Callens et al. (1995) who described the two radicals NOCORs and AICORS, Ishchenko et al. (2002) separated the AICORS in two species: one orthorhombic at gx=2.0030, gy=2.0015 and gz=1.9970 and an axial radical at gx=2.0023 and gz=1.9975. According to Ishchenko et al. (2002) the difference between the two radicals is the result of the rotating capacity of the axial radical when the O-O axis is parallel to the direction of the magnetic field (Figure 3.8). Brik et al. (1998) showed that NOCORs are significantly less stable than the AICORS species (Figure 3.9). Vorona et al. (2006) found a different stability of the two radicals, however, the kinetic properties slightly differ from those of Brik et al. (1998) and Scherbina and Brik. (2000), with a higher annealing temperature (between 250°C and 300°C) found by Vorona et al. (2006). Importantly, the three studies were performed on modern tooth enamel, which usually contains a different ratio of NOCORs and AICORS (after γ-irradiation between 65% and 80% of NOCORs in

Figure 3.8: representation of the rotating CO$_2$ in the hydroxyapatite crystal along the C axis (Ishchenko et al., 2002)
modern enamel (Vorona et al., 2006) against 40% in fossil enamel (Joannes-Boyau et al., 2009)).

![Graph showing ESR intensity vs temperature]

*Figure 3.9: Thermal stabilities of the two different types of CO2 radicals. NOCORS in red dot and AICORS in blue stars (Brik et al., 1998).*

### 3.3.3 AICOR/NOCOR: localisation in the enamel

The studies by Vorona et al. (2006) and Brik et al. (1998) concluded that NOCORS are transforming into AICORS after thermal treatment, thus explaining the increase of AICOR radical concentration between 200°C and 250°C (Figure 3.9 above). Nevertheless, both do not agree on actual processes that lead to the transformation of one form in to the other form. Brik et al. (1998) formulated the hypothesis of mass transfer process which consists of a mass transfer between organic and crystal phases. According to Brik (1998), thermal treatment anneals the organic phases and ordinates the nanocrystals within the enamel. This model is controversial and remains questionable, on the simple fact that the mass transfer would occur between radicals in two different domains (mineral and organic) of the enamel, which appears impossible to explain.

Callens et al. (1995) thought that the NOCORS were located at the surface of the crystal, while Ishchenko et al. (1999) argued that AICORS were located within the crystal. In a
more recent publication, Brik et al. (2000b) changed the location of the organic phases of the mass transfer process hypothesis to an organic phase 2 located at the surface of the nano-crystals. Nevertheless, the transfer process between organic and mineral phases remains problematic, even if the two phases are in direct contact.

Vorona et al. (2006) and Ishchenko et al. (2002) both postulated that the two radicals are in the same domain within the crystal lattice. According to the two studies, heating would evaporate water molecule surrounding the NOCORs, allowing the radical to rotate around its O-O axis, becoming then an AICOR. Water molecules are known to occur within crystals and enamel HAp (Murata et al., 1996). However, in this model the changes should be irreversible, while with more irradiation NOCORs can be regenerated. In this thesis we propose a new transfer process model between NOCORs and AICORs, and between the two AICRORs (PART 2: Results and Discussion, Chapter 15, p.240).

3.3.4 Formation of the CO$_2^-$ radicals

Organic materials are incorporated within the crystal during the amelogenesis, some radicals including COOH, CO$_2$ and CO$_3^{2-}$ molecules are described as precursor for the CO$_2^-$ radical responsible of the main ESR signal of tooth enamel (i.e. Callens et al., 1989, 1993; Moens et al., 1991, 1993, 1994; Brik et al., 2000c). The study by Brik et al. (2000c) described three processes for the formation of CO$_2^-$ radicals.

The first formation process is the delocalisation of a H$^+$ ion from the carboxyl group, COOH, by the mean of irradiation.

\[
-COOH + \sum \rightarrow -CO_2^- + H^+ \quad (3.2)
\]

where $\sum$ represent the energy given to the material by the mean of natural or laboratory irradiation, including $\gamma$-rays, $\beta$ and $\alpha$ particles, UV lights or X-rays. The influence of each
of these radiation sources (X-rays and α particles were not studied in this thesis) on the ESR signal of HAp have been investigated in this thesis, and correspond to separate chapters (*PART 3: Results and Discussion, Chapter 11, 12 and 13*).

The second process is the trapping of a free electron by the CO$_2^-$ radical, giving:

$$CO_2 + e^- \rightarrow CO_2^-$$  \(3.3\)

Brik et al. (2000c) distinguished two CO$_2$ precursors located in two separate domains, the organic phase and the crystal phase, leading with the same process to the NOCORs and to the AICORs formation, respectively.

The third process is the delocalization of an oxygen atom form the CO$_3^{2-}$ molecule induced by natural or laboratory irradiation.

$$-CO_3^{2-} + \Sigma \rightarrow -CO_2^- + O^-$$  \(3.4\)

Brik et al. (2000c) argued that the third mechanism can only be energetically achieved by γ rays and β and α particles.

Vorona et al. (2006) proposed a different model than the third process of Brik et al. (2000c), with the trapping of an electron by the CO$_3^{2-}$ group.

$$-CO_3^{2-} + e^- \rightarrow -CO_3^{3-} \rightarrow -CO_2^- + O^- + e^-$$  \(3.5\)

Vorona et al. (2006) argued that the third process of Brik et al. (2000c) is not realistic within the crystal, and that an intermediate state is necessary by the creation of a CO$_3^{3-}$ radical. This model requires far less energy to be achieved, and could be triggered by less energetic sources such as X-rays and UV.
Thus said, the models proposed for the formation of $\text{CO}_2^-$ radicals (especially eq 3.3) raises several concerns. One could argue that this process remains very unlikely, since no $\text{CO}_2$ molecule is supposedly trapped in the crystal, unless somehow the oxygen atom is "misplaced" in the process. A similar statement can be made for Brik et al. 2000c model (eq. 3.4), which appears highly unlikely, as single $\text{O}^-$ are not known to be stable ion. At last, one could ask our self, why most scientist privilege the formation of $\text{CO}_2^-$ radicals from organic matters exclusively, while $\text{CO}_3^-$ ions occurs in a greater number in the Hydroxyapatite crystal. Clearly, the complex process leading to the formation of $\text{CO}_2^-$ radicals appears far from solved and further studies are certainly required to shine light on this important mechanism.

### 3.3.5 Localisation of the $\text{CO}_2^-$ radicals

The HAp crystal $\text{Ca}_{10} (\text{PO}_4)_6 (\text{OH})_2$ offers two distinct sites A and B, on which the $\text{CO}_2^-$ radicals can be located within the crystal. The two sites A and B referring to a substitution by the $\text{CO}_2^-$ of a hydroxyl ion (HO$^-$) or the phosphorous group ($\text{PO}_4^{3-}$), respectively (refer to previous paragraph 3.2.1, p.69). The *substitution* B (Figure 3.10) is 9 times more likely to occur than the *substitution* A according to Elliott et al. (1985), Vugman et al. (1995), Chen et al. (2001) and Ishchenko et al. (2002). Nevertheless, this idea is still debated, with the possibility of radicals being adsorbed at the surface of the crystals (Callens et al., 1989; Rey et al., 1989). Brik et al. (1998 and 2000) goes around the problem by introducing several radicals each of which are created from different precursors, and located on several distinct sites such as organic matter 1 and 2 respectively within and at the surface of the crystal, or on the site A and B (described above) within elongated prism or in nano-crystal structure.
Figure 3.10: (a) Structure of the crystal hydroxyapatite close to the hexagonal c-axis. (b) Representation of the most common substitution: substitution of a phosphate by carbonate groups, this induces a charge compensating vacancy. (Vugman et al., 1995)
CHAPTER 4
4. ESR MEASUREMENTS OF FOSSIL TOOTH ENAMEL FRAGMENT

4.1 Sample Preparation

The initial step of sample preparation requires a fragment to be removed from the tooth. The use of natural cracks in the enamel offers the best opportunity to separate the fragment, causing minimal damage to the remaining tooth. Small fractures frequently occur on fossil tooth enamel caused by environmental processes during burial. Alternatively, if the sample does not possess any such fractures, the use of a diamond saw might be required to remove a fragment. For this project, fragments were removed from the Holon fossil bovid teeth using a saw since their particular structure had ensured the enamel was well protected against environmental processes. After removing the fragment, all organic material as well as cement and dentine was separated from the enamel using dentistry tools or a diamond saw. During these stages it is important not to apply the diamond saw too vigorously, so as to prevent a mechanically-induced ESR signal being created (Desrosiers et al., 1989).

Figure 4.1: Protocol to measure tooth enamel fragment with the ESR spectrometer, example of Holon sample. a) the enamel fragment can be removed from the tooth, either using natural fracture of by cutting the enamel with a saw. b) Mounting the fragment in three different Teflon older filled with low temperature melted parafilm. X orientation is perpendicular to the enamel/dentine boundary, Y orientation is parallel to the growth direction of the tooth and Z orientation is perpendicular to the two aforementioned orientations.
Powder samples afford an easier protocol for measurement, since by definition powder has a random orientation and can therefore be transferred to different tubes without concern. In contrast, tooth enamel fragments are strongly anisotropic, and in order to accurately compare spectra before and after thermal treatment or irradiation, the sample has to be positioned identically for each measurement. To overcome this technical requirement we specifically designed a Teflon holder filled with melted parafilm (at 80°C) that does not give any ESR signal. The fragment can then be pressed in to the parafilm, which after cooling retains an exact mould within which the fragment can be repositioned allowing for repeated measurements with minimal offset (Figure 4.1). When all experimentation has been completed the fragment can be reattached to the tooth, with little visible damage. As the impact on the fragment itself is minimal, this protocol is considered to be virtually non-destructive.

4.2 ESR measurements

The Teflon holder is inserted in the ESR cavity at the bottom of a quartzite tube. The cavity is then topped up with a programmable goniometre type ER 218PG1 that allows the rotation of the sample over 360° in the horizontal plane. The goniometre is associated with an ESR spectrometer ELEXYS E500 Bruker and X-band microwave bridge (frequency of ~9.5 GHz). Since the sample only rotates in the horizontal plane, three Teflon holders are necessary for each sample to measure the sample in every possible configuration (Figures 4.1 and 4.2). The fragment is mounted in three different orientations X, Y and Z (Figure 4.2), with the goniometer rotating 36 times through incrementing 10° steps, for each orientation. As a result, instead of a single powder spectra we obtain 108 spectra (Figure 4.3, below). When analysing powders, at least ten irradiation steps are required to obtain
a De. Using the same number of dose steps, our method produces at least 1000 spectra to analyse.

The three orientations (X, Y and Z) correspond to the rotation around the axis perpendicular to the DEJ (X), the rotation around axis parallel to the direction of the growth (Y), and the rotation around the axis perpendicular to the two other axes (Z). Unless otherwise stated, the following settings were used for all measurements in this thesis: 2 mW microwave power, 0.1 mT modulation amplitude, and 12 mT sweep width with a sweep time of 21 s. The spectra were accumulated from 50 consecutive measurements.

Figure 4.3: Illustration of 2D plots of stacked ESR spectra for X (A,C,E) and Z configuration (B,D,F) for the unheated (A,B), 15 min at 225 °C (C,D) and 1440 min at 175 °C (E,F) (Joannes-Boyau and Grün, 2009).
Experiments were carried out on fossil tooth enamel from the archaeological sites of Holon (Porat et al., 1999), Broken Hill (Woodward, 1921; Pycraft et al., 1928) and Irhoud (Smith et al., 2007). Modern incisor fragments for comparative purposes were removed from a tooth generously donated by Professor Rainer Grün.

4.3 Spectra decomposition

Single orthorhombic crystal angular measurements (rotated around the y or z axis) show a signal at different g-values, shifting from the parallel \( (g_{x,y}) \) to the perpendicular position \( (g_{x}) \). In contrast, enamel fragments are a partially ordered system and mostly only minor g-value shifts have been observed for the T1-B1 and B2 components. As mentioned in previous chapter, the ESR spectra of fossil tooth enamel is a complex composite signal of several radicals around \( g=2 \). In this study we developed two protocols to decompose the ESR spectra, one to separate NOCORs from AICORs and the other to separate all components included in the AICORs signal.

4.3.1 Estimation of NOCORs concentration

The first method (Method 1) was developed by Grün et al. (2008) and allows for the estimation of NOCORs concentration within the spectra. The starting point was the main difference that exists between the two radicals NOCORs and AICORs: the anisotropic properties. The NOCORs give rise to a spectrum that remains identical at every angle, while the AICORs are strongly influenced by the measuring angle. Grün et al. (2008) defines the composite total spectra \( S_T \) as a mix between the AICORs \( S_A \) and NOCORs \( S_N \) concentration. This can be simply expressed by the equation:

\[
S_T = S_A + \alpha S_N
\]

(4.1)

\( S_N \) is assimilated as a powder spectrum, nevertheless the radical concentration of \( S_N \) in the total spectrum varies, depending on the organic phases, crystal phases, the age of the sample and the burial conditions. Therefore, \( \alpha \) is a factor by which the powder spectrum
has to be multiplied to be equivalent to the radical concentration of NOCORs within the sample. For example, the amount of NOCORs in the Holon sample was estimated at between 9 to 13% of the total signal, and around 10% in the Broken Hill sample.

Figure 4.4: Comparison of the amount of NOCORs in the angular measurements (A.M.) at B2 minimum using the method by Grin et al. (2008), by subtracting the real powder spectrum (P.S) multiplied by a factor k and a simulated powder spectrum (S.P.S) multiplied by a factor K’. The residual 1 and 2 represent the amount of AICORs in the sample. The # symbol shows an incomplete fitting because of simulation imperfection. The use of PS or SPS induces a significant variation in the amount of NOCORs estimated, 43% to 37% respectively.

Because the B2 portion of the spectra is composed solely of NOCORs and AICORs without any other interfering radicals or signal, at certain angles, when B2 is minimum, the main component is considered to be the NOCORs. By subtracting αSN from the total spectrum, the remaining component will represent the AICORs. To estimate the amount of NOCORs, the B2 area has to reach a value of zero without deforming the shape of the region around g=2.004. A common mistake is to over-estimate the amount of NOCORs, which frequently results in observation of a depletion in the signal around g=2.004, as well as a bump in the B2 region (Figure 4.4).

The powder spectrum used to estimate the NOCORs proportion should always be obtained by either measuring a powder spectrum of the same enamel or by merging all
spectra from all three configurations, referred in herein as the ‘merged spectrum’. The use of any powder spectrum increases the error in the NOCORs concentration value. The shape of the powder spectrum can vary greatly between two fragments, especially on the T1-B1 and T1-B2 ratios. For example, the use of the actual powder spectrum in the estimation of NOCORs concentration for the Holon tooth gave a radical concentration of 9 to 13%, while the use of a random spectrum FN03 (powder spectrum obtained with from M. Duval, measured with the same equipment) gave 4 to 6%, and Hexian 1117 gave 12 to 17% of NOCORs.

Method 2 (Vorona et al., 2007)

The second method was originally developed by Vorona et al. (2007), though working independently R. Joannes-Boyau also developed and used a very similar technique without being aware of Vorona’s work. Both methods are based on the angular variation observed over 360° rotation. Vorona et al. (2007) based the method on the Δ factor independent of the enamel measured, but constrained by the irradiation source. Vorona et al. (2007) postulated that the ratio of NOCORs and AICORs would be identical in all enamel fragments, providing an example that UV generates 50% of AICORs and 35% for γ-irradiation. This matter is discussed in Chapter 11 later in this thesis (4. Results and discussion, p.177). The intensity measured corresponds to T1-baseline at a certain angle when the intensity is minimum and maximum.

Hence we can express this as:

\[
    k_{UV} = \frac{\Delta^{\gamma+UV} - \Delta^{\gamma}}{\Delta^{UV} - \Delta^{\gamma}} \tag{4.2}
\]

\[
    k_{\gamma} = \frac{\Delta^{UV} - \Delta^{\gamma+UV}}{\Delta^{UV} - \Delta^{\gamma}} \tag{4.3}
\]

where the value \( k \) corresponds to the amount of AICORs in the sample.
As noted above, the method developed by Vorona et al. (2007) is based on the assumption that $\Delta$ is constant. However, this thesis demonstrates that the ratios change radically from one tooth to another. This error may be the result of Vorona et al. (2007) undertaking all their experimentation on a modern sample, while we applied it also to a fossil sample. On the positive side, Vorona's technique allows us to estimate the contribution of each specific irradiation type though further investigation of it is required, as noted by Duval (2009).

**Method 3 (Joannes-Boyau et al., 2010b)**

The method proposed by Joannes-Boyau et al. (2010b), as stated previously, is also based on the angular variation of the sample, however, relies on the fact that the percentage of NOCORs in the sample will flatten the angular response of the total spectra (Figure 4.5). Each intensity (T1, B1, B2, T1-B1, T1-B2) is measured at each angle and plotted over 360°.

Figure 4.5 shows that the percentage of NOCORs in the sample diminishes the anisotropy, corresponding, after measuring the ideal orientation, to the plane defined by the rotation of AICORs in which the radicals are perpendicular and parallel every 90° to the magnetic field. The B2 region is supposedly free of AICORs when $g_z$ is parallel to the magnetic field, and the remaining signal belongs solely to the NOCORs. Therefore, the amount of NOCORs can be estimated using the Grün et al. (2008) method which subtracts the powder spectrum, or subtracts constant lines from the angular variation until the minimum B2 reaches zero. The percentage identified will therefore correspond to the
amount of NOCORs. Alas, the orientation of the crystal never reaches a 90° angle, and therefore, some AICORs always remain in the B2 region.

When comparing the results of Method 1, 2 and 3, it became apparent that the first method tends to over-estimate the amount of NOCORs because of misalignment problems with AICORs. Nevertheless, even if less accurate compared to Method 3 by Joannes-Boyau et al. (2010b) Method 1 is fast and sufficiently accurate. For example, the amount of NOCORs in the Holon sample was estimated by Method 1 to be around 11 to 13%, by Method 2 to be more than 20% and only 10% using Method 3. The discrepancy of ca 1 to 3% between Methods 1 and 3 is negligible when compared to other associated errors and to the time required to calculate it.

4.3.2 Simulated Annealing (SA) procedure

All spectrum decompositions and simulations were carried out on the measured derivative spectra. Initially we used Matlab software with the easyspin add-on (Stoll and Schweiger, 2006, 2007). For simplification, only line shapes defined by the first derivative of the Gaussian function were used, although there were indications that in some cases Lorentzian or Voigtian line shapes were perhaps more appropriate. Some apparent signals in the residual (measured minus simulated) spectra appear to be due to the error induced by the Gaussian approximation. After several subjective manual approaches to spectrum analysis, decomposition was optimised and automated using a simulated annealing (SA) procedure.

The name comes from annealing in metallurgy, a technique involving heating and controlled cooling of a material to increase the size of its crystals and reduce their defects. By analogy with this physical process, each step of the SA algorithm replaces the current solution with a random "nearby" solution, chosen with a probability that depends on the difference between the corresponding function values and on a global parameter T (temperature) that is gradually decreased during the process. The current solution changes
randomly when the temperature is high, and less when $T$ is close to zero, allowing the search of the global minimum misfit without getting stuck in local solutions. The Monte-Carlo optimisation method is a powerful tool when searching for globally optimal solutions amongst numerous local optima.

The SA procedure is an adaptation of the Metropolis-Hastings algorithm and can be described as a Monte Carlo method used for combinatorial optimisation problems (for details see Metropolis et al., 1953; Kirkpatrick et al., 1983; Černý, 1985; Mossegard and Sambridge, 2002; Bodin et al., 2009; Bodin and Sambridge, 2009). Our SA procedure is able to randomly generate a large number of synthetic spectra defined by a linear combination of four Gaussian lines. Each simulated spectra is compared to the measured spectra in terms of a least square misfit. The probability of changing the parameters is determined by the acceptance probability function $S(p,p',T)$, where $p$ and $p'$ representing the old and new parameters, respectively, defined by the variance ($\delta$) of the proposed parameters $p'$. The derivative Gaussian function of each radical is written:

$$R(i) = \frac{\alpha(\eta - i)}{\sqrt{(2\pi)\sigma^3}} e^{\frac{-(\eta-i)^2}{2\sigma^2}}$$  \hspace{1cm} (4.4)

where $(i)$ the number of points that defines the spectra, $\alpha$ the Gaussian amplitude, $\sigma$ the $g$-values or the channels and $\eta$ the width of the Gaussian.

The Gaussian lines had prescribed limits with respect to the $g$-value range so as to avoid unrealistic solutions outside the regions for the CO$_2^-$ radical in hydroxyapatite (see above). No restrictions were set on the intensity and maximum line width, however, a minimum width $(0.10 \text{ mT})$ was defined to avoid aberrations.
In order to assess the uncertainties of our decomposition approach, we firstly re-ran the Monte Carlo simulation on the same spectra with different initial parameters. However, the robustness of the fitting strategy resulted in very small differences. The changes in minimum and maximum g-values and widths range between -0.0002 and +0.0001 (average ±0.00004) and -0.03 and +0.03 mT (average ±0.01 mT), respectively. The changes in g-values are virtually within measurement error, and the line width changes are well below 5%. Most previous decomposition approaches (e.g. Jonas and Grün, 1997; Grün, 1998; Vanhaelewyn et al., 2000a) could not resolve closely overlapping signals. Hence, we consider our results as robust.

R₃ is a component of the orientated CO₂⁻ radicals that poses several problems (see Chapters 10 to 15). Recalling that enamel fragments are a partially ordered system with some preferential direction of the hydroxyapatite crystals, R₃ can be designated as a heterogeneous signal composed by misalignments of R₁ in the irradiation spectra and R₁ and R₂ in the natural. It would then consist of a semi-infinite series of Gaussian components with varying amplitudes between the T₁ and B₂ positions and can be described by the equation:

$$f(x) = \int_{a}^{b} A(\mu) \left( \frac{x - \mu}{\sigma^2} \right) e^{-\frac{(x - \mu)^2}{2\sigma^2}} d\mu$$  \hspace{1cm} (4.5)$$

where a and b are the positions of T₁ and B₂, \(\mu\) the centre, \(\sigma\) the width of the Gaussian line, and \(A(\mu)\) the amplitude function. A part of this equation is known as the error function (erf). This erf function has an influence on the line shape. For example, if \(A(\mu)\) is a linear function, \(f(x)\) may approximate a Gaussian function if the magnetic field is located between T₁ and B₂. Nevertheless, for the sake of effectiveness R₃ has been approximated by a simple Gaussian line, but should be endeavor in future simulation.
VARIANCE OF THE PROPOSALS

s_mu = 0.05;
s_sigma = 0.05;
s_A = 5000;

DATA=fopen('spectra.txt','r');
for i=1:600
    for n=1:nspec
        dat(i,n) = fscanf(DATA,'%f',1);
    end
end

START LOOPING OVER THE SPECTRAS

for sp=1:nspec
    Gaussian Fonction
    for i=1:600
        g(i)=A*(mu-1)/(sqrt(2*pi)*sigma^3)*exp((-mu-1)^2)/(2*sigma^2));
    end
    DEFINE LEAST SQUARE MISFIT
    misfit=0;
    for i=1:600
        misfit=misfit+f(i)^2;
    end
    START MCMC
    s = nb of samples
    for s=1:nsample
        \( R(i) \)
        mu_p=mu+randn*s_mu;
        sigma_p=sigma+randn*s_sigma;
        A_p=A+randn*s_A;
        Check Bounds
        out=1;
        if (mu_p>mu_max)||(mu_p<mu_min)
            out=0;
        end
        if (sigma_p>sigma_max)||(sigma_p<sigma_min)
            out=0;
        end
        if (A_p>A_max)||(A_p<A_min)
            out=0;
        end
        GET LIKELIHOOD
        misfit_p=0; for i=1:600
            g_p(i)=A_p*(mu_p-1)/(sqrt(2*pi)*sigma_p^3)*exp((-mu_p-1)^2)/(2*sigma_p^2));
            misfit_p = misfit_p + (g_p(i)-dat(i,sp))^2;
        end
        \end{verbatim}

Figure 4.6: SA procedure program on Matlab®, showing the protocol develop to decompose the AICORs of angular spectra of fossil tooth enamel measurements.
CHAPTER 5
5. URANIUM SERIES ANALYSES

To measure the $^{238}\text{U}$, $^{234}\text{U}$ and $^{230}\text{Th}$ isotopic concentrations for this research, we used an inductively coupled plasma mass spectrometer (ICP-MS) high resolution coupled with a multicollector NEPTUNE (MC-HR-ICP-MS), manufactured by Thermo Electron Corporation (Figure V.1).

5.1 LA-ICP-MS

As part of this research we developed a new mapping approach for U-series isotopic concentration analysis, using laser ablation ICP-MS. By doing so the sample preparation is reduce to a minimum. The sample is introduced in to a large ablation chamber (AC) specifically designed at the Research School of Earth Sciences at the Australian National University. The AC allows the investigation of large sized samples of fossils, minimising the impact of analysis. The sample is ablated by the laser and transport in to the ICPMS by a flow of argon (14 to 18 l/min) at atmospheric pressure, directly into the plasma torch (Figure 5.2), where the gas is vaporised, atomised and ionised. The plasma torch is composed of three concentric tubes made of quartz. The end of the torch is placed inside an induction coil supplied with a radio-frequency electric current. An electrical spark is applied that introduces free electrons into the argon gas stream. Electrons are accelerated by the radio frequency magnetic field of the induction coil in different directions according to the variation of the field. The collision between electrons in movement and argon ions generate plasma
reaching a few thousand degrees. A continuous flow of gas maintains the incandescence whilst simultaneously separating the plasma from the quartz tube preventing the torch from melting. The sample is ionised by the torch and introduced into the mass spectrometer. Only a small amount of the initial ions are introduced into the mass spectrometer, through the use of successive cones. The sample cone focuses the beam into the first vacuum chamber. The beam conserves its speed though the supersonic expansion. The second cone, the skimmer cone, modifies the shape of the beam (Figure 5.2).

ICPMS Neptune: Plasma Torch

![Diagram of plasma torch](image)

Figure 5.2: Detail of the plasma torch of the MC-ICP-MS Neptune. The sample is injected through the injector and mixed with the sample gas and the cooling gas to form the plasma that ionizes the sample. Then the ion beam travels across the cones, and through different lenses that focus and accelerate the ion beam.

The ion beam is later accelerated toward lenses located in the main vacuumed chamber, which focus and model the beam from a circular shape to a rectangular shape, before it enters the electrostatic analyser (ESA), which homogenizes the energetic distribution of the beam. The ESA serves to limit the polyatomic interferences introduced by the plasma torch to the mass spectrometer. The beam then travels toward the magnetic sector after being focused by the ESA.
The magnet separates the ions according to their mass and charge (m/z). Only the selected mass/charge ions will go through the magnet into the Faraday cups, which are aligned and mobile (Figure 5.4). The multicollector has another benefit: it reduces small fluctuations of the ion beam caused by the instability of the inductively coupled plasma source, which improves the precision of the isotopic ratio measurements. The NEPTUNE at RSES is equipped with eight faraday cups, associated with an ion counter. Additionally, a secondary electron multiplier (SEM) allows the detection of low intensity ions, which in our case is $^{230}$Th and $^{234}$U (Figure 5.3, above).

The SEM works as an amplifier of the signal: for each ion that hits the surface of the electrodes, several secondary electrons are emitted by those centred electrodes multiplying the signal. Unfortunately, some interference can be observed caused by the high intensity beam queue from low intensity neighbour isotopes. To avoid this problem, a retardation potential quadrupole (RPQ) sits forward of the SEM, and improves noticeably the sensitivity of the instruments, by rejecting every particle that has lost energy during the signal dispersion.
5.2 2D isotopic mapping of tooth enamel

A new *in situ* experimental protocol has been designed using laser ablation analysis (Grün et al., 2008b; Grün et al., 2008). The sample preparation involves cutting the tooth in half several times in order to obtain levelled surfaces. Samples are first analysed using the Finnigan VARIAN quadrupole ICP-MS. If the sample satisfies the requirement of a uranium concentration >1 ppm, analysis is carried out on the NEPTUNE LA-MC-ICP-MS. To measure low concentration isotopes ($^{234}$U and $^{230}$Th), we can interchange the central channel with 230 and 234 masses, using the so called *dynamic method*. The *static method* consists of measuring twice the same region, one with the $^{230}$Th in the central cup, and the second time with the $^{234}$U instead. The second method assumes that the sample is homogenous.

Fractionation occurring with laser ablation that consists of a systematic difference in the isotopic concentration between uranium and thorium is directly related to the sample
matrix and frequently causes major error (Figure 5.5). Therefore, to be able to reconstruct accurately the isotopic concentrations, a standard is used for correction. In this research we used a rhinoceros tooth (from the Hexian archaeological site in the Anhui Province in China) as a standard. To be able to use a material as a standard, the matrix must be similar to the sample analysed and U-series concentration and ratios had to be known. For the rhinoceros fossil tooth the U-series isotopes ratios were measured using a Finnigan TRITON Thermal Ionisation Mass Spectrometer (Figure 5.5). Grün et al. (2008) have mapped a 1.2 Ma old horse tooth, and reveal U-uptake and U-loss process in the tooth.

Figure 5.5: 412 ka old rhinoceros tooth from Hexian, in Anhui Province in China. The tooth is used as a standard for LA-MC-ICP-MS measurements (Eggins et al., 2003).

The horse tooth (Equus altidens) is from the archaeological site of Fuente Nueva III (Orce, Andalusia, Spain; Toro et al., 2003) and was analysed using the laser ablation ICP-MS protocol described above (for more details see Eggins et al., 2003, 2005). The sample was sliced in three transversal sections. In each section an area containing the three different dental tissues (dentine, cement and enamel) and measuring approximately 8 by
6 mm was analysed (Figure 5.6). The three sections were slightly polished to avoid any laser deviation. The measurements represent a total of 6000 to 6500 points of analysis per maps of 1 x 0.5cm.

![Diagram of horse tooth and sections](image)

**Figure 5.6: Schematic view (left) of the horse tooth from the archaeological site of Fuente Nueva III (Orce, Andalusia, Spain) and the three slices (top, middle and base) made for analysis. The three photos (middle) show the three slices top, middle and base, respectively, with the area (red rectangle) mapped for U-isotopes. Far right, the three pictures show the laser ablation tracks, and the three dental tissues (Grün et al. 2010).**

The study shows that the 2D mapping protocol give new insight for ESR/U-series combined dating, with the possibility of calculating the internal dose more accurately to use the fragment for ESR dose reconstruction. It is also important to mention that the U-isotopes migrating in the enamel are coming from the dentine and not directly from the environment, as observed by Grün et al. (2008). The complex pattern observed by Grün et al. (2008) and Duval et al. (2009) confirm that single track measurements raise serious concerns regarding whether the U-isotopic distributions are truly accurate (Figure 5.7). Single track measurements in view of the mapping protocol appear seriously constrained by the location of the analysis and could give strong variation for the internal dose estimation.
Figure 5.7: U-uptake (Left), the enamel plays the role of a barrier against diffusion. Uranium leaching (middle) is stronger next to the pulp cavity and the cement in direct contact with the soil. $^{230}\text{Th}^{234}\text{U}$ in equilibrium (right), (Grün et al. 2010).
CHAPTER 6
6. ARCHAEOLOGICAL SITES

6.1 Payre (France)

The archaeological site of Payre is located on the edge of the south-east gorge approximately 60 m above the Payre River tributary of the Rhône River, on the Rompon commune in Ardèche, France. The site comprises three caves, Payre I, II and III, which have been excavated regularly since 1990 (Combier, 1967; Moncel, 2003). The first excavation campaign took place in 1990, followed by three other 1994, 1995, and 1996 were most of the human remains of relevance to this thesis were found.

The site belongs to the Jurassic and Cretaceous complexes, which cover a large part of the eastern edge of the middle Rhône Valley (Debard, 1988). The three caves yielded a sequence of archaeological layers related to the middle Paleolithic, Chalcolithic and Mesolithic periods (Debard, 1988; Moncel et al., 2002; Moncel, 2004). According to Moncel et al. (2005) the caves show seasonal occupation with evidence of hunting of cervids, equids and bovids, as well as a lithic industry of local cherts belonging to the Middle Palaeolithic (Moncel, 2003).

The hominid remains from Payre, consisting of 14 teeth and a fragment of a parietal, were discovered in different archaeological levels (Moncel and Condeni, 1996, 1997). Five main levels (labeled from A to G inverse with increasing chronology) have been described as occurring over 5 m of sediments. The first two layers, A and B, did not contain any archaeological remains. Layers C to E contain large blocks of limestone
relating to the collapse of the cave’s roof, with one tooth each in Layer E and D. The final two layers, F and G, show multiple phases of human occupation - at least four in Layer G and two in Layer F. Layer G yielded the majority of the human remains recovered from Payre, including nine teeth and the parietal fragment, while three teeth were found in the human occupation phase of Layer F (Moncel and Condemi, 1996, 1997; Moncel et al., 2002).

Valladas et al. (2008) recently reviewed the dating studies that have been applied to the site. Several methods including ESR/U-series and thermoluminscence provided age estimations for occupation of the site within the range of 200 ka and 300 ka.

The U-series two-dimensional mapping analysis carried out in this thesis was on a fossil Neanderthal tooth (Valladas, 2008), found in the Level G, Layer D, Square 8, at a depth below surface of 400 cm, and was estimated as being between 200-230 ka old, using TL, ESR and U-series on flints and faunal remains (Moncel et al., 2002; Valladas, 2008).

6.2 Broken Hill (Zambia)

One of the most famous archaeological sites in the world is Broken Hill in Northern Rhodesia, Kabwe, Zambia. Here the famous Broken Hill 1 cranium, also known as the Kabwe skull, was found by Tom Zwiglaar in 1921, in the lead and zinc mine (Woodward, 1921; Pycraft et al., 1928). The description of the exact location is unfortunately doubtful and the skull’s association with the other hominid remains from the site (an upper jaw, a
sacrum, a tibia and two femur fragments) is unclear. The skull belongs to a fossil hominoid frequently classified as *Homo rhodesiensis* and could be from the Late Pleistocene age (Rightmire, 1993). The skull relates to a robust individual, with very large brow-ridges, with a broad face reminding of some of the *H. neandertalensis* characteristics, however, debates about the actual group are not yet resolved. Accurate dating of the skull appears complex, as the palaeoanthropological site has been destroyed inducing the layered dating impossible. AAR carried out directly on the Broken Hill skull estimated the age of the remains at 110 ka, substantially below the Late Pleistocene age estimations based the morphology (Bada et al., 1974; Woodwar, 1921).

For this study, three fragments were removed from the posterior crown of the upper right second molar of the Kabwe skull, designated by BHS, BHL and BHV.

### 6.3 Holon (Israel)

The archaeological site of Holon is located on the coastal plain near Tel Aviv, Israel. Here the first excavation commenced in the 1960s with three campaigns between 1963 and 1970 by Tamar Noy. A total of 260 m² was excavated producing typical Paleolithic artifacts and Middle Pleistocene fauna, including several hominid remains. Of a total of 1568 bones, more than 550 faunal remains were identified to species level, including the bones of dear, hippopotamus, gazelle, aurochs, turtle, and elephant. Despite the enormous quantity of fauna remains found at Holon, unfortunately none can serve as a biochronological indicator, since all animals were found throughout the Pleistocene to the Holocene. The mix of animals indicates a variety of landscapes from permanent deep-water, to forest-scrubland
including grassy steppes. Human occupation of the site is attested to by the presence of butchering traces on several bones, as well as an extensive artefact collection including choppers, double sidescrapers with Nahr Ibrahim truncation and hand axes belonging to a pseudo-Levalloisian industry. Anthropogenic marks have been found on 45 different bones, including striations, butchery cuts and indications for the disarticulation of limbs.

The archaeological remains at Holon were dated to approximately 200 ka through luminescence of the alkali feldspars from the sediments, while two teeth were directly dated by ESR methods using a linear uranium uptake model yielding an average age of 204 ka (± 16 ka) (Porat, 1999). According to the dates calculated the archaeological sites belong to the isotopic Stage 7, similar to the Acheulian sites of Yabrud I, Berekhat Ram and Tabun E.

Most of the experiments carried out on Holon remains in this thesis were carried out on bovid fossil teeth found during one of the excavations by Tamar Noy, referenced as 1556 and 1557 and estimated to be approximately 200 ka in age.

6.4 Jebel Irhoud (Morocco)

The archaeological cave site of Jebel Irhoud is located near Sidi Moktar, approximately 100 km west of Marrakech, Morocco. This site is well-known for several hominid fossil remains discovered since 1991, including portions of two adult skulls (Irhoud 1 and Irhoud 2), a child’s mandible (Irhoud 3), and a child’s humerus (Irhoud 4). Unfortunately however, only the juvenile humerus shaft (Irhoud 4) was excavated under using standard archaeological techniques. The first three remains (Irhoud 1, 2 and 3) were accidentally discovered during mining operations, thus their exact location and associations are not known. Until recently the site age had been based on the ESR dating of horse teeth from Level 17, estimated to be ca 130 ka using an early uptake model and ca 190 ka assuming a linear uptake (Smith et al., 2007). Accordingly the human remains would thus belong to the oxygen isotope Stage 5d-5c.
In 2004, a new study of Jebel Irhoud was undertaken to identify the remaining *in situ* deposits and thus guide potential future excavation programs. Two subsequent archaeological excavations in 2006 and 2007 produced new hypotheses regarding the source of the Mousterian Middle Paleolithic industries and the human remains. Until the study by Smith et al. (2007), the fossils from Jebel Irhoud had been considered to be North African Neandertals. Following the Smith et al. (2007) study they are now grouped with other early anatomically modern humans such as Omo Kibush and *Homo sapiens idaltu* from Ethiopia, Qafzeh and Skhul in Israel. The juvenile mandible Irhoud 3 has been shown to belong to an 8 year old hominid with similar development patterns to a European modern child (Smith et al., 2007). The Irhoud 3 fossil human remains are the oldest-known hominid with eruption and dental development similar to modern *Homo sapiens*.

The potential age of the sample, around 190 ka according to the originally undertaken ESR dating, would make the Irhoud 3 fossil a very significant find, with critical relevance in assessing the validity of the *out of Africa* model for modern human origins. This model suggests that modern human originates from Africa 200,000 years ago; potentially therefore, Irhoud 3 might be amongst the oldest anatomically modern humans in Africa and thus obtaining an accurate and precise age for it is critical.
PART 2
CHAPTER 7
High resolution analysis of uranium and thorium concentration as well as U-series isotope distributions in a Neanderthal tooth from Payre (Ardèche, France) using laser ablation ICP-MS

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Received 11 December 2007; accepted in revised form 5 August 2008; available online 22 August 2008

Abstract

We have mapped U (238U) and Th (232Th) elemental concentrations as well as U-series isotope distributions in a Neanderthal tooth from the Middle Palaeolithic site of Payre using laser ablation ICP-MS. The U-concentrations in an enamel section varied between 1 and 1500 ppb. The U-concentration maps show that U-migration through the external enamel surface is minute, the bulk of the uranium having migrated internally via the dentine into the enamel. The uranium migration and uptake is critically dependent on the mineralogical structure of the enamel. Increased U-concentrations are observed along lineaments, some of which are associated with cracks, and others may be related to intra-prismatic zones or structural weaknesses reaching from the dentine into the enamel. The uranium concentrations in the dentine vary between about 25,000 and 45,000 ppb. Our systematic mapping of U-concentration and U-series isotopes provides insight into the time domain of U-accumulation. Most of the uranium was accumulated in an early stage of burial, with some much later overprints. None of the uranium concentration and U-series profiles across the root of the tooth complied with a single stage diffusion-adsorption (D–A) model that is used for quality control in U-series dating of bones and teeth. Nevertheless, in the domains that yielded the oldest apparent U-series age estimates, U-leaching could be excluded. This means that the oldest apparent U-series ages of around 200 ka represent a minimum age for this Neanderthal specimen. This is in good agreement with independent age assessments (200–230 ka) for the archaeological layer, in which it was found. The Th elemental concentrations in the dental tissues were generally low (between about 1 and 20 ppb), and show little relationship with the nature of the tissue.

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1. INTRODUCTION

In recent years, micro-analytical techniques have greatly advanced through the development of in situ laser ablation sampling combined with inductively coupled plasma mass spectrometry analysis (LA-ICP-MS; Egins et al., 1998a,b). This technique is highly sensitive to uranium, allowing the analysis of sub-ppb concentrations with a spatial resolution of in the range of 100 by 10 µm.

The direct dating of human remains, that are older than 50 to 60 ka (the present uppermost limit for radiocarbon dating of bones: Higham et al., 2006; Jacobi et al., 2006), is confined to U-series and ESR (Grün, 2006). Both methods are seriously compromised by the fact that bones and teeth accumulate large amounts of uranium following their deposition in sediments. Over the past 30 years, a range of models have been developed to account for this uranium uptake and provide a basis for open system dating. The diffusion-adsorption (D–A) model, developed by Millard (1993) and Millard and Hedges (1996), refined by Pike (2000) and Pike et al. (2002) is the most sophisticated of these. It is based on a continuous diffusion of uranium from...
the outside of a bone or tooth towards the interior, and on the assumptions that the partitioning between the bone and solution (groundwater) and the U-concentration in the solution are constant. The bone or tooth is treated as a homogeneous medium. Under constant conditions, the cross-sections of bones that conform with the D-A diffusion model are expected to have both U-shaped U-concentration and apparent U-series age profiles, with the apparent ages at the surface being closest to the correct age of the sample. Deviations from the ideal profiles can be explained either by leaching or changes in the U-concentration in the solution. In the first applications of the D-A model, U-concentration profiles and U-series measurements were based on a few mechanically drilled samples, chemical separation of U and Th isotopes and their measurement either with an ICP or thermal ionisation mass spectrometer (e.g. Pike, 2000). Laser ablation ICP-MS provided a breakthrough in micro-scale U and U-series analyses and has subsequently been used to measure these isotopes continuously along profiles (Eggins et al., 2003, 2005). Grün et al. (1988) suggested combining U-series and ESR to simultaneously estimate the uranium diffusion process and age of a sample. The sensitivity of this combined approach could be checked by comparing ages based on continuous diffusion (Grün et al., 1988) and a single stage U-uptake (Grün, 2000). Laser ablation D-A U-series dating was recently applied to the human material from Tuinplaas (Pike et al., 2004), and combined U-series/ESR dating, based on laser ablation measurements, to Banyoles (Grün et al., 2006a) and Irhoud (Smith et al., 2007). The laser ablation scans on a dentine sample from Banyoles revealed that large contrasts in apparent U-series ages (57 and <2 ka) may occur within only a few hundred micrometers, a micro-distribution previously not thought possible.

Numerous faunal teeth from the Middle Palaeolithic site of Payre have been analysed for a range of isotopic techniques. We selected a Neanderthal tooth to evaluate and advance in situ analyses, including U-series, Sr, Ca and O isotopes. Preliminary results on oxygen isotope analysis using SHRIMP (with spot sizes of 35 μm diameter, about 2 μm deep, allowing a potential weekly to bi-weekly resolution for human molars) and Sr elemental distributions on this tooth using laser ablation were further reported by Grün et al. (2006b), maps of Sr isotopes by Aubert et al. (2007). In this paper, we present the first high resolution U (238U) and Th (232Th) elemental concentration and U-series isotope maps on a human fossil and discuss their implications for future dating studies.

The site of Payre is located 60 m above the Payre river, a small tributary of the Rhône river, on a cliff opening towards the southeast. Regular excavations have taken place since 1990 (Combier, 1967; Moncel, 2003). The site belongs to the Jurassic and Cretaceous complexes, which cover a large part of the right edge of the middle Rhône Valley (Debard, 1988). It yielded a sequence with different archaeological layers, numerous artifacts, and fauna remains (Debard, 1988; Moncel et al., 2002; Moncel, 2004). The human remains, consisting of 14 teeth and a fragment of a parietal, were discovered in the different archaeological levels (Moncel and Condomi, 1996, 1997).

The sedimentary sequence was about 5 m thick and composed of five main levels (from top to bottom: A-B, C-D, E, F, and G) each of them including sub-layers. Twenty-five to 60 m² have been excavated down to the substratum. Layers A and B were formed of karstic sediments and did not contain any archaeological remains. Layers C and D consisted of brown clay and stony sediments, and Layer E of large blocks of limestone indicating the collapse of the cave's roof. Layer F contained seven cyclic deposits of grey sediments and beds of rubble and clay. This layer contained four separate human occupation layers, which alternated with those indicating cave bear occupation. Layer G consisted of orange clays containing pebbles and blocks. It yielded two distinguishable phases of human occupation. This elastic sequence was underlain by a stalagmitic floor, which was subdivided into six separate units (H2 to H7). An additional stalagmitic unit (H1) was difficult to associate with the other sedimentary layers.

Most of the human remains were found in the oldest level G, which yielded nine teeth and one fragment of a parietal. These human remains were found close to each other. Four teeth belong to the oldest human settlement (sub-level G9) and the others, including the fragment of a parietal, to sub-level G8. Level F yielded three teeth, the Level E one, and the upper level D one (Moncel and Condomi, 1996, 1997; Moncel et al., 2002).

The human behaviour was the same in all archaeological levels. Seasonal occupations (evidence of hunting of cervids, associated to equids and bovids) were observed associated with a discordant debitage on flint, which came from local and semi-local outcrops. Other stones were also used, such as quartz, quartzite, basalt and limestone for a secondary flaking, hammers and pebble tools. The lithic assemblage belongs to Early Middle Palaeolithic, typical of Middle Rhône Valley and South France (Moncel, 2003). It is different from Orgnac 3, dated of MIS 9 and 8, where Levallois flaking was observed (Moncel et al., 2005).

The dating studies involving a range of methods has recently been summarised by Valladas et al. (2008). Layers D and E yielded ESR/U-series age estimates on faunal materials as well as thermoluminescence results on burnt flint in the vicinity of 150,000 years, while those on Layers F and G were in the range of 200,000–300,000 years without being distinguishable between these two layers. The underlying stalagmitic floor yielded a TIMS U-series ages in the range of 230–290 ka.

The Neanderthal tooth (#1) was found in Level G, Layer D, Square N8, at a depth of 400 cm below datum. Considering the recent dating results, its age can be expected to fall within a range of 200,000–230,000 years (the older age bracket provided by the more reliable TIMS dating results on the underlying speleothems).

2. EXPERIMENTAL

The tooth was cut into halves with a thin (100 μm) diamond saw (Fig. 1) along the buccal–lingual (cheek to tongue) axis. The enamel section analysed covers the lingual half (facing the mouth) of the tooth (for details on tooth histology see Hillson, 1986). One-half was imbedded in a
removable resin, which is required for SHRIMP oxygen isotope analysis. The imbedded sample was then mounted in a sample holder so that the sectioned surface lies in the focal plane of the laser. After analysis, the two halves can be glued back together with little visible damage. The greatest material loss derives from the cutting width.

The analyses were carried out using a custom-built laser sampling system interfaced between an ArF Excimer laser (193 nm; Lambda Physik LPX120i) and an ICP mass spectrometer. Elemental U and Th distributions were measured on a Quadrupole Varian-820, U and Th isotopic distributions on a multi-collector Finnegan Neptune. Details of this system and its capabilities have been described previously (Eggins et al., 1999a,b). In brief, it employs a single long-working distance lens to project and demagnifying (by a factor of 20) the image of a laser-illuminated aperture onto the sample surface, which enables a range of geometries to be ablated within bounding dimensions of between 1 and 400 μm. In this study, laser pulse rates of 10 Hz were employed with a fluence of 10 J/cm² (power density 0.3 GW/cm²), the latter resulting in removal of a uniform thickness layer (~200 μm) from the targeted sample site with each laser pulse. The in-house developed laser ablation cell produces very fast response times, which permits high spatial resolution analysis.

Data reduction followed established laser ablation ICP-MS protocols (after Longerich et al., 1996), using the international glass reference materials NIST SRM610 glass and a rhinoceros tooth (where the U-series ratios were established by repeated TIMS analysis) for external calibration, tailing correction and elemental fractionation. Mean background count rates measured with the ‘laser off’ were subtracted from all measured isotope intensities. All measured atomic ratios were converted into activity ratios, unless indicated otherwise.

3. RESULTS AND DISCUSSION

Prior to interpreting the results it should first be noted that the scans showed in various places small spots of anomalously high Th and/or U-concentrations (some are clearly visible in the resin, see e.g. Fig. 3: lower left area). These may either be due to small sample fragments dislodged by the laser pulsing, air bubbles in the resin and other voids that have been filled with some sample material during polishing.

In a first exploration of the U-distribution, 21 parallel scans were measured, covering a cross-section of occlusal and lingual enamel and the adjacent dentine (for the approximate position of Area 1 see Fig. 1). The laser tracks had a width of 85 μm, with a spacing of 100 μm (centre to centre). The measurements covered an area with a width of about 2085 μm and a length of about 5500 μm. Each scan consisted of 1100 individual measurements. These were smoothed using a sliding 20-point average. The data were then interpolated for a three-dimensional presentation using the commercial SigmaPlot (Ver. 19) software. There is a very large contrast between the U-concentrations in the dentine and enamel, uranium concentrations vary overall between about 1 and 40,000 ppb. A linear scale enhances the details in the U-distribution of the dentine while the U-concentrations in the enamel are indistinguishable from background (Fig. 2A). In contrast, a logarithmic scale enhances the details in the enamel (Fig. 2B). The U-concentration contrast between enamel and dentine is sharp, dropping from about 25,000 ppb in dentine to a few 100 ppb in enamel, by a factor of around 100. Much of the apparent width of this concentration slope is due to the spot size of the laser. A sharp concentration contrast (e.g. a step function in U-concentration) is widened (i) over the laser spot size of 85 μm, which corresponds to approximately 17 measurement cycles and (ii) by the averaging process, which widens the U-concentration transition by another 20 cycles. The observed U-decrease at the dentine-enamel junction (DEJ) takes place within 25 and 40 cycles, which is an indication that the U-concentrations drop instantly at the DEJ and demonstrates the extremely fast response of the RSES laser ablation system. Similar large concentration contrasts (~100) in the U-concentrations between dentine and enamel have previously been observed in a range of faunal teeth (Eggins et al., 2003).
Fig. 3 shows maps of the U and Th elemental distributions in the enamel. Th is predominantly enriched right at the surface of the enamel (Fig. 3C) and is there associated with detrital coatings (see Fig. 1). In the three-dimensional presentation, this thin veneer occurs as separate isolated cones, which is the result of rastering caused by the track width and interpolation strategies of the software. The Th concentrations on the occlusal surface are lower than on the lingual surface. This is partly due to the angle between the surface and the laser track. The ablation tracks passed the occlusal surface at approximately right angles, so that less volume of the thin surface layer was measured here than at the lingual surface, where the tracks passed the surface at shallow angles and partly ran along the surface (see Track 21 between Cycles 400 and 600 in Fig. 3C).

There is an approximately 1 mm wide rim on the outside of the tooth (between the surface and the dotted line in Fig. 3C) with enriched Th concentrations in the range of 10-20 ppb, irrespective of the nature of the dental tissue. Only near the occlusal surface of the tooth, is a minor contrast observable between the Th concentrations in the dentine (around 1-6 ppb) and enamel (around 0.3-1 ppb; see DEJ below Cycle 600). Relatively high Th concentration in enamel occur above Cycle 800, a region which also contains elevated U (Fig. 6) and Sr concentrations (Grün et al., 2006b; Aubert et al., 2007). Nevertheless, there does not seem to be any evidence for Th diffusion paths into the tooth, although it is clear that this tooth contains significantly higher Th concentrations than observed on modern humans, which average Th concentrations in bones in the sub-ppb range, as we have measured on modern teeth.

Uranium diffusion into the enamel did not follow a simple D-A model with constant conditions, which would have produced U-shaped U-distributions. In general it is expected that uranium migrates from the dentine side into the enamel, the surface being more or less impenetrable to U-diffusion as long as the outer non-prismatic layer is intact (e.g. Eggin et al., 2003). Starting from the DEJ, we have observed in many samples a more or less steady U-concentration decrease across the enamel layer, with increased U-concentrations right at the outer surface. The enamel of the Payre Neanderthal has on its outside a thin veneer of high U-concentrations, particularly at the base where up to 25,000 ppb is observed. Similar to the Th distribution, near the occlusal surface the U veneer is less pronounced with U-concentrations in the range of only 500 ppb. This may simply indicate variable amounts of detrital residue on the surface (see Fig. 1).

Within the enamel, the U-distribution varies by several orders of magnitude. The lowest concentrations are found near the occlusal surface where U-concentrations drop to less than 10 ppb, indicating little or no U-uptake. Elsewhere uranium has migrated into the enamel in a variety of modes. Firstly, U is concentrated along visible cracks. There is a clearly visible crack starting from the cusp of the dentine reaching to the surface (just above “1” in Fig. 3A and B). Uranium is also enriched along other linear features, as shown in Fig. 4. At least three different lineaments with enriched U-concentration are visible, all running at shallow angles to the surface and the DEJ. It becomes obvious that U-mapping is essential to understanding the mode of U-migration. If Track 4 was taken in isolation, significant U-diffusion normal to the surface could be postulated (Fig. 4C). Instead, U migrated within the enamel along a lineament, which forms a shallow angle to the surface. Given the observed concentration gradient, it seems that U migrated from the outer surface into the enamel. Whether this particular feature or the other lineaments are associated with cracks or inter-prismatic zones has yet to be confirmed. There is also a lineament of in-
increased U-concentration in the lingual enamel, perpendicular to the DEJ (Tracks 11–15, around Cycle 725, see Figs. 3B and 5B).

The most obvious feature in the enamel is a relatively large domain of greatly increased U-concentrations of up to 1500 ppb (around Cycle 500 in Tracks 9–14, Fig. 3B, enhanced in Fig. 5A). Uranium migrated from the dentine into the enamel along two pathways leading to a more or less constant enrichment in an area which reaches about 600 µm into the enamel and has a width of about 800 µm. From this area there is a series of smaller linear diffusion paths further into the enamel. This domain of high U can obviously not be explained by monotonic diffusion into a homogeneous layer, but more likely is due to a mineralogical change in this area or its subsurface. The SEM (Fig. 3A) indicates that this area is dominated by relatively large prismatic bundles, which perhaps allow preferential U-migration along wider intra-prismatic zones. Unfortunately, we cannot further investigate the mineralogical structure in this area, because the sample has been polished several times for subsequent analyses and due to the complexity of the three-dimensional structure of enamel (e.g. Macho et al., 2003). It is interesting to note that the enamel has also experienced a significant uptake of Sr. However, we do not find a similar distinct accumulation of Sr in this particular area (Aubert et al., 2007).

Near the base of the lingual enamel, U-concentrations drop from about 300 ppb close to the dentine to about 100 ppb close to the surface, implying that U-diffusion had penetrated the enamel over its whole thickness (Fig. 5B).

One can use the position of the Th peak to fix the boundary between detrital surface contamination and the enamel, on the assumption that there is no Th diffusion into the enamel. The comparison of the Th with the associated U-peak allows the assessment of any U-diffusion from the surface into the enamel. Fig. 6 shows one of the largest effects of U migrating perpendicular from the surface into the

Fig. 3. (A) SEM image of Area 1. (B) U-distribution. The solid lines indicate the enamel boundaries and lineaments of increased U-concentration. The squares indicate enhancements in Figs. 4 and 5. (C) Th distribution. The dotted line limits an area of higher Th concentration, which is unrelated to the dentine-enamel junction (DEJ).
enamel (Track 16). At the occlusal side of the enamel, the potential U-diffusion may be on a length scale of up to 30 µm and perhaps 60 µm at the lingual side. Correcting for the incident angle between laser track and enamel surface, and excluding the effect of lineaments reaching the surface (see Fig. 4C), we observe an average diffusion of uranium of about 5 µm (1 cycle) into the enamel at the occlusal surface (Tracks 1–15; between Cycles 130 and 190), and about 20 µm at the upper part of the lingual enamel (Tracks 16–20, Cycles 170–350) and 15 µm at the lower end (Tracks 9–20, Cycles 680–1100).

The uranium concentrations in dentine ranged from about 25,000 to 45,000 ppb (Fig. 7A). The higher concentrations are observed in the region that is not covered by enamel (see Track 1, Cycles 1050–1100 in Figs. 2A and 7). There is a general gradient from the centre of the dentine (Cycles 500–800, Tracks 1–5) towards the enamel. It is unclear whether this is the result of diffusion, or whether this is due to different adsorption capacity in different domains in the dentine. The increased U-concentration at the lower end of the dentine (Track 1, between Cycles 1050 and 1100) is due to diffusion into the dentine not covered with enamel (see Fig. 9, below). As mentioned above, the Th distribution in the dentine was not associated with the U-distribution, nor with the DEJ. The elemental U/Th ratio was in most areas well above 10,000, the lowest values in the range of 2600. This implies that any U-series age calculations on dentine are not affected by detrital Th contamination.

The U-concentrations in the enamel were too low to obtain meaningful U-series data using laser ablation. For the scanning of U-series isotopes, we therefore decided to investigate a cross-section of the root, most closely assembling a bone cross-section (see Fig. 1 for the approximate position of Area 2). Area 2 includes the part of the dentine where the uranium increased significantly in Area 1. 235U, 233U and 234Th were measured on the Neptune sector ICP-MS. The laser track width was 178 µm and track spacing 150 µm, during each measurement cycle the track advanced by 10 µm. The total area analysed was about 900 µm wide and 3000 µm long. Fig. 8 shows the effect of data reduction, after applying a 20 cycle sliding average, and calculating the mean value and error of the mean.
Fig. 5. (A) Details of increased U-concentration in the central part of the lingual enamel. (B) Details of increased U-concentration at the base of the lingual enamel.

Fig. 6. U and Th measurements along Track 16.
Fig. 7. Details of U (A) and Th (B) distributions in dentine. The outer enamel boundary is indicated by the dotted lines.

Fig. 9A shows an SEM photograph of the approximate dentine region of Area 2. This SEM was recorded some time after the U-series isotopes and subsequent analyses were carried out and shows an area that lies about 50 μm below the original tracks. Fig. 9B shows the 230Th distribution in Area 2. Th was adsorbed at the outside of the dentine and shows no sign of diffusion into the dental tissue. Inside the dentine, the elemental U/Th ratios were well above 10,000. U-series ratios and age estimates were calculated from the U maxima at the surfaces of the dentine (Fig. 9C-E). At the outside surface, elemental U/Th concentrations ratios were as low as 350, at the inside surface well in excess of 1000. This again implies that none of the U-series age calculations were affected by the presence of detrital 230Th, particularly those of Track 6 (see below).

The uranium concentrations in Area 2 ranged from about 27,000 to 39,000 ppb, similar to the concentrations in Area 1. It can also be seen that the higher U-concentrations in the dentine of Area 1 (Track 1, Cycles 1050–1100 in Figs. 2A and 7A) seems to have originated from a volume in the dentine that was not covered by enamel (see Tracks 3–6, Cycles 60–85 in Fig. 9C). The structure of U-distribution, however, is much more complex than in Area 1.

The U-concentrations show distinct peaks at the outside surface of the dentine, where it was not covered by enamel (Tracks 5 and 6, cycle 60–75, Fig. 9C). Tracks 1 and 2 show the gradual increase of the U-concentration from the DEJ, as observed for most of the dentine in Area 1. In Tracks 3 and 4, the U-maximum was located somewhat further to the inside, about 400 μm from the dentine outer surface. At the inside surface of the dentine, all tracks had their U-maximum at or near the surface. Tracks 1–5 had a second maximum further to the inside. U-series isotope ratios and ages were only calculated in the region between the U-concentration maxima on the outside and inside surfaces. This avoided any potential problems with detrital Th contamination.

The 234U/238U ratios (Fig. 9D) varied within a small band width, the average value being 1.202 ± 0.023 (1–σ standard deviation of all calculated values). The 230Th/238U ratios (Fig. 9E), on the other hand, varied greatly along and between the tracks. The same applies, of course, to the calculated apparent U-series age estimates (Fig. 9F). It can be seen, however, that there are some systematic patterns in the apparent U-series age estimates. Depending on the relationships between U-concentration and apparent U-series ages, we can distinguish four regions (I–IV, marked in Fig. 9C). In Region I, at and near the inner surface of the dentine, the highest U-concentrations are associated with the higher apparent U-series ages. This is generally expected from the predictions of the D-A model. The oldest ages occur at the inner surface (Cycles 240–260) and are steadily increasing from Track 1 to Track 6. Region II shows some distinct U maxima and minima, and these are inversely associated with U-series ages, i.e. in areas of relative high U-concentration, the ages are lower than in
areas with relatively low U-concentration. In the central Region III, the U-concentrations are lowest, but the apparent U-series ages are significantly higher than in the surrounding Regions II and IV. Region IV shows a similar pattern as Region II. The apparent U-series ages near the outer surface (Cycles 40–80, Region IV) are significantly lower than on the opposite side (Region I). When transferring these regions of distinctive U-concentration and U-series patterns onto the SEM image (Fig. 9A), it can be seen that Region I closely correlates to the darker rim around the pulp cavity (see Fig. 1), which consists of secondary dentine and/or weathered primary dentine. Region II seems to be dominated by tubules, while Regions III and IV are undistinguishable in the SEM characteristics with a non-directional patterns, perhaps caused by an overprint of secondary dentine or other secondary minerals.

To investigate whether there is a systematic relationship between U-concentration and apparent U-series age estimate, these two parameters were plotted on top of each other in Fig. 10A–F. In Region I, high U-concentrations are associated with high U-series ages (see Tracks 3–6). This is expected from the D–A model for the volumes at and near the surface. In most of the other areas, relatively high U-concentrations are associated with relatively low U-series ages and vice versa. Closer to the outer surface, U-series ages generally increase and are not immediately affected by the high concentrations at the outside (see Tracks 5 and 6, Cycles 60–70). However, the U-concentration peaks slightly further to the inside in Tracks 3 and 4, around Cycles 80–90, are clearly associated with significantly younger U-series ages, indicating that these peaks are the result of a relatively recent accumulation of U. The older apparent ages in the central part of the dentine (Region III in Fig. 9, Tracks 2–6, between Cycles 100 and 160) are clearly not associated with distinct minima in U-concentration.

None of the profiles show distributions in U-concentration and/or apparent U-series age estimates predicted from a single stage D–A model. The distributions of U-concentration and U-series ages in Regions II and IV point to at least two distinct phases of U-uptake. Nonetheless, if the age of the specimen is indeed somewhere in the 200–230 ka region, the age estimates near the inside surface and in some central parts come close to this expected age range, implying some early U-accumulation in these domains.

In order to gain some insight about the U-uptake history, it was assumed that the age of the tooth was 200 ka. This corresponds to a $^{230}\text{Th}/^{234}\text{U}$ ratio of about 0.875 for a $^{234}\text{U}/^{238}\text{U}$ ratio of 1.2. Using a simple two stage model of U-uptake, namely that the first stage of U-uptake was rapid around 200 ka ago and a second stage followed in recent times, the U-concentration distribution of the first stage of U-uptake can be calculated from the measured $^{230}\text{Th}/^{234}\text{U}$ ratios. For example, a data point with a $^{230}\text{Th}/^{234}\text{U}$ ratio of 0.5 would have acquired about 57% of its present day U-concentration 200 ka ago (this fraction has a $^{230}\text{Th}/^{234}\text{U}$ ratio of 0.875) and 43% most recently (with $^{230}\text{Th}/^{234}\text{U} = 0$, Fig. 10C–I). All profiles of these calculated initial U-concentrations show a more of less monotonic decrease from the inner to the outer surface, from about 30 to 20 ppm, respectively. This continuous concentration drop may well be associated with changes in the mineralogical composition of the dentine or size of its internal surface for U-adsorption. Only Tracks 5 and 6 have a distinct U-concentration peak at the outer surface, most
Fig. 9. Elemental, isotopic and age distributions in Area 2. (A) SEM of the dentine in Area 1, at about 50 μm below in depth. The large red square is for orientation subdivided into 50 cycle sections. Black lines indicate the boundaries of Regions I-IV (see C). (B) Th elemental distribution. (C) U elemental distribution, Regions I-IV indicate areas of different relationships between U-concentrations and U-series age calculations. (D) \(^{234}\text{U}/^{238}\text{U}\) ratios. (E) \(^{230}\text{Th}/^{234}\text{U}\) ratios. (F) U-series age estimates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)
Fig. 10. (A–F) Age and U-concentration along the individual Tracks 1–6. (G–L) Calculation of a two phase model of U-uptake. The early one at 200 ka ago, the latter recently. The modelled U-concentrations are derived from the difference of the measured $^{230}$Th/$^{234}$U ratio and that of 200 ka. The amount of late U-accumulation is the difference between the modelled and measured concentrations.

probably in the cement layer. On the whole, these calculated U-concentration profiles are closer to those expected from a single stage D-A model, than the measured ones. The second phase of U-accumulation is particularly strong.
in the outer volume (about Cycles 60–100) and around Cycles 180–220. It cannot entirely be excluded that the high ages of Region III and the low ages in the surrounding regions are the result of relatively recent U-leaching from Region III and re-deposition in Regions II and IV. However, this would require U-migration against the U-concentration gradient. One has to remember that U-redistribution must not necessarily only take place in the measured plane. U-injection may have originated in a volume above or below.

4. IMPLICATIONS FOR DATING

The U-concentration and U-series profiles measured in Area 2 of the dentine do not concur with a single stage D–A model. As such, this sample should be disregarded for dating (Pike, 2000; Pike et al., 2002). Nevertheless, some domains in the dentine yielded U-series results that are close to the expected age of the sample. In view that laser ablation provides insight into detailed U-migration, it is possible to investigate U-leaching and Th contamination. U-leaching would be expressed by a drop of U-concentration near the outside with an associated increase in the U-series age. This would generally occur similar to the pattern shown in Track 3 between Cycles 40 and 80 (Fig. 10C), except that here the mapping indicates a secondary overprint. Near the inner surface, there is no sign of U-leaching, so that the apparent U-series ages in Region I, around 200 ka, can be regarded as minimum age estimates for the tooth. The same restrictions (i.e. that the U-series results are only minimum age estimates) also apply to bones that concur the D–A model under constant conditions (see Grün, 2006).

Data from laser ablation tracks have also been used for assessing U-concentrations and U-series isotope ratios on dentine and enamel for ESR dating analysis (e.g. Grün, 2006; Grün et al., 2006a; Smith et al., 2007). For any open system modelling, bulk data are required, because ESR measurements do not allow for spatial resolution (at least for the spin concentrations usually found in fossil human teeth, see Oka et al., 1997). It is clear from the U-distribution in the enamel that for this sample, any single laser ablation track would yield U-concentrations that may be completely unrelated to the bulk U-concentration that is required for ESR dating. Similarly, the U-concentration distribution in the dentine is not homogeneous, and the critical $^{230}$Th/$^{234}$U ratios vary greatly. When laser tracks show such inhomogeneities, it seems necessary to carry out bulk analyses on the dental tissues that were used for ESR dating.

5. CONCLUSIONS

The detailed mapping of U-concentration data in dentine and enamel give insight into the U-migration patterns in these tissues. However, it is necessary to measure U-series isotope ratios to understand aspects of the time domain of these U-migration patterns. The U-uptake into the enamel does not follow any model that has been proposed so far. While some regions in the dentine may concur with a single stage D–A model, most regions do not. Laser ablation mapping allows the investigation of U-leaching and if this process can be excluded, the derived apparent U-series data provide a minimum age estimate for the specimen. Single tracks are critically dependent on the mineralogical structure of the measured tissue. The U-series results and their interpretation may vary greatly, depending on where the track was measured.

The areas measured here are still to small to obtain a clear picture how uranium behaved in this particular tooth. We shall attempt to provide a complete map of the whole cross-section. Even here, U-concentrations and isotopes will still be affected from the volumes below and above the measured cross-section. Although it is desirable to obtain three-dimensional distributions, this is clearly not feasible in view of the exceptional value of human fossils. Furthermore, it is also a question whether the detailed insights obtained from one tooth are applicable to any other teeth from the same site. Nevertheless, a complete map will only require about two to three days of measuring time, which is very short when comparing this to mechanical driling, isotope separation and subsequent TIMS measurements that used to be carried out for obtaining D–A data. The latter could only provide a few coarsely spaced measurements. It also seems necessary to find out where the uranium is located, i.e. whether it all accumulated on crystal surfaces or whether some of it was incorporated into the hydroxyapatite structure, for example replacing Ca ions.

U-concentration and U-series data derived from single laser ablation tracks may be a poor proxy for bulk data. When solution analysis is not an option, for example when analysing human fossils (Grün, 2006), laser ablation mapping should be carried out.

ACKNOWLEDGMENTS

We thank Stephen Eggins, RSES, for critical comments and advice during measurements. We thank Les Kinsley, RSES, for keeping the mass spectrometers running and Malcolm McCulloch, RSES, for finding measuring time for us. We thank the associate editor, Miryam Bar-Matthews, the referee, Alistair Pike, who identified himself, and two anonymous referees for their thoughtful comments. This study was funded through ARC grant DP 0664144 “Microanalysis of human fossils: new insights into age, diet and migration”.

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*Associate editor:* Miryam Bar-Matthews
CHAPTER 8
Two types of CO$_2^-$ radicals threaten the fundamentals of ESR dating of tooth enamel

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Received 14 April 2007; received in revised form 14 September 2007; accepted 28 September 2007
Available online 17 October 2007

Abstract

In ESR dating of tooth enamel, dose values are usually obtained from powdered samples. It has been shown that the qualitative response of enamel powder to environmental and laboratory dosing is closely similar, thus apparently validating ESR protocols for dose estimation. When working on human fossils, their cultural and scientific value prevents the powdering of any samples. Measurements are carried out on enamel fragments instead. In fragments, natural and laboratory irradiations cause significantly different ESR responses. These can be attributed to two distinct CO$_2^-$ radicals, one with apparently axial symmetry (oriented) and one that has no preferential orientation (non-oriented). The spectra of the naturally irradiated samples investigated here show a mix of about 90% of the oriented and about 10% of the non-oriented CO$_2^-$ radicals. In contrast, laboratory irradiation induces a mix of about 60% oriented and 40% non-oriented CO$_2^-$ radicals. Heating experiments show that the non-oriented CO$_2^-$ radicals are significantly less stable than the oriented. This fact on its own would have serious implications for dose estimations, implying massive underestimations. It turns out, however, that with the heating induced decline of the non-oriented CO$_2^-$ radicals, a larger number of oriented CO$_2^-$ radicals is created. It is presently unclear whether these two processes are directly connected. In spite of very large internal reorganisations during these processes, powder spectra are only little affected by heating, because the powder spectra of both CO$_2^-$ radicals are closely similar.

The maximum differences that are observed on powder spectra in post-irradiation heating experiments, are in the range of 3%, dose values may be affected by a similar amount. However, when using preheating steps, which would occur when applying post-irradiation heating protocols to single aliquots, the thermal behaviour of the irradiated samples is quite different. While the annihilation of the non-oriented CO$_2^-$ radicals has about the same decay rate in preheated and non-preheated samples, the increase of the oriented CO$_2^-$ radicals is less pronounced in the preheated samples. As a result, overall intensity changes become critically dependent on the preheating times. The observed increase in signal intensity is in contrast to long-term fading experiments that showed that the ESR intensity of irradiated samples actually decreased over 6–12 years after laboratory irradiation. In view of this complexity and the small amount of quantitative information available, it is presently not possible to recommend any pre- or post-irradiation protocols.

The good news for ESR dating is that any systematic errors introduced by a different mix of oriented and non-oriented CO$_2^-$ radicals in the natural and irradiation spectra seem relatively small (<5%). At present, the most reliable way for the dose estimation of fragments seems to measure the samples rotating around the three principal axes and to merge the spectra to produce a powder-equivalent spectrum and use this for the construction of the dose response curve.

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1. Introduction

ESR spectrometry has been widely applied in the assessment of past radiation doses in tooth enamel in archaeological and geological dating applications as well as accident dosimetry (Rink, 1997; Grün, 2000a, b, 2006a; Grün, 2006b). In this paper, all ESR measurements refer to tooth enamel unless noted otherwise. The main intensity of ESR spectra of powdered fossil samples is attributed to a CO$_2^-$ radical (Callens et al., 1987; Vanhaevel et al., 2000a, b). Grün (2006b) recently introduced a scaling procedure that allowed the rapid, qualitative analysis of the differences between the ESR spectra created through natural and laboratory irradiations. He found that the ESR powder spectra of the natural and laboratory
irradiated samples were closely similar, radiation insensitive components could easily be identified and removed. When working on enamel samples extracted from important human fossils, it is not possible to produce a powder prior to ESR measurement. Instead, the measurements are carried out on fragments (e.g. Grün, 1995; Grün et al., 2003). All dose estimations on such fragments have shown angular dependencies, which sometimes seemed negligible and at other times extreme (e.g. Grün et al., 2003; Robertson and Grün, 2000; see Fig. 1). The problems are caused by qualitative differences between the natural and irradiation induced spectra. However, spectrum deconvolution attempts led to results that were difficult to interpret (Robertson and Grün, 2000).

The CO$_2^-$ radical is primarily responsible for the ESR spectrum in tooth enamel, whose mineral phase is hydroxyapatite. The CO$_2^-$ radical has principal $g$-values at $g_2 = 2.0030$, $g_y = 1.9973$ and $g_z = 2.0015$ (e.g. Callens et al., 1998, 2002). In X-band measurements, the radical is usually interpreted to appear with an axial symmetry with principal $g$-values of around $g_\perp = 2.0025$ and $g_\parallel = 1.9973$ (e.g. Brik et al., 2000a; Vanhaelwenn et al., 2000a). Heating experiments (at 400 °C for 2–3 weeks) combined with ESR measurements on enamel plates led Callens et al. (1995) to speculate that hydroxyapatite contained two types of CO$_2^-$ radicals, one showing an axial symmetry and a second one without preferential orientation, i.e. assimilating a powder spectrum at all orientations. This was confirmed by X-band (Brik et al., 2000a) and Q-band studies (Vanhaelwenn et al., 2002). Further Q-band measurements implied that there may be two different axial CO$_2^-$ radicals with principle $g$-values at $g_\perp = 2.0030$ and $g_\parallel = 1.9971$ near liquid helium temperature as well as $g_\perp = 2.0027$ and $g_\parallel = 1.9974$ at room temperature (Vanhaelwenn et al., 2000b).

Brik et al. (2000b) described up to seven different CO$_2^-$ radicals in tooth enamel. These are supposed to have closely similar $g$-values, but significantly different thermal stabilities. Most would only provide small contributions to the overall signal intensity. In non-heated samples, the number is readily reduced to two, in moderately heated samples three may occur (one non-orientated, two orientated, the latter two may interact upon heating). In the following, the observations and discussions are reduced to two CO$_2^-$ radical types, orientated and non-orientated, both having identical $g$-values (one has to keep in mind that in reality there may be small differences in the $g$-values of different types of CO$_2^-$ radicals; see e.g. Callens et al., 1995; Vorona et al., 2007). These orientated and non-orientated CO$_2^-$ radicals can be identified in tooth enamel fragments, where the crystallites are partially ordered, because the orientated is influenced by the orientation of the crystal relative to the external magnetic field, while the non-orientated is not. Radicals with an axial $g$-tensor, present inside the apatite structure, have their parallel ($g_{\parallel}$) axis orientated along the c-axis of the crystal. When single crystals are mounted so that the c-axis (the direction of $g_{\parallel}$) is perpendicular to the external magnetic field, any signal occurring in the region of $g_{\parallel}$ is generated by paramagnetic centres and radicals other than orientated radicals. The same applies for the $g_\perp$ region when the c-axis is parallel to the external magnetic field. If those additional signals have the same $g$-values as the powder spectrum of CO$_2^-$, it is reasonable to infer that non-orientated CO$_2^-$ radicals are present. Because the powder spectra of both CO$_2^-$ radicals are nearly identical, such direct identifications cannot be carried out on powders. Nevertheless, both types have somewhat different responses to microwave power (Brik et al., 1998; Scherbina and Brik, 2000), which allows some indirect, non-quantitative observations in powders.

While the authors mentioned in the previous paragraphs suspected and pointed out that their findings may have implications for dose assessments in ESR dating, they never really addressed and quantified this problem for any geological samples. Thus, at the onset of this study, it was not clear how the occurrence of the axial, orientated and non-orientated CO$_2^-$ radicals would affect dose estimations on geological samples. The heating experiments by Brik et al. (1998) and Vorona et al. (2006) implied that the non-orientated CO$_2^-$ radicals were significantly less stable than the axial type and that the non-orientated type may at least partly convert into the axial. This would have serious implications for ESR dating. A two-component system, where one component is unstable over geological times,
will always yield age underestimations, if the unstable component is not removed or corrected for.

The mineral component of tooth enamel, hydroxyapatite, is secreted by cells called ameloblasts each of which produces a hydroxyapatite prism. These prisms consist of bundles of long, parallel crystals. The enamel prisms form successive, bend packages separated by brown Retzius striae. The general growth direction of the hydroxyapatite prism is perpendicular to the dentine–enamel boundary. However, in inter-prismatic zones, hydroxyapatite crystals may have perpendicular orientations. Near the surface of the tooth, ameloblasts form a non-prismatic surface zone. Perpendicular to the prisms, linear cross-striations indicate the daily tooth growth (FitzGerald, 1998; Risnes, 1998; Shellis et al., 1998), which varies in human molars between 2.5 and 6.5 μm (Dean, 1998). The internal crystal structure of hydroxyapatite is complex, forming rhombic prisms with vertical sides, which combine in packages that have complex three-dimensional structures (Macho et al., 2003). Because of this complex three-dimensional structure, an enamel fragment does not have the same properties as a single crystal, i.e. it is not possible to exactly identify the main crystal axes. Although the c-axis is more or less perpendicular to the dentine/enamel boundary, it is likely that some contributions of $g_{\perp}$ exist when the general prism growth direction is orientated perpendicular to the external magnetic field. Furthermore, according to Callens (personal communication), single crystals do not contain any non-orientated CO$_2^-$ radicals; they can only occur on surfaces or in inter-crystalline spaces.

Because of our earlier findings, that dose estimations are critically dependent on the orientation of the enamel fragments in the external magnetic field, as series of experiments were carried out to shed light on this problem. In the first experiment, the spatial dose response was investigated in detail. A set of tooth enamel samples was rotated around three perpendicular axes and measured over 360° in 10° steps. For each of these 108 orientations, a 17-step dose response curve was established. This experiment allowed us to identify and quantify the two different CO$_2^-$ radicals. In the subsequent post-irradiation annealing experiment, the thermal stabilities of the CO$_2^-$ radicals were assessed in natural and irradiated samples. This experiment was also carried out in view to establish a protocol that may allow the elimination of any unstable signal components. In a third experiment, the effect of pre-irradiation heating was investigated.

2. Experimental

Three small enamel fragments were removed from the posterior crown of the right upper second molar of the Broken Hill (BH) human skull, which was found near Kabwe in Zambia early last century (Woodward, 1921; Pycraft et al., 1928). The fragments were mounted in a paraffin mould in a sample holder (see Fig. 2), which can be inserted into a Bruker ER 218FG1 programmable goniometer. The first fragment (BHS, 9.4 mg) was mounted so that the dentine/enamel interface formed the rotational plane (i.e. the growth direction of the hydroxyapatite crystals was more or less perpendicular to the external magnetic field), the second fragment (BHL, 14.4 mg) was rotated around its occlusal surface and BHV (6.8 mg) was rotated around the third major axis, perpendicular to the previous two configurations. The samples were measured at each dose step at 10° angle intervals for 360°. ESR measurements were carried out on a Bruker ECS106 spectrometer with a 600 mT magnet and a rectangular 4102 ST cavity. The samples were recorded with the measurement parameters routinely applied at the ANU ESR laboratory: accumulation of between 200 (natural sample) and 25 scans (for the higher dose samples) with 0.1015 mT pp modulation amplitude, 20.48 ms conversion factor, 5.12 ms time constant, 1024 bit spectrum resolution (resulting in a total sweep time of 20.972 s), 12 mT sweep width and 2 mW microwave power. The enamel pieces were successively irradiated with a $^{137}$Cs-source for the following cumulative times: 0, 5, 15, 25, 35, 45, 55, 80, 120, 140, 160, 180, 200, 220, 240, 260 and 280 min. The total machine measurement time for each fragment was about 19 days. After measurement the background noise of the spectra was reduced through forward Fourier Transformation (FFT), removal of the high frequencies, and subsequent back transformation (see e.g. Grün and Clapp, 1996). The FFT spectra of BHS were lost in transfer from the ESR spectrometer. This causes a somewhat higher noise in the spectra (compare Figs. 3 and 6), but has no influence on the results of this study. The samples used in the annealing and preheating experiments were extracted from a bovid tooth from Holon (1557) and they were measured with the same conditions as the BH samples, except that a Bruker Elexys spectrometer was used. It may be noteworthy that UV exposure may also generate CO$_2^-$ radicals in tooth enamel (Vorona et al., 2007). Nevertheless, we are quite confident that this affected neither the natural nor the irradiated samples.

All spectra in this study were aligned on two lines that are attributed to methyl. These occur around channel 15 and 385 (see Fig. 3A) and have $g$-values of about 2.1060 and 1.9906, respectively. The positions of these lines were not dependent on the orientation of the enamel fragment relative to the external magnetic field. In order to find certain positions in the ESR spectrum, it is convenient to mark certain features. Three positions are labelled T1, B1 and B2 (see Fig. 3A), where the signal intensity between the top of T1 and the trough of B1 indicates a contribution of $g_{\perp}$ to the overall spectrum and B2 of $g_{||}$. The irradiation spectra also contain some small shoulders II–13 (see Fig. 3B). In the following, six types of spectra are discussed: (1) natural (not irradiated in the laboratory), (2) irradiated (labelled N + y, the sample is exposed to laboratory radiation and measured). The subtraction and scaling procedures of Grün (2006b) produce (3) an irradiation spectrum (by subtracting the natural spectrum.
Fig. 2. The three enamel fragments from the Broken Hill specimen used in this study. The top row shows the fragments in the sample holders, the lower row close ups. The dentine/enamel interface is indicated by the orange/brown colour. The black dots are markers for orientation.

from the spectra of the N + γ, e.g. Fig. 3B and E) and (4) a residual spectrum (by height normalising the natural and irradiation spectra and subtracting the latter from the natural spectra, e.g. Fig. 3C and F). The scaling procedure allows the analysis of the qualitative differences in the ESR response to natural and laboratory irradiation. Furthermore, to extract the spectrum of the orientated CO₂ radicals, a powder spectrum is subtracted from the natural and N + γ spectra, by minimising the B2 region. These are labelled (5) N-P and (6) N+γ-P.

Dose values were determined using a range of fitting procedures (for more details, see Grün, 2000c, 2002): fitting the natural spectrum into the N + γ, using a wide range between R1 and R4 (see Fig. 3D for the positions of the range markers R1–R4), a relatively narrow range around the central ESR peaks between R2 and R3, fitting the irradiation spectrum back into the natural and N + γ using the R1–R4 range, peak-to-peak intensities of T1–B1 and T1–B2, as well as the peak intensities of T1, B1 and B2, measured from the base line. In the context of this paper, the irradiation times were not converted into dose values to avoid any age implications for this palaeoanthropologically important sample. Dose values were estimated using a single exponential function and individual errors by Monte Carlo simulation (Grün and Brumby, 1994). Typical dose response curves of the different fitting strategies are shown in Fig. 5 (below). The scatter around the best fit was around 0.5–2.0% and the error in the individual dose estimations in the range of 2–5%.

3. Results and discussion

Because the experimental design of this study covers a diverse range, it seems most appropriate to report the results and discuss each experiment (irradiation, annealing, preheating) separately.

3.1. Irradiation experiments

For the discussion below it is helpful to use the terms “g_i in direction of the external magnetic field” or perpendicular to it. Because the enamel fragments do not have a single crystal symmetry, this means here that when g_i is parallel to the direction of the external magnetic field, then the ESR
spectra show a maximum in the B2 area. When \( g_z \) is perpendicular to the direction of the external magnetic field, the B2 area shows a minimum and the Ti–B1 peak is largest.

In the BHS configuration (rotation around the dentine/enamel interface), it is expected that \( g_z \) is perpendicular to the external magnetic field. Fig. 3 shows the spectra at 90° and 180°, which have the relatively largest differences in this data set (see Fig. 4). The natural and \( N + \gamma \) spectra are qualitatively very similar (see Fig. 3C and F), the differences being that (1) the outer methyl lines (see Fig. 3A) only appear in the natural spectrum (i.e., they are not radiation sensitive, see Fig. 3C and F); (2) there are two narrow peaks at channel 190 and 210, (labelled C1 and C2 in Fig. 3C), the former is the central methyl line at \( g = 2.0034 \), the latter is at \( g = 2.0023 \) and cannot directly be attributed; (3) the irradiation spectra have a significantly larger B2 component, which shows little angular dependence (see Fig. 3C and F). There are small apparent angular differences in the region of B1. At 180° the central line seems symmetrical (Fig. 3F), whereas at 90° there is a small interference in the range of channels 230–250 (Fig. 3C). This difference occurs in both the natural and the irradiation spectra. On the lower magnetic field side, there are three peaks in the irradiation spectra that do not appear in the naturals (labelled II–13, see Fig. 3B), corresponding to \( g \)-values of approximately \( g = 2.0115 \) (II, perhaps CO\(_2\) radical associated with water, Callens et al., 1998), \( g = 2.0093 \) (12), and \( g = 2.0076 \) (13). The amplitude of the residuals are in the range of 15% of the normalised natural and irradiation spectra.
Fig. 4 summarises the measurements of BHS. It is clear that the natural, as well as the irradiation spectra, contain a clear dip in the B2 position. The T1, B1 and B2 positions change neither in the natural nor the irradiation spectra (see Fig. 4A–F). The natural spectra show small, correlated variations in the intensity of T1 and B1, but virtually no change in B2 (see Fig. 4A–C). The irradiation spectra show similar variations in the T1 and B1 intensities, but somewhat more marked intensity changes in the B2 position (Fig. 4D and F). The main feature in the residual spectra (Fig. 4G–I) is the increased, angle dependent sensitivity in the B2 area. The two small central lines, C1

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Fig. 5. (A) Representative dose response curves for BHS at 90° (the spectra are shown in Fig. 3A). The first three dose response curves are constructed from partial spectrum fitting (natural spectrum fitting between R1–R4 and R2 and R3, fitting the irradiation spectrum between R2 and R3), the next two from peak-to-peak (T1–B1 and T1–B2) and the last three for each peak separately, measured from the base line (T1, B1 and B2). For positions of R1–R4, T1, B1 and B2 see Fig. 3. (B, C) Dose values for BHS for the eight fitting strategies at all angles. The numerical values in (C) are the averages with standard deviation of one fitting strategy over all angles. All numerical dose values in the diagram are dose equivalents expressed in minute radiation time. The colours in the legend indicate a range between the given and the next lower value. For example, yellow indicates values between 80 and 100.
(central methyl) and C2, occur at all angles and do not change their position.

Because the intensity measurements in the following sections are the result of various interferences, the dose estimations do not represent the best or correct dose estimate of the sample, but the mathematical result of constructing and fitting a dose response curve from interfered data. Thus these results are apparent dose values. Nonetheless, for linguistic simplification the adjective “apparent” has henceforth been dropped in this context. Fig. 5A shows typical dose response curves for BHS (measurement at 90°), following three main approaches, the details are outlined above, (1) spectrum fitting, (2) peak-to-peak fitting and (3) single peak intensities. The angular dose estimations are summarised in Fig. 5B and C. The spectrum fitting procedures yield dose estimations within a small range over the angles and the three approaches agreed with each other. The largest differences occur in the single peak dose estimations. Not surprisingly, the B2-peak results are significantly smaller than any of the other approaches, which is indicated by the much higher sensitivity of the B2 region in the irradiation spectra (Fig. 4G–I). The T1-peak yields dose values similar to the spectrum fitting procedures, while the B1-peak leads to somewhat higher dose values. The B1 estimations also show the widest standard deviation, which may be attributable to a varying interference of the C2-peak, or, perhaps, to an unidentified interference in channels 230–250 (causing the shape change of the natural and irradiation spectra at different angles). The peak-to-peak dose estimations resulted in intermediate dose values as is expected from the single peak values.

In the next configuration (BHL: rotation around the occlusal surface of the tooth), the ESR spectra are highly angular dependent (see Fig. 6). Note that the natural and irradiation spectra are exactly reproduced every 180° (Fig. 6A and D). As expected, the maximum intensities of T1–B1 (direction of the $g_z$) and B2 (direction of $g_y$) are offset by 90°, but neither signal disappears completely in the natural spectra (see also Fig. 7). Similar to the observations on BHS, the irradiation spectra show relatively larger B2 intensities where this peak is small in the natural spectra, at around 100° and 280°, compare Fig. 6B, C, E and 6I. Similarly, T1–B1 intensities are
Fig. 7. Measurement of BHL at angles, which show the greatest difference, at 100° (A–C) and at 190° (D–F). (A, D) N + γ spectra for selected dose steps. (B, E) Irradiation spectra. (C, F) Comparison of scaled natural and average irradiation spectra. The residual spectrum is offset for clarity.
relatively larger in the irradiation spectra where these are small in the natural spectra (e.g. around 10° and 190°). On the other hand, both T1-B1 and B2 are smaller in the irradiation spectra where these signals are large in the naturals. The maximum in the T1 region seems to shift. When \( g_r \) is perpendicular to the external magnetic field, the maximum is in channel 195 (largest signal in Fig. 6A). When the sample is rotated, the maximum first shifts up to channel 208, subsequently the top of the peak splits up into two smaller maxima (smaller signals in Fig. 6A). The one at channel 195 is associated with the central methyl line.

The region between B1 and B2 shows mostly positive residuals (i.e. the natural spectra lie above the irradiation spectra). This results in interesting patterns in the residual spectra (Fig. 6G-I), particularly a negative residual at the minima of the natural spectra, resulting in an angular meander between the B1 and B2 positions (Fig. 6H and I). Similar to BHS, the residual shows the central methyl line (C1) around channel 200 (Figs. 6C and 7F), as well as the radiation peaks I1-I3. The amplitude of the residuals are in the range of 25% of the normalised natural and irradiation spectra.

Fig. 7 shows the dose response of BHL at 100° and 190°, which are closest to \( g_r \) perpendicular and parallel to the external magnetic field, respectively. The peaks T1, B1 and B2 are all aligned in the natural and radiation spectra (Fig. 7A and B). There seem some interferences in the irradiation spectra of first two dose steps (Fig. 7B and E). At the 100° angle, the T1-B1 peak occurs only partially and seems altogether moved to the lower field side, at the 190° angle, B2 appears at higher field side for the first two dose steps. Because of the small increases in the overall intensity of the N + γ spectra in these first dose steps, the overall effect of these small shifts are negligible (however, worth pursuing in future studies). It is obvious, that the natural and irradiation spectra are significantly different (Fig. 7C and F). In both cases, the component that is supposedly perpendicular to the external magnetic field is more enhanced through irradiation than the one parallel to the magnetic field. The split in the natural spectrum of T1 at 190° (Fig. 7F) is due to the central methyl line. Although this line is not directly visible in the natural and N + γ spectra at 100°, the scaling procedure clearly separates this line out in the residual (at channel 200 in Fig. 7C), where it is clearly resolved with the same intensity as the methyl line at 190° (at channel 200 in Fig. 7F).

All dose estimations show strong angular dependencies, the values ranging from 17 to 144 min radiation time (Fig. 8A and B). There is a clear inverse relationship between the dose estimates derived from B1 and B2. When the doses derived from B1 have a maximum, the B2 doses have a minimum and vice versa. The dose values derived from spectrum fitting show a more muted angular dependency with the general trend following T1-B2(1) estimates. On the whole it seems impossible to obtain a meaningful dose value for the sample. In this configuration, the average dose values are about 10% smaller than those obtained from BHS.

The third configuration (BHV), rotation around the third major axis, yields results similar to those of the BHL configuration (see Fig. 9; and compare to Figs. 6 and 7).

Fig. 8. (A, B) Dose values for BHL for the eight fitting strategies at all angles. The numerical values in (B) are the averages with standard deviation of one fitting strategy over all angles. All numerical dose values in the diagram are dose equivalents expressed in minute radiation time. The colours in the legend indicate a range between the given and the next lower value.
again confirming that our assumptions concerning the axiality of the CO$_2^-$ radical and the enamel fragment structure are justified. When $g_c$ is parallel to the external magnetic field, the T1–B1 intensity is relatively smaller than in the same position in the BHL configuration. This is likely the result of a better orientation of the fragment. The dose values calculated from BHV show a very similar pattern to BHL, except that the average values are about 15% higher (compare Figs. 10 and 8).

In Fig. 11, all angular spectra are merged for the three configurations and then the spectra of the three configurations are weight normalised and merged again. Whilst the merged BHL and BHV spectra show still some significant residuals in the range between B1 and B2 (Fig. 11D and F), these are further reduced in the merged spectra that contain all angular measurements (Fig. 11G and H). The main features in the residual spectrum are the methyl radicals, including C1, the line at C2 as well as the small irradiation
features 11 and 12 (for positions C1, C2, 11 and 12, see Fig. 3C). While there are still dependencies of the dose results on the various estimation strategies using the merged spectra of the three different configurations, these differences disappear when the spectra of all three configurations are merged again (Fig. 12). The merged spectra of all three configurations, of course, should closely resemble a powder spectrum. To check for this, we can compare the merged natural and irradiation spectra with those of sample 2122. It was used in the scaling exercise of Grün (2006b) because the natural and irradiation spectra of this sample showed only minute qualitative differences (in the C2 region). Fig. 13 shows that both, the natural and irradiation spectra of the merged fragments and of the 2122 powder are closely similar. The residual of the natural spectra indicates some minor base line effects in 2122, the three lines of methyl in the fragments, and a small difference in the B2 position. The residual of the irradiation spectra shows even smaller differences. Most noteworthy is that the merged spectra, containing more than 100 different angular spectra for the natural and more than 1000 for the irradiation spectra, show no signs of line widening. This confirms the validity of our alignment procedure.

The question is where do all the huge residuals of the angular measurements come from? It is clear that the main feature of the merged ESR spectra as well as the powder spectra of 2122 are generated through CO$_3^+$ radicals. As mentioned above, these may occur in two configurations, orientated and non-orientated (Brik et al., 2000a; Callens et al., 1995; Vanhaelewijn et al., 2002). The angular measurement of a single crystal containing only orientated CO$_3^+$ radicals will produce spectra very similar to those shown for the natural samples (Figs. 4A-C, 7A-C, 9A and B). If the enamel fragment, with all crystallites aligned in the same direction, could be perfectly orientated, in the position $g_1$ parallel to the external magnetic field, the ESR spectrum would only contain the B2 dip, and when $g_1$ was perpendicular to the external magnetic field, only T1-B1. On the other hand, if the fragment contained only non-orientated CO$_3^+$ radicals, any angular measurement would result in a powder-like spectrum with the same intensity. As already mentioned, the powder spectra of both radicals are nearly identical. If the relative concentrations of the orientated and non-orientated CO$_3^+$ radicals in the natural and irradiation spectra are different, it is expected that the angular measurements show large residuals between the natural and irradiation spectra, particularly visible in the regions of $g_1$ and $g_2$ when these are perpendicular to the external magnetic field. In contrast, powder spectra would show no residuals at all. The measurements of BHS (Fig. 4) clearly indicate that this is the case. Here the fragment was rotated around the dentine-enamel boundary, i.e. $g_1$ was more of less perpendicular to the external magnetic field for all measurements. If we had only orientated CO$_3^+$ radicals in the sample, the small dip B2 would be the result either of our inability to orientate the tooth enamel fragment perfectly in the goniometer or that the piece of tooth enamel is indeed not a single crystal. Fig. 2 shows, for example, that the BHL fragment is slightly curved. Nonetheless, the irradiation spectrum should be the same as the natural. Instead, the B2 region is enhanced in the irradiation spectrum, clearly showing the
Fig. 11. Merged spectra for BHS (A, B), BHL (C, D) and BHV (E, F), separately and combined (G, H). Left: N + γ. Right: Scaled (normalized to the same intensity) natural and irradiation spectra. The residual spectra are offset for clarity. All residuals in the previous figures seem to cancel each other out. This means that the qualitative differences between the natural and irradiation spectra are caused by CO$_2$ radicals in general, but there must be at least two different types of CO$_2$ radicals involved with different relative distributions in the natural and irradiated spectra.
Fig. 12. Dose results for merged BH spectra (see Fig. 11). The numerical values are the average dose values and standard deviations from the eight fitting procedures for each measurement configuration. All numerical dose values in the diagram are dose equivalents expressed in minute radiation time. The colours in the legend indicate a range between the given and the next lower value.

Fig. 13. Comparison of merged BH natural and irradiation spectra (Fig. 11H) with the corresponding powder spectra of 2122, a sample that showed very few interferences (Grünewald, 2006a). The residuals are offset for clarity. There are only small qualitative differences between the merged BH spectra and the powder spectrum of sample 2122, which shows little differences between natural and irradiation spectra.

The presence of non-orientated CO$_2$ radicals. The same can be observed at the other configurations when $g_1$ is more of less parallel to the external magnetic field, as e.g. in Fig. 6D-F. Here, B2 is largest in the natural spectrum, but T1–B1 is significantly more enhanced than B2 in the irradiation spectrum. It can be concluded from these observations that the irradiation spectra have significantly higher proportions of non-orientated CO$_2$ radicals than the natural sample. This has very serious implications. Brik et al. (1998) and Vorona et al. (2005, 2006) showed that the two different CO$_2$ radicals have very different thermal stabilities, the non-orientated CO$_2$ radicals being significantly
less stable than the orientated (Fig. 14). A simple two-component system, which only contains a stable and an unstable component, will always lead to dose and, consequently, age underestimations. Applying this to the observed relative increase of non-orientated CO$_2^-$ radicals in the irradiation spectra, it can at this point of the investigation only be concluded that all ESR dose estimations would be underestimations, the magnitude of which could be large. However, the annealing results of Brik et al. (1998) also show that there may be a transfer from non-orientated to orientated CO$_2^-$ radicals (see Fig. 14: when the non-orientated CO$_2^-$ radicals decrease, there is a delayed increase of the orientated CO$_2^-$ radicals, until these are annealed at higher temperatures).

In the first instance, we can analyse the relative distribution of orientated and non-orientated CO$_2^-$ radicals in the natural and N + $\gamma$ spectra by using the B2 region. When $\gamma$ is perpendicular to the external magnetic field, B2 should be zero for the orientated radicals. In the first instance, all of the B2 intensity can be lumped together as non-orientated CO$_2^-$ radicals. For this, the merged, powder-equivalent spectrum is aligned and fitted into the natural and N + $\gamma$ spectra, so that the B2 region most closely assembles a background line (see Fig. 16C and G, below). From the remaining, reduced T1–B1 intensity, the apparent percentage of the orientated CO$_2^-$ radicals is calculated, i.e. (N + $\gamma$ - P)/(N + $\gamma$). From the radiation induced increase of the overall signal intensity, the apparent percentage of orientated CO$_2^-$ radicals in the irradiation component of the spectra is calculated. Because of the nature of the crystal complexity of the fragment and measurement conditions (see above), a certain amount of the B2 intensity is generated by the orientated CO$_2^-$ radicals, thus, the calculated percentages of the orientated CO$_2^-$ radicals are minimum (apparent) values. The subtraction procedure was carried out for all three configurations in the angular positions where B2 is smallest, which is at angles of 180° and 360° for BHIS (see Fig. 4), 100° and 280° for BHL (Fig. 6), and 80° and 260° for BHV (Fig. 9). The results are shown in Fig. 15. The apparent percentages of the orientated CO$_2^-$ radicals in all three natural fragments is close to 69%. This value continuously decreases with increasing irradiation to about 49%. When calculating the apparent percentages in the irradiation spectra, the first dose steps yield scattered results because of the small signal increases for these relatively small doses, see discussion above and Fig. 7B. The apparent average percentage of the orientated CO$_2^-$ radicals in the irradiation spectra from the third dose step onwards is 39.5 ± 1.5%. If the irradiation intensities were corrected, so that they contained the same 69.31% mix as the natural samples, dose estimations would roughly increase by between 30% and 100%, the precise amount would depend on the curvature of the dose response curves. However, before arriving at hasty conclusions, it is necessary to carry out annealing experiments to investigate the previously postulated transfer between the two radical types (Fig. 14).

3.2. Annealing experiments

It was not possible to obtain more material from the Broken Hill specimen for annealing experiments. Instead, we used a bovid tooth from the archaeological site of Holon (#1557; for more information on this site, the reader is referred to Perot et al., 1999). The bovid tooth has the additional advantage of being much larger than a human tooth, thus will provide a sufficiently large number of
samples for further studies, likely resulting in a whole Ph.D. thesis (RJB). The sample was irradiated with a dose corresponding to 100 min radiation. The measurements were similar to those of the BH sample. Here we focus on the measurements of the fragment lying on its dentine/enamel boundary, i.e., rotation more or less around $g_{0}$, resulting in spectra very similar to Fig. 4, measurement at 40° (minimum intensity of the natural B2 signal). The sample was also measured rotating around one of the other major axes, which produced spectra similar to those shown in Figs. 6 and 9.

The apparent percentage of the oriented $\text{CO}_2^-$ radicals in the natural of 1557 was 68.0% (BH: 68.5%) and in the N + $\gamma$ (100 min) 52.3% (BH: 53.0%), thus showing only minor differences in radical distribution to the Broken Hill samples. The annealing experiments were carried out at 125°C for between 0 and 275 h. This temperature was chosen because the earlier experiments of Brik et al. (1998) (Fig. 14) indicated that at this temperature the non-oriented $\text{CO}_2^-$ radicals would anneal while the oriented would not. For dating applications, moderate heating procedures may open the possibility of removing the less stable component while not affecting the stable.

Fig. 16 shows the spectra of the natural and irradiated samples. Both the natural and N + $\gamma$ spectra show an overall increase in the T1–B1 signal intensity with increasing heating time (Fig. 16A and E), and a small decrease in the B2 region. When scaling, the natural set (Fig. 16B) shows small overall qualitative differences, the wiggles in the residual spectra in the T1–B1 region are due to line widening, those on the B2 region to annealing of the non-oriented $\text{CO}_2^-$ radicals. The normalised N + $\gamma$ spectra (Fig. 16F) show a similar line widening in the central region, the shoulders on the low-field side disappear during the first annealing step, and the B2 region is first enhanced over the natural spectrum due to the irradiation. The B2 region of the N + $\gamma$ set approaches about the same intensity as the natural after 26 h annealing and a further decrease after 261 h. When subtracting the powder spectrum (Fig. 16C and G), the rate of signal increase is greatly enhanced with heating time, which is due to the decay of the non-oriented $\text{CO}_2^-$ radicals and the simultaneous increase of the oriented. Scaling (Fig. 16D and H) does not reveal any additional signals that may cause the increase of the T1–B1 region, apart from signal widening. The line widening may point to the occurrence of an additional $\text{CO}_2^-$ radical with slightly different g-values, as mentioned by Brik et al. (2000b), or perhaps other radicals, such as $\text{CO}_1^+$. 

Fig. 17A summarises the change of signal intensity and the percentage of the oriented $\text{CO}_2^-$ radicals in the natural and N + $\gamma$ spectra. Over the heating time of about 270 h, the signal intensity of the natural sample increased by about 20%. At the same time, the percentage of the oriented $\text{CO}_2^-$ radicals show an increase from about 68% to 79%. The N + $\gamma$ response is somewhat different, with a rapid increase of the signal in the first 3 h by about 13% after that the increase of N + $\gamma$ runs parallel to the natural. During the first 3 h, there is also a rapid increase of the percentages of the oriented $\text{CO}_2^-$ radicals, from about 52% to 62%. After heating of 26 h the percentage of oriented $\text{CO}_2^-$ radicals in the N + $\gamma$ is about the same as in the non-heated natural sample. After 261 h, the percentages of oriented $\text{CO}_2^-$ radicals in the natural and N + $\gamma$ are about the same. Fig. 17B gives some insight about the possible transfer mechanism. Here the amounts of oriented $\text{CO}_2^-$ and non-oriented $\text{CO}_2^-$ radicals in the heated samples are normalised on the percentages in the unheated sample (the percentages of both radical types add up to the signal intensities in Fig. 17A). Although the increase of the oriented $\text{CO}_2^-$, and decrease of the non-oriented $\text{CO}_2^-$ radicals is somewhat different in the natural and N + $\gamma$ samples, it is obvious, that decrease of the non-oriented $\text{CO}_2^-$ radicals can only be partly responsible for the increase of the oriented $\text{CO}_2^-$ radicals. In the natural sample, the overall increase of the oriented by 26 percentage points compares to a decrease of the non-oriented by 7, for the N + $\gamma$, the overall values are 50 and 18 and for the first 3 h, 19 and 5, respectively. This means that the decay of the non-oriented $\text{CO}_2^-$ radicals can contribute only a quarter of the increase of the $\text{CO}_2^-$ radicals in the natural and about 36% overall in the irradiated samples. In the irradiated samples, during the first hour of heating this contribution is highest at 50%. The first heating step is also associated with the disappearance of the low-field shoulders (see Fig. 16H), so that one may expect some complex interactions at the onset of heating (Sholom et al., 1998; Hayes and Haskell, 2000). The scaling procedures indicate that there is some line widening in the T1–B1 region, however, we cannot identify any additional signal that grows with temperature (e.g. other $\text{CO}_2^-$ radicals (Brik et al., 2000b), radicals associated with free carbon, which were described in enamel samples from Kangaroo Island (Grün, 2006b; Grün et al., 2006) or other non-specified organic radicals (Wieser et al., 2000)).

The data in Fig. 17A can now be used to estimate the correct percentages of oriented and non-oriented $\text{CO}_2^-$ radicals in the natural and irradiated samples. The apparent percentage values can be fitted with a single exponential curve (the same as used for fitting dose response curves). The annealing procedure will approximate an apparent percentage value of $78.8 \pm 0.5\%$ in both the natural and irradiated samples. This means that after sufficiently long heating periods to eliminate all non-oriented $\text{CO}_2^-$ radicals, the B2 region still contains about 20% of a component that has different kinetics to the non-oriented $\text{CO}_2^-$ radicals. This component can be attributed to the oriented type (see above). Combining these results with the estimated apparent percentages (Fig. 15), we can assess that the natural and irradiation spectra contain a mix of about 90:10 and 60:40 of oriented to non-oriented $\text{CO}_2^-$ radicals, respectively. Note that Vorona et al. (2007) found distinctively different proportions of oriented (about 20%) and non-oriented (about 80%)
Fig. 16. Response to post-irradiation heating. (A-D) Natural. (E-H) N+γ. (A, E) Normalised on non-heated spectrum. (B, F) Scaled to the same intensity, residuals are offset for clarity. (C, G) Subtraction of the merged, powder-like spectrum (so that B2 is minimised, this removes the non-orientated CO₂ radicals), normalised on the non-heated sample. (D, H) Scaled on the same intensity, residuals are offset for clarity. The residuals of the natural sample indicate peak-widening, which may be caused by additional radicals.
CO\textsubscript{2} radicals in irradiated modern tooth enamel plates. This may point to further complications during the fossilisation process.

Considering that radiation produces about 40\% of non-orientated CO\textsubscript{2} radicals, which is diminished to about 10\% (i.e. to one quarter) in the natural samples of 1557, the mean life of the non-orientated CO\textsubscript{2} radicals can be estimated to be about 25\% of the age of sample 1557 (Grün, 1985a, b), which is between about 200 and 300 ka (Porat et al., 1999; Grün, unpublished data). It follows that the mean life of the non-orientated CO\textsubscript{2} radicals is in the range of 50–75 ka. If the mean life and kinetics of the non-orientated CO\textsubscript{2} radicals could precisely be determined, the relative contributions of the non-orientated CO\textsubscript{2} radicals in the natural and irradiation spectra can, in principle, be used for age assessments without the determination of the dose rate (Grün, 1985a, b; Debuyst et al., 1984). However, the effective mean life is strongly dependent on long-term ambient temperature histories. Nevertheless, in those cases, where the environmental dose rate is difficult to reconstruct, even rough age assessments with this method may provide valuable chronological hints.

3.3. Preheating experiments

If post-irradiation heating protocols are developed for the dose assessment of fragments to eliminate a proportion of non-orientated CO\textsubscript{2} radicals in the irradiation spectra, then this post-irradiation heating step is a preheating step for the next irradiation. It is well known from luminescence studies that preheating (i.e. carrying out a heating step before irradiation), may lead to sensitivity changes (e.g. Wintle and Murray, 1998, 2000; Ward et al., 2003). The same could be expected for ESR measurements. To assess the effect of preheating, the natural sample that was heated for 268 h in the previous experiment, was irradiated with a dose corresponding to 100 min and subsequently heated again for between 0 and 268 h. Fig. 18 shows the spectra of this experiment. As before, the N + \gamma spectra increase with heating time (Fig. 18A), which is more pronounced when the powder spectrum is subtracted (Fig. 18C). In this experiment, the scaling procedure provides a clearer picture than the previous experiment (Fig. 18B and D). After the first hour, the low-field shoulders have disappeared. At the same time there is peak-widening in the T1–B1 region. This widening diminishes systematically from 4.3 h onwards. The B2 intensity approaches that of the pre-annealed natural spectra, which apparently contained about 78\% orientated CO\textsubscript{2} radicals, after 268 h (see Fig. 18C). There are some small changes in the upswing to T1 (around channel 485), but this may be a scaling artefact of a signal that is not affected by the heating procedure (compare this region in Fig. 18A and B to C and D).

Fig. 19 compares the results of the preheating/annealing experiment with those of the annealing experiment shown in Fig. 17B. After preheating, a heating induced increase of the orientated CO\textsubscript{2} radicals by 37 percentage points compares to a decrease of the non-orientated CO\textsubscript{2} radicals by 18\%, a potential 50\% contribution to the rise of T1–B1. The development of the apparent percentages of the orientated CO\textsubscript{2} radicals is closely similar to the previous annealing experiment. After about 28 h, the irradiated sample contains roughly the same distribution of orientated to non-orientated CO\textsubscript{2} radicals as the natural, non-heated sample. After 268 h, the distribution is similar to the preheated natural sample. The striking difference between the preheated and non-preheated sample is that the intensity of the preheated, N + \gamma spectrum increases by only 18.5\% after 268 h, very similar to the natural sample (19.5\%), whereas the non-preheated, irradiated sample increased by 33.0\%. The exact reason for this different behaviour of non-preheated and preheated samples eludes
Fig. 18. Response to preheating (268 h at 125°C), irradiation and post-irradiation heating. (A) N+γ, normalised on the sample without heat treatment. (B) N+γ, scaled to the same intensity. (C) N+γ minus powder (to remove the non-oriented CO\textsubscript{2} radicals) on the sample without heat treatment. (D) N+γ minus powder, scaled to same intensity. The spectrum range (compared to e.g. Fig. 16) was changed to accommodate the legends without losing spectral information. The residuals in (B) and (D) are offset for clarity.

Fig. 19. Normalised apparent percentages of the orientated and non-orientated CO\textsubscript{2} radicals of the N+γ spectra after preheating at 125°C for 268 h and subsequent post-irradiation heating. The data in Fig. 17B are shown for comparison.

Fig. 20. Normalised signal intensities of the merged (powder-equivalent) spectra of sample 1557 of the of the natural and N+γ spectra after heating at 125°C as well as the N+γ spectra after preheating and heating (see Figs. 17 and 19).

us thus far. One may speculate that preheating may lead to some reorganisation of the crystal structure and/or removes some of the crystal water from the sample.

We have to keep in mind, that so far the thermal behaviour of the two CO\textsubscript{2} radicals was investigated at one angle (40°) only. The question is what actually happens to the powder spectra? If a transfer from the non-orientated
to orientated $\text{CO}_2^-$ radicals contributes only about 30% to the increase of the orientated $\text{CO}_2^-$ radicals, powder spectra should show a general increase with heating time. Instead of using a powder, the measured angular spectra are merged including the set measured around the other main axis. In the non-preheated set, the irradiation increases the natural powder spectrum by a factor of 3.30, in the preheated by 3.27. This difference of less than 1% is within measurement uncertainty. The results of the heating experiments on the merged spectra are summarised in Fig. 20. The intensity changes of the natural spectra range between 0.5% and 2.0%. Although this presents the reproducibility of the ESR intensity measurements, these seems a slight increase, perhaps by around 1–2%. The $N + \gamma$ spectra jump by about 4% after the first hour of heating, but after this, change by less than 1%, well within the reproducibility of ESR intensity measurements. The preheated and irradiated samples show a slightly different thermal behaviour. After an initial increase by about 7%, they seem to exponentially decay to 97% of the initial signal intensity.

In order to establish whether there is a transfer mechanism between the two types of radicals, it would be vital to know where they are actually located within the tooth enamel. Brik et al. (2000a) presented one model where tooth enamel contained thin organic membranes, one separating hydroxyapatite prisms and prism bundles, the second forming thin layers around individual nanocrystals inside the prisms. The former harbours the non-orientated $\text{CO}_2^-$ radicals whilst the latter, being physically closer to the prisms, contains the orientated. Because of connections between these layers, one type can convert into the other. In a second model, Brik et al. (2000b) stipulated that heating may lead to a diffusion of $\text{CO}_2$ into the surface layers of the hydroxyapatite crystals, which may turn into orientated $\text{CO}_2^-$ centres after the capture of an electron, which may have been detrapped during the heating process. The Gent research group (e.g., Callens et al., 1987, 1995, 1998; Vanhaeylewijn et al., 2000b, 2002) concluded, however, that the orientated type was embedded in the structure of the hydroxyapatite crystals. Whilst they are quite certain that the non-orientated type was of orthorhombic nature (Vanhaeylewijn et al., 2002), they are non-committal, in which location it actually occurs. Vorona et al. (2006) suggested that both types of $\text{CO}_2^-$ radicals are located in the B-position of the hydroxyapatite, replacing a $\text{PO}_4^{3-}$ tetrahedron, the orientated type rotating around the O–O axis, while the non-orientated was somehow braked. They speculated that a transfer from non-orientated to orientated $\text{CO}_2^-$ type could be associated with a loss of water.

It is unclear in the model-I of Brik et al. (2000a), why the transfer is not reversible, i.e. it should reach a steady state. If the orientated $\text{CO}_2^-$ radicals are actually embedded in the crystal and the non-orientated are either on the surface of the crystals or close by, then it would be difficult to hypothesise that the orientated type actually converts into the non-orientated. Perhaps, both processes take place at the same time without being connected. This is supported firstly by our observation that the non-orientated type can, if at all, only provide about 30% of the increase of the orientated. This points to the fact that other factors contribute to the increase of the orientated $\text{CO}_2^-$ radicals. Secondly, we observe that after preheating and irradiation, the ratio of $(N + \gamma)/N$ as well as the relative distribution of the two types in the $N + \gamma$ is the same as in the non-pretreated. If heating involved a certain mass transfer (Brik et al., 2000a), or loss of water (Vorona et al., 2006) and precursors (Brik et al., 2000b), one would expect either a difference in radiation sensitivity or a somewhat different radiation induced radical distribution with less non-orientated $\text{CO}_2^-$ radicals. Thirdly, regardless of what one's attitude is towards the reliability of ESR dating, ESR age estimates are often close to independent age estimates and reasonable results have been obtained for samples older than 1 Ma (e.g. Chen et al., 2001; Curnoe et al., 2001; Huang et al., 1993; Schwarcz et al., 1994). It would be very unlikely that organic membranes would not deteriorate over time, which would go along with the chemical destruction of previously radiation induced $\text{CO}_2^-$ radicals. Depending on the preservation of the organic membranes, the radiation sensitivity of older teeth should be far less than that of young samples. Thus far, we have not observed any such obvious trends. We would conclude from our study that the membrane model is less likely than orientated $\text{CO}_2^-$ radicals being located within hydroxyapatite crystals. The suggestion of Vorona et al. (2006) that both radical types occupy the same position on the crystal lattice is difficult to reconcile with the observation of Callens (personal communication) that single crystals do not contain non-orientated $\text{CO}_2^-$ radicals of the type described in this paper. The non-orientated $\text{CO}_2^-$ radicals could either be located on or near the crystal surfaces or in other domains in tooth enamel, such as the non-prismatic surface zone (which could be checked by removing it), or inter-prismatic zones. Non-orientated hydroxyapatite micro-crystallites could provide a mineralogical matrix assimilating a powder, resulting in a power-like spectrum for the $\text{CO}_2^-$ radicals located in this matrix. If this was the case, any direct transfer from the non-orientated $\text{CO}_2^-$ radicals to the orientated is difficult to explain. The increase of the orientated $\text{CO}_2^-$ radicals with heating could either be explained by $\text{CO}_2$ diffusion into the surface layers of the hydroxyapatite crystals (Brik et al., 2000b), or that heating re-distributes charges so that the precursors of the orientated $\text{CO}_2^-$ radical can capture additional electrons, similar to the model proposed by Martinez et al. (2001) for the $\text{SO}_2^-$ radical in corals.

4. Summary discussion

The irradiation experiment shows clearly that tooth enamel contains two types of $\text{CO}_2^-$ radicals. Their relative distributions in the natural and irradiation ESR spectra are
quite different. The annealing experiments show that
annihilation of the non-oriented CO$_2$ radicals occurs
simultaneously with an increase of the orientated CO$_2$.
However, the decrease in the non-oriented CO$_2$ radicals
cannot be the only contributor to the increased signal
intensity in the T1-B1 region. There may be other centres
or radicals in the T1-B1 region that we have so far not
identified, or heating may re-distribute charges and create
paramagnetic centres from their precursors. Either effect
may be more pronounced at other angles, thus we will
continue to evaluate the whole present data set in detail.
Furthermore, kinetic studies may reveal, whether the
T1-B1 region of this sample (at this or other angles)
contains any components besides CO$_2$ radicals.
In this study, the natural samples contained about 90%
orientated CO$_2$ radicals, whereas laboratory irradiation
induced a mix of CO$_2$ radicals containing only about 60%
of the orientated. These relative distributions of the non-
orientated CO$_2$ in the natural and irradiation components
of the spectra can be used to assess the life time of the non-
orientated CO$_2$ radicals, which must be in the range of
many 10$^4$s. This means that different distributions are
expected to occur in samples with significantly different
ages to the ones studied here. More detailed annealing
experiments are required to determine the kinetic parame-
ters of both CO$_2$ types. The small shifts that have been
observed in the position of T1 (e.g. in Fig. 6A) may
perhaps indicate that $g_{\perp}$ splits up into $g_{\parallel}$ and $g_o$, or the
presence of other radicals (Brik et al., 2000b). This, as well
as the notion that the non-orientated CO$_2$ is orthorhombic
(Vanhaelwyyn et al., 2002), requires more detailed inves-
tigation. The relative distribution of the two types CO$_2$
radicals in irradiated modern teeth (Vorona et al., 2006)
is quite different than observed in this paper. This points to
further complications caused by the diagenesis of tooth
enamel during fossilisation.
For practical purposes, one ought to focus on the
thermal behaviour of the merged spectra. In spite of large
internal re-organisations, the merged (powder-equivalent)
spectra are reasonably light affected by the heat treatment.
On average, the N+$\gamma$, heated spectra are only up to 3%
larger then the equivalently heated natural spectra. This
does not necessarily mean that dose estimations are 3%
smaller. Depending on the kinetics of the other signals, and
the curve of the dose response curve, increased signal
intensity through heating may actually lead to increased
dose estimations. Sholom et al. (1998) indicated that more
moderate heating, 2.5 h at 90°C, annihilates all transient
signals. This, of course, has also to be investigated in more
detail.
The results of a long-term fading study showed that 12
years after the initial irradiation, the overall ESR
intensities had actually decreased by between 3% and
10%. Dose values increased accordingly by between 2%
and 6%, this increase was within expectations particularly
for the samples from Border Cave, where independent age
estimations are slightly older than the ESR data set (Grün
and Ward, 2002; Grün et al., 2003). This may imply that an
initial signal increase by moderate heating actually decays
over time. The preheated spectra first increase by 5% but
then decay over 268 h to 96% of the equivalently heated
natural sample. Although we can presently not explain this
behaviour, it is, perhaps by chance, in agreement with the
results of the long-term fading study.
To summarise, compared to non-heated irradiated
samples, the signal intensity of powder-equivalent N+$\gamma$
spectra increases by about 3% through heating. The long-
term fading study implies that, if heat treatment is carried
out, it should lead to smaller rather than larger signal
intensities. Therefore, we can presently not recommend any
pre- or post-irradiation heat treatments, neither for
multi- aliquot nor single aliquot measurements. For the
dose estimation of fragments it is highly recommended to
measure these over the three major axes (measurement
between 0° and 180° is sufficient, as the spectra repeat) and
merge the angular spectra to produce powder-equivalent
spectra. Measuring around one axis only may lead to
results that are somewhat different to those obtained from
rotations around all three main axes (see Fig. 12). The
overall systematic uncertainty in the dose estimation
should be reasonably small and is probably well within
the error of routine dose estimations.

5. Conclusions
On has to keep in mind that it is difficult to draw general
conclusions from the annealing experiments, in view of the
limited number of samples studied in detail so far ($n = 1$).
For the detailed study of the kinetics involved it is
absolutely essential that the spectra are properly aligned.
Our method of using methyl radicals is validated by the
fact that the merged spectra do not show any signs of line
widening.
We have shown that there are complex mechanisms
operating when irradiating and heating tooth enamel. The
heat induced annihilation of non-oriented CO$_2$ radicals
after natural and laboratory irradiation could contribute
about a third of the observed increase of the orientated
CO$_2$ radicals. Post-irradiation heating may enhance the
overall ESR intensities by a few percent and one may well
expect that dose values are affected on a similar scale.
Because this observation is in contrast to a long-term
annealing study, it is presently not possible to recommend
pre- or post-irradiation heating procedures, neither for
multiple nor single aliquot measurement protocols. For
dose evaluation of fragments, we recommend the incremen-
tal measurement of the sample rotated around the
three major axes between at least 0° and 180° and to merge
the spectra before assessing dose values.
It seems now necessary to investigate the kinetics of the
orientated and non-orientated CO$_2$ radicals, carefully
check whether there are other contributors (e.g. CO$_3^-$,
CO$_2^-$, etc.) to the ESR spectrum, assess the distributions of
the two radical types in samples of different age, and
evaluate how pre- and post-irradiation heating affects dose estimations.

Acknowledgements

This study was partly funded by ARC Discovery Grant DP0666084 “Out of Africa and into Australia: robust chronologies for turning points in modern human evolution and dispersal”. We thank E. Rhodes, RSES, for helpful comments. We are very grateful to Prof. F. Callens, Gent, for his constructive review of the paper. In addition, we thank an anonymous reviewer for his observations.

References


CHAPTER 9
Thermal behavior of orientated and non-orientated CO$_2$ radicals in tooth enamel

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**A R T I C L E   I N F O**

Article history:
Received 20 October 2008
Received in revised form
11 February 2009
Accepted 22 February 2009

Keywords:
ESR Dating,
Enamel,
CO$_2$-
Thermal transfer

**A B S T R A C T**

Isothermal heating experiments on enamel fragments show that there are two pronounced increases in the intensity of the orientated CO$_2$ radicals, after heating for 15 min at 225°C and 1440 min at 175°C. While the powder spectra virtually remained unchanged at these heating steps, it was found that the angular intensity variability in the fragments greatly increased. In general, it is not possible to explain these increases by transfers from non-orientated CO$_2$ radicals to orientated, because there are simply not enough non-orientated CO$_2$ radicals in the unheated sample. Instead we attribute the increased angular intensity variability by a higher crystallinity in certain domains in the enamel. It was found that after heating for 15 min at 225°C, newly occurring orientated CO$_2$ radicals had a somewhat different orientation to the unheated.

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1. Introduction

Non-destructive ESR analysis is carried out on enamel fragments instead of powders to minimize the impact of analysis on valuable archaeological samples. However, the ESR spectra of fragments have a high angular dependency which complicates their study and the establishment of experimental protocols. Previous investigations have shown that the angular irradiation response of fragments is different to that of powders due to the presence of two types of CO$_2$ radicals (orientated and non-orientated). The non-orientated (N-O) CO$_2$ radicals give rise to a powder spectrum of the same intensity at all angles. In contrast, the contribution of the orientated radical is changing at each angle, depending on the effective orientation in the enamel fragment (for more details see Grün et al., 2008). The growth of hydroxyapatite crystals in the enamel is complex, forming rhombic prisms with vertical sides, which combine in packages that have complex three-dimensional structures (Mchó et al., 2003). Because of this complex structure, an enamel fragment does not have the same properties as a single crystal, i.e. it is not possible to exactly identify the main crystal axes. Although the c-axis is more or less perpendicular to the dentine/enamel junction, it is likely that some contributions of $\beta_1$ exist when the general prism growth direction is orientated perpendicular to the external magnetic field.

When $\beta_1$ of the orientated radicals is largest (T1–B1 in Fig. 2), the $\beta_1$ position (B2 in Fig. 2) is dominated by the N-O radical.

Therefore, at this specific position it is possible to extract the component of the orientated CO$_2$ radicals, by fitting the B2 component of a powder spectrum into the B2 of the angular

Fig. 1. Measurement configurations.
Fig. 2. Stacked ESR spectra for X (A, C, E) and Z-configuration (B, D, F) for the unheated (A, B), 15 min at 225 °C (C, D) and 1440 min at 175 °C (E, F). The spectra of the Y-configuration are very similar to the X-configuration.
2. Material and methods

The experiments were carried out on tooth enamel of a fossil bovid from the archaeological site of Holon (Porat et al., 1999). This sample was already used in the study of Grün et al. (2008). A long lamella was separated from the tooth using a diamond saw, four small fragments removed (62.1, 51.2, 39.5 and 38.0 mg) and the rest of the lamella crushed into powder and divided into 24 aliquots. The fragments were used for isothermal cumulative treatment for 15, 45, 180, 1440 and 14,400 min at 175, 225, 250 and 300 °C, respectively. Powder aliquots were heated only once, for 15, 45, 180 and 1440 min at 50, 100, 125, 175, 225, 300 °C. The enamel fragments were mounted in three Teflon holders containing a Parafilm mold and were incrementally measured by rotating them around their three major axes. In the context of this paper we used the following configurations: X: rotation around the axis perpendicular to the dentine–enamel junction, Y: around the axis of tooth growth and Z: perpendicular to X and Y (Fig. 1). The sample holders were inserted in a Bruker ER 218PG1 programmable goniometer and measured with a Bruker Elexys E500 ESR spectrometer in 10° increments over 360° with the following measurement conditions: 2 mW microwave power, 0.1 mT modulation amplitude 12 mT sweep width with a sweep time of 21 s. The spectra were accumulated from 50 consecutive measurements. All spectrum
Fig. 4. Response to heating for 15 min at 225 °C: A–I: B2 peak. Angular variations normalised on the maximum intensity unheated, heated and residual (A–C). Unheated and heated in polar projection (D–F) and fitted angular intensity variations (G–I). J–K: T1–B1 peak. Angular variations normalised on the maximum intensity unheated, heated and residual (J–L), unheated and heated in polar projection (M–O) and fitted angular intensity variations (P–R).
simulations were carried out with the Matlab program with the easyspin add-on (Stoll and Schweiger, 2006, 2007).

3. Results and discussion

Fig. 2 shows the stacked spectra of the X and Z-configurations (the stack of the Y-configuration is very similar to X) for the natural sample and two selected heating steps where the largest intensity increases were observed (see Fig. 3), 15 min at 225 °C and 1440 min at 175 °C. In powders, it is thought that the main signal (the peak T1 to dip B1, see Fig. 2A) is dominated by radicals. The T1–B1 peak in powders (Fig. 3A) shows little response to heating up to 225 °C. Only after 1440 min, some of the signal is annihilated. At higher temperatures the signal rapidly decays. The merged ESR spectra of the fragments (the spectra of all angles of the three configurations are aligned and combined, see Grün et al., 2008), behave very similar to the powder (Fig. 3B).

The thermal response of the fragment is very different when specific angles are evaluated. After heating for 180 min, the N-O CO₂ radicals show significant annealing at 225 °C and have vanished by 250 °C (Fig. 3D). In contrast, the oriented CO₂ radicals begin to decrease at 225 °C and significant intensities can still be measured at 300 °C (Fig. 3C). Between 175 °C and 225 °C, the disappearance of the N-O CO₂ radicals seem to be related with a strong increases of the oriented CO₂ radicals. After isochronal heating for 15 min, the T1–B1 maximum position (orientated CO₂, Fig. 3C) shows an increase at around 225 °C which seems associated with a decrease in the B2 minimum position (non-orientated CO₂, Fig. 3D). At longer heating times, there is a second peak occurring 175 °C, which is particularly pronounced in the 1440 min set. This increase is also associated with decreases in B2. It has been speculated by various authors that increases in the orientated CO₂ radicals were due to a transfer of N-O CO₂ radicals. Brik et al. (1998, 2000a) postulated that a mass transfer between different physical domains of the enamel was responsible while Vorona et al. (2003) speculated that the removal of water molecules was involved.

The question is whether the transfer of non-orientated CO₂ radicals into orientated is quantitative and whether the newly created orientated CO₂ radicals are the same as those in the natural sample.

Fig. 4 summarises the thermal response of the T1–B1 and B2 peaks after heating for 15 min at 225 °C for all configurations. The B2 peak, which is only generated by CO₂ radicals, increases in its maximum and decreases in its minimum (Fig. 4A–F). The diagrams in Fig. 4 are normalised to the maximum intensity in the natural sample. It can be seen in Fig. 4A that there is an angular shift between the natural sample and the heated. This angular offset is particularly pronounced in the X-configuration where we observe a shift by ~26° (average determined from the two maxima and minima). In Y and Z-configurations it is 7° and ~5°, respectively. The error in these determinations is around 5°–10°. We can deduce from this behaviour that the newly created orientated CO₂ radicals have a different orientation in the enamel (Fig. 5). The angular intensity variations in the heated sample are the 96, 74, and 57% higher than in the natural in X, Y, and Z-configuration, respectively (Fig. 4G–I). A question arises why these increases are different for the different rotational planes? While the relative differences between the Y and Z-configurations could be attributed to the angular shift by about 26° in the X plane, it is difficult to explain why the angular intensity variation changes strongest in the X-configuration, where we actually observe the strongest angular change.

In addition, it is not possible to explain the relative increases exclusively by annihilation of N-O CO₂ radicals, because their maximum concentration cannot be higher than the ratio of the minimum over maximum B2 in the natural sample, 43% (see e.g. Grün et al., 2008). However, after heating the minimum B2 remained at 36%, which means that no more than 7% of the natural CO₂ radicals could have possibly been non-orientated CO₂ decaying. One way for explaining the increase in intensity variation is that the newly generated orientated CO₂ radicals occur in domains with higher crystallinity, either by moving into different domains, or by crystallisation of certain domains. Perhaps these more crystallised domains have a different orientation. After observing these dramatic changes in the fragment, the most surprising fact is that the power and merged spectra after heating for 15 min at 225 °C are virtually the same, indicating that the total concentration of CO₂ radicals did not change at all. At this stage it is not known which specific crystal domains change in crystallinity. We prefer not to resort to speculation and expect that XRD measurements will give further insights.

When evaluating the T1–B1 peak, we observe an angular offset in the newly generated T1–B1 intensity of ~30°, 7° and ~10° in X, Y and Z-configuration, which is quite similar to the B2 peak (Fig. 4J–O). The intensity variations of the heated sample are 95, 85 and 102% larger than in the natural in X, Y, and Z-configuration, respectively (Fig. 4P–R). In contrast to the B2 peak, some of the differences in the intensity variations could be explained by the angular shift, which should have the strongest effect on the
Fig. 6. Response to heating for 1440 min at 175 °C. A–I: B2 peak. Angular variations normalised on the maximum intensity unheated, heated and residual (A–C), unheated and heated in polar projection (D–F) and fitted angular intensity variations (G–I). J–R: T1–B1 peak. Angular variations normalised on the maximum intensity unheated, heated and residual (J–L), unheated and heated in polar projection (M–O) and fitted angular intensity variations (P–R).
Z-configuration and the least for Y. The relatively stronger increases in the T1–B1 peak (compared to B2) could be due to the fact that the T1–B1 position is interfered with by a range of other radicals, particularly those which are known to increase by heating, such as SO₂⁻ or CO₃²⁻ (Callens et al., 1995). Nevertheless, changes in the crystallinity are required to explain the increase in the angular intensity variations on the one hand, but the virtually overall unchanged powder spectra, on the other.

Carrying out the same approach for the B2 response to heating for 1440 min at 175 °C (Fig. 6), the angular shifts are 7°, 14° and −7° and the angular intensity variation increases through heating by 65, 35, and 77% in X, Y, and Z-configuration, respectively. For T1–B1 the angular shifts are 10°, 10° and −5° and the angular intensity variation increases through heating by 122, 63, and 99% in X, Y, and Z-configuration, respectively. Because of the errors involved in the assessment in the angular shifts it is not possible to unequivocally state that the newly created orientated CO₂ radicals have a different orientation to the natural. The changes in the angular intensity variations of B2 and T1–B1 are very similar to those at 225 °C, above. No more than 8% of the natural CO₂ radicals could have been non-orientated which decayed during the heating process. As the powder and merged spectra are again virtually unchanged, changes in the crystallinity are most likely responsible for the intensity variation changes.

4. Conclusions

In contrast to the finding of Brik et al. (2000a), we cannot explain the changes during heating by a transfer from non-orientated CO₂ radicals to orientated, because there are simply not enough non-orientated CO₂ radicals present in the natural sample to explain the changes in the apparent increase in orientated CO₂ radicals. We find that after heating for 15 min at 225 °C some of the non-orientated CO₂ radicals have a different orientation in the X-configuration. We could not verify this effect during the heating for 1440 min at 175 °C. The increases in the angular intensity variations can generally be attributed to increased crystallinity, without being able to explain the details and differences in the responses of the B2 and T1–B1 peaks.

Acknowledgement

We thank John Fitzgerald for advise and support of our studies. This study was partly funded by the ARC discovery projects DP06666084 Out of Africa and into Australia: robust chronologies for turning points in modern human evolution and dispersal and ARC DP066604144 Microanalysis of human fossils: new insights into age, diet and migration. The ANU Vice-Chancellor provided a travel grant to RJB to attend the 12th International conference on Luminescence and Electron Spin Resonance Dating, 18–22 September 2008, Beijing, China, where many invaluable discussions helped to understand this topic in more depth.

References

CHAPTER 10
Decomposition of the angular ESR spectra of fossil tooth enamel fragments

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Abstract

The ESR spectra of a fragment of fossil tooth enamel were measured by rotating it in 10 increments over 360° around its three major axes. We used a simulated annealing algorithm for the mathematical decomposition of the spectra. The results imply that the tooth enamel fragment contains at least two different types of oriented CO2 radicals, plus about 95% of a non-oriented CO2 radical. The oriented components were tentatively attributed to axial and orthorhombic CO2 radicals. Their explicit locations in the crystal domains of the tooth enamel remain unresolved.

1. Introduction

To minimize the impact of the analysis on important archaeological samples, non-destructive ESR analysis has been carried out on tooth enamel fragments rather than powders (Grün et al., 2006). Nevertheless, the use of fragments for dose reconstruction has strongly complicated the task due to the high anisotropy of tooth enamel crystal. Grün et al. (2008) showed that the angular irradiation response of fragments is different to that of powders due to the presence of at least two different types of CO2 radicals (oriented and non-oriented). The non-oriented CO2 radicals (NOCORs) give rise to a powder spectrum of the same intensity at all angles, while the intensities of the anisotropic CO2 radicals (AICORs) are angular dependent. Because of different relative distributions and thermal stabilities of these two types of CO2 radicals in the natural and irradiated specimen components, the calculated dose values become angular dependent.

AICORs may occur in two varieties: axial CO2 radicals (with θ = 1.9925 and θ = 1.9974, e.g., Callens et al., 1987; Ishchenko et al., 2002) occur through carbonate substitution of the PO4 tetrahedron (e.g., Vugman et al., 1995). Their axial symmetry is explained by rotation around the O-O axis which is in direction of the mineralogical c-axis of the hydroxyapatite crystals. Orthorhombic CO2 radicals (with θ = 2.0030, 2.0015 and 1.9973, respectively, e.g., Callens et al., 1987; Ishchenko et al., 2002; Rudko et al., 2007) are associated with carbonate substitution of (OH)−. Their explicit location is disputed. Some speculated that they are located at the surface of the hydroxyapatite crystals (Callens et al., 1995; Brik et al., 2000, 2005), while others argued them to be in the same position as the axial CO2 radicals (Ishchenko et al., 2002; Vorona et al., 2005).

At specific angles, when the θ = 2.0030, 2.0015 or 1.9973 of the AICORs are largest (T1–B1 dominant, Fig. 1), the b1θθ components, which correspond to the B2 dip (Fig. 1C), are mainly composed by NOCORs. Using these specific positions, the fitting of a powder spectrum (obtained by merging all angles of con gurations) into the spectra allows the estimation of the amount of NOCORs in the sample. The NOCORs are then subtracted at every angle. Nonetheless, some B2 component of the AICORs may also be removed with the subtraction of the NOCORs. One has to keep in mind that the T1–B1 peak may consist of a range of radicals (methyl, CO32−, CO2, CO2 etc., for a compilation see Callens et al., 1998; Vanhaecke et al., 2000b). Only CO2 radicals, which may occur as orthorhombic, axial or non-oriented types, have any signal intensity in the B2 region through their b1θθ or b2θθ components. Tumbling CO2 radicals give rise of an isotropic line at g = 2.0006 (Callens et al., 1987; Debuys et al., 1993; Vanhaecke et al., 2000b, Vorona et al., 2006).

Thus far, all papers dealing with the description and interpretation of the ESR spectra of enamel fragments focussed on only specific angles, particularly when the peaks in the T1–B1 and B2 positions were largest or smallest. The spectra recorded for intermediate angles were usually not further discussed. However, these are important for validating proclaimed interpretations. For example, we found that it was not possible to t intermediate spectra of irradiated fossil tooth enamel with a linear combination of the natural signal and a NOCOR powder spectrum. This means that there are more than just two radicals involved in the natural and/or irradiated spectra of fossil tooth enamel. This was already
pointed out in the heating experiments by Joannes-Boyau and Grün (2009). In this paper, we present the decomposition of all ESR spectra of a fossil tooth enamel fragment that was rotated around its three major axes in 10 increments.

2. Materials and methods

In this paper, X, Y and Z denote configurations, x, y and z the main axes of the measured fragment (Fig. 2). T1, B1 and B2 are positions in the measured or simulated ESR spectra (Fig. 1), R1, R2, R3 and B2 tted Gaussian components (Fig. 4).

The experiments were carried out on a tooth enamel fragment of a fossil bovid from the archaeological site of Holon (Porat et al., 1999). A long lamella was separated from the tooth using a dentist’s diamond saw and series of consecutive fragments were cut and used for a range of heating and irradiation experiments (e.g. Grün et al., 2008; Joannes-Boyau and Grün, 2009).

The fragment (H5) was successively mounted into three separate Te on holders containing Para Im moulds. This allowed for the rotation of the fragment around its three major axes along with the incremental measurements of ESR spectra. We used the following configurations: X: rotation around the axis perpendicular to the dentine—enamel junction, Y: around the axis of tooth growth and Z: perpendicular to X and Y (Fig. 2). The sample holders were inserted in a Bruker ER 218PG1 programmable goniometer and measured with a Bruker Elexys E500 ESR spectrometer in 10 increments over 360 with the following measurement conditions: 2 mW microwave power, 0.1 mT modulation amplitude 12 mT sweep width with a sweep time of 21 s. The spectra were accumulated from 50 consecutive measurements.

All spectrum decompositions and simulations were carried out on the measured derivative spectra. Initially we used the Matlab software with the easyspin add-on (Stoll and Schweiger, 2006, 2007). For simpli cation, only line shapes de ned by the rst derivative of the Gaussian function were used, although there were indications that in some cases Lorentzian or Voigtian line shapes were perhaps more appropriate. Some apparent signals in the residual (measured minus simulated) spectra seemed to be due to
the error induced by the Gaussian approximation. After several subjective manual approaches to spectrum analysis, decomposition was optimised and automated using a simulated annealing (SA) procedure. SA is a Monte Carlo method used for combinatorial optimisation problems (for details see Metropolis et al., 1953; Kirkpatrick et al., 1983; Cerný, 1985; Mosegaard and Sambridge, 2002). Our SA procedure is able to randomly generate a large number of synthetic spectra demanded by a linear combination of four Gaussian lines. Each simulated spectra is compared to the measured spectra in terms of a least square fit. The particular advantage of the SA algorithm is the search of the global minimum fit without getting stuck in local solutions. As we shall see below, SA can resolve completely overlapping signals, which are very difficult to decompose with alternative decomposition approaches.

The Gaussian lines had prescribed limits with respect to the $g$-value range to avoid unrealistic solutions outside the regions for the CO$_2$ radical in hydroxyapatite (see above). No restrictions were set on the intensity and maximum line width, however, a minimum width (0.10 mT) was needed to avoid aberrations.

3. Results and discussion

ESR spectra of fragments differ from those of powders due to the pronounced anisotropy of the CO$_2$ radical (e.g. Fig. 1). The angular variations of the ESR spectra vary from one conformation to the other. The anisotropy of the measured spectra of the Z-conformation (Fig. 1B, Table 2) is significantly larger than of the other two conformations. The X-conformation shows the smallest anisotropic effects (Fig. 1A), while the Y-conformation appears to show a mixed pattern of the other two.

3.1. Spectrum decomposition

When a single orthorhombic crystal is rotated around the $y$ or $z$ axis, a signal will appear at different $g$-values, shifting from the parallel ($g_{xy}, g_{xz}$) to the perpendicular position ($g_{y}$), as shown in Fig. 3. In contrast, enamel fragments are a partially ordered system and mostly only minor $g$-value shifts have been observed for the T1–B1 and B2 components. This could be the result of a combination of interfering oriented and non-oriented radicals or multiple orientations of the main CO$_2$ radical. Spectrum decomposition should help the understanding of the behaviour of known radicals that are supposedly involved in the spectra.

The ESR spectra of tooth enamel have a range of non-CO$_2$ components, such as dimethyl radicals. To subtract these, a merged spectrum was produced (the average of all measured spectra). In the range used for decomposition, the measured spectra contain a dimethyl triplet and two wide lines (Fig. 4A). W1, around $g_{/2} 2.0061$, was also described by Grün (2002) but could not be attributed to a specific radical. W2, around $g_{/2} 2.0051$, relates most likely to a combination of SO$_2$ and CO radicals which occur at $g_{/2} 2.0056$ and $g_{/2} 2.0047$, respectively (Bouchez et al., 1988; Schramm and Rossi, 2000; see also the fitting of Grün, 2002, his Fig. 1G and H). The intensity of the central dimethyl line was derived from the next two outer lines assuming a septet with
The intensity distribution of a Pascal triangle. None of these components exhibited any quantifiable angular variation, thus they were assumed to be isotropic. The two wide lines were combined with the dimethyl lines into an iso-combined (IC) spectrum (Fig. 4A) which has an intensity of about 25% of the merged spectrum (Fig. 4A). However, most of the IC intensity lies outside the range of CO₂ radicals and will have little effect on the decomposition results. The IC spectrum was subtracted from all angular measurements.

While the X-con guration can be reasonably fitted with just three components (R₁, R₂, and B₂, see Fig. 4B), the Y- and Z-con gurations require an additional component (R₃) to obtain a satisfactory match between experimental spectrum and simulation. The introduction of R₃ resulted in a division of the original B₂ signal that was abnormally wide and intense (compare the fitted results in Fig. 4B and C). In this context we prefer the use of B₂ rather than R₄, since all our previous papers uses B₂ for the dip around g ≈ 1.9976 (see also Fig. 1C).

At this stage, we did not subtract a NOCOR component from the measured spectra, as this does not have an in uence on the angular behaviour of the various components (it only causes constant offsets). In the rst instance, its maximum intensity can be derived from the minimum B₂ position in X-con guration and cannot exceed 13% of the total radical concentration.

3.2. Decomposition of the spectra of the three configurations

The description of the results starts with the Z-con guration, which showed the largest angular changes. The radical concentrations were derived from the double integration of the tted lines to account for changes in the line width (Table 2 and Figs. 5L, 6L and 7L). The angular variation is expressed in the difference between maximum and minimum concentration divided by the average concentration of the tted component (C_max - C_min)/C_avg. For features in the measured ESR spectra, T₁-B₁ and B₂, it was not possible to carry out double integrations, their angular variations were derived from their intensities (Table 1). The results for the various tted components are listed in Table 2.

3.2.1. Z-configuration

The Z-con guration shows the largest angular dependency of the measured spectra between the T₁-B₁ maximum and the B₂ maximum 90° later (see Fig. 5A, H and I), with large angular variations in the T₁-B₁ and B₂ intensities of 0.60 and 0.74, respectively. Fig. 5 shows the results of the tting. Generally, there is a very good match between the measured and tted spectra (Fig. 5A-C, top row). The intensities in the residual stack (measured minus tted spectrum Fig. 5C) are well below 5% of the measured spectra (Fig. 5A). The results on the individual components are shown in the rows below.

As expected from the measured spectra, B₂ shows the largest radical concentrations (0.55) of all con gurations and large angular variations (0.54, see Fig. 5H,I). In contrast to the behaviour in the
other configurations, \( B_2 \) changes its line width (between 0.33 and 0.42 mT) and \( g \)-value (between 1.9981 and 1.9987) considerably (see Fig. 5C and Table 2). This has some influence on the calculation of the \( B_2 \) concentrations. As a result, the angular variability of the \( B_2 \) component is somewhat smaller than expected from the intensity changes in the \( B_2 \) position.

\( R_1 \) and \( R_2 \) show very similar angular behaviours, with their maxima and minima offset from \( B_2 \) by about 90°. The main intensity changes in \( T_1 - R_1 \) (0.60) are due to the angular response of \( R_1 \) (0.56) while \( R_2 \) shows significantly less angular variation (0.29). Similar to \( B_2 \), the line width of \( R_1 \) changes significantly (0.28 to 0.36 mT), but its \( g \)-value remains within a narrow range (2.0025–2.0027).
R₁, R₂, and B₂ show a 180° symmetry, which is expected from an oriented CO₂ component. In contrast, R₃ shows a pronounced 90° symmetry (Fig. 5I). While this is surprising, the stack of the measured spectra shows a widening of the T₁–B₁ peak with a 90° symmetry (arrows in Fig. 5A).

The sum of the radical concentrations of the four tted components (Fig. 5L) shows some angular dependency (0.2). However, any other combination (i.e., excluding one of the tted components) would lead to larger angular variations. We therefore conclude that all tted components are associated with CO₂ radicals.

3.2.2. Y-configuration

The angular responses in the Y-configuration are rather similar to Z (Fig. 6). T₁–B₁ and B₂ vary by 0.62 and 0.53, respectively. The differences between Y and Z for B₂ are smaller variations in width and g-value as well as a signi cantly smaller average intensity. R₂ shows a larger angular variation (0.49) and R₃ has a somewhat smaller average concentration and converts into a 180° symmetry. R₁ and R₂ show similar angular behaviours and their maxima and minima are offset by 90° from B₂. The angular response of R₃ is similar to B₂. The intensity variations in T₁–B₁ are caused in equal measures by the variations in R₁ and R₂. The
The X-con guration shows the smallest angular variability in T1—B1 and B2 of 0.31 and 0.40, respectively (Fig. 7). R1, R2 and B2 show the smallest variations in line widths and angular variations. R2 has its largest intensity while R1 and B2 their smallest. R3 has its largest line width. The variations in T1—B2 are caused in equal measure by R1 and B2 (the latter has a smaller angular variation, but much higher concentrations). As in the previous con gurations, R1 and R2 show similar angular behaviours and their maxima and minima are offset by 90° from B2 and R3. The sum of the four components shows a small angular dependency of 0.1.

It is perhaps noteworthy that any peak-to-peak measurements on powders for geochronology purposes or retrospective dosimetry should be carried out on T1—B2, as their interdependency minimises the angular variability of Alcos (see last column in Table 1). Nevertheless, further studies are required on dose reconstruction to be able to propose a clear statement on peak-to-peak measuring protocols.
3.3. Uncertainties

In order to assess the uncertainties of our decomposition approach, we firstly ran our Monte Carlo simulation several times on the same spectra with different values of initial parameters (i.e., initial g and width values, seed of the random number generator). Relatively small differences were observed resulting from the robustness of the T2g strategy. Secondly, a simulation was carried out on a second fragment (H4) immediately adjacent to the fragment discussed in this paper (H5). The results are summarised in Table 3. In spite of using different moulds resulting in somewhat different measurements positions in the cavity, the simulation results on the three con gurations are very comparable. The changes in minimum and maximum g-values and widths range between 0.0002 and 0.0001 (average 0.00004) and 0.03 and 0.03 mT (average 0.01 mT), respectively. The changes in g-values are virtually within measurement error, and the line width changes are well below 5%. Changes in the minimum and maximum radical concentrations as well as changes angular variation are signi cantly larger, reaching up to 36% (average 9%) and 46% (average 20%), respectively. These large changes in the intensity derived parameters are not entirely surprising, considering discontinuous nature of the radical concentration curves (Figs. 5l, 6l and 7l). More importantly, the average relative radical concentrations of R1, R2 and R3 and B2 changed only to a maximum of 9% with an average of 1.3%. The relative contributions of R1, R2 were 36.64 and 37.63 for H5 and H4, respectively. This is particularly remarkable as R1 and R2 completely overlap. Most previous decomposition approaches on X-band (e.g., Jonas, 1995; Grün, 1998; Vanhaevelyn et al., 2000a) could not resolve closely overlapping signals. Altogether, we consider our results as robust.

3.4. Discussion

From the roughly 90 offsets between R1, R2 maxima and minima from B2, we conclude that R1 and R2 are AlCOR components. Based on its g-values, B2 is either g5 or g7 of axial or orthorhombic CO2 radicals, respectively, or a combination of both. Note that the g-value of g5 or g7 is measured at the position of the dip, around 1.9972 (as indicated Fig. 1) whilst the g-values for the B2 component is the position of the zero-passing (maximum of the absorption line) with g-values around 1.9985. R1 may represent g8 of orthorhombic and Rg 1 of axial CO2 radicals.

A question remains with respect to the nature of R3. The fact that R3 is needed for minimising the angular dependency of the sum of all fitted components points to anisotropic CO2 radicals. To recapitulate, enamel fragments are a partially ordered system with some preferential direction of the hydroxyapatite crystals, but there is also a significant number of crystals in random orientation (Macho et al. 2003). What is seen in the measurements are the extreme values of the preferential directions (g5, g7, and g8) whilst the other orientations are lumped into R3, see also Liidja (2001). These may arise from the misalignments of the preferential mineralogical axes with the x, y, and z-axes of the fragment, but also from misalignments of certain crystal domains with the preferential mineralogical axes. It is interesting to note that the fitted parameters of R3, apart from its width in X-con guration, hardly change in the three con gurations. Its average g-value of 2.0005 0.0001 is indistinguishable from the average value of isotropic CO2 radicals in hydroxyapatite (see above).

If R3 is the composite of all misalignments, it would consist of a semi-in nite series of Gaussian components between the T1 and B2 positions. If there is no spatial preference, R3 would basically present a Gaussian error function. While such a series is dif cult to simulate, it can be approximated by a Gaussian line. However, if the misalignments are not randomly distributed (see Fig. 8B, below), the shape of R3 may change with angle (see e.g. Fig. 9B, below) and its Gaussian approximation may create mathematical artefacts. Our decomposition approach does not allow an accurate assessment of the line shape of R3. As a result, it cannot entirely be excluded that R3 is part of misaligned crystal domains or an artefact of the assumption that R3 has a Gaussian line shape.

Two previous observations on enamel fragments of this tooth support the existence of two oriented CO2 components. Firstly, heating experiments showed angular shifts of the T1-B1 maximum position by about 30 (Joannes-Boyau and Grün, 2009), which could be most readily explained by a transfer of orthorhombic to axial CO2 radicals. Secondly, we found that it is not possible to detect the irradiation spectra of fossil tooth enamel fragments with a linear combination of the spectra of the natural sample and isotropic CO2 radicals. This should be possible if only one oriented and one non-oriented type of CO2 radicals werepresent...
Table 3
Results of the decomposition of enamel fragment H4, using the SA protocol with a mix of four Gaussian lines.

<table>
<thead>
<tr>
<th>Z-configuration</th>
<th>Minimum g-value</th>
<th>Maximum g-value</th>
<th>Minimum width (mT)</th>
<th>Maximum width (mT)</th>
<th>Minimum radial conc.</th>
<th>Maximum radial conc.</th>
<th>Angular variation</th>
<th>Average radical conc.</th>
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<td>R1</td>
<td>0.0225</td>
<td>0.0227</td>
<td>0.28</td>
<td>0.35</td>
<td>0.28</td>
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<th>Maximum width (mT)</th>
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<th>Maximum radial conc.</th>
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<td>0.0023</td>
<td>0.41</td>
<td>0.42</td>
<td>0.60</td>
<td>0.72</td>
<td>0.18</td>
<td>0.67</td>
</tr>
<tr>
<td>R3</td>
<td>0.0003</td>
<td>0.0006</td>
<td>0.47</td>
<td>0.54</td>
<td>0.62</td>
<td>0.77</td>
<td>0.22</td>
<td>0.69</td>
</tr>
<tr>
<td>R2</td>
<td>1.9984</td>
<td>1.9987</td>
<td>0.39</td>
<td>0.42</td>
<td>0.19</td>
<td>0.38</td>
<td>0.49</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Fig. 8: A: The orientation of each radical has been estimated using the angular variation response. Each intensity maximum of each configuration corresponds to the strongest projection of the vector (reflecting the radial orientation and intensity) on the opposite plane parallel to the rotation plane. B: Directions of R1, R2, R3 and B with respect to the main axes of the fragment and their relative position (B). The grey circle corresponds to the plane perpendicular to the enamel/dentine junction.

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Fig. 9. Rotation of the fragment around an axis perpendicular to B, and the resultant of R, and R₂. A: Subtraction of the 9° powder spectrum in B2 minimum position. B: Rotation from T1–B1 maximum to the B2 maximum. C: 3D view of stacked spectra. D: map of intensities of stacked spectra.
present with different ratios in natural and irradiated spectra (Grün et al., 2008). The ts are particularly poor at intermediate angles between the T1–B1 and B2 minima and maxima.

Q-band studies on enamel powders found additional lines in the region of the g $\frac{4}{2}$ 2.0007 (Jonas and Grün, 1997; Skinner et al., 2001), which can be attributed to tumbling CO$_2$ radicals (see above). In addition, the intensity in the B2 position could not be tted by a simulation of axial CO$_2$ radicals (Jonas and Grün, 1997). Q-band studies on fragments of fossil tooth enamel clearly shows the presence of orthorhombic CO$_2$ radicals (Bouché et al., 1988; Rossi and Poupeau, 1990), which (i) had a somewhat different orientation to the axial CO$_2$ radicals and (ii) were thermally less stable than the axial (and tumbling) CO$_2$ radicals (Rossi and Poupeau, 1990). Taken all lines of evidence into consideration, we are con dent that B2 represents a component of orthorhombic CO$_2$ radicals.

For the two fragments analysed, the ratio between R1;R2 is around 36:64. Thus far, we do not have any data for fossil tooth enamel. Vorona et al. (2006) found a relative distribution between axial and orthorhombic CO$_2$ radicals in irradiated modern tooth enamel of approximately 20:75. They showed that orthorhombic CO$_2$ radicals could convert into axial by heating. One would expect that the same happens over geological time scales of hundreds of thousands of years.

We can now use the measurements of the three con gurations to calculate the main directions of the tted components (Fig. 8A). Firstly, there is a clear separation between R1 and R2 of about 23 (Fig. 8B). This is very similar to the angular separation of 30 of CO$_2$ components observed in previous heating experiments (Joannes-Boyau and Grün, 2009). The angle between R1 and R2 is 76 and between R1 and B2 is 86. The angles are di erent from 90 because B2 is a composite. The calculated angle between B2 and the resultant vector of R1 and R2 is 86. The direction of R3 (Fig. 7A) is perhaps less meaningful, but may indicate a general preferential direction of the misaligned crystal domains.

Subsequent to these calculations, we inserted the enamel fragment into the goniometer so that it was rotated around the plane of the B2 vector and the resultant vector of R1 and R2 (Fig. 9). After subtraction of a powder spectrum with 9% of the total radical concentration (Fig. 9A), we can clearly observe a behaviour that is closer to that described for single crystals (compare Figs. 3 and 9B). T1–B1 and B2 intensities can be virtually eliminated at G. and 90°, respectively. However, in these two positions, the ESR spectrum cannot be tted with a single Gaussian line, both require at least two components. This clearly indicates a large ‘misalignment’ component, as simulated by Liidja (2001), see his Fig. 13. At intermediate angles, a wider line occurs which resembles R3 with a 90 symmetry, peaking between the T1–B1 and B2 maxima (compare to Fig. 5I). From these measurements we can also conclude that the maximum contribution of the NO currents is 9%. This agrees well with a value of 10% from the annealing experiments on another fragment of this tooth (Grün et al., 2008).

We also tried to decompose this data set. The problem of R2 not being of Gaussian line shape (particularly obvious between 170 and 208 in Fig. 9B) made it impossible to track R1 and R2 when their intensity decreased. Instead, R3 was widening which then had a knock-on eect on the calculation of the line width of B2. We are continuing to work on this problem.

At this stage it is not possible to deduce whether the R1 and R2 components are located in the same or different mineral domains, e.g. inside or on the surface of crystals with di erent sizes average crystal sizes etc. If orthorhombic CO$_2$ radicals can be transferred into axial (as observed by Vorona et al., 2006) then these should be located in the same crystal domains.

4. Conclusions

We have developed a rapid method for the decomposition of the angular spectra of tooth enamel. The simulated annealing procedure seems particularly well suited to decompose overlapping signals, such as R1 and R2. All spectra can be satisfactorily decomposed using four Gaussian Lines, R1, R2, R3 and B2. All tted components seem related to anisotropic CO$_2$ radicals. R1 has been tentatively related to the g$_{\perp}$ of orthorhombic and R2 to g$_{\|}$ of axial radicals. B2 is a combination of g$_{\perp}$ and g$_{\|}$ of the two anisotropic CO$_2$ radicals. R3 presents an envelope for misalignments, resulting from angles between the dominant axial directions and the principal axes of the fragment as well as misalignments of various crystal domains with the dominant axial directions. We found an angle of 23 between the directions of R1 and B2. The occurrence of two different anisotropic CO$_2$ radicals in fossil tooth enamel is supported by Q-band studies on fossil enamel fragments (Bouché et al., 1988; Rossi and Poupeau, 1990) and our earlier observations on the ESR response to heating (Joannes-Boyau and Grün, 2009) and that the irradiation spectra of irradiated fossil tooth enamel fragments cannot be tted by a linear combination of the spectra of the natural sample and isotropic CO$_2$ radicals.

Our decomposition approach will now allow the investigation of the irradiation e ects in fossil tooth enamel and assess their relevance for dose response and dating.

Acknowledgments

We thank G. Lidia, Talinn for providing us with one of his reprints that was difficult to obtain and N. Manson, Research School of Physical Sciences, ANU, for helpful comments. We are grateful to F. Callens and Henk Vrielinck, Gent, for their thorough advice in the earlier stages of this study. Aspects of this study were supported by ARC grant DP0664144 Microanalysis of human fossils: new insights into age, diet and migration.

References


CHAPTER II

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CHAPTER 11
Decomposition of the laboratory gamma irradiation component of angular ESR spectra of fossil tooth enamel fragments

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1. Introduction

This paper follows our earlier studies on the spectrum decomposition of fossil tooth enamel fragments (Joannes-Boyau et al., submitted for publication), where we introduced an automated simulated annealing (SA) procedure for the mathematical decomposition of the ESR spectra. This allowed, for the first time, to analyse all angular spectrum measurements of a tooth fragment rotated around its three major axes. The decomposition of all natural spectra required a minimum of four Gaussian lines, $R_1$, $R_2$, $R_3$, and $B_2$. All fitted components seemed to be related to anisotropic CO$_2$ radicals (AICORs). $R_1$ was tentatively related to the $g_z$ of orthorhombic and $R_2$ to $g_x$ of axial radicals. $B_2$ was regarded as a combination of $g_y$ and $g_z$ of the two AICORs. $R_3$ presents an envelope for misalignments, resulting from angles between the dominant axial directions and the principal axes of the fragment as well as misalignments of various crystal domains with the dominant axial direction. The ratio between $R_1$ and $R_2$ was around 35:65 and there was an angle of around 23° between the directions of $R_1$ and $R_2$.

As already pointed out by Grün et al. (2008), the natural and irradiation components in the ESR spectra of fossil teeth are distinctively different in angular measurements of tooth enamel fragments, while virtually the same for powders. They found that the natural sample had about 10% of the non-oriented CO$_2$ radicals (NOCORs), while the irradiation component had around 40%. However, the irradiation component could not be fitted by a linear combination of the natural component and NOCORs, which means that there are additional differences between the natural and irradiation components of the ESR spectra (e.g., through a different distribution of orthorhombic and axial radicals). In this paper, we decompose the laboratory irradiation components of the ESR spectra (henceforth, irradiation spectra) of a tooth enamel fragment and compare the results with those from the natural sample.

2. Material and ESR measurements

The experiments were carried out on a tooth enamel fragment of a fossil bovid from the archaeological site of Holon (Porat et al., 1999). A long lamella was separated from the tooth using a dentist’s diamond saw and series of consecutive fragments were cut and used for a range of heating and irradiation experiments (e.g., Grün et al., 2008; Joannes-Boyau and Grün, 2009; Joannes-Boyau et al., submitted for publication).

The methodology follows that established by Joannes-Boyau et al. (submitted for publication). X, Y and Z denote configurations, x, y and z the main axes of the measured fragment (Fig. 1). $T_1$, $B_1$ and $B_2$ are positions in the measured or simulated ESR spectra (see Fig. 2B), $R_1$, $R_3$, $R_4$ and $B_2$ fitted Gaussian components (Fig. 4D–G, see below). The concentrations for the radicals were derived from the double integration of the fitted lines to account for changes in the line width. For the features in the measured ESR

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spectra, $T_1-B_i$ and $B_2$, it was not possible to carry out double integrations; their angular variations were derived from their intensities.

The fragment (H4) was successively mounted in three separate Teflon holders each containing a Parafilm mould, which allowed the incremental measurement of the fragment by rotating it around its three major axes. We used the following configurations: X: rotation around the axis perpendicular to the dentine-enamel junction, Y: around the axis of tooth growth and Z: perpendicular to X and Y (Fig. 1). The sample holders were inserted in a Bruker ER 210PG1 programmable goniometer and measured with a Bruker Elekys E500 ESR spectrometer in 10 increments over 360° with the following measurement conditions: 2 mW microwave power, 0.1 mT modulation amplitude 12 mT sweep width with a sweep time of 21 s. The spectra were accumulated over 50 consecutive measurements. The sample was measured before and after irradiation with a $^{137}$Cs-source for 100 min, which corresponds to an approximate dose of 187 Gy.

3. Spectrum decomposition

In the first step, the angular irradiation spectra were extracted by subtracting the natural spectra from the natural plus gamma irradiated. It is very important that the spectra are well aligned otherwise artefact signals are introduced. The methyl lines were used as markers for alignment (see Grün et al., 2008).

The ESR spectra of tooth enamel have a range of non-CO$_2$ components, these have to be removed before decomposition. Non-radiation sensitive isotropic lines, which occur in the natural spectra (Fig. 2A), are automatically removed by the subtraction of the natural spectrum. For example, the irradiation spectra do not contain methyl lines. In addition, three wide lines were identified in the irradiation spectra, $W_1$ centred at $g=2.0115$ with a line width ($hw$) of 0.3 mT, $W_2$ with $g=2.0081$, $hw=2.3$ mT and $W_3$ $g=2.0072$, $hw=0.7$ mT. These were combined into a single IC line and subtracted from all irradiation spectra (see Fig. 2B and C). The isotropic components of the natural spectra do not occur in the irradiation spectra, i.e. they are not radiation sensitive.

In the next step, the non-oriented CO$_2$ radicals (NOCOR) were subtracted. We estimated that the remaining irradiation spectra consisted 39% of NOCORs, indicating a more than threefold increase over the natural spectra. A similar contribution was found by Grün et al. (2008). The remaining spectra are thought to contain only anisotropic components, at this stage all attributed to NOCORs.

Fig. 1. Direction of axes and configurations used for the measurement of the tooth enamel fragment.

Fig. 2. Decomposition of the measured spectra. A: isotropic lines in the natural spectra (from ). B: position of the isotropic lines which were combined (IC) and subtracted from all measured irradiation spectra. C: decomposition of a measured irradiation signal showing all components extracted, the natural (nat), non-oriented CO$_2$ radicals (PS) and isotropic lines (IC) are combined (IC+PS+Nat), and the four main anisotropic components $R_i$, $R_2$, $R_3$ and $B_2$. 

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Fig. 3. Stacks of natural spectra (row A), natural plus γ (row B), irradiation (row C), natural anisotropic (row D) and irradiation anisotropic spectra (row E) for the three configurations X, Y and Z.
These anisotropic components of the irradiation spectra were decomposed with an automated simulated annealing (SA) procedure which is particularly well suited to separate completely overlapping signals (Joannes-Boyau et al., submitted for publication). To keep the results comparable with the previous study, the spectra were initially decomposed with four Gaussian lines, which had the same prescribed g-value range limits to avoid unrealistic solutions outside the regions for the CO₂ radical in hydroxyapatite. No restrictions were set on the intensity, while the line widths were kept between 0.10 and 2 mT to avoid aberrations. Fig. 2C shows the composite of all subtracted components, as well as the four Gaussian lines R₁-R₃ and B₂.

4. Results and discussion

Fig. 3A and B shows the stacks of the natural and irradiated ESR spectra (natural + γ) for the different configurations. The angular variations in the measured ESR spectra (defined as the difference between maximum and minimum radical concentration or peak intensity divided by the average, (cmax−cmin)/cav) vary from one configuration to the other. In Z-configuration (Table 1), the angular variation is significantly larger than of the other two configurations. The X-configuration shows the smallest anisotropic effects, while the Y-configuration appears to show a mixed pattern of the other two. The angular variations in the natural spectra are generally higher than in the irradiated (Table 1). The irradiation components, obtained after subtraction of the natural from the irradiated spectra, are shown in Fig. 3C. Their angular variability is also generally smaller than of the natural spectra.

Fig. 3D and E shows the natural and irradiation spectra after subtraction of the isotropic lines and NOCORES. There are striking differences between the anisotropic components of the natural and irradiation spectra. The subtraction of the NOCORES reveals that the irradiation spectra have actually much higher angular variations in T₁−B₁ than the natural spectra, but somewhat less in B₂ (see Table 1). The T₁−B₁ region is generally narrower in the irradiation spectra, particularly in the X and Y-configuration.

Fig. 4 shows the results of the fitting of the anisotropic components of the irradiation spectra in Z-configuration. The quality of the simulation is shown in the stack of the residuals (Fig. 4C). They reach a maximum of 7% in the Z-configuration and an average of 4.2%, while in Y and X the maxima are 4.7% and 5.1% and the averages 3.2% and 3.1%, respectively. These values are somewhat higher than for the natural spectra, which had maxima of 3.5%, 4.1% and 3.2% and averages of 2.9%, 3.3% and 2.8% in X, Y and Z configurations, respectively. This is not entirely surprising since extracting the irradiation spectrum includes subtracting the natural spectrum, which may create alignment problems.

The middle row in Fig. 4 shows the intensity and line-width changes for the four Gaussian components and the lower row examples of the signal intensity along with the plot of radical concentrations. The results for the decomposition of the anisotropic components of the irradiation spectra are tabulated in Table 2. For comparison, the data of the natural spectra are shown in Table 3. The lower row in Fig. 4 shows some selected fitting examples along with the angular changes in the radical concentrations. For the comparison between the natural and irradiation components, this plot is the most informative. All measurements are summarised in Fig. 5.

All fitted components show higher angular variability in the irradiation component than in the natural for all configurations. The general angular patterns for R₁, R₂ and B₂ are approximately the same in the natural and irradiation components, i.e. their maxima and minima occur at approximately the same angles. However, there is a significant change in B₁. The angular behaviour shifts by 90° in X and Y-configuration and to a 90° symmetry in Z. This is however, the exact behaviour of R₃. This could indicate that the original component R₃ is either too small to be distinguishable from R₁ or that it does not exist at all. As pointed out before, the problem with R₁ is that it actually only approximates a Gaussian line. As a result, it would not be surprising that the prescription of an additional line could create fitting artefacts. The same does not apply to the decomposition of the natural spectra, where R₂ has an inverse behaviour to R₁ (see Fig. 5) and the region of T₁−B₁ cannot be fitted without the introduction of R₃. The X-configuration clearly shows that the radical concentration in R₃ has an effect on B₂, because angular response (or the lack thereof in X-configuration) of B₂ cannot be explained just through the angular behaviour of R₁, because B₂ would have to show an inverse concentration relationship to R₁.

To check whether R₂ can be omitted altogether, the anisotropic components of the irradiation spectra were fitted with only three components. The residuals have maximum deviations of 6.1% in the Z-configuration and 5.4% and 4.9% in Y and X, while the respective averages are 3.9%, 3.3% and 3.4%. Overall, these approximations to the measured spectra are virtually the same as for 4 Gaussian lines, which means that the introduction of an additional component had no influence on the quality of the fitting results, a first indication that R₂ does not exist as an independent component.

The results for the Z-configuration are shown in Fig. 6. While the results for R₁ are virtually unchanged, B₂ shows systematic g-value shifts (see lower row in Fig. 6). These may be the result of the pronounced 90° symmetry of R₂. Comparing the two fitting runs (Fig. 7), the angular response and radical concentrations of R₁ are virtually unchanged in all three configurations. There are some small differences in the angular behaviour of B₂ in X-configuration, but remains nearly unchanged in Y and Z. The main difference occurs for R₃ in Z-configuration, where the use of 4 Gaussian lines seemed to have split the angular behaviour into a highly anisotropic component (R₃) and less anisotropic (R₂).

In contrast to the decomposition of the natural spectra, where the use of only three Gaussian components lead to a significant and unrealistic widening of R₁ and B₂ (Joannes-Boyau et al., submitted for publication), the fitting of the irradiation spectra with three components has a negligible effect on the line widths of R₁ and B₂ (compare Tables 2 and 4) and.

Considering that (i) the introduction of a new component does not provide a better fit to the measured spectra, (ii) the angular behaviour of R₂ observed in the natural sample does

<table>
<thead>
<tr>
<th>Table 1 Angular variations.</th>
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<tr>
<td></td>
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<tr>
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<tr>
<td>Z</td>
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<tr>
<td>Y</td>
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<tr>
<td>X</td>
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<tr>
<td>Natural+γ</td>
</tr>
<tr>
<td>Z</td>
</tr>
<tr>
<td>Y</td>
</tr>
<tr>
<td>X</td>
</tr>
<tr>
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<tr>
<td>Z</td>
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<tr>
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<td>Z</td>
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<td>Y</td>
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<tr>
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<td>X</td>
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</table>
not occur in the irradiation spectra, and (iii) the line widths of $R_1$ and $B_2$ hardly change, one can only deduce that either the natural $R_2$ component does not exist in the irradiation spectra at all or that it is so small that the fitting approach cannot identify it.

Joannes-Boyau et al., (submitted for publication) assessed uncertainties by running SA simulations several times on the same spectra with randomly chosen values for the initial parameters. Differences in the range 1–2% were observed for the estimation of intensities, and 4–5% for radical concentrations.
Table 2: Results of the decomposition of the anisotropic components in the irradiation spectra with four components.

<table>
<thead>
<tr>
<th>Minimum g-value angle</th>
<th>Maximum g-value angle</th>
<th>Minimum width (mT)</th>
<th>Maximum width (mT)</th>
<th>Minimum radical conc. angle</th>
<th>Maximum radical conc. angle</th>
<th>Angular variation</th>
<th>Average radical conc.</th>
<th>Relative radical conc. (%)</th>
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<tr>
<td>Z-configuration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R₁ 2.0025</td>
<td>0.0205</td>
<td>279</td>
<td>0.31</td>
<td>0.41</td>
<td>240</td>
<td>0.24</td>
<td>80</td>
<td>0.72</td>
</tr>
<tr>
<td>R₂ 2.0014</td>
<td>0.0205</td>
<td>270</td>
<td>0.51</td>
<td>0.54</td>
<td>20</td>
<td>0.20</td>
<td>0.02</td>
<td>140</td>
</tr>
<tr>
<td>R₃ 2.0002</td>
<td>0.0210</td>
<td>150</td>
<td>0.20</td>
<td>0.45</td>
<td>40</td>
<td>0.02</td>
<td>0.02</td>
<td>110</td>
</tr>
<tr>
<td>R₄ 1.9989</td>
<td>2.0027</td>
<td>150</td>
<td>0.20</td>
<td>0.45</td>
<td>40</td>
<td>0.02</td>
<td>0.02</td>
<td>110</td>
</tr>
</tbody>
</table>

Table 3: Results of the decomposition of the anisotropic components in the natural spectra (from Joannes-Boyau et al. submitted for publication).

<table>
<thead>
<tr>
<th>Minimum g-value angle</th>
<th>Maximum g-value angle</th>
<th>Minimum width (mT)</th>
<th>Maximum width (mT)</th>
<th>Minimum radical conc. angle</th>
<th>Maximum radical conc. angle</th>
<th>Angular variation</th>
<th>Average radical conc.</th>
<th>Average radical conc.</th>
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<tr>
<td>R₁ 2.0025</td>
<td>0.0205</td>
<td>279</td>
<td>0.31</td>
<td>0.41</td>
<td>240</td>
<td>0.24</td>
<td>80</td>
<td>0.72</td>
</tr>
<tr>
<td>R₂ 2.0014</td>
<td>0.0205</td>
<td>270</td>
<td>0.51</td>
<td>0.54</td>
<td>20</td>
<td>0.20</td>
<td>0.02</td>
<td>140</td>
</tr>
<tr>
<td>R₃ 2.0003</td>
<td>0.0206</td>
<td>200</td>
<td>0.20</td>
<td>0.45</td>
<td>40</td>
<td>0.02</td>
<td>0.02</td>
<td>110</td>
</tr>
<tr>
<td>R₄ 1.9982</td>
<td>0.0208</td>
<td>270</td>
<td>0.20</td>
<td>0.45</td>
<td>40</td>
<td>0.02</td>
<td>0.02</td>
<td>110</td>
</tr>
</tbody>
</table>

keeping in mind that the latter is based on intensity of line and width squared. For a second assessment of the uncertainties in the simulation, the decomposition was carried out on the irradiated spectra that had only the NOCORs and the isotropic lines subtracted (i.e. natural plus gamma minus isotropic lines). This allows the comparison of the behaviour of Gaussian components from the decomposition of the (natural) irradiated spectra with the combined, separate results of the natural and irradiation components (Fig. 8). Normalising on the combined averages of the components, deviations between 3.4% and 8.5% are observed. Using three Gaussian components, a somewhat better agreement is achieved for R₁ (6.1%) and R₂ (6.2%). The latter is probably caused by the given g-value changes in the fitting of the irradiation spectra (see Fig. 6F). However, the major features, such as the angles of the maxima and minima, or the symmetry (180° versus 90°) remain unchanged.

Our decompositions show that there is a profound difference between the natural and irradiation spectra in fossil tooth enamel:

- the natural spectra contain a 9% component of NOCORs, the irradiation spectra 39%;
- the methyl line and other isotropic lines which occur in the natural spectra are not irradiation sensitive;
- the irradiation spectra contain three additional wide lines;
- the angular variation of all fitted components is significantly higher in the irradiation spectra than the natural;
- the natural spectra have a R₁/R₂ ratio of 35:65, the irradiation spectra do not contain an identifiable component that shows an angular behaviour as R₂ in the natural spectra.

The observations on the various isotropic lines have been reported by many authors. The occurrence of two different AICORs in fossil tooth enamel was described in the two previous studies of Joannes-Boyau and Grün (2009) and Joannes-Boyau et al., (submitted for publication) and is supported by Q-band studies on fossil enamel fragments (Bouchet et al., 1988, Rossi and Poupeau, 1990). The Q-band experiments also demonstrated that axial and orthorhombic CO₂ radicals had different orientations and that the orthorhombic radical was less stable than the axial. Vorona et al. (2006) suggested that in modern teeth orthorhombic radicals could convert into axial by heating.

The higher angular variations of the irradiation spectrum are most probably the result that only one anisotropic radical is
Fig. 5. Summary of the angular behaviour of the anisotropic components of the natural spectra as well as the irradiation spectra decomposed with four Gaussian components.
present. The overlapping of two radicals in the same region, particularly when having somewhat different orientation will always lead to a muting of angular response. The observed increase in angular variability after heating was tentatively attributed by Joannes-Boyau and Grün (2009) to a higher crystalinity. It seems, however, that rather than changing the crystal properties, heating reduces the mix of different CO\textsubscript{2} radicals, as a consequence leading to higher angular variabilities.

It is reasonable to assume that low dose rate natural radiation generates a similar mix of radicals as high dose rate laboratory irradiation. However, the geological aging of the sample incurs various annealing and transfer processes. Clearly, a large proportion of the NOCORs disappear, while virtually all \( R_2 \) (axial) components appear. At this stage, it is not possible to speculate whether NOCORs, \( R_1 \) radicals, or both lead to the formation of \( R_3 \) over time. It is also not clear whether there are transfers between the isotropic components and the various CO\textsubscript{2} radicals. The understanding of the formation and transfer processes that lead to the observed mix of the CO\textsubscript{2} radicals in fossil tooth enamel is essential for the reliable application of ESR dating.

If the kinetics of the decay of orthorhombic CO\textsubscript{2} radicals and formation of the axial were known, their ratio could be used for rough age assessments. While this will not approximate the precision of conventional ESR dating, it may be helpful in cases their dose rate assessments are impossible, e.g. when working on archaeological collections.

\( R_3 \) is a problematic component and its nature remains unclear. \( R_3 \) is needed for minimizing the angular dependency of the sum of all fitted components. The behaviour of \( R_3 \) indicates that \( R_3 \) is a component related to the ALCORs (see Fig. 5, Xconfiguration). Keeping in mind that enamel fragments are partially ordered systems with some preferential direction of the hydroxyapatite crystals, \( R_3 \) can be designated as a heterogeneous signal composed by misalignments of \( R_1 \) in the irradiation spectra and \( R_1 \) and \( R_2 \) in the natural. \( R_3 \) would then consist of a semi-infinite series (in the case of discrete values (1.1)) of Gaussian components with varying amplitudes between the \( T_1 \) and \( T_2 \) positions and can be described by

\[
f(x) = \sum_{\mu} A(\mu) \left( \frac{\mu - x}{\sigma^2} \right)^{n/2} e^{-\frac{(\mu - x)^2}{2\sigma^2}} d\mu,
\]

with \( a \) and \( b \) the positions of \( T_1 \) and \( T_2 \), \( \mu \) and \( \sigma \) are the centre and the width of each Gaussian lines, respectively. \( A(\mu) \) is the amplitude function of each integrated Gaussian lines.
Both $\chi$ and $\mu$ are independent spatial variables on the $g$-value axis. Eq. (1.1) gives the intensity of $R_1$ at any $\chi$ value that the variable can take along the $g$-value axis. For a given $\chi$, $f(\chi)$ is the sum of all the values taken by Gaussian functions (with different $\mu$ centres) at this specific $\chi$ position. A part of this equation is known as the error function (erf). This erf function has an influence on the line shape. For example, if $A(\mu)$ is a linear function, $f(\chi)$ may be approximated by a Gaussian function if the magnetic field is located between $T_1$ and $B_2$. In future simulations, we will endeavor to add this function to our simulation program.

5. Conclusions

This study shows that the radiation response of fossil tooth enamel is significantly more complicated than previously thought. Apart from a very high proportion of AlCOX, the irradiation spectra do not contain any identifiable axial CO$_2$ component, which, on the other hand, dominates the natural spectra. Keeping in mind that various ESR dating attempts have been accompanied with some degree of success, it seems that most of the radicals are converted from one type to another without much reducing the overall radical concentration. To enhance our understanding of potential...
Table 4
Results of the decomposition of the anisotropic components in the irradiated spectra with three components.

<table>
<thead>
<tr>
<th>Minimum g-value angle</th>
<th>Maximum g-value angle</th>
<th>Minimum width (mT) angle</th>
<th>Maximum width (mT) angle</th>
<th>Minimum radical conc. angle</th>
<th>Maximum radical conc. angle</th>
<th>Angular variation</th>
<th>Average radical conc.</th>
<th>Relative radical conc. (%)</th>
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<tr>
<td>R₁ 2.0023 210</td>
<td>2.0031 120</td>
<td>0.32 340</td>
<td>0.44 240</td>
<td>0.37 110</td>
<td>0.90 180</td>
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<td>0.62</td>
<td>0.53</td>
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<td>0.30 260</td>
<td>0.44 210</td>
<td>0.27 190</td>
<td>0.82 240</td>
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<td>0.28 160</td>
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</table>

Fig. 8. Comparison of angular components from the decomposition of the natural+irradiation spectra and the sum of the separate decompositions of natural and irradiation spectra.

transfer processes occurring between radicals, an upcoming kinetics study appears critically required now.

Acknowledgments

We thank N. Manson, Research School of Physical Sciences, ANU, for helpful comments. We are grateful to F. Caliens and Henk Vrielinck, Gent, for their thorough advice in the earlier stages of this study. RG is grateful to the Institut des Sciences humaines et sociales du CNRS, Bordeaux, and the Laboratoire d’Anthropologie des populations du Passé, Université de Bordeaux I, for their kind hospitality in the writing-up stage of this manuscript. Aspects of this study were supported by ARC DP0664144 Microanalysis of human fossils: new insights into age, diet and migration.
References


CHAPTER 12

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CHAPTER 12
Decomposition of UV induced ESR spectra in modern and fossil dental enamel fragments

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(Received 11 December 2009; in final form 22 April 2010)

Abstract
Using an automated simulated annealing (SA) procedure, spectrum decomposition on angular measurements of tooth enamel fragments shows that UV irradiation of modern human and fossil bovid samples results in distinctively different ESR spectra. In the fossil sample, UV irradiation generates qualitatively identical spectra to natural. The amounts of non-oriented CO₂ radicals in the modern and fossil samples are about 35% and 9%, respectively. The two oriented CO₂ radicals, R1 and R2, attributed to orthorhombic and axial types, show a ratio of 64:36 in the modern and 34:66 in the fossil sample. R1 is also observed in the natural fossil sample, while the axial type was either absent or too small to be identified in a γ-irradiated fossil sample. We could not observe a measurable UV induced signal after 7 months of sunlight and laboratory light exposure, respectively. The clear difference between γ and UV induced signal raises the possibility of using UV lights for dating protocols.

Introduction
Radiation induced signals in tooth enamel can be used for the evaluation of past radiation doses in retrospective dosimetry (e.g. Ikeya et al. 1984; Romanyukha et al. 1994), and dating (e.g. Grün, 1989, Grün et al. 2008). These two application areas differ with respect to the dose ranges evaluated and the age of the materials. While retrospective dosimetry deals with a dose range from a few mGy to 5 or 10 Gy on modern teeth, dating deals with old teeth (usually between several thousand and up to several million years old) and a dose range from a few Gy to several thousands of Gy. The radiation induced signals in fossil teeth are qualitatively different from those of modern teeth as any unstable signals in fossil teeth have partly or completely faded over geological times.

Previous studies suggested that the main ESR signal generated by gamma radiation can be attributed to two categories of CO₂ radicals, one anisotropic (AICOR) and the other with no preferential orientation, also called non-oriented CO₂ radicals (NOCOR) (e.g. Callens et al. 1995; Brik et al. 2000; Ishenko et al. 2002; Grün et al. 2008; Joannes-Boyau and Grün, 2009). In fossil teeth it was found that 2% of NOCORs were present in the natural sample, while 40% were present in the laboratory γ-irradiation component (Joannes-Boyau et al. in press, submitted). Those values differ from modern teeth, which have up to 80% NOCORs after γ-irradiation (Vorona et al. 2007; Rudko et al. 2007). However, the calculation method used for these studies differs from ours which could lead to systematic errors. Two studies by Joannes-Boyau et al. (in press, submitted) demonstrated that two types of AICORS (axial and orthorhombic) contribute to the ESR signal of fossil tooth enamel. In the γ-induced spectra, the axial form (R2) was undetected (either absent or negligible) and only the orthorhombic form (R1) contributed to the anisotropic ESR spectral components (Joannes-Boyau et al. in press).

Studies on retrospective dosimetry suggested that UV may contribute significantly to the overall ESR intensity (Lidja et al. 1996; Nilsson, 2001; El-Faramawy 2005). Brik et al. (1998) and Vorona et al. (2007) showed that UV induced spectra in modern teeth contained significantly less NOCORs than those by γ-irradiation. However, the relative depletion of NOCORs could have been the result of a combination of UV radiation and heating during the experiment. UV exposure is usually associated with significant heating but it is not known whether samples were cooled during these experiments. The occurrence of methyl radicals in the ESR spectra of Nilsson (2001) indicates a possible thermal influence, but these radicals could have been induced by UV irradiation itself.

The aim of the present study was to assess the influence of UV light on fossil teeth enamel, focusing particularly on dose estimation.
T1-B1 and B2, it was not possible to carry out double integrations; their angular variations were derived from their intensities (for more details see Joannes-Boyau et al., in press, submitted).

Both fragments H2 and MH were each mounted in three separate Teflon holders containing a Parafilm mould and were incrementally measured by rotating them around their three major axes. We used the following configurations: X: rotation around the axis perpendicular to the dentine-enamel junction, Y: around the axis of tooth growth and Z: perpendicular to X and Y (Fig. 1). The sample holders were inserted in a Bruker ER 218PG1 programmable goniometer and measured with a Bruker Elexys E500 ESR spectrometer in 10° increments over 360° with the following measurement conditions: 2 mW microwave power, 0.1 mT modulation amplitude 12 mT sweep width with a sweep time of 21 s. The spectra were accumulated over 50 consecutive measurements. The sample was measured before and after irradiation.

The fragments were exposed to UV light for 168 hours using a Hoenel UVASpot 400T lamp emitting UVA/B at 400 W producing incident energy of 5.0±0.1 mW cm² at the sample location. The temperature was recorded in 2 min intervals with a thermobg controller near the surface and at the base of the fragments, located near the cooling plate. The measured temperatures were 21±2°C at the base of the sample and 33±2°C at the top. The γ-irradiation was carried out with a 137Cs-source for 100 min, which corresponds to an approximate dose of 187 Gy (the experiment is described in Joannes-Boyau et al., in press).

**Extraction of the isotropic components of the UV induced spectra**

The first step in spectra decomposition of H2 consists of subtracting the natural signal from the UV irradiated spectra. The alignment of the two spectra is the key to avoid artefact signals, for that matter the isotropic methyl lines are used as markers for alignment. Previous studies by Joannes-Boyau et al. (in press, submitted) have shown that non-CO₂ components are found in the natural signal and in the irradiated signal (Fig. 2a). Those signals described as isotropic lines have to be removed before decomposing the ESR spectrum at each angle. In the fossil sample, the isotropic lines in the UV-induced spectra were found to have a similar signature as in the natural (combined into one signal, named Isotopic Combined signal (IC)) (Fig. 3), but increased with UV exposure. No methyl line was created by UV exposure. A wide line, W₂, at g=2.0051 (see Joannes-Boyau et al. submitted),
Figure 2: Decomposition of the measured spectra (for more details see Joannes-Boyau et al. in press). A: (Top) Isotropic lines in the natural spectra. A simulated signal is fitted into the natural measured spectra to extract the isotropic component (IC is the residual offset for clarity). (Bottom) Position of the isotropic components that when merged form the isocombined spectra (IC). B: (Top) Comparison of the measured and simulated spectra. The residual is offset for clarity and corresponds to the subtraction of the simulated spectra from the measured. (Bottom) Decomposition of the measured spectra using the SA decomposition with four Gaussian components representing the anisotropic lines R₁, R₂, R₃, and R₄.

Figure 3: Comparison of the isotropic signal found in the modern and fossil tooth natural spectra IC (from Joannes-Boyau et al., submitted) (a), fossil tooth UV irradiation spectra (b), modern human tooth UV irradiation spectra (c) and modern human tooth natural spectra (d) respectively. The symbol S indicates the unknown isotropic line that appears with UV exposure described in the text with a g-value around g=2.0093.

Figure 4: Comparison of the influence of different exposure on the merged ESR signal of modern human tooth enamel fragment. (a) Native signal N.S.; (b) 7 months laboratory lights (c) 7 months sunlight exposure (d) 168 hours UV lamp exposure.
attributed to a combination of \( \text{SO}_2^- \) and \( \text{CO}^+ \) radicals, increased. \( \text{SO}_2^- \) - radicals which occur at \( g=2.0056 \) are most likely responsible for this increase (Bouchez et al. 1988; Schramm and Rossi 1999, see also the fitting of Grün 2002). The \( \text{SO}_2^- \) radical is known to be temperature sensitive. It is likely that the \( \text{SO}_2^- \) radicals were created at the surface of the sample where the temperatures were slightly higher due to the UV exposure. A new isotropic line (S) at \( g=2.0093 \), with a line width of around 0.1 mT (Fig. 3) was created by UV radiation. This new, unknown component does not interfere with the main signal and fades rapidly (its intensity is negligible after three months storage at ambient temperature). The NOCORs were removed using the same amount found in the natural signal corresponding to 9% of the total intensity. After subtracting all aforementioned components (natural, isotropic lines and NOCORs), only UV-induced anisotropic components remain.

**Spectrum Decomposition**

The anisotropic components of the irradiation spectra were decomposed with an automated simulated annealing (SA) procedure which is particularly well suited to separating overlapping signals (Fig. 2b) (for more details see Joannes-Boyau et al. in press, submitted). SA is a Monte Carlo method used for combinatorial optimisation problems (for details see Metropolis et al. 1953; Kirkpatrick et al. 1983; Černý 1985; Mossegard and Sambridge 2002; Bodin and Sambridge, 2009). The spectra were decomposed with four Gaussian lines which had the same prescribed \( g \)-value range limits used in the previous study to avoid unrealistic solutions outside the regions for the \( \text{CO}_2^- \) radicals in hydroxyapatite (see above). No restrictions were set on the intensity although the line widths were kept between 0.10 mT and 2 mT to avoid aberrations. Our SA procedure is able to randomly generate a large number of synthetic spectra defined by a linear combination of four Gaussian lines. Each simulated spectra is compared to the measured spectra in terms of a least square misfit.

**Results and discussion**

The modern human tooth (MH) contains a wide, isotropic signal centred at \( 2.0044 \pm 0.0005 \) prior to any irradiation, also called the native signal (NS) (Ljdić et al. 1996; see Figs. 3 and 4). Due to the intensity and width of the signal compared to the background, the measured \( g \)-value is not very precise. The spectra confirm that the enamel fragment was not exposed to any measurable ionising radiation. The ESR spectra after 168 hours UV exposure is qualitatively different from the natural, as the shapes of the two spectra clearly differ (Fig. 4).

**Figure 5.** Angular variation of ESR signals induced by UV exposure in a modern human fossil tooth enamel fragment. Summary of all decomposition results of the radical concentration (obtained by double integration) variation over 360° (a) \( X \)-configuration, (b) \( Y \)-configuration and (c) \( Z \)-configuration of \( R_1, R_2, R_3 \) and \( B_2 \) component. Note that on the \( Z \)-configuration \( R_3 \) shows a 90° symmetry.
Table 1: Results of the decomposition of the anisotropic components of the modern tooth after UV-irradiation

The native signal grows slightly with irradiation, at the same time that CO$_2$ radicals appear in the spectra. Subtracting the native signal (multiplied by a factor 1.2) plus an isotropic component (IC MH in Fig. 3) which is similar to the one used in the natural sample from Holon (IC Natural, see Joannes-Boyau et al. submitted), yields typical CO$_2$ radicals. The amount of NOCORs is around 35%.

Fig. 5 and Table 1 summarise the angular response of the four UV generated Gaussian components in MH. We observe an overall $R_1$/$R_2$ ratio of 62.3. The maxima and minima of $R_1$ and $R_2$ in the various configurations are offset by 0°, 0° and -30° in X, Y and Z-configuration, respectively, indicating different orientations of the radicals within the crystal structure. Similar values were found by Joannes-Boyau and Grün (2009) in the sample from Holon with offsets of -26°, 7° and -5° ± 10° in X, Y and Z-configurations, respectively. The angular differences between $R_1$ and $R_3$ of the two teeth point to differences in the tooth formation of the two species.

The results obtained on MH are similar to those described in the literature (e.g. Cailes et al. 1995, 1998, Lidija et al. 1996; Nilsson et al. 2001; El-Fararawy 2005; Vorona et al. 2007; Rudko et al. 2007). The exception is that no methyl radicals were found in our measurements, contrary to Nilsson et al. (2001). This makes us confident that temperature was well controlled by the cooling plate used in the present study and that the anisotropic signal extracted from the fossil tooth is UV rather than temperature induced. After 3 months, the UV induced spectra show major changes. The NOCORs have faded by 15% of their intensity but still represent 30% of the initial spectra. The T1-B1 region is slightly shifted to higher g-values and shows a depletion of 3 to 5% of its original signal. The ratio between $R_1$/$R_2$ has changed to 59:41 respectively. The ratio disparity after 3 month could be attributed to the disappearance of some of the $R_1$ species and at the same time the appearance of new $R_2$ radicals. However, because of the small amount of variation (between 3 to 5%), we cannot conclude that any transfer process took place within the 3 months.

The fossil bovid tooth

In Z-configuration the angular variations of the natural and UV spectra show little difference while those of the y-spectra are significantly muted (Fig. 6). This can be attributed to the much higher contribution of the NOCORs in the y-component (around 40%) compared to the natural (around 9%, see Joannes-Boyau et al. in press). This also implies that the relative distributions of the NOCORs in the natural and UV components are approximately the same; otherwise the angular variation would change dramatically such as the y-induced variation, since NOCORs remains constant at all angles (see Joannes-Boyau et al., in press).

Fig. 7 compares a natural spectrum to those of the y- and UV components. While it is not possible to scale the natural spectrum to fit the y-component (Fig. 7a), due to a distinctly different distribution of AICORs (Joannes-Boyau et al in press), the natural spectrum can be easily scaled into the UV component (Fig. 7b). This implies a closely similar distribution of the different types of CO$_2$ radicals in the natural and UV components. Scaling the natural spectrum by a factor of 1.75 into the natural + UV, leaves small residuals with signal intensities of 5.6%, 5.0% and 4.4% in X, Y and Z configuration, respectively (Fig. 8). These residuals are approximately the same as when using multi-component decompositions of spectral components (Joannes-Boyau et al. in press, submitted).
Figure 6: Angular variation of the natural, UV induced and γ-induced spectra in Z-configuration on fossil tooth enamel fragments.

Table 2: Angular variation

<table>
<thead>
<tr>
<th></th>
<th>T1-B1</th>
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<td>X</td>
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</tr>
<tr>
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<td></td>
<td>X</td>
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<tr>
<td></td>
<td>X</td>
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</table>

Fig. 9 shows the stacks of all spectra of the anisotropic components of the natural sample as well as of the UV and γ-induced (see also Tables 2 to 4). The γ-irradiation stacks, especially the Y-configuration, show a very different pattern to the corresponding UV stacks (Fig. 8, middle column, rows B and C). The T1-B1 complex is significantly narrower in the γ-irradiation spectra than in the other two. The angular variations of the T1-B1 and B2 positions (marked in Fig. 6) of the γ-irradiation spectra are significantly more pronounced in all configurations than of the natural and UV spectra (Table 5). At the same time, it is unclear why UV exposure induces a smaller amount of NOCORs than γ-irradiation. Perhaps this is due to the energy difference between UV emissions (in the range of 3 to 10 eV) and γ rays (>600 keV). So far no explanation can be clearly proposed, however, local saturation of radicals could induce significant differences in the signals.

Fig. 10 and Tables 2 to 4 summarise the results of the decomposition. Note that the R2 component was not identifiable in the γ-irradiation spectra (Joannes-Boyau et al. in press) while it is present in both natural and UV spectra. The g-values of the four components are in a similar range and do not show any significant shifts for the natural and UV irradiated signals. Their line widths appear slightly narrower in UV than in the natural, but this parameter always shows the largest deviations in repeated SA runs (Joannes-Boyau et al. submitted). The average and relative radical concentrations of natural spectra.
Figure 8: Stacks of fossil bovid enamel natural spectra multiplied by a factor of 1.75 (row A), natural + UV irradiation (row B) and residual from the subtraction of the natural (x1.75) from the natural + UV irradiation (row C) for the three configurations X, Y and Z, column 1, 2 and 3 respectively.
Figure 9: Stacks of fossil bovid enamel: natural spectra (row A), natural anisotropic (row B), natural + UV irradiation (row C), UV exposure anisotropic spectra (row D) and γ-irradiation anisotropic spectra (row E) for the three configurations X, Y and Z, column 1, 2 and 3 respectively.
Figure 10: Summary of the angular behaviour of the anisotropic components of the natural spectra as well as the UV irradiation spectra decomposed with four Gaussian components and with the γ-irradiation spectra decomposed with three Gaussian components (see Joanes-Boyau et al., in press).
Table 3: Results of the decomposition of the anisotropic components in the UV spectra

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<tr>
<th>Minimum g-value</th>
<th>Angle (°)</th>
<th>Maximum g-value</th>
<th>Angle (°)</th>
<th>Minimum width (mT)</th>
<th>Angle (°)</th>
<th>Maximum width (mT)</th>
<th>Angle (°)</th>
<th>Angle (°)</th>
<th>Minimum radical conc.</th>
<th>Angle (°)</th>
<th>Maximum radical conc.</th>
<th>Angle (°)</th>
<th>Angle Variation</th>
<th>Average radical conc.</th>
<th>Relative radical conc. (%)</th>
</tr>
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<td>70</td>
<td>2.0029</td>
<td>250</td>
<td>0.26</td>
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<td>0.32</td>
<td>50</td>
<td>0.14</td>
<td>30</td>
<td>0.33</td>
<td>170</td>
<td>0.64</td>
<td>0.22</td>
<td>14.6</td>
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<td>2.0024</td>
<td>240</td>
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<td>300</td>
<td>0.31</td>
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<td>0.55</td>
<td>180</td>
<td>0.31</td>
<td>0.46</td>
<td>30.6</td>
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<td>R2 2.0005</td>
<td>120</td>
<td>2.0006</td>
<td>300</td>
<td>0.39</td>
<td>100</td>
<td>0.51</td>
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<td>0.47</td>
<td>150</td>
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<td>0.55</td>
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</tr>
<tr>
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<td>330</td>
<td>1.9989</td>
<td>250</td>
<td>0.29</td>
<td>210</td>
<td>0.42</td>
<td>80</td>
<td>0.17</td>
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<td>0.40</td>
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<td>0.28</td>
<td>18.7</td>
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</tbody>
</table>

Table 4: Results of the decomposition of the anisotropic components in the natural spectra

<table>
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<th>Angle (°)</th>
<th>Maximum g-value</th>
<th>Angle (°)</th>
<th>Minimum width (mT)</th>
<th>Angle (°)</th>
<th>Maximum width (mT)</th>
<th>Angle (°)</th>
<th>Angle (°)</th>
<th>Minimum radical conc.</th>
<th>Angle (°)</th>
<th>Maximum radical conc.</th>
<th>Angle (°)</th>
<th>Angle Variation</th>
<th>Average radical conc.</th>
<th>Relative radical conc. (%)</th>
</tr>
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<tbody>
<tr>
<td>R1 2.0026</td>
<td>20</td>
<td>2.0029</td>
<td>300</td>
<td>0.26</td>
<td>0</td>
<td>0.33</td>
<td>110</td>
<td>0.14</td>
<td>120</td>
<td>0.29</td>
<td>190</td>
<td>0.62</td>
<td>0.23</td>
<td>13.9</td>
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<tr>
<td>R6 2.0018</td>
<td>220</td>
<td>2.0023</td>
<td>250</td>
<td>0.36</td>
<td>220</td>
<td>0.39</td>
<td>100</td>
<td>0.56</td>
<td>110</td>
<td>0.59</td>
<td>200</td>
<td>0.60</td>
<td>0.47</td>
<td>31.5</td>
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</tr>
<tr>
<td>R2 2.0004</td>
<td>280</td>
<td>2.0010</td>
<td>10</td>
<td>0.60</td>
<td>180</td>
<td>0.51</td>
<td>240</td>
<td>0.48</td>
<td>10</td>
<td>0.72</td>
<td>250</td>
<td>0.62</td>
<td>0.57</td>
<td>38.1</td>
<td></td>
</tr>
<tr>
<td>B1 1.9984</td>
<td>270</td>
<td>1.9989</td>
<td>150</td>
<td>0.31</td>
<td>200</td>
<td>0.42</td>
<td>310</td>
<td>0.16</td>
<td>190</td>
<td>0.34</td>
<td>290</td>
<td>0.62</td>
<td>0.25</td>
<td>16.5</td>
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are somewhat different to the UV induced (compare Table 2 with 3). The angular variations for all components of UV spectra were slightly higher than the natural, but were smaller than the γ-irradiation spectra (compare Tables 2, 3 and 4). This clearly implies that UV radiation causes a significantly different radical distribution in the enamel than γ-irradiation. The fading observed on the UV irradiated sample for modern human tooth after three months is not as evident for the fossil bovid tooth. The difference in signal intensity between the last UV exposure and the fading test conducted 3 months later is in the range of 3 to 4% of the total intensity. Prima facie, it appears that the diminution is induced by the fading of some NCOs. However, the spectra depletion falls into the error range and therefore does not allow any affirmation.

Average R1/R2 ratios in natural and UV components are different in the three configurations (compare Tables 2 and 3). Nonetheless, when normalising on the total radical concentration, R2/R3 is virtually the same as the UV ratio (34.66).

Based on the ESR intensity of the fragment, we have estimated that the UV lamp is the γ-equivalent of 3.1 ± 0.3 mg/kg/min. Since the natural and UV spectra show closely similar radical distributions (in contrast to γ-irradiation), UV irradiation could be more suitable than γ-irradiation for the establishment of dose response curves in ESR dating. While fading, unknown intensity attenuation and energy calibration will complicate this approach at the present time, systematic correlations between UV exposure and the equivalent dose from γ-irradiation could lead to the use of photons instead of γ-rays for dating purposes. Further, studies should be undertaken on the effect of UV on the equivalent dose (DE) assessment, however, because UV irradiation is a very slow process, local saturation could happen during exposure. For that reason, a specific protocol should be designed to measure the sample while irradiating, which would complicate the experiment greatly.

The high radical concentrations induced by UV raise the possibility that sunlight or laboratory light induced radicals may interfere with dose estimations.
However, intermittent exposure to daylight for more than 50 years while laughing about ones own bad jokes did not induce any measurable CO\textsubscript{2} radicals in MH1, neither did exposure to indirect sunlight or laboratory light over 7 months in the fossil sample (Fig. 4). Samples are normally shielded behind window glass, known to block UV, and it is therefore unlikely that archaeological samples are exposed to direct UV sunlight over extended periods of time.

**Summary**

UV and γ-irradiation induce very different compositions of CO\textsubscript{2} radicals in tooth enamel. In a modern sample, UV generated 35% of NOCORs and a mix of 64:36 of orthorhombic to axial radicals. In the fossil sample, UV generated 9% NOCORs and a mix of 34:66 of orthorhombic to axial radicals. While there are some differences between the natural and UV components in the various configurations, the overall radical distribution of the UV and the natural is the same. This is in contrast to γ-irradiation component of the fossil sample, which had about 40% AICORs and no axial radicals. While the UV components in the modern samples showed strong fading over three months (18 to 20% of the spectra) with possible transfer process between R\textsubscript{1} and R\textsubscript{2}, the fading in the fossil sample was small (3 to 4%).

Blocked sunlight and laboratory light exposure over 7 months had no measurable influence on the samples.

**Conclusions**

Like γ-irradiation, UV irradiation induces significant differences between modern and fossil samples. While this change must have an impact on dose estimations, this aspect has never been systematically investigated in ESR dating. In the fossil sample, UV generates a similar mixture of CO\textsubscript{2} radicals as found in the natural while the γ-irradiation response is completely different. This could make UV irradiation the choice for the establishment of dose response curves.

**Acknowledgements**

We are very grateful to T. Bodin, Research School of Earth Sciences, Australian National University (ANU), Canberra, for helping with the design of the simulating annealing program used for this work. We thank N. Manson, Research School of Physical Sciences, ANU, for helpful comments. We are grateful to F. Callens and H. Vrielinck, Gent, for their considered advice in the earlier stages of this study. Aspects of this study were supported by the ARC funded DP0664144 Microanalysis of human fossils: new insights into age, diet and migration. We thank Kathryn Fitzsimmons, RSES, for critical comments.

RG is grateful to the Institut des Sciences humaines et sociales du CNRS, Bordeaux, and the Laboratoire d'Anthropologie des populations du Passe, Université de Bordeaux I, for their kind hospitality in the writing-up stage of this manuscript. RJB and RG would like to thank Jean-Jacques Bahain at the Institut de Paléontologie Humaine for reviewing the paper in such a short period of time.

**References**


Joannes-Boyau, R., Bodin, T., Grün, R. (submitted) Decomposition of the angular ESR spectra of fossil tooth enamel fragments. Radiation Measurements


Reviewer
Jean-Jacques Bahain
CHAPTER 13
Decomposition of beta-ray induced ESR spectra of fossil tooth enamel

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ABSTRACT

Two fossil tooth enamel fragments were irradiated with beta rays, one through the outer surface, the other through the dentine-enamel junction. The angular ESR spectra of the two fragments were decomposed using an automated simulated annealing (SA) procedure, which is particularly well suited to separate overlapping signals.

Beta irradiation generated different qualitative and quantitative responses to previous gamma irradiation experiments. Similar to gamma rays, the beta irradiation created both non-oriented and oriented CO₂ radicals. In contrast to gamma irradiation, which only created orthorhombic oriented CO₂ radicals, both axial and orthorhombic CO₂ radicals were extracted after beta irradiation. Furthermore, gamma irradiation created significantly more non-oriented radicals than beta irradiation. Alongside, the radial distribution created by beta irradiation resembled that of the natural sample, which had been exposed to environmental irradiation over several hundreds of thousands of years. The natural sample contained 4% non-oriented CO₂ radicals and a mixture of orthorhombic to axial CO₂ radicals in the ratio of 35:65. The beta induced spectra of the fragment irradiated through the outer surface contained 9% non-oriented CO₂ radicals and a mixture of orthorhombic to axial CO₂ radicals in the ratio of 45:55, while for the other sample these values were 15% and 50:41, respectively. The angle between the axial and orthorhombic CO₂ radicals is around 231° in both natural and beta irradiation components. This indicates that the radicals produced by the different irradiation modes are located in the same positions in the hydroxyapatite crystals. The higher percentage of non-oriented CO₂ radicals closer to the dentine-enamel junction points to interprismatic zones for their possible location.

1. Introduction

Direct ESR dating of fossil human remains is only carried out on tooth enamel, because all other skeletal tissues, such as bone, dentine and cement, are significantly more geochemically altered during burial than enamel (Ikeya, 1982; Rink, 1997; Grün and Schwarcz, 1987; Grün, 2006). During the burial time, environmental radiation, mainly from K, U and Th decays, generates ESR signals in the enamel. These have mainly been attributed to CO₂ radicals (Vanhaeylewijn et al., 2000), as well as several interfering radicals (methyl, CO₂, CO, CO₂, etc., for a compilation see Callens et al. 1998; Vanhaeylewijn et al., 2000, 2002). The archaeological dose is generated by a mix of a, b particles and g-rays emitted by the above mentioned radioactive isotopes during the burial time, plus cosmic rays (Grün, 1989).

In recent studies on tooth enamel fragments (Joannes-Boyau et al., 2010a), we have found that fossil samples contain three different types of CO₂ radicals: non-oriented CO₂ radicals (NOCORs), giving rise to a powder spectrum at all measurement angles (e.g. Callens et al., 1995; Brik et al., 2000a; Ishchenko et al., 2002), and two anisotropic CO₂ radicals (AICORS)—one most likely orthorhombic and the other axial (Liitja, 2001; Vorona et al., 2005, 2006). While the NOCORs are by definition identical at all angles, the two oriented radicals are strongly anisotropic (Joannes-Boyau et al., 2010a, b). Until the study by Joannes-Boyau et al. (2010b), it was assumed that both oriented radicals would respond equally to thermal treatment and/or irradiation. This is clearly not the case. UV exposure and gamma irradiation experiments have shown that irradiation responses of all CO₂ radicals in tooth enamel require independent investigations (Joannes-Boyau et al., 2010b; Joannes-Boyau and Grün, 2009, 2010). In a natural fossil sample, the contribution of the non-oriented radicals to the overall intensity was around 9% and the ratio of orthorhombic to axial radicals was 36:64 (Joannes-Boyau et al., 2010a). In contrast, the gamma irradiation component of the same sample contained 40% NOCORs and a mix of 25% of orthorhombic to axial radicals (Joannes-Boyau et al., 2010b).

In retrospective dosimetry and dating, the qualitative effects of gamma and beta irradiations are usually assumed to be the same because the energy transfer in gamma radiation occurs through the production of secondary electrons (Attix, 1968). However, external beta and gamma radiation of fossil teeth will affect different
domains in the enamel. The energy of gamma irradiation will be more or less evenly distributed in the enamel (provided that charged particle equilibrium is assured) while beta irradiation will mainly affect domains close to the surface of the enamel because of the strong attenuation of beta particles in enamel (Brennan et al., 1997; Marsh et al., 2002). The effect of alpha irradiation is highly localised (because of the short, 40 mm range of alpha rays, Grün, 1987). The spatial distribution of alpha generated radicals is governed by the location of uranium in the enamel (fossil enamel is virtually free of Th and K). The U-distribution depends on weathering (Eggins et al., 2003) and mineralogical weaknesses in the enamel (Grün et al., 2008a). There are structural differences between the volume close to the outside of the enamel, which contains a zone with non-preferentially orientated hydroxyapatite crystals, and the volume close to the dentine-enamel junction, which contains strongly orientated crystals, but wider inter-prismatic zones (Hillson, 1986). As a result, one may expect different radiation responses in these domains. To study these possible differences, two enamel fragments were irradiated with a beta source, one through the outer surface (buccal-enamel boundary), the other through the dentine-enamel junction. This paper offers for the first time to investigate the influence of beta irradiation on the distinctive species of CO₂ radicals that compose the ESR spectra of tooth enamel.

2. Materials and methods

The experiments were carried out on a fossil bovid from the archaeological site of Holon (Porat et al., 1999). A long lamella was separated from the fossil tooth using a dental diamond saw and a series of consecutive fragments was extracted and used for a range of heating and irradiation experiments (e.g. Grün et al., 2008b; Joannes-Boyau and Grün, 2009, 2010; Joannes-Boyau et al., 2010a, b).

Two adjacent fragments (HD and HS) were cut from the enamel lamella and irradiated with a calibrated ⁹⁰⁹Sr/⁹⁰⁸Y beta source of a Riso reader with an activity of 40 mCi generating an approximate surface dose of 180 Gy (for more details see Bätter-Jensen et al., 2000). HD (31.5 mg) was irradiated with the dentine-enamel junction facing the source while HS (29.4 mg) was irradiated from the opposite side. Gazu et al. (2006) experimentally determined the dose attenuation for α₂₀₂ dosimeters exposed to a Riso reader ⁹⁰⁹Sr/⁹⁰⁸Y beta source. They found that the dose distribution could be approximated by a simple exponential function, \( D(\gamma) = D_0 e^{-\gamma D_0} \), where \( D_0 \) is the surface dose and \( \gamma \) the depth in millimeters. The beta irradiation will affect different domains in the enamel plates because of the strong beta attenuation; at a depth of 1300 nm (the average thickness of HD and HS) the surface dose is attenuated to about 6%.

For comparison, the g-irradiation was carried out with a ¹³⁷Cs- source for 100 min, corresponding to an approximate dose of 780 Gy (this experiment is described in Joannes-Boyau et al., 2010b).

The methodology established by Joannes-Boyau et al. (2010a, b) was used for the present study. X, Y and Z denote configurations, with the main axes of the measured fragment (Fig. 1). T1, B1 and B2 are positions in the measured or simulated ESR spectra (for their location see Fig. 3), and R1, R2, R3 and R4 are the fitted Gaussian components. The radical concentrations were derived from the double integration of the fitted lines to account for changes in the line width. For the features in the measured ESR spectra, T1–B1 and B2, it was not possible to carry out double integrations; their angular variations were derived from their intensities (for more details see Joannes-Boyau et al., 2010a, b).

Both HS and HD were successively mounted into three separate Teflon holders containing Parafilm moulds. This allowed for the rotation of the fragments around their three major axes along with the incremental measurements of ESR spectra. The following configurations were used: X: rotation around the axis perpendicular to the dentine–enamel junction, Y: around the axis of tooth growth and Z: perpendicular to X and Y (see Joannes-Boyau et al., 2010a, b). The sample holders were inserted in a Bruker ER 218PG1 programmable goniometer and measured with a Bruker Elexys E500 ESR spectrometer in 101 increments over 360° with the following measurement conditions: 2 mW microwave power, 0.1 mT modulation amplitude and 12 mT sweep width with a sweep time of 21 s. The spectra were

![Fig. 1. Direction of axes and configurations used for the measurement of the tooth enamel fragments (from Joannes-Boyau and Grün, 2009).](image)

![Fig. 2. Comparison of the combined isotropic lines (IC).](image)
accumulated over 50 consecutive measurements. The fragments were measured before and after irradiation.

The anisotropic components of the irradiation spectra were decomposed with an automated simulated annealing (SA) procedure that is particularly well suited to separating overlapping signals (Joannes-Boyau et al., 2010a, b; Bodin and Sambridge, 2009). The spectra were decomposed with four Gaussian lines, which had the same prescribed g-value range limits used in the previous study to avoid unrealistic solutions outside the regions for the CO₃ radicals in hydroxyapatite. No restrictions were set on the intensity although the line widths were kept between 0.10 and 2 mT to avoid aberrations (for more details see Joannes-Boyau et al., 2010a).

Fig. 3. Comparison of the stacked (natural+b) ESR spectra of the HS (left) and HD (right) fragments in X, Y and Z-configurations.

3. Results and discussion

In the following sections, the data are discussed without explicit uncertainties. These are partly difficult to assess and could be determined only through repeated analysis on the same fragments and repeated analysis of the same experiment on different fragments. From the repeated decomposition analyses of natural fragments in our various experiments, we estimate that ESR intensity measurements have uncertainties of less than 0.5%, the decomposed radial intensities at a certain angle of about 5–10%, and the radial intensities in the merged spectra of about 3–5%. The uncertainties in the assessment of the angles between radicals range between 21 and 101.

3.1. Isotropic lines and non-oriented CO₂-radicals

The spectrum decomposition of the ESR signals in the two fragments followed the protocol described in Joannes-Boyau et al. (2010a). All spectra were aligned on the methyl line to avoid an artefact signal caused by misalignments. First, the natural signal was subtracted from the irradiated spectra at each angle. Second, non-CO₂, isotropic components were separated from the spectra (Fig. 2). Beta irradiation gave rise to the same isotropic lines found in the natural signal (see Joannes-Boyau et al., 2010a), but with higher intensities. In contrast to gamma irradiation, beta irradiation had considerable impact on the intensity of these non-CO₂ radicals. The methyl lines increased by 150% and 200% in HS and HD, respectively. W₃, a radical found at around g₃=2.0061 (line width lw=1.52 mT) in the natural spectra (Grün, 2002; Joannes-Boyau et al., 2010a), became narrower (lw=1.05 mT) but remained at the same g-value. The increase in peak-to-peak intensity of W₃ was about 185% in both HS and HD. This was the result of the line width changes, while the radical concentration remained constant. This may mean that either no radicals were created by irradiation or that the nature of the line changed. The radical concentration of a second wide line, W₄, at g₄=2.0051 (see Joannes-Boyau et al., 2010a,b), increased with b-irradiation by 10% and 40% in HS and HD, respectively. W₅ was slightly wider in the natural spectra (lw=0.37 mT) compared to the beta induced in HS and HD (lw=0.35 mT). W₅ was attributed to a combination of SO₂ and CO radicals (for more details see Bouchez et al., 1988; Schramm and Rossi, 2000; Joannes-Boyau et al., 2010a).

HD and HS were best fitted with 19% and 9% NOCOR concentrations, respectively. The higher NOCOR concentrations closer to the dentine–enamel junction may indicate that the NOCOS are located in the inter-prismatic zones. After subtracting the above mentioned signals (natural, isotropic lines and NOCORS), only the b-induced AICORS remain in the spectra.

3.2. Natural + beta

Stacks of spectra of both HS and HD fragments (natural+b) are shown in Fig. 3. There are obviously considerable differences between the beta induced spectra of the two fragments. After beta irradiation, HS shows significantly less angular variation (difference between the maximum and minimum intensity/concentrations divided by the average intensity/concentration) in T1−B1 in the X-configuration (0.17) than in the natural (0.29). In contrast, the angular variation in HD increases (0.46) over the natural (Fig. 3 and Table 1). In Y- and Z-configurations, HS shows much stronger angular variations than the natural, and they are also more pronounced than that of the g-irradiation, while the angular variations of HD in Y- and Z-configurations are considerably smaller than the natural (Table 1). The most striking differences between HS and HD lie in the spectral range between T1−B1 and B2 in Y and Z-configurations. HS shows an expected 180° periodicity, while HD has a pronounced 90° periodicity. This was also observed in the gamma irradiated sample (Joannes-Boyau et al., 2010b). The two fragments show clearly different angular responses with respect to anisotropy, periodicity and orientation (Fig. 3).

One has to keep in mind that most of the radicals created by beta irradiation of HS are close to the outer surface of the enamel fragment and of HD are close to the dentine–enamel junction. In contrast, the radicals in the natural and those generated by gamma irradiation are averaged over the whole volume of the enamel fragments. This means that the radicals observed after two beta irradiations are located in distinctively different domains in the enamel and either of these have significantly different spatial distributions to the natural as well as gamma induced radicals.

Changes in angular variation are usually the result of different relative amounts of AICORS in the spectra (Joannes-Boyau et al., 2010a,b). Their presence leads to muddled angular variability. The HS fragment in X-configuration has, however, low B2 intensities (Fig. 3) indicating low overall NOCORS concentrations. In Y and Z-configurations, the angular variability is much larger than that of the natural. Indeed, the fitted NOCORS concentrations of the natural and HS are the same (9%). Thus, the muted angular variability of HS in the X-configuration cannot be attributed to a higher amount of NOCORS, but is more likely due to the effective orientation of αₖ or γₖ of the AICORS (axial and/or orthorhombic) created during beta radiation being closer to the perpendicular, relative to the rotational plane. In other words, the enamel domain, in which the AICORS were produced, has a different effective orientation to the fragment as a whole. As a result, when rotating in Y- and Z-configurations, the AICORS (axial and/or orthorhombic) can either be close to parallel or perpendicular to the magnetic field, leading to maximum angular variability. The effective orientation of the beta generated AICORS in HD must be different from HS (see below).

HD shows generally smaller angular variations than the natural (Table 1). This is partly due to a higher amount of NOCORS (19%). Furthermore, the effective orientation of the generated AICORS may be more misaligned relative to the rotational planes.

### Table 1

<table>
<thead>
<tr>
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<th>T1−B1</th>
<th>B2</th>
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<tbody>
<tr>
<td>Natural</td>
<td>Z</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>Y</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>X</td>
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<tr>
<td>HS Natural+b</td>
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<tr>
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<tr>
<td>Natural (anisotropic)</td>
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<tr>
<td></td>
<td>Y</td>
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<tr>
<td></td>
<td>X</td>
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<tr>
<td></td>
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</table>

Natural AICORS spectra were obtained by subtracting the isotropic and NOCOR components from the natural spectra. The
beta AICOR spectra resulted from the subtraction of the natural, isotropic and NOCOR components from the natural+beta spectra. Fig. 4 shows the stacks of natural and beta AICOR spectra of HS and HD along with a γ-irradiation AICOR spectrum stack (Joannes-Boyau et al., 2010b). The natural AICOR spectra of HD and HS are virtually indistinguishable, indicating that the

Fig. 4. Comparison of the stacked anisotropic ESR spectra after subtraction of ICS (see Fig. 2) from the natural and (IC+natural) from the irradiated samples.

spatial and relative distributions of the various CO$_3$ radicals in both fragments are closely similar. It perhaps also confirms the good reproducibility of our measurements and analytical approach for extracting AICOR components (see above).

For both fragments, the natural spectra are considerably different from those of the b-induced in periodicity and orientation. HS shows significantly less angular variation in the X-configuration than the natural, but much more in Y and Z.

The respective Y and Z stacks of both fragments are similar. This is surprising, particularly for HD with complex angular responses (Fig. 4C). HS shows its strongest anisotropy in the Z-configuration. Rotation by 90° transforms a spectrum with dominating T1–B1 and negligible B2 to a spectrum dominated by the B2 spectrum and almost no T1–B1 (Fig. 4B). This indicates that g$_x$ and/or g$_z$ of the axial and/or orthorhombic radicals lie in the rotational plane. Such ideal angular variations were also described by Joannes-Boyau et al. (2010a), where care was taken to orientate the effective hydroxyapatite long-axis of the fragments to the rotational plane of the measurements.

The b-induced intensities of the merged spectra, HD$_m$ and HS$_m$, increase by the same amount (13% calculated by double integration). However, their shapes are noticeably different (Fig. 5), particularly in the relative positions of B1 and B2. The main feature in the residual can be explained by the presence of tumbling CO$_3$ radicals in HS, which occur at g$\approx$2.0007 and have been observed in fossil tooth enamel (Bouchez et al., 1988; Grün, 2002). Other features in the residual are caused by relative line widening in the T1–B1 region in HD and perhaps an incomplete subtraction of NOCORs from HD, resulting in a slightly larger B2 component. However, the latter two effects could also be caused by the different orientations of the hydroxyapatite crystals relative to the rotational planes.

For the extraction of R$_1$ and R$_2$, which are thought to represent the orthorhombic and axial CO$_3$ radicals, respectively, each anisotropic spectrum was decomposed using the methods of Joannes-Boyau et al. (2010a). The results are shown in Fig. 6. Beta-irradiation creates both R$_1$ and R$_2$ (Fig. 6), similar to the natural. The R$_1$ : R$_2$ ratios in HS and HD are 45 : 55 and 59 : 41, respectively. These results are quantitatively different from those of the natural (35 : 65) and qualitatively different from those in gamma induced spectra, where R$_2$ could not be be not detected at all (Joannes-Boyau et al., 2010b). On the other hand, UV irradiation through the outer surface (Joannes-Boyau and Grün, 2010), which also involves strong attenuation, yielded 9% NOCORs and an R$_1$ : R$_2$ ratio of 64 : 36, similar to the results of HS.

Fig. 5 shows that the beta induced spectra of HS and HD have significantly different patterns in their angular variations in the Gaussian components. The offsets between R$_1$ and R$_2$ of the natural and HD in the X-, Y- and Z-configurations are 201°, 201° and 01°, respectively. The offsets between R$_1$ and R$_2$ of the natural and HS are more pronounced, being 301°, 301° and 201°, respectively. Except for R$_1$ and R$_2$ of HS in the Z-configuration, none of the stacks of angular measurements vary between zero and a maximum value. Instead, at all angles there is a substantial minimum radical intensity. This may partly be due to changes in preferential direction of the hydroxyapatite crystals within the volume affected by beta irradiation (see below), but may also result from decusation of the hydroxyapatite crystals in tooth enamel (Korvenkontio, 1934; Wahlert, 1989; Sander, 1997; Smith and Talfoore, 2008; Smith, 2008). As a result, there will be a substantial number of smaller crystallites oriented into the direction of the magnetic field, even when the preferential crystallite direction is perpendicular to it. Decusation has usually a preferential orientation and as a result, its effect may be observed in two rotational planes, but not in the third. The exact nature of the decusation in our samples is at present not known.

Fig. 7 shows the projections of the preferential orientations of R$_1$, R$_2$ and B$_2$ onto the Y–Z plane. The results of the two natural samples were very similar and were averaged. As expected, the orientations of the components of the natural samples lie between the orientations of the respective components of the two beta-irradiated samples. The angles between the beta components of HD and HS are around 501° (R$_1$), 271° (R$_2$) and 301° (B$_2$), indicating a change in the preferential direction of the hydroxyapatite crystallites of around 30–40° from the volume close to the dentine–enamel junction to the volume close to the outer surface.

The angles between R$_1$ and R$_2$ in the naturals are 231° (HD) and 251° (HS) and in the beta components 251° (HD) and 221° (HS). This is in close agreement with our previous measurement of 231° (Joannes-Boyau and Grün, 2010) as well as the angle of 301° deduced from heating experiments (Joannes-Boyau and Grün, 2009). The results of the latter experiment did not involve any decomposition procedures. The reproducibility of the calculations of the angle between R$_1$ and R$_2$ indicates that the two radicals are located at intrinsic positions in the hydroxyapatite crystallites.

Heating experiments on gamma irradiated samples show that heating (i) increases the methyl lines (Grün and Invernati, 1985), (ii) causes a transfer of orthorhombic CO$_3$ radicals into axial (Vorona et al., 2005, 2006) and (iii) shows that NOCORs are thermally less stable than AICORs (Brik et al., 1998, 2000b). In principle, the results of the beta irradiation (increased methyl intensity, relatively low NOCOR concentrations and a significant amount of axial CO$_3$ radicals) could be obtained by gamma irradiation and subsequent heating. Our model of the rise reader does not allow in-situ measurement of the temperature during irradiation, because the sample holder rotates before it reaches the irradiation position. In a subsequent experiment, crystal bond grains were placed under an enamel sample. Their melting during beta irradiation indicated that temperatures of at least 85°C were reached during the experiment. It may well be that temperature reached at the surface was somewhat higher. This requires quantification in future experiments using a stationary beta source and thermocouples embedded into the enamel fragments. One should keep in mind, however, that the radical distributions of the UV induced spectra were even closer to those of the naturals. In the UV experiment, a thermocouple was placed next to the surface of the sample and did not yield readings above 35°C (Joannes-Boyau and Grün, 2010).

At this moment we can only speculate that the qualitative differences between the radiation sources are due to surface interactions (e.g. backscattering). Both beta and UV were directly
aimed at the outer surfaces of the enamel fragments and both attenuate strongly with depth (see above). During gamma irradiation, charged particle equilibrium was ensured through an Al holder and the sample was rotated in front of a relatively large source, resulting in even irradiation from all sides. The high energy of the gamma rays (662 keV from the $^{137}$Cs source) ensures that only small attenuation effects occur in an enamel layer of 1300 nm thickness (in the region of 2x, Airken, 1985).

4. Conclusions

Beta irradiation induces ESR signals in fossil tooth enamel, which are qualitatively and quantitatively different from $\gamma$-irradiation. Beta irradiation resulted in the creation of both $R_1$ and $R_2$ radicals while gamma irradiation resulted in the formation of $R_1$ only radicals. Furthermore, the $\beta$-induced mix of signals in HS and HD are different from each other and from the natural. This can be attributed to the different mineralogical properties of the domains in which the radicals are produced. Thus far, all experiments yielded an angle of about 23° between $R_1$ and $R_2$, indicating that the two radicals are located in intrinsic positions within the hydroxyapatite crystals. The NOCOR concentration differences between HS and HD could point to their location in interprismatic zones, which are more abundantly close to the dentine–enamel boundary. Further studies are required to investigate the effects of $\alpha$- and $\gamma$-irradiation in different enamel domains as well as thermal treatment to observe the transfer processes. This study alone points to serious problems related to the estimation of the equivalent dose and consequently dating. Experiments should now be carried on a
Fig. 7. Projections of the preferential orientations of $R_1$, $R_2$ and $R_3$ onto the Y-Z plane. The results of the two natural samples were averaged.

wide range of specimens (from bovid to carnivores, from animals to human and from incisors to molar) of different ages to observe variability within samples and age.

Acknowledgments:

We are very grateful to T. Bodin, Research School of Earth Sciences, Australian National University (ANU), Canberra, for help with the design of the simulating annealing program used for this work. We thank N. Manson, Research School of Physical Sciences, ANU, for helpful comments. We are grateful to F. Callens and H. Vrielinck, Gent, for their considered advice in the earlier stages of this study. Aspects of this study were supported by the ARC funded DP0664144 Microanalysis of human fossils: new insights into age, diet and migration. We thank Tegan Kelly, RSES, for corrections and comments on the manuscript. R.G. is grateful to the Institut des Sciences Humaines et Sociales du CNRS, Bordeaux, and the Laboratoire d’Anthropologie des Populations du Pasquier, Université de Bordeaux I, for their kind hospitality in the writing-up stage of this manuscript. R.J.B. would like to thank Iain McCulloch and Kathryn Fitzsimmons from RSES ANU, for the irradiation of samples. We thank the referees for their insightful comments, which helped improve this paper.

References

CHAPTER 14
Research Paper

A comprehensive model for CO$_2^-$ radicals in fossil tooth enamel: Implications for ESR dating

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1. Introduction

ESR analyses on valuable archaeological or palaeoanthropological specimens are carried out on enamel fragments instead of powders to minimize the impact of the measurements. However, the ESR spectra of fragments have a high angular dependency, which complicates their study and the establishment of experimental protocols for dose estimation and dating (e.g. Grün et al., 2008a).

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doi:10.1016/j.quageo.2010.09.001

Most of the ESR intensity of fossil tooth enamel has been attributed to a range of CO$_2^-$ radicals (Brik et al., 2000a,b; Vanhaecke et al., 2000) and several interfering radicals mainly isotropic (dimethyl, CO$_2^-$, CO$_2^-$, CO$_2^-$, etc., for a compilation see Callens et al., 1998; Vanhaecke et al., 2000, 2002). The CO$_2^-$ radicals occur in two distinct states, the first have no preferential direction within the enamel (isotropic, non-oriented CO$_2^-$ radicals. NOCOR), the second is oriented (anisotropic CO$_2^-$, AICOR, e.g. Callens et al., 1995; Brik et al., 2000a,b; Ishchenko et al., 2002; Grün et al., 2008a; Joannes-Boyau and Grün, 2009). NOCORs give rise to a powder spectrum of the same intensity at all angles. Vanhaecke et al. (2002) argued that these CO$_2^-$ radicals were
orthorhombic. NOCORs are believed to be imbedded in randomly organised crystals that give at once a mix of all orientations (Roveni et al. 2009) or associated with organic membranes of nano-crystals (Brik et al. 2000b). Extreme heating (400 °C over two to three weeks) destroys the precursors for the NOCORs (Callens et al. 1995; Vanhaevelyn et al. 2002), i.e. they cannot be regenerated with subsequent irradiation. This observation indicates an association with organic radicals or crystal water.

In contrast, the contribution of AICORs to the overall ESR spectra is changing at each angle, depending on the effective orientation in the enamel fragment (for more details see Grün et al. 2008a; Joannes-Boyau and Grün 2009). AICORs may occur in two varieties. Axial CO$_2$ radicals (with g$_x$ around 2.0025 and g$_y$ around 1.9974, e.g. Callens et al. 1987; Ishchenko et al. 2002) occur through carbonate substitution of the PO$_4$ tetrahedron (Yugman et al. 1995). Their axial symmetry is explained by rotation around the 0–0 axis which is in direction of the mineralogical c-axis of the hydroxyapatite crystals. Orthorhombic CO$_2$ radicals (with g$_x$, g$_y$, and g$_z$ around 2.0030, 2.0015 and 1.9973, respectively, e.g. Callens et al. 1987; Ishchenko et al. 2002; Rudko et al. 2007) are associated with carbonate substitution of (OH$^-$). Their explicit location is disputed. Some speculated the axial CO$_2$ radicals were located at the surface of the hydroxyapatite crystals (Callens et al. 1995; Brik et al. 2000b), while others argued them to be in the same position as the axial CO$_2$ radicals (Ishchenko et al. 2002; Vorona et al. 2005, 2006).

Because of different relative distributions and thermal stabilities of NOCORs and AICORs in the natural and irradiated spectrum components, calculated dose values become angular dependent (Grün et al. 2008a).

We have carried out series of irradiation experiments on a fossil bovid tooth from the archaeological site of Holon (Porat et al. 1999). The ESR spectra were decomposed with an automated simulated annealing (SA) procedure (see below). The angular anisotropic spectra could only be fitted with four Gaussian components ($R_0$, $R_1$, $R_2$, and $B_2$; see Joannes-Boyau et al. 2010a,b). Depending on their g-values, $R_1$ was associated with orthorhombic CO$_2$ radicals, $R_2$ with axial, and $B_2$ with the combination of g$_x$ and g$_y$ of the aforementioned anisotropic radicals. $R_0$ is thought to represent the sum of AICOR misalignments (Liija 2001).

We found the following:

- The natural spectra contained about 9% NOCORs. The remaining AICORs consisted of a mix of orthorhombic to axial radicals of 35-65. The g$_x$ orientations of the orthorhombic and g$_y$ of the axial radical formed an angle of about 25° (Joannes-Boyau et al. 2010a).

- The gamma induced (by approximately 780 Gy) spectrum components contained about 40% NOCORs and the AICORs consisted exclusively of orthorhombic radicals. We could not detect any axial CO$_2$ radicals (Joannes-Boyau et al. 2010b).

- UV induced spectra (irradiation through the buccal–enamel boundary, BEB) contained 9–10% NOCORs and a mix of 36-64 orthorhombic to axial CO$_2$ radicals. The angle between the two AICOR species was 24° (Joannes-Boyau and Grün, 2010).

- Beta irradiation through the BEB produced 9–12% NOCORs and a mix of 45-55 orthorhombic to axial AICORs with an angle of about 25° between them.

- Beta irradiation through the dentine–enamel junction (DEJ) resulted in 19% NOCORs, an orthorhombic to axial mix of 59-41 and an angle of about 23° (Joannes-Boyau and Grün, in press).

From these experiments, we concluded that the constant angle between the two AICOR species indicates that these are located within the hydroxyapatite crystals. The complete lack of axial CO$_2$ radicals in the gamma induced spectra suggested that the orthorhombic CO$_2$ radicals partly convert into axial during geological times, which would mean that the orthorhombic are less stable than the axial radicals. Both UV and beta radiation are strongly attenuated by the enamel layer, thus their results are dominated by the volumes close to the surface that was exposed to the radiation source. In contrast to gamma radiation, both UV and beta radiation generated axial radicals, and also significantly less relative amounts of NOCORs. The UV experiment was temperature monitored, we considered it unlikely that irradiation associated with concurrent heating were the cause for the qualitative and quantitative differences between gamma irradiation on the one hand and UV and beta on the other. The higher relative NOCOR concentrations of the beta irradiation through the DEJ implied that these were perhaps located in intrapristine zones or associated with organic-rich domains that are found in the enamel close to the DEJ (Suga et al. 1987; Balasse, 2003; Smith and Tafforeau, 2008; Smith, 2008; Smith et al. 2008).

In this paper, we follow up the beta irradiation experiments (Joannes-Boyau and Grün, in press). Since higher energy gamma rays (here 662 keV of a $^{137}$Cs source) attenuate little over the enamel thickness (by only around 1–2%, Atken, 1985), energy transfer is more or less constant in the different domains of the enamel. For the investigation of the gamma response in the outer and inner volumes of tooth enamel, these domains were physically separated before irradiation (Fig. 1). In addition, we carried out annealing experiments to shed light on the possible transfer mechanisms between the different types of CO$_2$ radicals and their relative thermal stabilities. From our irradiation and heating experiments, a comprehensive model on CO$_2$ radicals in fossil tooth enamel will be developed.

2. Materials and methods

The present study follows the methodology established by Joannes-Boyau et al. (2010a,b). X, Y and Z denote configurations, x, y and z the main axes of the measured fragment and T$_x$, B$_x$ and B$_z$, are positions in the measured or simulated ESR spectra (Fig. 2). $R_0$, $R_1$, $R_2$, $R_3$ and $B_2$ are the fitted Gaussian components. The radical concentrations were derived from the double integration of the fitted lines to account for changes in the line width. For the features

![Fig. 1. Photo of the enamel fragment of Holon before separating the two fragments HS and HD. On the picture the HD fragment shows parallel striation most likely the Hunter-Schreger bands (HSB) (Wahlert, 1989).](image-url)

Please cite this article in press as: Joannes-Boyau, R., Grün, R., A comprehensive model for CO$_2$ radicals in fossil..., Quaternary Geochronology (2010), doi:10.1016/j.quageo.2010.09.001
in the measured ESR spectra, it was not possible to carry out double integrations for $T_1$ and $B_2$; their angular variations were derived from their intensities (for more details see Joannes-Boyau et al., 2010a).

The experiments were carried out on the same fossil bovid that was used in all other experiments (see first-authored publications by Joannes-Boyau). A long lamella was separated from the fossil tooth using a dental diamond disk and a series of consecutive fragments were extracted and used for a range of heating and irradiation experiments (see above). One fragment was subdivided lengthwise (see Fig. 1, the connecting middle section was later removed). HD (37.9 mg) corresponds to the layer next to the DEJ and HS (27.9 mg) to the layer next to the BEB.

The sample received a geological dose of around 270 Gy, as estimated on the powder. The two fragments will have received different natural beta dose rates because of the relatively high U concentrations in the dentine (56 ppm, for a laser ablation scan of sample 1556, see Eggins et al., 2003, their Fig. 4) and low dose rates from the sediment (1.6 ppm U, 2.4 ppm Th and 0.42% K, 10% water). The U-series data on the dentine (Eggins et al., 2003, their Table 1) can be used to calculate the closed system U-series dose from the dentine, which is 52 Gy for the whole width sample, 13 Gy for HS and 102 Gy for HD. The average sediment beta dose to the total thickness is around 10 Gy, 21 Gy to HS and 4 Gy to HD. U concentrations in the two fragments were determined with laser ablation, resulting in 0.26 ± 0.04 ppm in HS and 0.13 ± 0.05 ppm in HD. This

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would result in alpha doses of 2.8 and 1.4 Gy in HS and HD, respectively. Altogether, the whole fragment (=powder) will receive a combined alpha and beta dose of about 64 Gy while HS would receive around 37 Gy and HD 105 Gy. One would expect a dose value of about 245 Gy in HS and 310 Gy in HD, i.e. the geological dose in HD should be around 25% higher than in HS. The two fragments were each mounted consecutively into sets of three separate Teflon holders containing Parafilm moulds. In this way the fragments could be incrementally measured by rotating them around their three major axes (see Fig. 2). We used the following configurations: X: rotation around the axis perpendicular to the DEJ (and BEB), Y: around the axis of tooth growth and Z: perpendicular to X and Y (see Joannes-Boyau and Grün, 2009). The sample holders were inserted in a Bruker ER 2180P1 programmable goniometer and measured with a Bruker Elexys E500 ESR spectrometer in 10° increments over 360° with the following measurement conditions: 2 mW microwave power, 0.1 mT modulation amplitude, 12 mT sweep width with a sweep time of 21 s. The spectra were accumulated over 50 consecutive measurements. The fragments were measured before and after irradiation. Both fragments were irradiated using a 137Cs source for 100 min, which corresponds to an approximate dose of 780 Gy. Thermal treatment was carried out in a furnace with a first heating step of 1 h at 90 °C (T0), then for isothermal cumulative treatment for 45 (T1), 120 (T2), 1440 (T3) and 14,400 (T4) min at 125 °C. All spectrum decompositions and simulations were carried out on the measured derivative spectra. Decomposition was optimised and automated using a simulated annealing (SA) procedure. SA is a Monte Carlo method used for combinatorial optimisation problems (for details see Metropolis et al., 1953; Kirkpatrick et al., 1983; Černý, 1985; Mossegaard and Sambririge, 2002; Bodin and Sambririge, 2009). The SA procedure is able to randomly generate a large number of synthetic spectra defined by a linear combination of four Gaussian lines. Each simulated spectra is compared to the measured spectra in terms of a least square misfit. The particular advantage of the SA algorithm is the search of the global minimum misfit without getting stuck in local solutions. As we shall see below, SA can resolve completely overlapping signals, which are very difficult to decompose with alternative decomposition approaches.

It is estimated that the measurement uncertainty is less than 1%, positioning of the samples in the cavity may contribute about 1%. The uncertainties in the estimation of angular radial intensities from the SA method are in the range of 5–10%, and in radial intensities of the merged spectra in one configuration around 1–3%. The Gaussian lines had prescribed limits with respect to the g-value range to avoid unrealistic solutions outside the regions for the CO2 radicals in hydroxyapatite (see above). No restrictions were set on the intensity and maximum line width, however, a minimum width (0.10 mT) was defined to avoid aberrations. While it could be argued that the restriction to Gaussian lines is oversimplifying the nature of CO2 radicals in hydroxyapatite, our approach will lead to a comprehensive model for CO2 radicals in fossil tooth enamel (see below). Due to the close similarity of Gaussian and Lorentzian peak shapes, one would expect the same qualitative results with minor quantitative differences (Grün, 1998). This will be verified with alternative fitting models in future studies.

3. Results and discussion

Two experiments were carried out in this paper, firstly gamma irradiation, secondly isothermal heating at 125 °C. The experiments and their implications are discussed separately followed by a synthesis of all our irradiation and heating experiments in the development of a comprehensive model for CO2 radicals in fossil tooth enamel. In order to compare radiation effects, non-CO2 components were subtracted and the ESR intensities of the merged spectra of the various fragments were then weight normalised. Since the merged and NOCOR spectra are very similar, the intensities can be readily assessed via the T1–β g-peak-to-peak intensities. The overall ACOR intensity is simply derived from the total minus NOCOR intensity. The relative orthorbomic/axial contributions were inferred from the R1–R2 ratios (see below). All intensity values for the discussion below are listed in Table 1.

3.1. Natural and gamma irradiation

The weight normalised natural ESR intensities for HS (158 a.u.) and HD (169 a.u.) were very similar. This difference of around 6% is most likely the result of different external and internal dose rates (see above). The gamma sensitivities of HS and HD are radically different, the same gamma dose induced 446 a.u. in HS and 683 a.u. in HD, i.e. the overall signal intensity of HS increased by around 280% and of HD by 400%. The beta experiment showed normalised natural intensities of 166 and 168 a.u. in the two natural samples (covering the whole thickness of the enamel) and relative overall increases of around 290% (irradiation through the BEB) and 320% (irradiation through the DEJ).

3.1.1. Non-CO2 components

Several non-CO2 components occur around g = 2 in the ESR spectra of fossil tooth enamel. Most of them are located outside the g-values range of the CO2 radicals (approximately 2.0060–1.9970). Some, however, can interfere greatly with the main signal and influence the peak-to-peak intensity reading from the T1–β positions.

Isotopic non-CO2 components were extracted from the spectra (Fig. 3) following Joannes-Boyau and Grün (2009) and Joannes-Boyau et al. (2010b) methodology and results. All isotopic lines are combined into one isotopic combined spectrum (IC), which was subsequently subtracted from all angular spectra. The natural IC is closely similar in both fragments and composed of dimethyl triplet lines and two wide lines already described by Joannes-Boyau et al. (2010a). W1 (around g = 2.0061) could not be attributed to a specific radical, W2 (around g = 2.0051) relates most likely due to a combination of SO2 and the CO− radicals, which occur at g = 2.0056 and g = 2.0047, respectively (Bouchez et al., 1988; Schramm and Rossi, 1999). The intensity of the central dimethyl line was derived from the next two outer lines assuming a septet with intensity distribution of a Pascal triangle. None of these components exhibited any quantifiable angular variation, thus, they were considered to be isotropic.

ESR intensities of the isotopic components found in the natural spectra did not increase with laboratory irradiation. The influence of gamma irradiation differs between the fragments (Fig. 3). The gamma induced IC is composed of three wide lines in the irradiation spectra already described by Joannes-Boyau et al. (2010b) in both fragments HD and HS, with W1 centred at g = 2.0115 with a line width (lw) of 0.3 mT, W2 with g = 2.0081, lw = 2.3 mT and W3 with g = 2.0072, lw = 0.7 mT. The g-values are identical for both fragments but there are small fluctuations in line width. The W1 intensity in HD is twice that of HS. The W3 intensity in HD is more than double as intense as in HS. It is likely that the DEJ plays a role in the amount of organic radicals.

3.1.2. NOCORs

Both natural samples of HS and HD contained about 1% NOCORs (14 and 15 a.u.), the same as measured for the fragments with the whole enamel thickness. The gamma irradiation spectra of HD contained 435 a.u. NOCORs (64%) while HS only around 109 a.u.
### Table 3

Weight normalised intensities.

<table>
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<th>$R_1$</th>
<th>$R_2$</th>
<th>$R_3$</th>
<th>$B_2$</th>
<th>$B_3/R_3$</th>
<th>NOCORs</th>
<th>Orthorhombic</th>
<th>Axial</th>
<th>Total</th>
<th>%NOCOR</th>
<th>%Ortho</th>
<th>%Axial</th>
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<td>0.09</td>
<td>0.56</td>
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<td><strong>Beta irradiation</strong></td>
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<td>1.17</td>
<td>55</td>
<td>166</td>
<td>0.10</td>
<td>0.57</td>
<td>0.33</td>
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</table>

(249) This compares with an average value of 40% for the whole thickness of the enamel (Grün et al., 2008a; Joannes-Boyau et al., 2010b). These values correspond to an eight-fold NOCOR concentration increase in HS and a 29-fold increase in HD. These large differences in the NOCOR concentrations (the equivalent of around 300 a.u.) account for most of the differences in the overall gamma induced ESR intensity (around 240 a.u.) between HS and HD. The gamma results confirm the observation of the beta experiment, that irradiation generates more NOCORs in the volume closer to the DEI than in the volume close to the BEB. The relative NOCOR concentrations of the gamma irradiation are significantly higher than those observed after UV and beta irradiation (see above).

Considering that the natural spectra of HS and HD, and indeed of all other natural fragments separated from this specific tooth, contained a very similar amount of NOCORs (9-10%), there is a clear indication that two groups of NOCORs must exist, one relatively stable (of the type being present in the natural sample), the other unstable, mainly produced in the volume close to the DEI.

#### 3.1.3. AICORs

The anisotropic irradiation spectra (subtracting the natural, gamma IC and NOCORs from the natural + gamma spectra) were decomposed as outlined by Joannes-Boyau et al. (2010a,b). $R_1$, $R_2$, $R_3$ and $B_2$ were determined for each angular measurement for the three configurations and their intensities plotted versus the measured angle (Figs. 4 and 9 below). The results for the gamma induced AICORs in HS and HD are essentially the same as observed for the whole fragment: gamma irradiation only generates $R_1$ radicals, $R_2$ radicals are either absent or below the detection limit of the SA method. The $R_1$/$R_2$ ratios in the gamma irradiation component are 1.82 and 1.97 for HS and HD respectively. All other fragment have $R_1$/$R_2$ ratios between 1.69 and 1.94. On the other hand the $R_2$/$R_3$ ratios in the samples containing $R_2$ range between 1.06 and 1.62. This may indicate that the $R_1$ component is dominated by misalignments of $R_1$.

The AICOR concentrations of HS and HD correspond to about 143 a.u. and 154 a.u. In the respective irradiation spectra, the intensities are 337 a.u. and 249 a.u., corresponding to AICOR increases by 235% and 160%, respectively. In contrast to the disparate gamma response, beta irradiation enhanced the AICORs nearly uniformly, 200% by irradiation through the BEB and 210% through the DEI.

The analyses of the minima and maxima allow the plot of the vectors of the components (Fig. 5). The directions of $R_1$ in the natural and irradiation spectra are essential the same. $R_2$ is not present in the irradiation spectra. There are small directional changes in $B_2$, which can be attributed to its composition as a composite of $g_0$ of $R_1$ and $g_1$ of $R_2$. Because there is an angle of 23° between $R_1$ and $R_3$ (see above), the direction of $B_2$ depends on the relative contributions of $R_1$ and $R_2$, which are different in the natural (35.6°) and irradiation spectra (100°).

The natural and gamma irradiation components of HS and HD indicate a significant directional change of the hydroxyapatite crystals in the two domains. The angles between the natural
components of HD and HS are around 54° (R1), 31° (R2) and 51° (B2); 63° (R1) and 56° (B2) between the irradiated components, indicating a change of the overall preferential direction of the hydroxyapatite crystallites of around 35°-45°. These values are similar to those obtained from the beta measurements where angles between 30° and 40° were found between the respective AICOR components close to the BEB and DEJ (Joannes-Boyau and Grün, in press).

3.1.4. Implications of the irradiation experiments

Considering that all natural fragments, whether taken from different domains or over the whole enamel thickness, have very similar R1/R2 and NOCOR:AICOR ratios and absolute intensities, it is expected that their dose response is very similar. For beta irradiation this is more or less the case, but definitely not for gamma irradiation (Fig. 6). Apart from not producing any R2-type CO2 radicals, the AICOR and NOCOR responses of HD and HS are completely different. The only explanation that can be found for the even distributions in the natural fragments is that a considerable amount of irradiation generated NOCORs will convert into AICORs over time. As strange as it seems, the effect of beta irradiation seems to correspond to gamma irradiation plus radical transfer which leads to annealing of unstable NOCORs and the conversion of R1 into R2-type CO2 radicals. It was not possible to measure the beta irradiation temperature directly with a thermocouple, the sample holder of the Risø reader rotates the sample before it arrives in the irradiation position. Instead crystallography grains were placed under an enamel sample. Their melting indicated that temperatures of at least 85 °C were reached during beta irradiation. It may well be that the temperature reached at the surface was somewhat higher. This requires quantification in future experiments using a stationary beta source and thermocouples embedded into the enamel fragments. One should keep in mind, however, that the radical distributions of the UV induced spectra were even closer to the naturals. The UV experiment was monitored with two thermocouples and only slightly elevated temperatures were recorded (Joannes-Boyau and Grün, 2010).

3.2. Heating

Heating experiments were carried out to gain insights firstly into the transfer of NOCORs to AICORs and of R1 into R2-type CO2 radicals. The problem with the heating experiments is that it is not possible to distinguish between the natural and irradiation induced CO2 radicals. This would entail the production of a second set of fragments.

The first thermal treatment, 1 h at 90 °C, was originally designed to remove the isotropic unstable component created by laboratory irradiation (Sholom et al., 1998). The subsequent heating steps were carried out at 125 °C. This temperature was chosen because it seems to affect the NOCORs while having less influence on AICORs (Birk et al., 1998; Grün et al., 2008a).

3.2.1. Non-CO2 components

Fig. 7 shows that the first heating step of 1 h at 90 °C removed all gamma induced isotropic lines, but did not create any further lines. The subsequent four 125 °C heating steps increased the intensity of dimers in both HS and HD (compare Fig. 7A and B) and generated a small isotropic component around g = 2.0059 that is most likely due to SO2 radicals. These are known to be temperature and dose dependent (Bouchet et al., 1988; Schramm and Rossi, 1999; Martinez et al., 2001; Joannes-Boyau and Grün, 2009). The SO2 radicals appear immediately after the first 125 °C heating step in HD while they only appear after 240 h in HS. This difference is an additional indication that the enamel close to the DEJ contains more organic radicals than the domains close to the BEB. The overall intensities of the temperature induced lines are small, with nearly negligible intensities. The IC spectrum of each temperature step was subtracted from all angular measurements.

3.2.2. NOCORs

Table 1 shows that the thermal treatment had little influence on the angular variation of HS, with less than 10% change between the natural non-heated and the longest heating step. On the other hand, HD is more affected by temperature and shows a strong angular variations after 2 h at 125 °C (Table 1). Fig. 8 shows the response of the NOCORs to laboratory irradiation and thermal treatment (TT). In HD, thermal treatment reduces the amount of
NOCORs from 435 a.u. to 340 a.u. (a 22% reduction), while in HS from 109 a.u. to 92 a.u. (a 16% reduction, see Fig. 8).

The behaviour of the fragment from the previous annealing study of Joannes-Boyau and Grün (2009), representing the whole enamel thickness, is similar to the combined behaviour of HS and HD (Fig. 8). It clearly shows that most of the gamma induced unstable NOCORs are located in HD, most likely associated with organic radicals in the enamel close to the DEJ.

3.2.3. **AICORs**

Laboratory irradiation and thermal treatment have a strong influence on the angle at which the $T_1-B_1$ maximum intensity occurs. This angle is shifting after irradiation in both fragments, but particularly in the HD sample. The $T_1-B_1$ maximum angle is by definition not influenced by isotropic lines or by the NOCORs, but by the ratio between the two $R_1$ and $R_2$-type $CO_2^-$ radicals. As gamma irradiation only produces $R_1$-type radicals, the $R_1:R_2$ ratio...
changes. After heating, the maximum drifts back to its original position, indicating a gradual transfer from $R_1$ to $R_2$.

The spectrum decomposition of the laboratory irradiation and the last temperature step of HS are shown in Fig. 9. The $R_2$ component is not detectable until the fragments were heated for 2 h at 125 °C. The radical concentration of $R_1$ is diminishing while the radical concentration of $R_2$ is increasing. Note that $R_1$ changes little, perhaps an indication that it also contains some contributions from $R_2$-type radicals. Fig. 10 summarises the thermal responses of the NOCORs and AICORs. It can clearly be seen that while $R_1$ decays $R_2$ increases. The overall AICOR concentration remains more or less constant which indicates a quantitative transfer from one AICOR into the other. The reduction of the total signal intensity is mainly caused by the decay of NOCORs and a very small component of AICORs. There is no quantifiable transfer from unstable NOCORs to stable AICORs.

In the previous studies by Joannes-Boyau et al. (2010a,b) and Joannes-Boyau and Grün (2010, in press), the angle formed between the two $R_1$ and $R_2$-type $CO_2$ radicals remained the same after gamma irradiation (natural + gamma irradiation), UV exposure and $\beta$-irradiation. Thermal treatment appears to have no effect on the angles between $R_1$ and $R_2$, 25° and 24° in HD and HS, respectively. This further confirms that both radicals are in the same crystal prisms and that thermal treatment induces the transfer from one species to the other by rotating or twisting the radical main axis. In principle, it should be possible to calculate the energy that has to be introduced into the system to induce the rotation, analogous to the methyl rotation in alanine single crystal (Miyagawa and Itoh, 1962).
As \( B_2 \) is a mix of axial and orthorhombic \( CO_2 \) radicals, the goniometer angle at which the maximum \( B_2 \) intensity angle occurs varies with irradiation and thermal treatment. The maximum \( B_2 \) intensity occurred at 85° and 91° in the naturals of HD and HS, respectively, at 89° and 94° in the irradiated fragments and back to 88° and 92° after thermal treatment.

3.3. Summary of irradiation and heating observations

From the irradiation and heating experiments on fragments of sample 1556, the following observations can be made:

1. All natural enamel fragments from sample 1556 representing the full enamel thickness or the domains close to the DEI and BEB have closely similar absolute radical intensities as well as NOCOR: AICOR and \( R_1:R_2 \) ratios.
2. The occurrence of significant amounts of NOCORs in the natural sample indicates that these have a considerable stability.
3. Gamma irradiation only produces \( R_1 \)-type radicals and NOCORs.
4. Gamma irradiation produces significantly less AICORS close to the DEI than close to the BEB.
5. Gamma irradiation produces significantly more NOCORs close to the DEI than close to the BEB.
6. In contrast to sample 1556, gamma irradiation created \( R_2 \)-type \( CO_2 \) radicals in a fossil human sample (see below).
7. Beta irradiation produces \( R_1 \) and \( R_2 \)-type radicals and NOCORs.
8. Beta irradiation produces about the same amount of AICORS when irradiating through the BEB and DEI.
9. Beta irradiation produces more NOCORs when irradiating through the DEI.
10. Beta irradiation produces significantly less NOCORs than gamma irradiation.
11. The composition of UV irradiation spectra is closely similar to the natural (Joannes-Boyau and Grün, 2010).
12. The irradiation response implies that \( R_1 \) consists mainly of misalignments of \( R_1 \).
13. The fact that the natural and beta irradiation compositions are close, but the gamma irradiation compositions are not, implies that a significant amount of NOCORs is transferred into AICORS over time.

Fig. 7. Merged isotope lines for HD and HS for natural, gamma irradiated and subsequently heated fragments HD and HS. Cumulative heating steps: \( T_0 = 1 \text{ h at 90°C} \), \( T_1 = 45 \text{ min at 125°C} \), \( T_2 = 2 \text{ h at 125°C} \), \( T_3 = 24 \text{ h at 125°C} \), \( T_4 = 340 \text{ h at 125°C} \).

Fig. 8. Heating response of the NOCORs.
Fig. 9. Summary of the angular behaviour of the anisotropic components of the natural and irradiation component spectra of HS as well as the spectra of the highest heating step.

14. Heating shows a more or less quantitative transfer from $R_1$-type to $R_2$-type radicals.
15. Heating did not cause any quantifiable change in $R_3$, indicating that $R_3$ has also $R_2$ components.
16. There is no transfer from unstable NOCORs to AICORS.
17. The angle between $R_1$ and $R_2$ does not change during any of the above experiments.
18. Heating leads to a stronger angularity of $T_1 - B_1$ (Joannes-Boyau and Grün, 2009).
19. Grün et al. (2008a) showed that the concentrations of unstable NOCORs were the same for samples that were irradiated and those that were preheated and then irradiated. This casts serious doubt on the mass transfer model of Brink et al. (2000a).

3.4. A model for CO$_2$ radicals in fossil tooth enamel

3.4.1. Stable and unstable NOCORs

The occurrence of NOCORs in the natural spectra and the observation that unstable NOCORs do not convert into AICORS, require postulation two different NOCOR species. One stable, being able to convert quantitatively into AICORS (for the following it is assumed that this transfer is 1:1), the other unstable. The latter component decays over geological times and does not convert into any further measurable signals. The unstable NOCORs are neither eliminated by prescribed annealing steps (e.g. Sholom et al., 1998) nor very long storage times (several years) between irradiation and measurement (Grün and Ward, 2002).
The gamma irradiation experiments require that stable NOCORs convert into AICORs, at least in HD. If the AICOR ratio of the natural and irradiation spectra is approximately the same for HD and HS, one would expect 363 a.u. AICORs in HD, compared to 249 a.u. measured. In other words, HD requires an amount of stable NOCORs which later convert into 114 a.u. AICORs. In addition, it can be expected that the irradiation components contain similar NOCOR:AICOR ratios to the naturals (corresponding to 36 a.u. in HD and 33 a.u. in HS). This means that the unstable NOCORs correspond to 270 a.u. in HD and 61 a.u. in HS. After 240 h at 125 °C, 95 a.u. are annealed from HD and 17 a.u. from HS. This corresponds to 35% and 28% of the unstable NOCORs. Since some of the NOCORs may still be stable and convert into AICORs later, it is assumed that the relative amount of annealed NOCORs is the same for HD and HS. A short iteration shows that there further stable NOCORs in the fragments corresponding to 17 a.u. in HD and 16 a.u. in HS. The final results are that 58% (253 a.u. of 435 a.u.) of the gamma induced NOCORs in HD are unstable and 42% in HS (45 a.u. of 109 a.u.). The average is 50%.

At this stage, the heating experiments show that unstable NOCORs do not transfer into AICORs. However, the existence of the stable NOCOR component as well as their transfer into AICORs is thus far not conclusively proven.

3.4.2. AICOR response to different types of radiation

Beta irradiation created nearly an even $R_1$:$R_2$ distribution. If this is caused by thermal transfer, it would correspond to a significantly higher temperature than 125 °C, because the annealing of the gamma irradiated sample did not reach an even distribution after 240 h, and the beta irradiation took only 100 min. While it may be assumed that irradiation at higher temperatures may be somewhat different to low temperature irradiation with subsequent annealing, it is difficult to explain the qualitative differences between gamma and beta irradiation. Perhaps there are local heating effects at the very surface. However, TL studies show 110 °C peaks in 100 μm quartz grains which argues against such an explanation. It seems that some of the NOCORs in the beta irradiated samples are either not converted into AICORs or are unstable, particularly in the sample irradiated through the DEI. Additional annealing
experiments on the beta irradiated samples are required to confirm either hypothesis.

UV irradiation generated spectra that show virtually the same composition as the natural. Here, overall heating can be excluded as the sample was monitored during the irradiation. The differences between non-attenuated gamma irradiation and strongly attenuated beta and UV irradiation are perhaps due to changes in the cross sections for radical production as a result of competition in the volumes close to the surface. If this was the case, the effect should be dependent on the applied laboratory dose rate.

The qualitative and quantitative responses to alpha irradiation are thus far completely unknown and require urgent attention, because the values used for age calculations (e.g. 0.13 ± 0.02, Grün and Katzenberger-Apel, 1994) are based on powder experiments.

4.3. Location of CO₂ radicals

The close association between R₁ and R₂ in the heating experiments as well as the constant angle between the two species indicates that these are located in the same crystal domains. The heating experiments of Callens et al. (1993) and Vanhaecke et al. (2002) indicated that the orthorhombic CO₂ radicals are located within the hydroxyapatite crystals. The quantitative transfer from R₁ to R₂-type radicals supports the hypothesis that both are in equivalent positions rather than in different domains, perhaps associated with water molecules (Ishchenko et al., 2002; Vorona et al., 2005).

If the stable NOCORs transfer quantitatively into AICORs (probably first into R₁-type CO₂ radicals) they are probably located in the same domains as well. Both unstable and stable NOCORs seem preferably to be associated with organic-rich domains near the DEI. The occurrence of two types of NOCORs (instead of one) may have caused some of the controversies in the discussions about the location of the NOCORs as well as postulated mass transfer processes (Brik et al., 2000a; Grün et al., 2008a). As extreme heating seems to destroy all NOCOR precursors (Callens et al., 1993; Vanhaecke et al., 2002), it may well be that the unstable NOCORs are associated with organic radicals and the stable with water.

The nature of R₂ is still not well resolved. While the gamma irradiation seems to indicate a dominance from R₁, the annealing does not confirm this. Nevertheless, the observation that heating increases the angularity of the T₁–B₁ position could be explained that a part of the R₁ radicals is transferred into R₂ rather than another R₁ component of R₂.

3.5. Implications

While our model cannot explain all reported effects, it can now serve as a basis to understand a range of observations such as outlined below. Problem specific experiments can now be set up to verify the various effects and to refine the model. The occurrence of the unstable NOCORs has serious implications for published as well as future ESR dating projects.

3.5.1. Dose response

The dose response of NOCORs and AICORs may significantly differ. As long as both are reasonably sensitive to laboratory irradiation, dose response curves of the combined signal (i.e. in powdered samples) can be fitted with a single saturating function. However as soon as one of the components shows strong saturation effects, the dose response cannot be fitted with a single saturating function and it may be necessary to use additional terms or saturating functions. This will be of particular importance of samples that had received a high environmental dose and require large additional laboratory doses. The AICOR–NOCOR saturation characteristics may well be responsible for the necessity of using two saturating functions for the samples from the Orce sites (Guadix-Baza basin, Spain) which are supposed to be older than one million years and had received doses in excess of 1000 Gy (Duval et al., 2009).

3.5.2. Failed isochrons

Several attempts to use ESR isochron dating (Blackwell and Schwarze, 1993; Blackwell et al., 2001) failed because many samples actually show somewhat lower dose values in the domains with higher U concentration (Grün, unpublished data). Since U-diffusion is often proceeding from the dentine (Grün et al., 2008b), higher U concentrations are found in domains with higher percentage of unstable NOCORs, thus combining increased U concentrations with larger dose underestimations.

3.5.3. Systematic age underestimations

At several sites, systematic age underestimations have been observed when ESR results were compared to independent age estimates, e.g. Border Cave in the range of 5–15 Gy (Grün, 2006), or Tabun in the range of 30–90 Gy (compare Grün and Stringer, 2000 with Mercier et al., 1993). At this stage it is difficult to speculate about the effect of NOCORs at given sites, because there is reason to believe that the NOCOR concentration could be species specific (e.g. our first assessment of human materials shows that gamma irradiation can create R₂-type radicals), it could depend on the temperatures at the site as well as the age of the samples. Modern and fossil teeth show different behaviours (compare the NOCOR results of Grün et al., 2008a and this paper with Vorona et al., 2007).

As such, there are no easy solutions or applicable correction factors to estimate the influence of unstable NOCORs on published ESR age results. Fragments from each site have to be analysed to assess the amount of NOCORs.

3.5.4. Sites with seemingly correct ESR ages

The apparent explanation for systematic age underestimations of course fails for the sites where the apparent ESR age estimates agree well with the independent results. For example, the site of Skhul is only a few hundred metres away from Tabun (discussed in the previous paragraph). Here, ESR and TL age estimates agreed with each other (compare Grün et al., 2005 with Mercier et al., 1993). It is difficult to argue that teeth from two sites in close proximity and age should show significantly different behaviours with respect to the NOCORs. However, a closer examination of the age errors may well allow the reconciliation of these two apparently discrepant data sets.

3.5.5. Sites with ESR age overestimations

Our experiments did not reveal any processes that could lead to age overestimations. Thus, we are still unable to find an explanation for the two distinctively different ESR and OSL data sets for the Mungo 3 burial (Thorne et al., 1999; Bowler and Magee, 2000; Gillespie and Roberts, 2000; Grün et al., 2000; Bowler et al., 2003; Olley et al., 2006).

3.5.6. Published results on human samples

It may well not be possible to carry out annealing experiments on the human samples (fragments) that were analysed in the past. Nevertheless, it is possible to assess the NOCOR contributions in the dose response curves by analysing the ESR spectrum stacks. For samples that were measured in Y or Z type configurations, the angularity of the B₁ position can be used to estimate the NOCOR contribution. For samples that were rotated around the hydroxypatite growth direction (e.g. Border Cave 5, Grün et al., 2003), this may not be possible.
3.5.7. Sample preparation

The immediate lesson from the experiments is that the domains close to the dentine are to be avoided. They are prone to high NOCER concentrations in the gamma induced spectra as well as elevated U concentrations. However, teeth where the outer surface is weathered may experience extreme U-uptake from the outside (Legtis et al., 2003). It seems therefore advisable to firstly scan the enamel with laser ablation for the U-distribution. Secondly, if no significant U-uptake took place through the BEB, discard about 2/3 of the enamel thickness close to the DEU and use the volume close to the BEB for dose estimation. This will require a two-layer beta dose rate estimation for the contributions of the uranium in the discarded enamel and the uranium in the dentine (Brennan et al., 1997, 2000).

3.5.8. Estimation of NOCERs in powder samples

The routine measurement of fragments is time consuming with respect to the measurement, but particularly with respect to data evaluation. Furthermore, it will be difficult to automate these measurements as it is possible for powders (Grün and Clapp, 1996). At the moment it is necessary to develop recipes for the assessment of unstable NOCERs in powders. This can be done in the first place through long annealing times at relatively low temperatures on fragments, to evaluate the annihilation of unstable NOCERs, $R_1$ to

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Fig. 11. Analysis of the BH fragments. (A) Angularity of $B_2$. (B) Dose response of NOCERs. (C) Natural + gamma at the angle of maximum $B_2$ intensity. (D) Gamma irradiation spectra at the angle of maximum $B_2$ intensity. (E) Merged gamma irradiation spectra. (F) $R_1$, $R_2$, and $B_2$ components of gamma irradiation spectra. $R_1$ is not shown for clarity.

Please cite this article in press as: Joannes-Boyau, R., Grün, R., A comprehensive model for CO$_2$ radicals in fossil..., Quaternary Geochronology (2010), doi:10.1016/j.quageo.2010.09.001
3.6. Application of the CO₂ model to the tooth fragments of the Broken Hill specimen

The analysis of the enamel fragments of the Broken Hill (BH) specimen (Grün et al., 2008a) was the motivation to further investigate the behaviour of NOCORs and AICORs in fossil tooth enamel. All fragments were irradiated using a ¹³⁷Cs source with cumulative irradiation times of 0, 5, 15, 25, 35, 45, 55, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, and 280 min (Grün et al., 2008a). The analysis of the spectrum stacks is exactly the same as described for sample 1556 above. The fragment was initially measured to avoid any irreversible damage to the sample, such as powder analysis.

Fig. 11 summarises the essentials of the detailed analysis of the natural and gamma induced ESR spectra. The angular response of the B₂ position (Fig. 11A) is used to evaluate the NOCOR concentrations in the gamma induced spectra. The NOCOR concentrations in BH steadily increase from around 15% of the total signal intensity in the natural to around 40% in the highest irradiated sample. All three fragments yielded closely similar results (Fig. 11B).

Fig. 11C and D show the dose response of BH in Y-configuration at the angle where the natural T₁ intensity is smallest. The natural spectra show a double peak in the T₁ position and a relatively large dip at B₂. The T₁ double peak is the result of a combination of R₁ and R₂ radicals which have a ratio of around 40:60. The natural - + gamma spectra appear similar to the natural (Fig. 11C). However, a closer inspection of the irradiation spectra (Fig. 11D) shows that gamma irradiation mainly created R₁ radicals. Fig. 11E and F show the analyses on the merged spectra. Irradiation is dominated by the production of R₁ radicals. However, in opposite to the irradiation experiments on sample 1556, a certain number of R₂ radicals are created as well. In the highest irradiated sample (natural - + gamma), the R₁:R₂ ratio increased to around 65:35. The B₂ intensity lies in the middle of the two respective 90° component of R₁ (g₂) and R₂ (g₃) (Fig. 11F) and offers the smoothest dose response, since its determination is not affected by the problem of decomposing partly overlapping signals.

Because of the similarity in age and general behaviour to irradiation and heating between the fragments of BH and Holon (Grün et al., 2008a) it is assumed that the split of unstable to stable NOCORs is the same in BH as for 1556. Fig. 12 summarises the dose response of all components and Table 2 provides the results of the fitting with a single saturating function. Between 5 and 55 min, the NOCORs dose response seems supralinear. However, in the same region the data points of the AICORs are somewhat elevated, so that this apparent supralinearity is more likely the results of a slightly insufficient mathematical separation of NOCORs from AICORs. The saturation intensities and doses of the total AICORs and total NOCORs dose response curves are very similar. The conventional T₁-B₂ peak-to-peak D₀ value corresponds to 64.6 ± 0.6 min irradiation time while the AICORs result in 99.4 ± 3.9 min and the total NOCORs in 18.3 ± 1.7 min. Using a 50:50 stable to unstable NOCOR distribution, the best dose estimation corresponds to 82.7 ± 1.3 min irradiation time, around 30% higher than using the conventional approach.

4. Summary

The irradiation and heating experiments show that gamma irradiation of fossil tooth enamel generates large amounts of unstable NOCORs that are neither eliminated by prescribed annealing steps nor very long storage times between irradiation and measurement. A significant portion of these NOCORs does not seem to transfer into more stable radicals. This has significant implications for ESR dating, implying systematic age underestimations.

The overall effects of UV and beta irradiation seem to correspond to gamma irradiation with contemporary heating leading to the annealing of unstable NOCORs and conversion of stable NOCORs and R₁-type radicals into R₂-type radicals. This could perhaps be explained by the elevated temperatures which apparently occur during the beta irradiation, but the UV experiment was monitored and only slightly elevated temperatures were recorded. Our decomposition results imply that fossil tooth enamel contains four different types of CO₂ radicals. Two of them are non-oriented (stable and unstable NOCORs), two are oriented (orthorhombic and axial AICORs). While it is possible to distinguish the
total amount of NOCORs as well as the two AICORs, we have not yet been able to distinguish the two NOCOR radicals.

5. Conclusions

Our results imply that published ESR dating results will usually be age underestimated. However, we strictly warn against a naïve approach by simply multiplying all published ESR dating results by a given factor. There are indications that different teeth yield different gamma responses. For example, a fossil human incisor from Irboud yielded qualitatively and quantitatively different results from the molars of Broken Hill. Both are somewhat different from the large bovid from Holon (production of $R_2$-type radials).

The same can perhaps be expected for different teeth (incisors versus molars) and different species (humans, bovids, horses, bears, etc.).

Our results require a large series of experiments to resolve some of the issues raised in this paper. In particular, it is required to analyse teeth of roughly the same age from different regions of the world (to assess species and temperature dependencies), and teeth of significantly different age (to assess the effective thermal stability of the NOCORs in the natural). Furthermore, temperature controlled beta irradiation experiments need to be carried out along with beta irradiation with significantly different dose rates.

There is also an urgent need to re-assess alpha irradiation effects (on thin slices). Heating experiments at higher temperatures are required to understand the kinetics of all CO2 radicals (see Brik et al., 1997).

Furthermore, it seems advisable to utilise the different microwave saturation characteristics for the identification of specific CO2 radicals (Scherbina and Brik, 2000; Vanhaevelyn et al., 2002).

Based on the experiments on the fragments, new protocols for ESR dating of powders will be developed.

Acknowledgments

We are very grateful to T. Bodin, Research School of Earth Sciences, Australian National University (ANU), Canberra, for helping with the design of the simulating annealing program used for this work and L. Kinsley, RSES, for his assistance with the laser ablation measurement. We thank N. Mansor, Research School of Physical Sciences, ANU, for helpful comments. We are grateful to F. Callens and H. Vrielinc, Gent, for their considered advice in the earlier stages of this study. Aspects of this study were supported by the ARC fund DP0664144 Microanalysis of human fossils: new insights into age, diet and migration. RG is grateful to the Institut des Sciences humaines et sociales du CNRS, Bordeaux, and the Laboratoire d'Anthropologie des populations du Pasé, Université de Bordeaux I, for their kind hospitality in the writing-up stage of this manuscript. The SA program can be obtained by contacting JRB by email at renaud.joannes-boyaux@anu.edu.au or RG at rainer.grun@anu.edu.au.

Editorial handling by: R. (Bert) Roberts

References


CHAPTER 15
Dose assessment of a fossil tooth fragment from Jebel Irhoud (Morocco) using the SA decomposition model

1. Introduction

Direct ESR dating of human remains is complicated by the fact that dose assessments have to be carried out on fragments, instead of powders to minimize the impact of ESR analyses on valuable archaeological or palaeoanthropological specimens. Alas, the complex crystal organisation of fragments lead to a high angular dependency of the ESR spectra (see Figure 1), which complicates their study and the establishment of experimental protocols for dose estimation and dating (e.g. Grün et al., 2008).

The main ESR signal in fossil tooth enamel is generated by a range of CO$_2^-$ radicals (Brik et al. 2000a; Callens et al. 1998), which are interfered with several other radicals, mainly isotropic (methyl, CO$_3^{3^-}$, CO$^-$, CO$_2$ etc., for a compilation see Callens et al. 1998; Vanhauweyn et al. 2000a; 2000b; 2002). Two distinct groups of CO$_2^-$ radicals occur. The first group has no preferential direction within the enamel (non-oriented CO$_2^-$ radicals, NOCORs) giving rise to a powder spectrum of the same intensity at all angles. Radicals of the second group is oriented (anisotropic CO$_2^-$ radical, AICOR). For more details see Callens et al. (1995), Brik et al. (2000b), Ishchenko et al. (2002), Grün et al. (2008) and Joannes-Boyau and Grün (2009).

The NOCORs are believed to be imbedded in randomly organised crystals that give at once a mix of all orientations (Roveri et al., 2009) or associated with organic membranes of nanocrystals (Brik et al. 2000a). In contrast, the contribution of AICORs to the overall ESR spectra is changing at each angle, depending on the effective orientation in the enamel fragment (Grün et al. 2008, Joannes-Boyau and Grün, 2009).

Joannes-Boyau and Grün (2010c) developed a comprehensive model for CO$_2^-$ radicals in fossil tooth enamel. Two types of NOCORs and two types of AICORs were described. The NOCORs were distinguished by their thermal stability. An unstable component was preferably found near the dentine-enamel junction (DEJ) probably in the organic phase. More stable NOCORs occurred in the interprismatic domain (or inter-row sheet, Sander, 1997), closer to the buccal-enamel boundary (BEB). AICORs occur in two varieties as well: stable CO$_2^-$ radicals (also denoted R$_2$) (with $g_\perp$ around 2.0025 and $g_\parallel$ around 1.9974; Callens et al. 1987, Ishchenko et al. 2002) and the somewhat less stable axial radicals (with $g_{30}$, $g_{32}$ and $g_\parallel$ around 2.0030, 2.0015 and 1.9973, respectively; Callens et al. 1987, Ishchenko et al. 2002, Rudko et al., 2007). Contrary to the NOCORs, the two AICORs occur in the same crystal domains, based on a constant angle in the orientation between the two radicals (Joannes-Boyau and Grün, 2010c).

In a series of previous studies, we have decomposed the ESR angular response of fossil enamel fragment using an automated simulated annealing (SA) procedure (for more details see Joannes-Boyau et al., 2010a, b). The angular anisotropic spectra could only be fitted with four Gaussian components (R$_1$, R$_2$, R$_3$ and B$_2$, see Figure 1B). Depending on their g-values, R$_1$ was associated with orthorhombic CO$_2^-$ radicals, R$_2$ with the axial form and B$_2$ with the combination of $g_\parallel$ and $g_2$
of the aforementioned anisotropic radicals. \( R_3 \) is thought to represent the sum of AICOR misalignments (Lloidja 2001).

The studies were carried out on a bovid tooth from the archaeological site of Holon (Porat et al. 1999) and can be summarised as follows:

- The natural spectra contained about 9% NOCORs. The remaining AICORs consisted of a mix of orthorhombic to axial species with a ratio of 35:65 (Joannes-Boyau et al. 2010a).
- Beta irradiation through the DEJ resulted in 19% NOCORs, an orthorhombic to axial mix of 59:41 and an angle of about 23° (Joannes-Boyau and Grün, 2010b).
- Beta irradiation through the BEB produced 9% NOCORs and a mix of 45:55 of orthorhombic to axial AICORs and an angle of about 25° (Joannes-Boyau and Grün, 2010b).
- UV irradiation through the BEB generates a mix of \( \text{CO}_2^+ \) radicals that is closely similar to the natural sample.
- Gamma irradiation of the entire fragment induces the creation of only orthorhombic radicals and about 40% NOCORs. (Joannes-Boyau et al. 2010a).
- Gamma irradiation of an enamel layer close to the DEJ resulted in 57% NOCORs, and the creation of only orthorhombic radicals. (Joannes-Boyau and Grün, 2010c).
- Gamma irradiation of an enamel layer close to the BEB resulted in about 15% to 18% NOCORs, and the creation of only orthorhombic radicals. (Joannes-Boyau and Grün, 2010c).
- Thermal annealing experiments on gamma irradiated samples show that the NOCORS close to the DEJ are less stable than those close to the BEB, neither convert into AICORS. Furthermore, orthorhombic AICORs are converted into axial by heating and also most probably during geological burial (Joannes-Boyau and Grün, 2010b).

Because of different relative distributions and thermal stabilities of NOCORs and AICORs in the natural and irradiated spectrum components, the calculated dose values are angular dependent (Grün et al. 2008) and may result in an underestimation of the geological dose of the sample, consequently of its age.

In this paper, we apply the new model developed by Joannes-Boyau and Grün (2010b) to a tooth fragment of the human remains from Jebel Irhoud (Morocco, see Smith et al. 2007).

2. Materials and methods

The present study follows the methodology established by Joannes-Boyau et al. (2010a). The fragment was longer than 8 mm and it could only be rotated around its growth axis, which corresponds to the Y-configuration in our other papers (Figure 1A). T1, B1 and B2 are positions in the measured or simulated ESR spectra (Figure 1C). \( R_1, R_2, R_3 \) and \( B_2 \) are the fitted Gaussian components (Figure 1D). The radical concentrations were derived from the double integration of the fitted lines to account for changes in the line width. For the features in the measured ESR spectra, T1-B1 and B2, it was not possible to carry out double integrations; their angular variations were derived from their intensities (for more details see Joannes-Boyau et al., 2010a, b).
Figure 1: (A) orientation of the measurements. (B) Stack of the Y-configuration angular variation of IR (left in 3D; right top view). (C) Decomposition of the angular spectrum where the B2 intensity was largest (G10). (D) components found in the decomposition spectrum.

On Jebel Irhoud 3, a juvenile mandible that belongs to an 8 years old human child (Smith et al. 2007), measurements were carried out on an incisor enamel fragment (IR). The fragment was mounted in a Teflon holder containing a parafilm mould. Because of its large size it could not be measured by rotating it around its other two main axes.

At the time of measurements, (Smith et al. 2007) the fragment has been incrementally measured by rotating it around only one axis the Y-configuration (see Figure 1). The sample holder has
been inserted in a Bruker ER 218PG1 programmable goniometer and measured with a Bruker ECS106 ESR spectrometer in 10° increments over 360° with the following measurement conditions: 2 mW microwave power, 0.1 mT modulation amplitude 12 mT sweep width with a sweep time of 21 s. The spectra were accumulated over 50 consecutive measurements.

The fragment was irradiated using a 137Cs-source with cumulative dose steps of 0, 8, 58, 106, 155, 203, 298, 397, 494, 590, 780 and 969 Gy (for more details see Smith et al. 2007). The measurements on Irrhoud resulted in 12 stacks with a total of 432 spectra.

All spectrum decompositions and simulations were carried out on the measured derivative spectra. Decomposition was optimised and automated using a simulated annealing (SA) procedure. SA is a Monte Carlo method used for combinatorial optimisation problems (for details see Metropolis et al. 1953; Kirkpatrick et al. 1983; Černý 1985; Mossgard and Sambridge 2002; Bodin and Sambridge, 2009). The SA procedure is able to randomly generate a large number of synthetic spectra defined by a linear combination of four Gaussian lines. Each simulated spectra is compared to the measured spectra in terms of a least square misfit. The particular advantage of the SA algorithm is the search of the global minimum misfit without getting stuck in local solutions. As we shall see below, SA can resolve completely overlapping signals, which are very difficult to decompose with alternative decomposition approaches.

The Gaussian lines had prescribed limits with respect to the g-value range to avoid unrealistic solutions outside the regions for the CO3− radical in hydroxyapatite (see above). No restrictions were set on the intensity and maximum line width, however, a minimum width (0.10 mT) was defined to avoid aberrations.

3. Results and Discussion

3.1 Non-CO3− components

Several non-CO3− components occur around g=2 in the ESR spectra of fossil tooth enamel. Most of them are located outside the g-values range of the CO3− radicals (approximately 2.0060 to 1.9970). Some, however, can interfere greatly with the main signal and influence the peak-to-peak intensity reading from the T1-B1 position; these have to be removed before the SA decomposition. The isotropic lines occurring in the natural spectra of IR are the same as identified in our previous experiments (Joannes-Boyau and Grün (2009): a central methyl line as well as two wide lines, W1, around g=2.0061 (could not be attributed to a specific radical), and W2, around g=2.0051, most likely related to a combination of SO3− and CO− radicals which occur at g=2.0056 and g=2.0047, respectively (Bouchez et al. 1988, Schramm and Rossi 1999). These lines are not radiation sensitive and are automatically removed from the irradiation component when subtracting the natural spectrum from irradiation spectra.

The irradiation spectra are obtained by subtracting the natural from the natural + gamma spectra. With gamma irradiation a range of new isotropic lines occur. It is not clear why IR does not show the usual irradiation lines W1 and W2 (Joannes-Boyau et al., 2010a) but only W3 at g=2.0071, lw=0.9mT. This isotropic line interferes little with the main ESR signal, but was removed anyway prior to decomposition.
Figure 2: Evolution of the angular variation over 360° of IR with irradiation

3.2 NOCORs

The angular variations decrease with increasing irradiation (Figure 2). This is a direct effect of the increasing relative amounts of NOCORs by the gamma irradiation (Joannes-Boyau et al. 2010a). The amount of NOCORs responsible for the diminution of anisotropy can be easily estimated through a linear combination of the measured natural+gamma spectra and a negative powder spectrum until the angular variation of the natural spectra is obtained. The relative NOCORs concentrations in natural+gamma spectra increase dramatically in the first irradiation steps, but then saturates at around 300 Gy (Figure 3A). Nevertheless the radical concentration is constantly increasing with irradiation (Figure 3B). The relative NOCORs concentration in the natural is similar to the Holon and Broken Hill fragments (Joannes-Boyau and Grün 2010a, b). Between 750 and 780 Gy, the relative and absolute amounts of NOCORs in the irradiation components differ for the three fossil fragments of Holon (780 Gy, 39% NOCORs), IR (780 Gy, 30% NOCORs) and Broken Hill (750 Gy, 30% NOCORs). The observation of Vorona et al. (2005; 2006) of a constant percentage of NOCORs in the gamma component of modern human teeth does not apply to fossil tooth enamel. Diagenetic process or loss of organic maters could explain potentially explain the dissimilarities.
Figure 3: Dose response of (A) relative, (B) absolute NOCORs concentration in the total signal of IR

Nevertheless, the behaviour of NOCORs in BH is very similar to IR, with the same relative amount of NOCORs after around 750 Gy.

3.3 AICORs

The direction of the AICORs seems to lie nearly exactly in the rotational plane, which is expressed by a near zeroing of the T1 intensity at certain angles (Figure 5 A and B). Because only one orientation was measured, it is not possible to calculate the angle formed between the prisms and the DEJ, however, according to the angular response, we can speculate that the prisms would form a 90° angle with the DEJ.

Figure 5 summarizes the behaviour of IR with irradiation. The T1-B1 peak in the natural+gamma spectra is clearly shifting from $g=2.0023$ to $g=2.0032$ (Figure 4A), which corresponds to a transfer from a peak dominated by R2 to a peak dominated by R1. On the other hand, when looking at the irradiation component (Figure 4B), the peak at $g=2.0033$ believed to be linked to R1 is negligible in the first irradiation steps and then strongly increases while the peak at $g=2.0023$ shows almost no increase.

Figure 4C shows the influence of irradiation on the merged spectra at each irradiation steps, while Figure 4D shows the decomposition of the above spectra using the SA procedure. The natural contains mainly R2 radicals, with a R1:R2 ratio of around 30:70. As demonstrated in previous study (Joannes-Boyau et al., 2010a) gamma irradiation induces the creation of the orthorhombic radicals. At the last irradiation step the R1:R2 ratio is around 78:22 (in the natural+gamma spectra).

The variation in the R1:R2 ratio induces the T1-B1 peak shift previously described, caused by the preferential creation of R1. However, in contrast to Holon sample (Joannes-Boyau et al., 2010c) not only R1 appears to be radiation sensitive, R2 increases also with irradiation. Figure 5E shows the behaviour of R1, R2 and B2 with irradiation, normalised to the maximum radical concentration of R1. R1 increases by 4000%, from 0.025 a.u. to 1 a.u, while R2 increases by 480%, from 0.058 to 0.28.
Figure 4 Dose response at the angle of the maximum of B2 (G10) (A) the natural+gamma ESR spectra. (B) gamma component. (C) Merged spectra. (D) Dose response of R1, R2 and B2 (R3 is not shown for clarity) with their respective g-values. (E) Dose response of the radical concentrations of R1, R2 and B2.
3.4. Dose Estimation

The heating experiments of Joannes-Boyau and Grün (2010b, c) implied a quantitative transfer from orthorhombic to axial AICORs. They also speculated that this applies during storage over geological times. If this assumption is correct, then \( R_1 : R_2 \) ratio should not have any influence on the dose estimation, and consequently on the age calculated. Nevertheless, Joannes-Boyau and Grün (2010b) have also shown that two different NOCORs, one stable and the other one unstable are occurring with irradiation.

![Graph](image)

*Figure 5: Dose response of conventional T1-B2 peak-to-peak intensity, AICORs, NOCORs, stable NOCORs and Unstable NOCORs; (left) considering that the relative amount of stable NOCORs remains constant in the gamma induced ESR spectra through irradiation steps; (right) considering a 50:50 split between stable and unstable NOCORs in the gamma induced ESR spectra.*

The amount of NOCORs increases with irradiation (Figure 3B, above). Firstly, an iterative approach can be applied. If the dose response is entirely that of the AICORs, then the sample may have received a dose as high as 365 Gy (see Figure 5 and Table I). Assuming that the different types of radiation behave similar in nature (i.e. there is no heating effect during beta irradiation (Joannes-Boyau and Grün, 2010b), 365 Gy would have generated around 730 a.u. NOCORs. Of these, 150 (20.5 %) have remained in the natural. Using a short iteration shows that around 22.5% of the gamma induced NOCORs remained in the sample. Applying this percentage in the dose response a dose value of 340±23.5 Gy is obtained. However, this calculation explicitly assumes that beta (and alpha) irradiation behaved quantitatively the same as gamma irradiation and that no NOCORs converted into AICORs. Because the mix of \( \text{CO}_2 \) radicals in IR and Broken Hill are closely similar, one can follow the conclusions of Joannes-Boyau and Grün (2010b) who deduced a 50:50 distribution for stable to unstable NOCORs based on the comparison of gamma and beta dose response as well as annealing. This will result in a dose value of 292±16 Gy which is around 30% higher than the published T1-B2 estimate (Smith et al. 2008).
<table>
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Table 1: Fitting parameters for Figure 5.

4. Conclusions

The Irhoud sample shows atypical non-CO$_2^-$ components concentration especially after laboratory irradiation with only one irradiation sensitive lines W$_1$. The relative NOCOR concentration is different from a sample from Holon, but closely similar to BH. The angular measurements show a higher angle between the HAp prisms and the DEJ, with what appears to be almost perpendicular average orientation.

The SA decomposition of IR shows that in contrast to the sample from Holon, R$_2$-type radicals are generated by gamma irradiation. For that reason, each fossil fragment appears different and should be evaluated independently. The unstable gamma induced NOCORs may contain a stable component between 22.5% and 50%. As a result, the correct dose for this specimen may be as much as 50% higher than previously published.
PART 3

Previous models used to explain U-series sample dating variability, such as the D-A model (Pye et al., 2000), suggest directional impacts such as that from Pears (Chapter 7). Most of the restrictions that control the D-A model, including sample orientation, apply to the non-destructive ESR analysis protocol (Coffin, 2008). However, the benefits of the new two-dimensional mapping protocol is that it provides insights into dissolution within the tools that yield potentially unstable bones matching the expected age. Simultaneously, the last 67 years of discussion effort in determining maximum burning and potential burial contamination that single-track management would be unlikely to reveal. Understanding the uranium distribution within the tools' context is also valuable for ESR U-series correlation dating. The spatial analysis of the entire dataset, especially subject to the fragmental data for ESR dating, appears to overcome known problems of varved U-concentrations in these materials. In light of the data obtained from the U-series sample, one single butanol extraction would likely increase the error for age calculation and internal error measurement, since repeated ESR measurements does not allow spatial replication (Oiba et al., 1997). The varying heterogeneity of the uranium and thorium distribution within bone samples clearly shows the necessity of performing bulk analysis.
SYNTHESIS

This chapter presents a series of summaries that pull together the work presented in previous chapters. Each section below refers to a specific chapter, outlining explicitly and clearly the improvements made in the discipline via the research reported herein. The synthesis gives a broad overview of new mapping protocols for U-series and ESR/U-series combined dating, and for the non-destructive ESR analysis of tooth enamel.

THE BIG PICTURE

Previous models used to estimate U-series sample dating suitability, such as the D-A model (Pike, 2000), would disregard samples such as that from Payre (Chapter 7). Most of the restrictions that constrained the D-A model, including sample minimum age, apply to the two-dimensional mapping protocol (Grün, 2006). However, the benefit of the new two-dimensional mapping protocol is that it provides insights into domains within the tooth that yield potentially suitable dating areas matching the expected age. Simultaneously, the use of laser ablation offers to investigate uranium leaching and potential thorium contamination that single track measurements would be unlikely to reveal. Understanding the uranium distribution within the tooth domains is also valuable for ESR/U-series combined dating. The spatial analysis of the entire domain, especially adjacent to the fragment used for ESR dating, appears to overcome known problems of unrelated U-concentration for dose assessment. In light of the map obtained of the Payre sample, one single laser ablation track would likely increase the error for age estimation and internal dose assessment, since regular ESR measurements does not allow spatial resolution (Oka et al., 1997). The strong heterogeneity of the uranium and thorium distribution within tooth samples clearly shows the necessity of performing bulk analysis.
in preference to single laser ablation tracks in order to obtain an accurate dose assessment.

If accurate semi non-destructive internal dose estimation appears achievable with the use of laser ablation mapping of large portions of the fossil tooth, the establishment of non-destructive protocols for ESR dating raises several problems. The annealing experiments (Chapter 8) conducted on tooth fragments instead of powder have shown the absolute need for the proper alignment of spectra. The methodology developed to measure fragments using specifically designed Teflon holders filled with melted parafilm offers for the first time an accurate non-destructive protocol. At the same time, the use of methyl radicals for spectra alignment is validated by the fact that the merged spectra do not show any signs of line widening. We have shown that complex mechanisms are operating on irradiated and heated tooth enamel. Natural spectra show a mix of two CO$_2^-$ radicals species, one anisotropic (the AICORs) and the other one with no preferential orientation (the NOCORs). Their relative distributions in the natural and irradiation ESR spectra are quite different. The annealing experiments show that annihilation of the NOCORs occurs simultaneously with an increase of the AICORs at some angles, while the overhaul intensity remained the same. However, the decrease in the NOCORs cannot be the only contributor to the intensity increase in the T1–B1 region. The natural sample (Chapter 9) contains approximately 90% of AICORs, whereas laboratory irradiation induced a different ratio of CO$_2^-$ radicals with ca 40% of NOCORs.

The ESR angular response had to be decomposed since the merged spectra (Chapter 10) show little variation after thermal treatment or irradiation while T1 maximum intensity angle is shifting. Two distinct AICORs, R$_1$ orthorhombic and R$_2$ axial, were separated from the signal, with slight differences in their average orientation. At the same time, we cannot explain the changes during heating by a transfer from NOCORs to AICORs, because there are simply not enough of the first species present in the natural sample to explain the changes in the apparent increase in AICORs.

Chapter 11 describes the development of a rapid method for the decomposition of the angular spectra of tooth enamel. The simulated annealing (SA) procedure seems
particularly well suited to decompose overlapping signals, such as R₁ and R₂. All spectra can be satisfactory decomposed using four Gaussian Lines, R₁, R₂, R₃ and B₂. All fitted components seemed related to anisotropic CO₂⁻ radicals. R₁ has been tentatively related to the gₓ of orthorhombic and R₂ to g₅ of axial radicals. B₂ is a combination of g₉ and g₂ of the two anisotropic CO₂⁻ radicals. R₃ presents an envelope for misalignments, resulting from angles between the principal axes of the fragment as well as misalignments of various crystal domains. At first R₃ was thought to be related to both R₁ and R₂, however as demonstrated in Chapter XV, R₃ has been proven now to only relate to R₁. We found that the average orientations of R₁ and R₂ are offset by an angle of 23⁰. The occurrence of two different anisotropic CO₂⁻ radicals in fossil tooth enamel is further supported by Q-band studies on fossil enamel fragments (Bouchez et al., 1988; Rossi and Poupeau, 1990) and our earlier observations on the ESR response to heating (Joannes-Boyau and Grün, 2009). The gamma irradiation induced signal is significantly more complicated than previously thought. Apart from a very high proportion of NOCORs (40%) induced by irradiation, the spectra do not contain any identifiable axial CO₂⁻ component, the latter of which dominates the natural spectra. Since natural and laboratory irradiation induces significant differences in the overall radical composition, ESR dating accuracy on tooth enamel could be severely reduced.

The influence of gamma-irradiation on teeth was compared in Chapters 12 and 13 to UV and beta irradiation, respectively. Both irradiations induce very different signals than that found after gamma-irradiation. In a modern sample, UV generated 35% of NOCORs and a mix of 64:36 of orthorhombic to axial radicals. In the fossil sample, UV generated 9% NOCORs and a mix of 35:65 of orthorhombic to axial radicals. While there are some differences between the natural and UV components in the various configurations, the overall radical distribution of the UV and the natural is the same. This is in contrast to γ-irradiation component of the fossil sample, which had approximately 40% AICORs and no axial radicals. While the UV components in the modern samples showed strong fading over three months (18 to 20% of the spectra) due to a possible transfer process between R₁ and R₂, the fading in the fossil sample was small (3 to 4%).
β-irradiation (Chapter 13) induces the creation of both $R_1$ and $R_2$ radicals, contrary to γ-irradiation. The β-induced signal from the HS and HD fragments are different not only from each other, but also from the natural. It appears that the irradiation does not induce all the differences, many of which appear to lie within the crystal structure of the tooth itself. The $23^\circ$ angle between $R_1$ and $R_2$, calculated from the natural, is reproducible in both fragments HS and HD, indicating that the angle is induced by the radicals' organisation within the hydroxyapatite crystal. The difference in concentration of NOCORs in the two fragments could be explained by the DEJ, described as an amorphous layer. At the same time, we suggest that some of the NOCORs could also be found in the inter-prismatic phase that occurs in between prisms.

Chapter 14 presented a comprehensive model for $CO_2^-$ radicals in fossil tooth enamel. The crystals were found to be composed by four different $CO_2^-$ radicals Two AICORs ($R_1$ and $R_2$) and two NOCORs (stable and unstable) with different arrangement within the enamel domains. The DEJ is thought to be the preferential domain for the least stable of the two NOCORs. Nevertheless, the other, more stable, NOCORs are likely sitting in the interprismatic phase (or interrow sheet) known to be a mix of nano-crystals in random orientation. Two domains with different prismatic organisation were identified. Apart from the orientation of the prisms, significant differences between the two layers HD and HS was found in terms of NOCORs radical concentration, and kinetics behaviour of radicals. The SA simulation undertaken on both fragments after laboratory γ-irradiation and thermal treatment shows that a transfer process occurred between the two AICORs, transforming the $R_1$ species (orthorhombic) into the $R_2$ form (axial). The orientation offset between the two radicals of $\sim 23^\circ$ indicates a possible transformation of the main axis of the radical by heat. A transfer process between stable NOCORs and AICORs could potentially occur, however, no strong evidence has been found to support this hypothesis. The exact influence of unstable NOCORs on dating will depend on the amount of radicals created by irradiation, however, will generally lead to an underestimation of the sample age, by increasing the slope of the equivalent dose curve. For example, the Broken Hill sample shows that the original estimated dose was
underestimated by 30%. Unfortunately, the amount of total NOCORs is not consistent for all samples. For example, the Irhoud sample appeared to have fewer NOCORs than BH and therefore fewer unstable NOCORs.

Chapter 15 was focused on the study of the ESR fragment of important hominid remains from Jebel Irhoud (Morocco) using the new SA procedure. The Irhoud sample shows atypical non-CO$_2^-$ components concentrations, especially after laboratory irradiation, with only one irradiation sensitive line $W_{13}$. The relative NOCORs concentration differs from the relative amount observed in the Holon sample from 22% to 39% after ca 780 Gy gamma irradiation. The angular measurements show a higher angle between the HAp prisms and the DEJ, with what appears to be almost perpendicular average orientation. The SA decomposition of the merged spectra of IR has shown a very different repartition of AICORs ratios than the one observed in the Holon sample after irradiation. The $R_1:R_2$ ratio of 39:61 in IR is similar to the Holon fragment and in accordance with a potential transfer process occurring between $R_1$ and $R_2$ over the burial time. Nevertheless, the influence of irradiation on the AICORs differs from Holon with the increase of $R_2$ over the dose steps. For that reason, each fossil fragment appears different and should be evaluated independently. The unstable NOCORs induce a 30% underestimation of the age of the remains, by increasing the slope of the dose response curve. The revised dose for the Irhoud fossil tooth fragment would correspond to 292±16 Gy (instead of 250±20 Gy Smith et al., 2008). This is substantively different to the original dose estimation. By this mean Jebel Irhoud fossil remains potentially represent the oldest anatomically modern humans found in Africa.
CONCLUSIONS

In this concluding chapter I offer an overview of the outcomes from the research presented in the preceding chapters. The improvements made in U-series and ESR dating through this research are summarised, showing significant progress in our understanding of dating techniques suitable for establishing robust chronologies for hominin remains and therefore understanding hominid evolution.

The use of isotopic two-dimensional mapping for U-series dating represents a paradigm shift in comparison to the traditionally utilized single track analysis. The ability to overcome the strong heterogeneity of the uranium distribution within the dentine and the enamel allows us to dramatically improve the dating accuracy. Isotopic mapping not only causes minimal damage to the sample but additionally allows us to gain insight on the uranium uptake history of the sample. The latter has been demonstrated to be much more complex than previously described simple models such as early or linear uptake. While previous methods of U-series dating used an inaccurate average uranium and thorium concentration to calculate the internal dose, the two-dimensional mapping protocol offers for the first time the possibility of accurately calculating the dose next to and within the fragment used for ESR measurements. By doing so, the age obtained independently with the U-series mapping can be compared to the ESR age obtained on the enamel fragment, thereby it offers the ability to compare the two ages and assess potential problems of the dating.

The use of a single tooth fragment instead of a powder offers non-destructive ESR dating for hominin remains. The protocol developed allows the accurate measurement of a fragment without limitation to the irradiation steps or thermal treatment. Nevertheless, fragment analysis revealed complex patterning that had to be resolved. The use of a Monte Carlo simulated annealing procedure allowed the decomposition of the composite
ESR spectra obtained when measuring tooth fragments. It was found that four different CO$_2^-$ radicals two anisotropic (AICORs) and two with no preferential orientation (NOCORs) are responsible for the total ESR spectra. The study has shown that all CO$_2^-$ radicals have different kinetic properties as well as different responses to irradiation. While UV exposure resulted in a similar signal to the natural in terms of the AICORs:NOCORs ratio, beta and gamma radiation induces very different mix of CO$_2^-$ radicals. Only 10% of NOCORs were identified in the natural spectra, while 40% and an average of 15% NOCORs were found in the gamma and beta-induced spectra. The beta-induced spectra appear to be similar to the gamma-induced signal with thermal treatment. An experiment using resin on a fragment during irradiation revealed traces of melting, indicating potential temperature increase at the surface of the sample with irradiation. Thermal treatment experiments have demonstrated the existence of a complex transfer process between the CO$_2^-$ radicals. The orthorhombic radical (R$_1$) transfers in to the axial species with time; nevertheless, since the transfer appears total, the ratio of the AICORs has no influence on dating results. Because a potential transfer process occurs between the stable NOCORs and the AICORs, the stable NOCORs must be included in the dose response curve. This implies that only the unstable NOCORs are responsible for major age underestimation by increasing the slope of the dose response curve. For example, the dose underestimation of the Jebel Irhoud specimen was calculated to be 30%. By this mean Jebel Irhoud specimen represent the oldest modern human remains found in Africa, reinforcing the Out of Africa hypothesis.
RECOMMENDATIONS FOR FUTURE WORK

The 2D isotopic mapping protocol developed through this research provides insight into the history of the mobility of U-isotopes within a fossil tooth sample. The protocol could also be applied to a wider range of sample materials, including corals and shells. Thompson and Goldstein (2005) suggested that corals can no longer be considered as a closed system for uranium series, since complex biological and physical process lead to uranium mobility (Flor and Moore, 1977; Bard et al., 1991). However, their approach, consisting of single track laser-ablation analysis, raises concerns about the accuracy and relevance of U-series dating of diagenetic coral structures. Making high resolution U-series maps using the protocols developed in this thesis will facilitate the identification of uranium leaching and uptake that may have occurred due to increasing levels of acid in coastal waters. Thus, the superposition of 2D isotopic maps has unparalleled potential for the geochemical investigation of naturally occurring isotopes and anthropogenic impact on coral reefs. Nevertheless, the sample areas measured in this thesis were too small to develop a model for uranium uptake in tooth tissues. In light of observing uranium distribution within the dentine and around the DEJ, we can speculate that data derived from single laser ablation tracks may be considered a poor proxy for dose assessment. Laser ablation mapping should be carried out to overcome dose estimation errors, especially when extrapolated for ESR dating. Furthermore, such mapping should also be carried out on valuable archaeological human remains for which solution ICPMS analyses are not a viable option owing to their destructive impact.

The distribution of uranium-isotopes should be investigated at a nano-scale to enhance our knowledge of the incorporation pattern. The use of NanoSims has the potential to reveal complex U-mobility process operating within tooth enamel. This could have a major impact on U-series dating and ESR/U-series combined dating, and thus our understanding of the chronological framework for hominin evolution.
The SA procedure developed to decompose the angular response of fossil tooth enamel should be applied to a wider range of significant archaeological samples. This could lead to improvements in the accuracy of dating, but could also enhance our understanding of diagenetic process potentially occurring during burial.

Further studies are required to be able to understand more completely the interactions between enamel domains and their role in the stability of radicals. First we suggest that the evaluation of alpha irradiation on the different radicals should be investigated along with the dose rate effect of beta irradiation. Secondly, we suggest that a fossil enamel fragment large enough to be divided in four segments (DEJ, mature enamel near the DEJ, Mature enamel near the BEB, and the BEB) could then be subjected to laboratory irradiation and thermal treatment, which would allow researchers to accurately identify the impact of irradiation and to establish the kinetic behaviour of the different radicals with respect to their domains. The kinetics should be carried out at low temperatures (between 80°C and 110°C) over a long thermal treatment time (few months) to be able to accurately describe transfer process and kinetic behaviour of each CO₂⁻ radical species.

A systematic evaluation of the amount of unstable NOCORs should be conducted to potentially define a general behaviour for rapid age correction. At the present point no easy solution can be proposed to correct for age underestimation, and archaeologist should be warn against simply multiplying ESR results with a factor of 1.3. The development of a fast and accurate protocol for powder dose assessment should be one of the top priorities in future research on ESR dating of tooth enamel.

Significant fossil remains for hominid evolution should be re-investigated, especially when the estimated age using surrounding sediments or ESR dating are disputed, such as the Qesem Cave remains (Israel), Border Cave (South Africa), Tabun (Israel) or the Liujiang skull (China) (Grün, 2006; Grün and Stinger, 2000; Shen et al., 2002).

Finally, further investigations into the potential use of the ratio between orthorhombic and axial as a fast age estimation technique would be valuable. The ratio of AICOR in
fossil teeth could be used analogous to AAR dating, though unfortunately would also suffer from the same limitations of the latter technique. Nevertheless, the ratio does not require dose assessment, and thus could offer independent age estimation, especially for samples from museum collections with little information about the provenance of the specimens, i.e. the burial conditions are unknown and cannot be reconstructed.
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APPENDIX
Appendix A

List of Publications
# PUBLICATION LIST

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List of publication written during and included in this PhD where R. Joannes-Boyau was first or second-author. (⭐️ R. Joannes-Boyau along with co-author; (*) graciously lend the fossil (M.H. Moncel and C. Stringer); (”) Co-author helped developing the SA procedure for decomposing the ESR spectra (T. Bodin).
Appendix B

Tables of radicals' g-values, width, intensity and average radical concentration for all Holon fragment (H3, H4, H5, H6) used in Joannes-Boyau et al. (2009, 2010, in press) and Joannes-Boyau and Grün (2010).
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Appendix C

Tables of AICORs g-values, width, intensity, angular variation and average radical concentration with respective angles, for natural and irradiated Holon fragment (HD and HS) used in Joannes-Boyau and Grün (submitted B)
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Appendix D

Illustration of the S.A. procedure used to decompose and extract radicals composing the ESR spectra of fossil fragments. Angle G8 of the H3 fragment in the natural in X-configuration.
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Appendix E

Conference posters

LED (China, 2009)
AAA (AUS, 2008)
APLED (India, 2009)
INQUA (AUS, 2006)
Natural and irradiated fossil teeth contain two types of CO₂ radicals (orientated and non-orientated). Experiments on a non-irradiated bovid tooth from the archaeological site of Holon show that heating creates another orientated CO₂ radical, perhaps as the result of the annihilation of the non-orientated CO₂. We speculate that the orientated CO₂ centres in the natural sample are orthorhombic and the newly created axial is rhombic.

Non-destructive ESR analysis was carried out on enamel fragments instead of powders (Fig. 1). Because of the high angular dependency, the ESR spectra (Fig. 2A, B) are incremented by rotating the fragments around their three major axes.

Enamel ESR spectra are dominated by two types of CO₂ radicals: a non-orientated (N-O; giving rise to a powder spectrum at all angles) and an orientated, changing with each angle. While their powder spectra are virtually identical, they can be distinguished in fragments. The contribution of the orientated CO₂ radical to the overall spectrum is largest where B2 dip is the smallest (Fig. 2A) and smallest where B2 is largest (Fig. 2B). These specific positions are used to investigate the thermal behaviour of the different CO₂ radicals (Fig. 3).

A similar thermal response is observed in some B2 positions (Fig. 4D), but other B2s show markedly different thermal behaviour with only small increases (Fig. 4C). Because only the g of CO₂ radicals give rise to any ESR signals in the B2 region, this different thermal behaviour allows only one conclusion: there must be two different types of orientated CO₂ radicals.

The ESR response to thermal treatment: maximum of T1-Bi (A, B) and B2 (CO₂) in fragments.

The direction of g for these two orientated CO₂ radicals can now be determined from the angular response of the natural (B2 ann) and heated (B2 max increase) samples, respectively. The effects are about 19°, 30° and 30° (Fig. 5). It is not clear in which domain of the enamel these newly created orientated CO₂ radicals occur.

The angular spectra can be decomposed into their different components (Fig. 6). The natural, unheated spectrum of the B2 Minimum in H configuration (see Fig. 2A) is dominated by the T1-Bi of the orientated component, the powder spectrum of the N-O CO₂ plus minor contributions from an isotropic line around 2,000 G (o), a wider line around 2,000 G (organics*), and the methyl radical (Fig. 6; Left diagram). After heating for 1440 min at 175°C, the T1-Bi is reduced by 70% while at the same time the powder component decreases by 82%. The two isotropic lines have disappeared, while three new isotropic lines occurred, two narrower at ~2,000 G (SO₂) and g=2.0055 (diamagnetic CO₂), as well as a wide line centred at 2,083 G (methyl). The methyl lines increased by 25% (Fig. 6; Right diagram). This effect can be used to identify archaeological heating. The simulation of these components are a very close match for the measured spectra.

CONCLUSIONS

Heating demonstrated the presence of two orientated CO₂ radicals in tooth enamel. The question is whether the heating induced component is also present in the unheated sample. The next step of our studies will be the investigation of irradiated samples (recent and fossil) to quantify the relationship between the non-orientated and orientated CO₂ radicals. We hope that beta irradiations will give insights in which enamel domains the various CO₂ radicals are located.
DIRECT DATING OF HUMAN REMAINS BY NON-DESTRUCTIVE ESR AND U-SERIES ANALYSIS

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Direct dating of bones and teeth older than 50 ka can only be carried out using ESR and/or Uranium series analysis. These methods are extremely useful since they can date samples up to 500 ka and 2 Ma respectively, though damage to valuable samples has previously been an issue, especially for ESR measurements. Recently, two new protocols have been developed at the Research School of Earth Sciences that are considered virtually non-destructive, but at the same time give a significant improvement of the understanding of the migration history and on the ESR compounds itself. The combined use of U-series and ESR dating is rather complex, but considerably improves the accuracy of the age estimation and perhaps even the dating range.

ESR dating of fossil tooth enamel fragments

Non-destructive ESR analyses are carried out on enamel fragments instead of powders to minimize the impact of sampling on valuable archaeological material (Grün et al., 2007). A small fragment is removed from the fossil tooth using natural cracks to be measured with an ESR spectrometer. After analysis, the fragment is replaced on the tooth and becomes virtually unnoticeable.

Figure 1: (A) After being removed from a tooth along natural fractures, enamel fragments are mounted in three Teflon holders. H: rotation around the axis perpendicular to the enamel surface. V: rotation around the axis parallel to the enamel surface. W: perpendicular to H and V. (B) and (C) 380 degree Scan ESR spectra of the H and L configuration.

Because of the high angular dependency of the hydraphosphate crystals of which tooth enamel is composed, fragments have to be measured in three configurations H, L and V to obtain every crystal orientation (Fig.1A). The ESR spectra recorded are complex (Fig.1B, C) and their study has led to the development of new techniques to extract information. The suspected presence of at least three CO radicals (two orientated and one with no preferential orientation) has raised the problem of possible overestimation of age calculation. With the new fragment approach we were able to identify the thermal stability differences of the orientated and non-orientated CO radicals (Fig.2A) (Joannes-Boyau and Grun, submitted). At the same time, the two orientated CO radicals have been identified and their orientations characterized (Fig.2B).

Figure 2: (A) Thermal stability of the orientated and non-orientated radicals. (B) Directions of the orientated radicals are consistent with crystallographic analysis. (C) Spectroscopic sensitivity profiles of radicals that compose the ESR signal of a carved fragment.

New spectrum decompositions carried out on angular responses show a very complex signal, reflecting the presence of several radicals with distinctive thermal stabilities and radiation sensitivities (Fig.2C). We are confident that this new approach will lead to significantly improved dating accuracy and may potentially also increase the dating range of human fossil teeth.

U-series mapping of fossil tooth

A tooth is a "open system" for uranium, gaining uranium from the burial environment. Our ability to date human remains relies entirely on our understanding and modeling of the uranium uptake history. A new protocol has been developed using in situ laser ablation sampling combined with ICP-MS (inductively coupled plasma mass spectrometry) analysis that allows mapping of an entire tooth or sections thereof (Fig.3A, B). This new technique is applied directly on the fossil without chemical preparation. It minimizes damage and can be considered virtually non-destructive.

Figure 3: (A) Half of the tooth being measured. (B) Robotic cell linked to the ICP-MS at ANU.

Mapping of tooth U/Th concentrations (Fig.4A,B) and ratios (Fig.4C,D,E) has significantly increased our understanding of U-migration history. The heterogeneity of U/Th ratio repartition within the sample indicates a complex uptake pattern and raises questions regarding age estimations with single point measurements. Maps (Fig.4F,G,H) clearly illustrate different domains of the tooth that give very different apparent age estimations (Grün et al., 2008).

At the same time, the high precision analysis on the enamel and surrounding dentine allows precise calculation of the internal dose, especially within and in the direct surrounding area of the fragment used for ESR analysis. As a result, ages can be mapped across the entire tooth (Fig.4I) and compared to the age found with the ESR calculation, increasing the accuracy of the dating.

Figure 4: (A) UES3 picture of the sample mapped. (B) Map of the U concentration in the mapped and the dentine. (C) UES3 picture of the same corresponding to the ratio maps (D) and (E) and the age map. (F) Map of the U concentration in the mapped tooth. (G) Map of the U concentration in the mapped tooth. (H) Map of the U concentration in the mapped tooth. (I) Map of the U concentration in the mapped tooth.
Decomposition of the natural and laboratory irradiation components of angular ESR spectra of fossil tooth enamel fragments

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The Australian National University, Canberra, ACT 0200, Australia

ABSTRACT
Spectrum decomposition of the angular measurements of fossil tooth enamel fragments using an automated simulated annealing (SA) procedure shows that the mix CO₂-radicals generated by laboratory irradiation is significantly different to that of the natural sample. The naturally irradiated sample contains about 19% of non-oriented CO₂-radicals and a mix of 25.65 armorphous, 38% axial CO₂-radicals. In contrast, laboratory Irradiation generated about 48% of non-oriented CO₂-radicals and a large amount of armorphous CO₂-radicals, while we failed to detect any axial CO₂-radicals. The results indicate that the optical aging of the sample incurs various annealing and transfer processes; their precise nature is yet unknown. Nevertheless, the understanding of the formation and transfer processes that led to the observed mix CO₂-radicals in fossil tooth enamel is essential for the reliable application of ESR dating.

Non-destructive ESR analysis is carried out on enamel fragments instead of powders. Because of the high angular dependency, the ESR spectra (Fig. 2A,B) are incremented measured by rotating the fragments around their three major axes.

Figure 1: After being removed from a tooth along natural fractures, enamel fragments are mounted in three silver holders containing Paraffin wax perpendicular to the enamel surface. Parallel to X, Y, around the axis of tooth growth, and Z, perpendicular to X and Y.

Enamel ESR spectra are dominated by two types of CO₂-radicals: non-oriented (N.O.) giving rise to a powder spectrum at all angles, and orientated, changing with each angle. While their powder spectra are virtually identical, they can be distinguished in fragments.

The natural CO₂-radical to the overall spectrum is large, but B₂ is the smallest, with B₂ largest (Fig. 2A) and smallest where B₂ is largest (Fig. 2B).

Figure 2A, B: Stacked ESR spectra of the angular measurements in X and Z configurations, B₂ minimum and maximum are used to identify the orientated and non-orientated CO₂-radicals.

Natural and irradiation spectra are separated by subtracting the natural from the natural + y (see Figure 4A to C). Then isotopic components have to be identified and subtracted (Figure 3), plus the non-oriented CO₂ radial components are subtracted (99% and 48% for the natural and irradiation spectra, respectively). Left are the isotopic components of the natural (Figure 4D) and irradiation spectra (Figure 4C). While the natural and irradiation spectra look quite similar (Figure 4A to C), the isotopic components are obviously different, indicating a different mix of radicals.

Figure 3: Identification of the isotopic components (A,B) and fitted Gaussian components (C,D).

X-configuration Y-configuration Z-configuration
A: Natural
B: Natural plus Y
C: Irradiation
D: Natural minus isotopic and non-oriented
E: Irradiation minus (isotopic and non-oriented)

The natural spectra can be decomposed with an overall contribution of 99% non-oriented CO₂-radicals and 1% armorphous and 65% axial CO₂-radicals.

Figure 4: Stacked spectra
The natural spectra could be decomposed with four Gaussian components. R₁, R₂, R₃, and R₄, R₅, R₆, R₇, R₈, R₉, and R₁₀, R₁₁, R₁₂, R₁₃, R₁₄, R₁₅, R₁₆, R₁₇, R₁₈, and R₁₉.

Figure 6: Decomposition of the anisotropic natural and irradiation spectra with four Gaussian components.

Figure 7: Decomposition of the irradiation spectra with three and four Gaussian components.
The irradiation spectra can be decomposed with an overall contribution of 40% non-oriented CO₂-radicals and the anisotropic component consists entirely of armorphous CO₂-radicals.

CONCLUSIONS
This study shows that the radiative response of fossil tooth enamel is significantly more complicated than previously thought. Apart from a very high proportion of non-oriented CO₂-radicals, the irradiation spectra do not contain any identifiable axial CO₂-radical component, which, on the other hand, dominates the natural spectra. Keeping in mind that various ESR dating attempts have been accompanied with some degree of success, it seems that most of the radicals are converted to one type in another without much reducing the overall radical concentration. To understand these transfer processes will be subject of an upcoming kinetics study.
ABSTRACT

Detailed studies on tooth enamel fragments have shown that the ESR spectra are significantly more complex than previously assumed. Because of unstable components, it was initially suspected that all ESR age estimations could be underestimated by more than 30%. However, detailed annealing experiments imply that this effect has only a small effect (2 to 6%).

In ESR dating of tooth enamel, the estimation of the dose value, D, is usually obtained on powdered samples. The qualitative dose response of enamel powder to environmental and laboratory radiation is closely similar (Figure 1). The spectra are generated by an axial CO$_2$-radical.

To investigate this problem, measurements were carried out on enamel fragments, which were rotated around their three principal axes in 10° increments (Figure 4).

Figure 5 shows the stacked ESR spectra for two of the main axes (because of the axial symmetry of the CO$_2$-radical the main axes, L and V, yield very similar ESR responses).

The overall results can only be explained that the contributing paramagnetic radicals are CO$_2$. However, these occur in two configurations. The first one is an axial (orientated) nature and gives rise to angularly dependent ESR spectra. The second one is a non-orientated radical giving rise to a powder spectrum at all angles. The problem is that the relative concentrations of these two radicals is different in the naturally (90:10) and laboratory irradiated (80:20) components of the ESR spectra, respectively.

It has been shown on modern teeth, that these two radicals have different thermal behaviour (Figure 7), the non-orientated CO$_2$-radical being considerably less stable. The implication is that all ESR dose estimates may be seriously underestimated, perhaps by more than 30%.

Detailed heating experiments, however, show that whilst the non-orientated CO$_2$-radical anneals, the orientated increases. As a consequence, the overall effect on the total signal and dose estimation is rather small (2 to 6%).

CONCLUSIONS

Our results present the starting point of detailed studies of tooth enamel fragments, which involve the precise description of the kinetics of the paramagnetic centres and the identification of all radicals involved in the ESR spectra of fossil teeth. In spite of the complexity of the ESR spectra, we are confident that the overall effects on dating are reasonably small.