Research paper

Two types of CO_2^- radicals threaten the fundamentals of ESR dating of tooth enamelRainer Grün^{a,*}, Renaud Joannes-Boyau^a, Chris Stringer^b^a*Research School of Earth Sciences, The Australian National University, Canberra, ACT 0200, Australia*^b*Department of Palaeontology, Natural History Museum, Cromwell Road, London SW7 5BD, UK*

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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 (orientated) and one that has no preferential orientation (non-orientated). The spectra of the naturally irradiated samples investigated here show a mix of about 90% of the orientated and about 10% of the non-orientated CO_2^- radicals. In contrast, laboratory irradiation induces a mix of about 60% orientated and 40% non-orientated CO_2^- radicals. Heating experiments show that the non-orientated CO_2^- radicals are significantly less stable than the orientated. 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-orientated CO_2^- radicals, a larger number of orientated 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-orientated CO_2^- radicals has about the same decay rate in preheated and non-preheated samples, the increase of the orientated 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 orientated and non-orientated 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;

IAEA, 2002). 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; Vanhaelewyn 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

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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 $g_x = 2.0030$, $g_y = 1.9973$ 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; Vanhaelewyn 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 (Vanhaelewyn 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 (Vanhaelewyn 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 allow 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,

Fig. 1. Angular dose estimations for an enamel fragment from Border Cave (A) and Irhoud (B). Data from Grün et al. (2003) and Smith et al. (2007).

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 $g_{\parallel}$ 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 parafilm mould in a sample holder (see Fig. 2), which can be inserted into a Bruker ER 218PG1 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 dosed 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 Eleksys 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 $g_{\parallel}$. The irradiation spectra also contain some small shoulders I1–I3 (see Fig. 3B). In the following, six types of spectra are discussed: (1) natural (not irradiated in the laboratory), (2) irradiated (labelled N+ γ , 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 $N + \gamma$, 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 CO_2^- radicals, a powder spectrum is subtracted from the natural and $N + \gamma$ spectra, by minimising the B2 region. These are labelled (5) N-P and (6) $N + \gamma$ -P.

Dose values were determined using a range of fitting procedures (for more details, see Grün, 2000c, 2002): fitting the natural spectrum into the $N + \gamma$, 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 $N + \gamma$ 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 palaeoanthropologi-

cally 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 “ $g_{\parallel}$ 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 $g_{\parallel}$ is parallel to the direction of the external magnetic field, then the ESR

Fig. 4. Summary of the natural (A–C), irradiation (D–F) and residual spectra (G–I) of BHS measured at 10° steps over 360°. The diagrams give different viewing angles to the stacked spectra.

spectra show a maximum in the B2 area. When $g_{\parallel}$ is perpendicular to the direction of the external magnetic field, the B2 area shows a minimum and the T1–B1 peak is largest.

In the BHS configuration (rotation around the dentine/enamel interface), it is expected that $g_{\parallel}$ 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 + γ 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 I1–I3, see Fig. 3B), corresponding to g -values of approximately $g = 2.0115$ (I1, perhaps CO_3^- radical associated with water, Callens et al., 1998), $g = 2.0093$ (I2), and $g = 2.0076$ (I3). The amplitude of the residuals are in the range of 15% of the normalised natural and irradiation spectra.

Fig. 3. Measurement of BHS (rotation around the dentine/enamel interface) at angles which show the greatest difference, at 90° (A–C) and at 180° (D–F). (A, D) N + γ spectra for selected dose steps. (B, E) Irradiation spectra (subtraction of the natural spectrum from N + γ). (C, F) Comparison of scaled (normalised on the same intensity) natural and average irradiation spectra. The residual spectrum, obtained by subtracting the scaled irradiation spectrum from the natural is offset for clarity. T1 and B1 denote the position of the $g_{\perp}$ component of the CO_2^- radicals, B1 of the $g_{\parallel}$. R1–R4 indicate various widths used for spectrum fitting, I1–I3 transient signals in irradiated samples, C1 and C2 small peaks in the residual spectra. C1 is the central methyl line, the next low/high field pair is indicated in (A). All spectra were aligned on the low-field methyl line.

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

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 $g_{\perp}$) and B2 (direction of $g_{\parallel}$) 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 6F. Similarly, T1–B1 intensities are

Fig. 6. Summary of the natural (A–C), irradiation (D–F) and residual spectra (G–I) of BHL measured at 10° steps over 360°. The diagrams give different viewing angles to the stacked spectra. The colours in (A, D, G) repeat every 180°.

Fig. 7. Measurement of BHL at angles, which show the greatest difference, at 100° (A–C) and at 190° (D–F). (A, D) $N+\gamma$ 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 $g_{||}$ 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 $g_{||}$ 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 $N+\gamma$ 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 $N+\gamma$ 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(!) 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.

Fig. 9. Summary of the natural (A, B), irradiation (C, D) and residual spectra (E, F) of BHV measured at 10° steps over 360° . The diagrams give different viewing angles to the stacked spectra. The colours in (A, C, E) repeat every 180° .

again confirming that our assumptions concerning the axially of the CO_2^- radical and the enamel fragment structure are justified. When $g_{\parallel}$ 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

Fig. 10. (A, B) Dose values for BHV 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.

features I1 and I2 (for positions C1, C2, I1 and I2, 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_2^- radicals. As mentioned above, these may occur in two configurations, orientated and non-orientated (Brik et al., 2000a; Callens et al., 1995; Vanhaelewyn et al., 2002). The angular measurement of a single crystal containing only orientated

CO_2^- 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 $g_{\parallel}$ parallel to the external magnetic field, the ESR spectrum would only contain the B2 dip, and when $g_{\parallel}$ was perpendicular to the external magnetic field, only T1–B1. On the other hand, if the fragment contained only non-orientated CO_2^- 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_2^- 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 $g_{\parallel}$ and $g_{\perp}$ 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. $g_{\parallel}$ was more or less perpendicular to the external magnetic field for all measurements. If we had only orientated CO_2^- 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: $N + \gamma$. Right: Scaled (normalised 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 (Grün, 2006b). 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 has shown little interferences between natural and irradiation spectra.

presence of non-orientated CO_2^- radicals. The same can be observed at the other configurations when $g_{\parallel}$ is more or 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

Fig. 14. Isochron (1 h) annealing. Data from Brik et al. (1998).

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 $\text{N}+\gamma$ spectra by using the B2 region. When $g_{\parallel}$ 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 $\text{N}+\gamma$ 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. $(\text{N}+\gamma\text{-P})/(\text{N}+\gamma)$. 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

Fig. 15. Apparent percentages of orientated CO_2^- radicals in the $\text{N}+\gamma$ spectra (open symbols) and irradiation spectra (closed symbols). The percentages are obtained by scaling and subtracting the merged spectrum (Fig. 11G and H) from the $\text{N}+\gamma$ and irradiation spectra (those that have the smallest B2 intensity, see Figs. 4, 6 and 9) until B2 is minimised.

positions where B2 is smallest, which is at angles of 180° and 360° for BHS (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 $39.5 \pm 1.5\%$. 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 Porat 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 $g_{\parallel}$, 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 orientated CO_2^- radicals in the natural of 1557 was 68.0% (BH: 68.5%) and in the $\text{N}+\gamma$ (100 min) 52.5% (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-orientated CO_2^- radicals would anneal while the orientated 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 $\text{N}+\gamma$ 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-orientated CO_2^- radicals. The normalised $\text{N}+\gamma$ 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 $\text{N}+\gamma$ 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-orientated CO_2^- radicals and the simultaneous increase of the orientated. 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 CO_2^- radical with slightly different g -values, as mentioned by Brik et al. (2000b), or perhaps other radicals, such as CO_3^{3-} .

Fig. 17A summarises the change of signal intensity and the percentage of the orientated CO_2^- radicals in the natural and $\text{N}+\gamma$ 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 orientated CO_2^- radicals show an increase from about 68% to 79%. The $\text{N}+\gamma$ response is somewhat different, with a rapid increase of the signal in the first 3 h by about 13%,

after that the increase of $\text{N}+\gamma$ runs parallel to the natural. During the first 3 h, there is also a rapid increase of the percentages of the orientated CO_2^- radicals, from about 52% to 62%. After heating of 26 h the percentage of orientated CO_2^- radicals in the $\text{N}+\gamma$ is about the same as in the non-heated natural sample. After 261 h, the percentages of orientated CO_2^- radicals in the natural and $\text{N}+\gamma$ are about the same. Fig. 17B gives some insight about the possible transfer mechanism. Here the amounts of orientated CO_2^- and non-orientated 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 orientated CO_2^- , and decrease of the non-orientated CO_2^- radicals is somewhat different in the natural and $\text{N}+\gamma$ samples, it is obvious, that decrease of the non-orientated CO_2^- radicals can only be partly responsible for the increase of the orientated CO_2^- radicals. In the natural sample, the overall increase of the orientated by 26 percentage points compares to a decrease of the non-orientated by 7, for the $\text{N}+\gamma$, the overall values are 50 and 18 and for the first 3 h, 19 and 6, respectively. This means that the decay of the non-orientated CO_2^- radicals can contribute only a quarter of the increase of the 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 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 orientated and non-orientated 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-orientated CO_2^- radicals, the B2 region still contains about 20% of a component that has different kinetics to the non-orientated CO_2^- radicals. This component can be attributed to the orientated 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 orientated to non-orientated CO_2^- radicals, respectively. Note that Vorona et al. (2007) found distinctively different proportions of orientated (about 20%) and non-orientated (about 80%)

Fig. 16. Response to post-irradiation heating. (A–D) Natural. (E–H) $N+\gamma$. (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 CO_2^- 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.

Fig. 17. (A) Apparent percentages of orientated CO₂⁻ radicals and normalised signal intensities of the natural and N + γ spectra after heating at 125 °C. (B) Normalised apparent percentages of the orientated and non-orientated CO₂⁻ radicals. The percentage values are obtained by subtracting the merged, powder-like spectrum, so that B2 is minimised (see Fig. 16C and G).

CO₂⁻ 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 CO₂⁻ 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 CO₂⁻ 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 CO₂⁻ radicals is in the range of 50–75 ka. If the mean life and kinetics of the non-orientated CO₂⁻ radicals could precisely be determined, the relative contributions of the non-orientated CO₂⁻ 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 CO₂⁻ 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 + γ 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 in 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 CO₂⁻ 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 CO₂⁻ radicals by 37 percentage points compares to a decrease of the non-orientated CO₂⁻ radicals by 18, a potential 50% contribution to the rise of T1–B1. The development of the apparent percentages of the orientated CO₂⁻ 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 CO₂⁻ 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 + γ 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) $N+\gamma$, normalised on the sample without heat treatment. (B) $N+\gamma$, scaled to the same intensity. (C) $N+\gamma$ minus powder (to remove the non-orientated CO_2^- radicals) on the sample without heat treatment. (D) $N+\gamma$ 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_2^- radicals of the $N+\gamma$ 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 natural and $N+\gamma$ spectra after heating at 125 °C as well as the $N+\gamma$ 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_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 CO_2^- radicals contributes only about 30% to the increase of the orientated 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, there seems a slight increase, perhaps by around 1–2%. The $\text{N}+\gamma$ 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 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 CO_2 into the surface layers of the hydroxyapatite crystals, which may turn into orientated 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; Vanhaelewyn 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 (Vanhaelewyn et al., 2002), they are non-committal, in which location it actually occurs. Vorona et al. (2006) suggested that both types of CO_2^- radicals are located in the B-position of the hydroxyapatite, replacing a 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 CO_2^- type could be associated with a loss of water.

It is unclear in the model-1 of Brik et al. (2000a), why the transfer is not reversible, i.e. it should reach a steady state. If the orientated 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 CO_2^- radicals. Secondly, we observe that after preheating and irradiation, the ratio of $(\text{N}+\gamma)/\text{N}$ as well as the relative distribution of the two types in the $\text{N}+\gamma$ is the same as in the non-preheated. 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 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., 1995; 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 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 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 CO_2^- radicals of the type described in this paper. The non-orientated 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 CO_2^- radicals located in this matrix. If this was the case, any direct transfer from the non-orientated CO_2^- radicals to the orientated is difficult to explain. The increase of the orientated CO_2^- radicals with heating could either be explained by 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 CO_2^- radical can capture additional electrons, similar to the model proposed by Martinez et al. (2001) for the SO_2^- radical in corals.

4. Summary discussion

The irradiation experiment shows clearly that tooth enamel contains two types of 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-orientated CO_2^- radicals occurs simultaneously with an increase of the orientated CO_2^- . However, the decrease in the non-orientated 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 a. 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 parameters 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_x and g_z , or the presence of other radicals (Brik et al., 2000b). This, as well as the notion that the non-orientated CO_2^- is orthorhombic (Vanhaelewyn et al., 2002), requires more detailed investigation. 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 little affected by the heat treatment. On average, the $\text{N} + \gamma$, heated spectra are only up to 3% larger than 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 curvature 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 $\text{N} + \gamma$ 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

One 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-orientated 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 incremental 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_3^{3-} , 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.

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