Calcif Tissue Int (1987) 41:124–129

Calcified Tissue
International

The Contribution of CO_3^{3-} and CO_2^- to the ESR Spectrum Near g = 2 of Powdered Human Tooth Enamel

F. J. Callens,[1] R. M. H. Verbeeck,[2] P. F. A. Matthys,[1] L. C. Martens,[3] and E. R. Boesman[1]

[1]Laboratory of Crystallography and Study of the Solid State; [2]Laboratory for Analytical Chemistry; and [3]Department of Preventive Dentistry, State University Gent, Belgium

Summary. The ESR spectrum near g = 2 of powdered human tooth enamel from upper central incisors and lower canines was studied as a function of microwave power, irradiation, and storage time. The results clearly demonstrate that the ESR spectrum is composite with at least five paramagnetic species contributing to the signal. The main stable component is assigned to CO_2^-. Two other components arise from CO_3^{3-} radicals, one of which is demonstrated to be the same center as is present on a phosphate site in sodium- and carbonate-containing calciumapatite.

Key words: Tooth enamel — ESR spectrum.

In the last few years the ESR spectrum of human tooth enamel has been extensively studied. The spectrum of enamel blocks around g = 2 was measured by Cevc et al. [1–3] and later by Martens et al. [4–6] to determine the so-called R-value. According to Cevc et al. this R-value was related to the microcrystal alignment and to caries susceptibility. Differences were found in R-value, depending on tooth type and tooth position; the enamel from sound teeth was also found to have higher R-value than that from carious teeth [1–6].

Such variation of a phenomenological parameter like R clearly demonstrates the variability of the ESR signal. Knowledge of the nature of this signal is a prerequisite for a systematic evaluation of the qualitative and quantitative relationship between the ESR signal around g = 2 and the chemical and crystal-chemical properties of tooth enamel.

Although several research groups have studied the ESR properties of powdered enamel, the identification of the asymmetric signal near g = 2 is still controversial. This signal has either been assigned to CO_3^{3-} [1–7] or CO_2^- [8–9]. Doi et al. [7] also found an isotropic center with g = 2.0019, which remained unidentified. On the other hand, for synthetic carbonated calciumapatites it was shown in a previous study [11] that the ESR signal around g = 2 is the composition of several powder spectrum contributions arising from different paramagnetic species. On this basis the possibility cannot be excluded that this signal in the ESR spectrum of tooth enamel is also composite.

For this reason the dependency of the ESR signal near g = 2 of powdered tooth enamel on microwave power, amount of irradiation, and storage time was studied as a first step. In order to minimize the effect of biological variations within a group of teeth of the same type and position, pooled samples were used. Moreover, the dependency of the signal on the parameters mentioned above was investigated for the enamel of central upper incisors and lower canines which show clearly different R-values [5].

By comparing and subtracting the digitized spectra, in some cases an isolated component is obtained. In spite of some successes obtained by numerical manipulations of ESR curves, it is clear that it is still useful to characterize an experimental curve by a restricted number of phenomenological parameters, such as Cevc's R-value. This certainly applies if trends in and correlations between ESR signals from biominerals are to be determined.

Send reprint requests to Dr. R. M. H. Verbeeck, Research Associate N.F.S.R., Laboratory for Analytical Chemistry, State University Gent, Krijgslaan 281-S12, B-9000 Gent, Belgium.

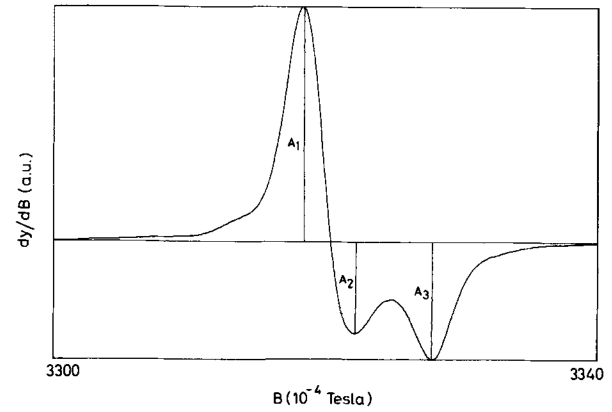

Fig. 1. Definition of R- and S-value. $R = A_3/A_1$; $S = A_2/A_1$.

Figure 1 shows how R is defined; in addition we introduce an analogous parameter, the S-value.

$$R = A_3/A_1 \quad (1)$$

$$S = A_2/A_1 \quad (2)$$

By means of this R- and S-value a complete survey of the experimental results can be given more easily.

Materials and Methods

Mature human tooth enamel samples were obtained from permanent upper central incisors and lower canines which were extracted from adult patients for prothetical or periodontal reasons. Only intact teeth with clinically sound enamel were used in this study. After cleaning with a water-cooled rotating small bristle and a nonabrasive polishing paste, the teeth were stored until further use.

For the ESR recordings, a small enamel piece of approximately 1 × 1 × 3 mm was cut from the buccal surface of each tooth using a water-cooled diamond saw. Each enamel piece was X-irradiated, powdered by hand grinding with an agate mortar and pestle, and pooled according to the tooth type. The ESR spectrum of the powdered enamel sample pooled from the incisors and that pooled from the canines was then recorded. After 6 months storage, the spectrum of each sample was recorded again with and without an additional irradiation of 1 hour.

In the course of this study the teeth, the enamel pieces, as well as the pooled enamel powders, were always stored in closed vessels under an atmosphere saturated with water vapor at room temperature in the presence of thymol as a preservative against bacterial growth.

The samples were X-irradiated for 1 hour at room temperature with a Au-anticathode 2.5 kW tube operated at 60 kV and 40 mA. ESR spectra were recorded using a Varian E-line X-band spectrometer. In each case the microwave power was varied from 200 mW down to 0.0002 mW (60 dB). The magnetic field was modulated at 10 kHz with an amplitude of 1 Gauss. All spectra were stored in digital form [10] so that they could be normalized to the same frequency (9.3 GHz). Corrections were also made for the finite sweeprate which shifts the stored spectrum towards higher magnetic field values. The magnetic field was measured with a Bruker NMR BNM12, and calibrated using DPPH at 0.1 mW (g = 2.0036).

Table 1. S- and R-values of the powdered samples of sound upper incisors as a function of microwave power (Pwr), storage time, and additional irradiation

	1 h XRT		After 6 months		1 h extra XRT	
Pwr (mW)	S_1	R_1	S_2	R_2	S_3	R_3
200	0.506	0.496	0.408	0.369	0.504	0.498
100	0.466	0.488	0.390	0.375	0.461	0.490
50	0.443	0.492	0.385	0.390	0.436	0.493
20	0.424	0.500	0.388	0.412	0.419	0.503
10	0.421	0.509	0.396	0.426	0.419	0.512
5	0.425	0.518	0.406	0.438	0.423	0.522
2	0.438	0.537	0.422	0.456	0.435	0.536
1	0.456	0.548	0.430	0.461	0.448	0.548
0.5	0.476	0.561	0.441	0.468	0.462	0.558
0.2	0.504	0.577	0.450	0.473	0.472	0.570
0.1	0.517	0.589	0.465	0.477	0.484	0.576
0.05	0.536	0.596	0.470	0.483	0.495	0.581
0.02	0.552	0.602	0.476	0.484	0.510	0.590
0.01	0.570	0.604	0.488	0.496	0.525	0.591
50 dB	0.646	0.624	0.547	0.519	0.572	0.602
60 dB	0.868	0.694	0.717	0.572	0.831	0.696

Results

In Table 1 a survey of the R- and S-values of the upper incisors sample is given as a function of microwave power. The effect of storage time at ambient conditions and additional irradiation (XRT) is also presented. Similar results for the lower canines sample are given in Table 2. In Tables 3 and 4, Δ_1 refers to the difference $R_2 - R_1$ (or $S_2 - S_1$) and Δ_2 refers to the difference $R_3 - R_1$ (or $S_3 - S_1$). As a typical example, the R- and S-value of canine powder 6 months after irradiation is presented in Figure 2.

In the low power range (from 60 dB up to 10 mW) the R- and S-value decrease with increasing incident power. At higher power levels, a minimum is found in some cases. With the exception of the 50 dB and 60 dB recordings, the reproducibility is certainly better than 0.01 both for R and S. We conclude that the power dependency is clearly marked, and also depends strongly on storage time and additional irradiation. Generally, as storage time increases, R and S decrease. For the canines, however, in a large power range the R-value is nearly constant. After an additional irradiation, the R-values are restored within 0.02 units; the S-value does not recover equally well, especially at low power levels.

Comparing the R- and S-values for upper incisors

Table 2. S- and R-values of the powdered samples of sound lower canines as a function of microwave power (Pwr), storage time, and additional irradiation

	1 h XRT		After 6 months		1 h extra XRT	
Pwr (mW)	S_1	R_1	S_2	R_2	S_3	R_3
200	0.531	0.495	0.398	0.428	0.510	0.491
100	0.489	0.482	0.382	0.439	0.466	0.479
50	0.463	0.483	0.377	0.456	0.438	0.479
20	0.446	0.490	0.379	0.480	0.426	0.489
10	0.443	0.496	0.387	0.495	0.427	0.500
5	0.451	0.510	0.399	0.512	0.434	0.512
2	0.469	0.528	0.414	0.529	0.449	0.528
1	0.487	0.540	0.429	0.543	0.461	0.539
0.5	0.509	0.554	0.442	0.551	0.477	0.552
0.2	0.543	0.573	0.458	0.567	0.493	0.561
0.1	0.560	0.578	0.468	0.566	0.502	0.565
0.05	0.581	0.584	0.475	0.576	0.519	0.575
0.02	0.590	0.571	0.481	0.586	0.534	0.571
0.01	0.611	0.584	0.500	0.579	0.551	0.587
50 dB	0.652	0.589	0.553	0.606	0.602	0.618
60 dB	0.910	0.704	0.744	0.719	0.832	0.655

Table 3. Differential table for the powdered samples of sound upper incisors

	Δ_1		Δ_2	
Pwr (mW)	ΔS_1	ΔR_1	ΔS_2	ΔR_2
200	−0.133	−0.067	−0.021	−0.004
100	−0.107	−0.043	−0.023	−0.003
50	−0.086	−0.027	−0.025	−0.004
20	−0.067	−0.010	−0.020	−0.001
10	−0.056	−0.001	−0.016	+0.004
5	−0.052	+0.002	−0.017	+0.002
2	−0.055	+0.001	−0.020	0.000
1	−0.058	+0.003	−0.026	−0.001
0.5	−0.067	−0.003	−0.032	−0.002
0.2	−0.085	−0.006	−0.050	−0.012
0.1	−0.092	−0.012	−0.058	−0.013
0.05	−0.106	−0.008	−0.062	−0.009
0.02	−0.109	+0.015	−0.056	0.000
0.01	−0.111	−0.005	−0.060	+0.003
50 dB	−0.099	+0.017	−0.050	+0.029
60 dB	−0.166	+0.015	−0.078	−0.049

and lower canines, some remarkable differences are found. The S-value of lower canines is higher than for upper incisors, after a first irradiation, whereas the R-values are nearly equal in the whole power range considered. After 6 months, the S-value is identical for both tooth types. The R-value of lower canines is stable, whereas a strong decrease (about 0.10) is found for the upper incisors. After an additional irradiation, the original situation is almost restored, but the difference in S-value between the canine and incisor sample has decreased.

Table 4. Differential table for the powdered samples of sound lower canines

	Δ_1		Δ_2	
Pwr (mW)	ΔS_1	ΔR_1	ΔS_2	ΔR_2
200	−0.098	−0.127	−0.002	+0.002
100	−0.076	−0.113	−0.005	+0.002
50	−0.058	−0.101	−0.007	+0.002
20	−0.036	−0.088	−0.005	+0.003
10	−0.025	−0.083	−0.002	+0.003
5	−0.020	−0.080	−0.002	+0.004
2	−0.016	−0.081	−0.003	−0.001
1	−0.026	−0.087	−0.008	0.000
0.5	−0.035	−0.093	−0.014	−0.003
0.2	−0.054	−0.104	−0.032	−0.007
0.1	−0.052	−0.112	−0.033	−0.013
0.05	−0.066	−0.113	−0.041	−0.015
0.02	−0.076	−0.118	−0.042	−0.012
0.01	−0.082	−0.108	−0.045	−0.013
50 dB	−0.099	−0.105	−0.074	−0.022
60 dB	−0.151	−0.122	−0.037	−0.002

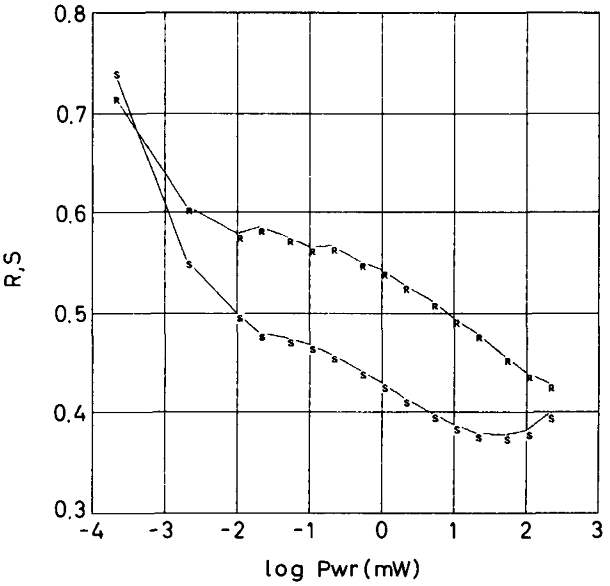

Fig. 2. Plot of the R- and S-value as a function of the logarithm (base 10) of the microwave power (spectra 6 months after irradiation).

In Figure 3, the spectra of the incisor and canine samples are compared after a second irradiation. There is no doubt that these spectra cannot be distinguished without an accurate computer analysis. By a least squares method the proportionality factor between the two curves is determined and the differential spectrum is calculated. The result is also shown in Figure 3.

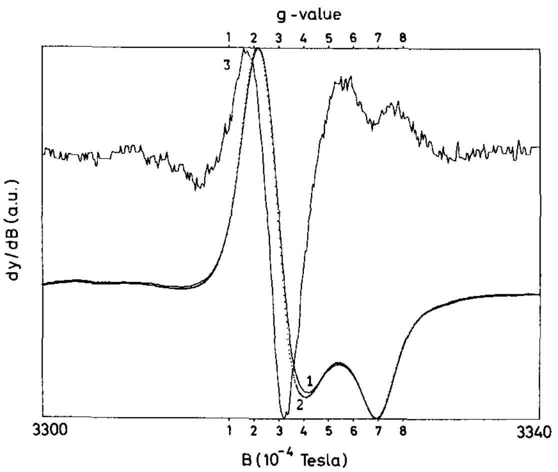

Fig. 3. (1) Upper incisor ESR spectrum at 5 mW after 2nd irradiation; (2) lower canine ESR spectrum at 5 mW after 2nd irradiation; (3) differential spectrum. The notation 1, 2, . . . 8, referring to special points of the curves used here, is the same for Fig. 4 (at ν = 9.3 GHz). 1. B = 3315.0, g = 2.00446; 2. B = 3317.0, g = 2.00326; 3. B = 3319.0, g = 2.00205; 4. B = 3321.0, g = 2.00084; 5. B = 3323.0, g = 1.99964; 6. B = 3325.0, g = 1.99844; 7. B = 3327.0, g = 1.99723; 8. B = 3329.0, g = 1.99603.

The position (g ~2.0030) and shape of this differential spectrum agree with those of the signal obtained for a synthetic sodium- and carbonate-containing apatite, as described in Callens et al. [11] (see also curve 1 in Fig. 7a). It was shown that in the latter case the signal is caused by two similar CO_3^{3-} radicals, one of which is situated on a phosphate site of the apatite lattice. Only at low microwave power can it be seen that this signal has further substructure. The amplitude of these CO_3^{3-} contributions is at least 10 times smaller than that of the main signal in the ESR spectrum of tooth enamel near g = 2.

In the same way, a signal similar to the differential spectrum in Figure 3 results from the difference of two spectra of canines, taken after 6 months storage and an additional irradiation (Fig. 4). In Figure 4 two additional components are visible. In the low field region an unstable center (with $g_x \cong 2.0123$ and $g_z \cong 2.0059$) and a decay time of appproximately 1 day is present, while in the high field region another unidentified center was found which is probably isotropic at g = 1.9974. At first sight it is surprising that the latter has a reversed polarity, but this is readily explained by the following example. Suppose spectrum 1 and 2 are both superpositions of component A and B, where spectrum 1 contains component A in a higher amount than spectrum 2, while the opposite is true for component B. Subtraction of spectrum 2 from 1 will yield a curve wherein component A has a positive sign, while component B will appear with a reversed polarity. Further experiments are in progress to study these as yet unidentified radicals in more detail.

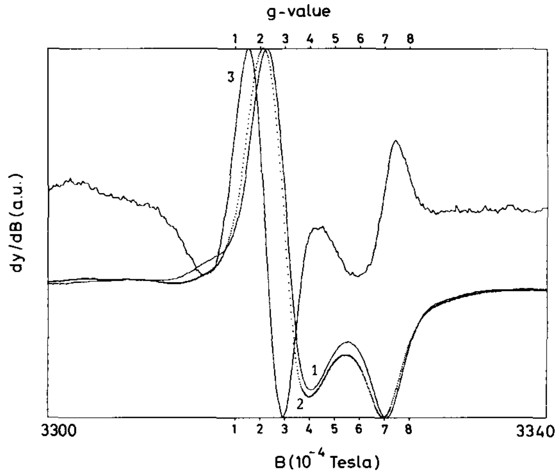

Fig. 4. (1) Canine ESR spectrum at 1 mW after 6 months; (2) canine ESR spectrum at 1 mW after 2nd irradiation; (3) differential spectrum.

Discussion and Conclusions

From the ESR observations on powdered enamel in this study, the following facts are clear.

The asymmetric signal near g = 2 in these samples is a composite signal. In fact, a single radical on a specific lattice position cannot give rise to a dependency of its ESR signal on the parameters considered as reflected by the variation of R- and S-values.

As shown by a previous study of the R-value of enamel blocks [5, 6] and as suggested by the difference in R- and S-values between upper central incisors and lower canines in this study, the ESR spectrum may depend on tooth type and position. We emphasize that individual differences within one class of teeth may also occur. This is subject to a further study.

Considering the above two observations, it must be noted that differences in R- and S-values are not due only to differences in the microcrystal alignment in enamel blocks [5, 6] and/or in the chemical composition of the enamel crystallites. The present results clearly indicate that the R- and/or S-values are also determined by the relative contributions of the components of the ESR signal. Hence, before a distinct and unambiguous correlation can be made between the individual constitutional properties of tooth enamel and each of the components of its

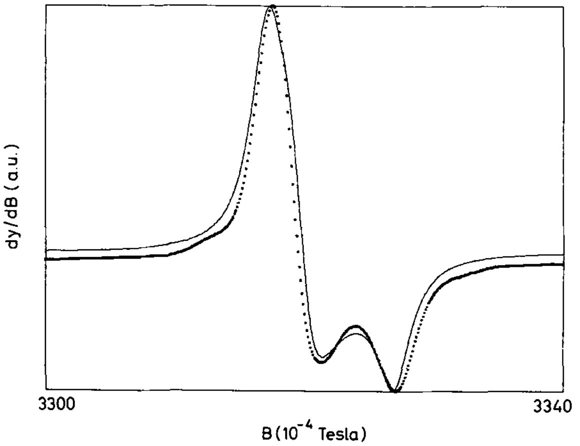

Fig. 5. Calculated (–) CO_2^- powder spectrum (Lorentzian) as compared to the canine spectrum (. . .) at 10 mW (after 6 months).

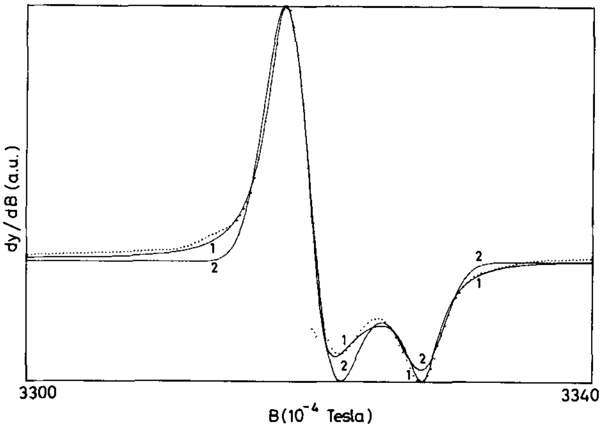

Fig. 6. Lorentzian (1) and Gaussian (2) fitting to the canine powder spectrum (. . . .) at 10 mW (after 6 months). For the Lorentzian fitting g_x = 2.0030, g_y = 2.0019, g_z = 1.9971, C = 2.64.

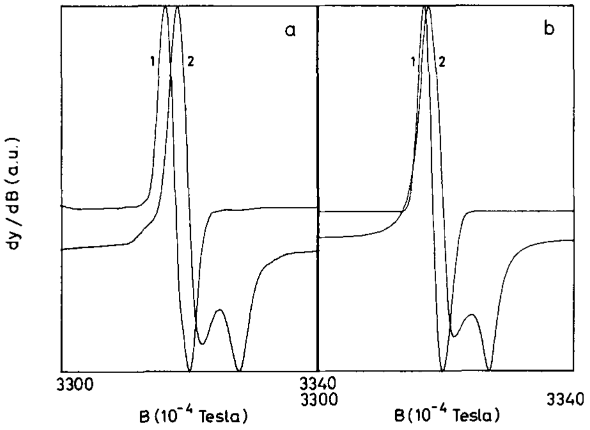

Fig. 7a. Curve 1: experimental spectrum for CO_3^{3-} found in synthetic sodium- and carbonate-containing apatite [11]; curve 2: experimental spectrum for CO_2^- found in tooth enamel powder. **(b)** Curve 1: calculated spectrum for CO_3^{3-} [13]; curve 2: calculated spectrum for CO_2^- [12].

ESR signal near g = 2, the nature of the paramagnetic species and their line parameters must be known.

Until now at least five components were found which contribute to the ESR signal of tooth enamel near g = 2, i.e., three components to be ascribed to two CO_3^{3-} radicals and a CO_2^- radical (see the next to paragraphs) and two components arising from two as yet unidentified paramagnetic species. Of the latter, one is probably isotropic at g = 1.9974 whereas the other is only stable during 1 day and has approximate g-values of g_x = 2.0123 and g_z = 2.0059.

Plausible arguments are given above and in another paper [11] to identify two species, which contribute only a small fraction (maximum 10%) to the overall ESR signal, as CO_3^{3-}-radicals. These components, the relative importance of which depends on microwave power and other parameters (see Figs. 3 and 4), are stable during several months, but their intensities nevertheless decrease in a significant way. In Callens et al. [11] we estimate g_x = 2.0044 and g_z = 2.0020 for the CO_3^{3-} radical on a phosphate site in the apatite lattice, and g_x = 2.0039 and g_z = 2.0014 for the other CO_3^{3-} radical.

The behavior of the CO_3^{3-} signals contrasts with that of the main signal, which remains constant for years. The CO_3^{3-}-radicals being identified, we believe that the assignation of CO_2^- to the main signal, as stated by several authors [8, 9], is correct. This is supported by averaging the CO_2^- g-tensor [16], measured in calcite by Marshall et al. [12] (g_x = 2.00320, g_y = 2.00161, g_z = 1.99727, C = 2.5), since this yields a curve which nearly approximates the experimental ESR spectrum of powdered human tooth enamel (see Fig. 5). The parameter C is proportional to the square of the linewidth, which equals 1.12 Gauss for this particular simulation [16].

The CO_3^{3-}-contributions to the main signal are eliminated at relatively high microwave power levels. However, at the highest levels available in standard ESR spectrometers (viz. 200 mW), the as yet unidentified high field signal (see Fig. 4) perturbs the CO_2^- component. From extensive trial and error we conclude that the fitting of the recorded ESR signal of powdered human tooth enamel is most satisfactory near the 10 mW microwave power level. Typical fittings, based on a Gaussian and Lorentzian line shape (the latter clearly yielding the best fitting in these experimental conditions) are shown in Figure 6. A striking graphical illustration of the above conclusions is shown in Figures 7a and 7b, the former representing the experimental contributions of the CO_3^{3-} and (by subtraction) the CO_2^- components, the latter displaying calculated orientational averages based on CO_3^{3-} and CO_2^- in calcite [12, 13].

We are convinced that the analysis of our experiments leaves little doubt with regard to the identification of the main signal (CO_2^-) and a smaller CO_3^{3-} contribution. Nevertheless, several questions remain unanswered; among these, identification of two weaker components is still lacking. Dependence of R-values (and other parameters) on tooth type and/or tooth position also ought to be more systematically studied. Finally, the question raised by Geoffroy and Tochon-Danguy [15] whether the CO_2^- molecular ions are present in the bulk or in surface locations, is still unsettled. Further experiments aimed at solving these problems are under way.

Acknowledgment. Part of this study was supported by the Fund for Joint Basic Research (Belgium).

References

1. Cevc P, Schara M, Ravnik C (1972) Electron paramagnetic resonance study of irradiated tooth enamel. Radiat Res 51:581–589
2. Cevc P, Schara M, Ravnik C, Skaleric U (1976) Study of the arrangement of crystallites in irradiated human enamel by electron paramagnetic resonance. J Dent Res 55:691–695
3. Cevc G, Cevc P, Schara M, Skaleric (1980) The caries resistance of human teeth is determined by the spatial arrangement of hydroxyapatite microcrystals in the enamel. Nature 286:425–426
4. Martens LC, Verbeeck RMH, Matthys PFA, Driessens FCM (1984) The use of electron spin resonance on human tooth enamel in relation to caries susceptibility. J Biol Buccale 12:27–36
5. Martens LC, Verbeeck RMH, Callens FJ, Matthys PFA, Boesman ER, Dermaut LR (1985) Electron spin resonance spectra of sound human tooth enamel. J Biol Buccale 13:347–353
6. Martens LC, Verbeeck RMH, Callens FJ, Matthys PFA, Driessens FCM, Dermaut LR (1986) The microcrystal alignment in human tooth enamel from carious and non-carious teeth in relation to caries susceptibility. J Biol Buccale 14:65–68
7. Doi Y, Aoba T, Okazaki M, Takahashi J, Moriwaki Y (1979) Analysis of paramagnetic centers in X-ray-irradiated enamel, bone and carbonate-containing hydroxyapatite by E.P.R.-spectroscopy. Calcif Tissue Int 28:107–112
8. Bacquet G, Quang Truong VO, Vignoles M, Trombe JC, Bonel G (1981) ESR of CO_2^- in X-irradiated tooth enamel and A-type carbonated apatite. Calcif Tissue Int 33:105–109
9. Geoffroy M, Tochon-Danguy HJ (1982) ESR identification of radiation damage in synthetic apatites: a study of the ^{13}C-hyperfine coupling. Calcif Tissue Int 34:S99–S102
10. De Keersgieter A, Callens FJ, Baroen M, Matthys PFA, Boesman ER (1985) ESR of isolated Mn^{2+} centers in alkali chlorides. Phys Stat Sol (b) 129:297–301
11. Callens FJ, Verbeeck RMH, Matthys PFA, Martens LC, Boesman ER, Driessens FCM (1986) The ESR spectrum near $g = 2$ of carbonated calciumapatites synthesized at high temperature. Bull Soc Chim Belg 95:589–596
12. Marshall SA, Reinberg AR, Serway RA, Hodges JA (1964) Electron spin resonance absorption spectrum of CO_2^- molecule-ions in single crystal calcite. Mol Phys 8:225–231
13. Serway RA, Marshall SA (1967) Electron spin resonance absorption spectra of CO_3^- and CO_3^{3-} molecule-ions in irradiated single-crystal calcite. J Chem Phys 46:1949–1952
14. Meriaudeau P, Vedrine JC, Ben Taarit Y, Naccache C (1974) Electron paramagnetic resonance studies of CO_2^- radials absorbed on MgO. Identification and structure of the species using ^{13}C and ^{17}O labelling. J Chem Soc Faraday Trans I 71:736–748
15. Geoffroy M, Tochon-Danguy HJ (1985) Long-lived radicals in irradiated apatites of biological interest: an e.s.r. study of apatite samples treated with $^{13}CO_2$. Int J Radiat Biol 48: 621–633
16. Callens FJ, Verbeeck RMH, Matthys PFA, Martens LC, Boesman ER (1986) Electron spin resonance absorption of centres with an orthorhombic g-tensor in polycrystalline substances. Phys Stat Sol (a) 94:267–274

Received September 16, 1986, and in revised form January 20, 1987.