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Additional Criteria for EPR Dosimetry using Tooth Enamel

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Currently, EPR measurements are based on the assumption that odontogenesis (the series of events between the bud formation stage until the complete maturation of the tooth) is finished as soon as the tooth erupts. Consequently, it is also assumed that the hydroxyapatite concentration of the enamel (source of free radicals) does not depend on tooth age. However, the present work provides evidence that odontogenesis does not end after tooth eruption but continues for several years after eruption. Fifty-nine molars and pre-molars were analyzed by EPR spectroscopy. Tooth enamel samples were irradiated with different doses of γ radiation from a ^{60}Co source. The resulting EPR signals were evaluated in terms of posteruption tooth age and tooth position. It was found that, except for wisdom teeth, the concentration of the dosimetric EPR free radicals increased with tooth age after eruption and became constant after a certain period. A mathematical equation was developed to describe this effect as a function of tooth age, tooth position and applied dose. The results suggest that EPR measurements obtained on young teeth should be interpreted carefully unless data are available that would allow one to describe the effect of posteruptive enamel maturation on the EPR estimated dose quantitatively. Little or no correction is needed for older teeth. Since only a limited number of young teeth were available for the present study, further studies are needed to clarify the situation and quantify this effect. © 2007 by Radiation Research Society

INTRODUCTION

For many decades, electron paramagnetic resonance (EPR) has been the only physical method to assess the dose from ionizing radiation to calcified tissues retrospectively. EPR has contributed to the dose assessment after radiological accidents and to radioepidemiological studies. EPR was used to reconstruct the exposure of, e.g., the atomic bomb survivors in Hiroshima and Nagasaki (*1*), the liquidators in Chernobyl (*2*), populations of the contaminated regions in the Southern Urals (*3*), and residents of the Techa River area (*4*). EPR was also used to estimate the background radiation levels of a population living in uncontaminated regions (*5, 6*).

EPR dosimetry relies on the detection of paramagnetic centers (molecules or atoms with unpaired electrons) induced in calcified tissue by ionizing radiation. In the case of dental tissues, the most suitable material for dose reconstruction is hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$. During mineralization of hydroxyapatite crystals, both the phosphate and the hydroxyl ions are occasionally substituted by CO_3. After exposure to ionizing radiation, many types of free radicals with different thermal stability were identified (*7*). Among the more abundant stable radicals is CO_2^-, which is the main contributor to the EPR signal. The number of CO_2^- free radicals and the intensity of the EPR signal are directly proportional to both the radiation dose and the concentration of hydroxyapatite and thus the concentration of the carbonate ions. Although hydroxyapatite is found in all parts of the human tooth (enamel, dentin, root), it is the dominant compound in the enamel (approximately 96% per weight). Therefore, research on EPR dosimetry has concentrated on tooth enamel.

The success of EPR using teeth for retrospective radiation dosimetry motivated further development for low-dose measurements (below 20 cGy). This objective was achieved in individual laboratories (e.g. *8, 9*) and internationally in three intercomparison studies (*10–12*). Most of these efforts sought to establish a methodology for tooth preparation and to identify those parameters important for EPR spectrometry and EPR signal analysis programs (e.g. *13–15*). In spite of these efforts, discrepancies were found in the EPR estimated doses for different teeth, even if they were extracted from the same individual and passed through the same protocol of preparation, radiation exposure, and EPR signal analysis. This means that there must be some additional factors related to the nature of the teeth themselves that influence the concentrations of the induced free radicals and consequently the EPR dose assessments.

The aim of the present work was to investigate the following: Is there any dependence of the hydroxyapatite compounds on the age of the teeth? If the answer is positive, how large is the effect on the results of the EPR measure-

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ments? To achieve this goal, EPR measurements of molar and pre-molar teeth extracted from individuals who were not exposed to any occupational or accidental radiation dose were analyzed.

EXPERIMENTAL PROCEDURE

Fifty-nine permanent teeth were used for the present study. Twenty-four molar and pre-molar tooth samples were collected from two different cities in Egypt, Cairo and El-Calubia City. Both are located in the Nile Delta region and are 60 km from each other. The samples were collected within 3 months in an area of about 50 km^2 in Cairo by two dentists and in an area of 100 km^2 in El-Calubia City by one dentist. For the study, only those samples in the best physical condition were selected. The samples included teeth from different tooth positions, and the teeth were not older than 42 years. In addition, 35 permanent molar teeth from 21 individuals living in six different cities in India were used. Five of these cities are located within a distance of 1,290 km, while the sixth town is located in the far south of India. The teeth were identified and extracted after medical indication by one dentist in New Delhi in 1999. Detailed information on each sample such as tooth position, age and donor gender was recorded. To our knowledge, none of the samples had been exposed to medical or occupational radiation.

The crown of each tooth was cut off with a circular saw blade after removal of any tooth filling and signs of disease by a drill. To eliminate the organic dentin compound from the crown, the following chemical procedure was used:

1. Washing with acetone for 5 min in an ultrasound bath after rough removal of the dentin by the drill.
2. Washing with 0.1 *M* Titriplex III for 15 min and with 5 *M* NaOH for 15 min in the ultrasound bath.
3. Rinsing with isopropanol and drying under vacuum for 30 min at 40°C.
4. Gently crushing the enamel pieces obtained to 125–600-μm grain size.
5. Etching the net grains with 20% acetic acid.
6. Rinsing the grains with isopropanol and drying under vacuum for 1 day at 40°C.

The crushed enamel grains (typical mass: 50–100 mg) were put into a quartz tube with an internal diameter of 3 mm. The EPR spectra were recorded at room temperature with a Bruker ESP 300E spectrometer operating in X-band. The experimental parameters of the spectrometer were as follows: microwave power: 25.3 mW; modulation frequency: 50 kHz; modulation amplitude: 0.145 mT; receiver gain: 1.25×10^5; conversion time: 81.92 ms; time constant: 163.84 ms; magnetic-field sweep: 5 mT; sweep time: 83.89 s; number of scans: 40.

After measuring the baseline EPR signals, the grainy tooth samples were irradiated with 10 cGy, 50 cGy, 1 Gy and 10 Gy of γ rays from ^{60}Co (Type Eldorado) at a dose rate of 5 cGy/min. The irradiations and measurements were done at the GSF-National Research Center for Environment and Health close to Munich, Germany.

The system was calibrated using a pool of old molars extracted for medical reasons from different positions in German donors. Grains from their enamel (grain size: 65–125 μm) were irradiated with known doses of γ radiation, and the resulting EPR signals were measured using the same procedure used in the present study.

The EPR signal of each individual tooth enamel sample was measured before and after irradiation. The intensities of the background EPR signal and the dosimetric CO_2^- signal were determined by deconvolution of the EPR spectra with the first derivative of a Gaussian function (*16*). Using a special software routine including a dose calibration curve for the deconvoluted EPR spectrum (*17*), the absorbed doses in the investigated samples were calculated.

Each EPR reading was repeated three times. Due to their larger uncertainties, the background and 10 cGy irradiation EPR values were not used for the present analysis.

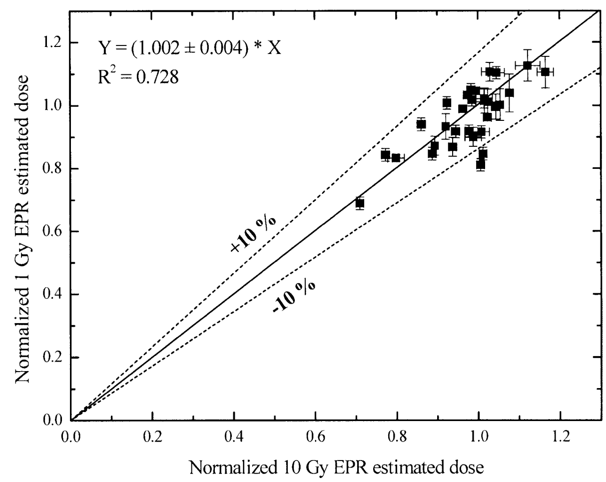

FIG. 1. Comparison of EPR estimated doses normalized to the average dose obtained for samples older than 35 years that were irradiated with 10 and 1 Gy. If outliers outside the 10% standard deviations (dashed lines) were excluded, the slope and the correlation coefficient were 1.014 ± 0.004 and 0.836, respectively.

RESULTS AND DISCUSSION

Comparison of Results Obtained after Irradiation with 50 cGy, 1 Gy and 10 Gy

To investigate the consistency of the measurements, the results obtained after irradiation with 50 cGy and 1 Gy were compared with those obtained after irradiation with 10 Gy. For this comparison, all data were normalized to the average dose obtained for samples older than 35 years. It was decided to consider the results for 50 cGy and 1 Gy as outliers if they did not agree within 15% (50 cGy) or 10% (1 Gy) with the results obtained after the 10-Gy irradiation of the same sample. The values of 10% and 15% were chosen because typical standard deviations of EPR measurements for doses above 1 Gy were reported to be about 10% and those for low doses less than 50 cGy to be about 15% (e.g. *9, 13*). For the current individual EPR results, 5% (comparison of 1 Gy and 10 Gy) and 8% (comparison of 50 cGy and 10 Gy) of the measurements were considered as outliers and were not used for further analysis. The improvement of the data before and after application of this approach is shown in Figs. 1 and 2.

Figure 1 shows that the normalized EPR results obtained after irradiation with 1 Gy are consistent with those after irradiation with 10 Gy; the slope is close to one (1.002 ± 0.004) and the correlation coefficient is 0.728. Every data point in these figures represents six individual measurements: e.g., in Fig. 1, three EPR measurements of a sample irradiated with 1 Gy and three additional EPR measurements of the same sample irradiated with additional 9 Gy, to obtain 10 Gy in total. Figure 1 shows that only two values had to be considered as outliers, while nine cases were identified as outliers when the 50-cGy and the 10-Gy data were compared (Fig. 2). When these outliers are ignored, the results do not change greatly for 1 Gy (slope:

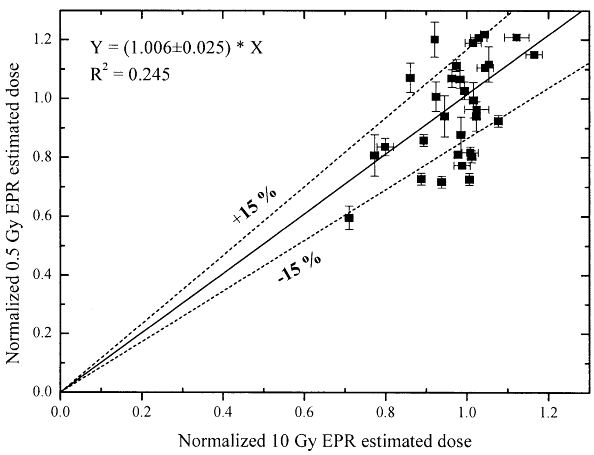

FIG. 2. Comparison of EPR estimated doses normalized to the average dose obtained for samples older than 35 years that were irradiated with 10 Gy and 50 cGy. If outliers outside the 15% standard deviations (dashed lines) were excluded, the slope and the correlation coefficient were 1.014 ± 0.004 and 0.798, respectively.

TABLE 1
Eruption Ages of the Tooth Types

Tooth position	Tooth location	Primary teeth eruption age (months)	Permanent teeth eruption age (years)
Central incisor	1-upper	8–12	7–8
	1-lower	6–10	6–7
Lateral incisor	2-upper	9–13	8–9
	2-lower	10–16	7–8
Canine	3-upper	16–22	11–12
	3-lower	17–23	9–10
First premolar	4-upper	—	10–11
	4-lower	—	10–12
Second premolar	5-upper	—	10–12
	5-lower	—	11–12
First molar	6-upper	13–19	6–7
	6-lower	14–18	6–7
Second molar	7-upper	25–33	12–13
	7-lower	23–31	11–13
Third molar (wisdom tooth)	8-upper	—	17–21
	8-lower	—	17–21

1.014 ± 0.004; correlation coefficient: 0.836). The correlation coefficient improved significantly for 0.5 Gy from 0.245 to 0.798, with the slope of the curve still being very close to one (from 1.006 ± 0.025 to 1.014 ± 0.004).

Dependence of the EPR Signal on Posteruptive Tooth Age for all Tooth Positions Combined

To investigate whether the EPR signal depended on posteruptive tooth age, the 10-Gy data obtained for a specific tooth position were normalized to those from very old teeth at the same position. The posteruptive tooth age was calculated as the difference between the date of tooth extraction and the mean date of tooth eruption, which depended on the tooth position (see Table 1) (*18*). The normalized EPR signals were then plotted in Fig. 3 (positions 4–7). It should be noted that Fig. 3 shows data for teeth from different positions, which may show different EPR sensitivities to γ radiation. This could be one reason for the data spread observed. Wisdom teeth (position 8) were excluded from this figure, because they did not show any dependence on posteruptive tooth age (see below). The results of the three repeated EPR measurements performed on the same sample are shown individually to demonstrate the reproducibility of the EPR method.

From Fig. 3 it appears that the EPR-estimated doses increase with the increasing age of the sample and become independent of age after a certain time. This means that the CO_2^- free radical concentrations measured after irradiation with 10 Gy are different in the enamel of different teeth. The same behavior was found after irradiation with 1 and 50 cGy (data not shown). These results might be attributed in part to the difference in tooth age and/or in tooth position (see below).

The measured EPR-estimated dose appears to becomes independent of age after a tooth eruption age of about 30 years. At younger ages, the EPR estimated dose is smaller and reaches about 80%, on average, of the normalized radiation dose at a posteruptive tooth age of about 10 years. Unfortunately, only few young samples were available. It can also be seen in Fig. 3 that the scattering between the normalized EPR values is lower for old teeth, while it appears to be greater for younger teeth. This can be attributed in part to the effect of the tooth position on the estimated EPR doses, since each tooth position has its own tooth eruption age (see the next section).

Dependence of the EPR Signal on Posteruptive Tooth Age: Tooth Positions Analyzed Separately

Since it could not be ruled out that part of the data scattering observed in Fig. 3 is due to the fact that all tooth

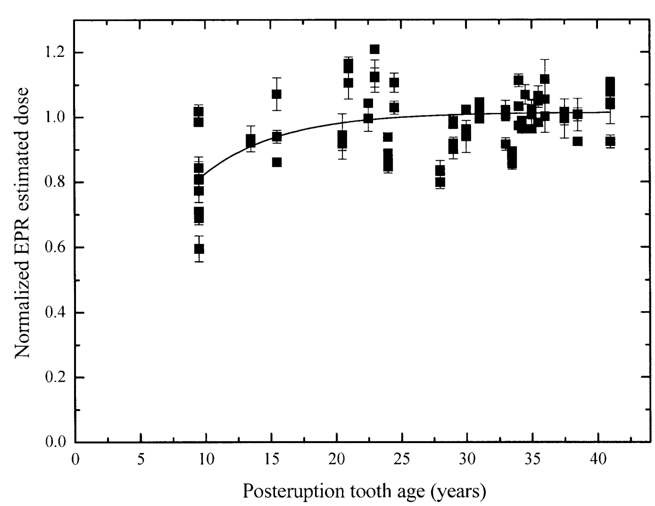

FIG. 3. EPR estimated doses for the inner teeth (molars and premolars, positions 4–7) as a function of posteruption tooth age. For each sample, the results of three repeated EPR estimated dose measurements are shown after irradiation with 10 Gy, normalized to the average dose obtained for samples older than 35 years; the solid line serves as a guide for the eye.

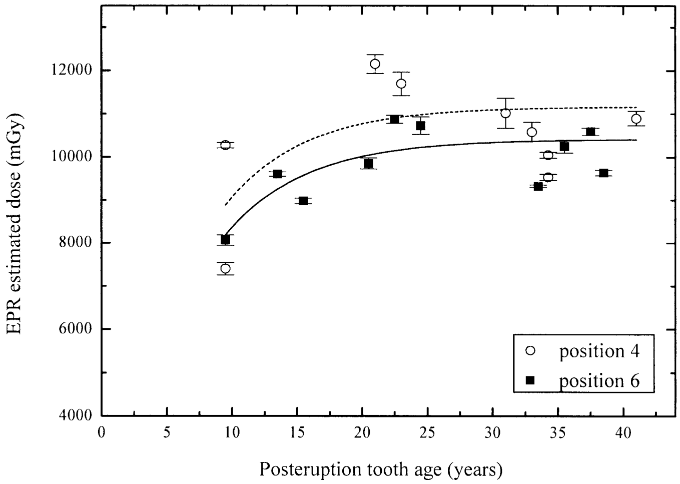

FIG. 4. Measured EPR estimated doses after irradiation with 10 Gy for enamel from first premolar position 4 (○) and from first molar position 6 (■) as a function of the posteruptive tooth age. Each data point represents the mean of three repeated measurements on the sample. The solid and dotted lines are the least-squares fits using Eq. (1). The resulting fit parameters are given in Table 2.

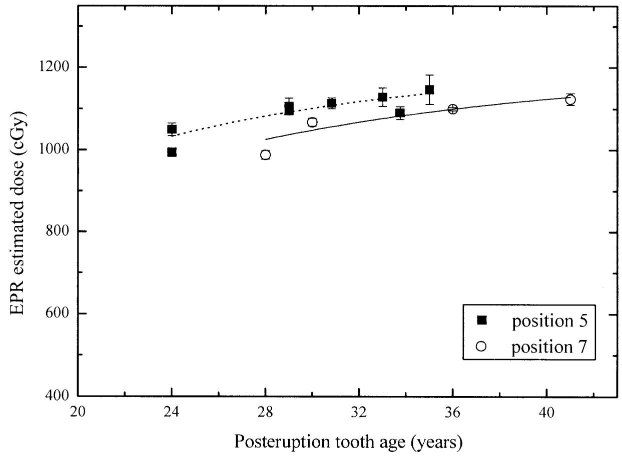

FIG. 5. Measured EPR estimated doses after irradiation with 10 Gy for enamel from second premolar position 5 (■) and from second molar position 7 (○) as a function of the posteruptive tooth age. Each point represents the mean of three repeated measurements on the sample. The solid and dotted lines are the least-squares fits using Eq. (1). The resulting fit parameters are given in Table 2.

positions were combined in the figure, the same analysis was also done separately for the different tooth positions. To discuss the results shown in Figs. 4–6 more quantitatively and to fit the data by means of weighted least-squares regressions, a mathematical equation was devised to express the relationship between the measured EPR-estimated dose D (Gy) and the posteruptive tooth age (A_t) in years as

$$D = (a + b \times D_a) \times [1 - \exp(-c \times A_t)], \qquad (1)$$

where a, b and c are constants (which may depend on the tooth positions, see below) and D_a is the radiation dose (in Gy). The results are shown in Figs. 4–6, and the resulting values for a, b and c are tabulated in Table 2 for two different scenarios. In these figures, only the mean values of the three EPR measurements performed on the sample are shown. For the first scenario, the EPR estimated dose was calculated without subtraction of the baseline EPR estimated dose. This method is important in the case of persons who were exposed occupationally or who lived in regions in which the EPR background signal could not be quantified. For the second scenario, the values of the parameters a, b and c were evaluated after subtraction of the background signal.

The data shown in Figs. 4–6 still show some spread even though they are shown for each position separately. Therefore, a different EPR radiation sensitivity of teeth from different positions is not the only source of uncertainty in the data. Additional sources of uncertainty involved in EPR dosimetry have been reported, such as variations in the sensitivity of the EPR spectrometer with time and variations in the organic content of enamel. The measurement procedure itself might also induce some uncertainties due to, for example, variations in the location of the sample inside the EPR spectrometer or variations in the grain size of the samples. A comparison of Figs. 1 and 2 also suggests that the measurement uncertainties become larger with lower radiation dose. Detailed discussions of the uncertainties involved in EPR dosimetry can be found in refs. (*5, 9, 14*).

In spite of the uncertainties, it appears from Fig. 4 that there may be a trend that suggests lower EPR values for lower posteruptive tooth ages for positions 4 and 6, although only a few samples with a young tooth eruption age were available. For positions 5 and 7, however, there are insufficient data on tooth samples with young posteruptive tooth age to draw any definite conclusion. For position 8, there were young tooth samples available (posteruptive tooth ages: 9–41 years), and it is evident from Fig. 6 that no trend with posteruptive tooth age could be observed.[2]

For a more quantitative analysis, the data in Fig. 4 were fitted using Eq. (1). For the first premolar position (position 4), the estimated EPR dose increases with posteruptive tooth age (which ranges from 9.5 to 41 years), and the correlation coefficient is 0.424. The corresponding coefficients are 0.650 and 0.502, respectively, when the data obtained after irradiation of position 4 with γ-ray doses of 50 cGy and 1 Gy were analyzed. With these fit parameters it can be shown that the fitted curves reach 95% of their final level after 15.8 years (10 Gy) and 17.7 years (1 Gy) and reach 90% of the final level after 17.5 years (50 cGy). Thus a time of 17.0 ± 1.0 years (mean ± standard deviation) can be interpreted as a typical time after eruption at which the mineralization process is complete for position 4. This means that the mineralization process is complete for the premolar position 4 at an approximate donor age of 27.8 ± 1.4 years. The same analysis can be used for the first molar position (position 6), where the posteruptive tooth

[2] The numbering system used to specify certain tooth positions is as denoted in Table 1.

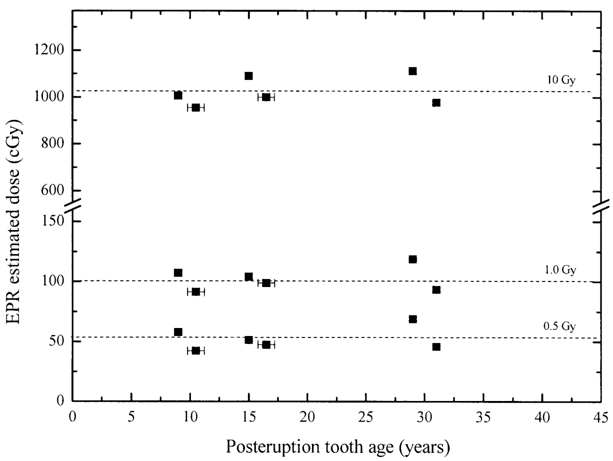

FIG. 6. Measured EPR estimated doses after irradiation with 50 cGy, 1 Gy and 10 Gy for enamel from wisdom teeth as a function of the posteruptive tooth age. Each point represents the mean of three repeated measurements on the sample. The dotted line represents the mean value of all EPR estimated doses at the given radiation dose.

age of the available teeth ranged from 9.5 to 38.5 years. The results in Fig. 4 indicate that 95% of the final level is reached at a posteruptive tooth age of about 16.0 ± 2.4 years. The correlation coefficients for the fits are 0.491, 0.331 and 0.837 for 50 cGy, 1 Gy and 10 Gy, respectively. This means that the mineralization process was finished for the first molar position 6 at an approximate donor age of 22.5 ± 2.4 years.

It is important to note that the overall results do not change significantly if the outliers as defined above are included in the analysis. In some cases, the uncertainties associated with the fit parameters become larger due to the increased scattering of the data. In other cases, however, the uncertainties become smaller, since one of the outliers excluded was from a young tooth eruption age (11 years). For example, the fitting correlation coefficient at position 4 is improved for a dose of 1 Gy, from 0.502 to 0.566.

Unfortunately, the teeth available for the second premolar and second molar positions (positions 5 and 7) were not appropriate to perform a similar analysis, because the youngest teeth were 24 and 23 years old, respectively. The curves in Fig. 5 may also suggest some trend with time, i.e., an increase of the EPR estimated dose with increasing posteruptive tooth age. However, this increase is not significant without young samples. On the other hand, Fig. 6 shows that the EPR estimated dose for wisdom teeth does not depend on the posteruption tooth age. This was observed for all doses.

From these results, two major conclusions can be drawn: (a) Maturation of molar tooth enamel may not be finished after tooth eruption; if so, the mineralization process continues for a certain maturation time after eruption. Consequently, the concentration of hydroxyapatite depends on age, and so does the number of radiation-induced CO_2^- free radicals. (b) The maturation time may be different for the different molar positions; the corresponding values as suggested by the data presented here are given in Table 2.

The results presented here are consistent with those of Stiefel and Binus (*19*), who discussed the interplay between de- and remineralization exchange processes that occur as soon as the tooth enters the oral cavity and how these processes mask the layers of the maturation process. During this period of exchange, the potassium phosphate of the enamel is replaced by a similar amount of hydroxyl and fluorohydroxyl apatite, with adaptation of the organic matrix. Robinson *et al.* (*20*) explained how this period of exchange is increased due to the presence of fluoride. These findings were verified by Almy,[3] who determined the differences in bond strength (the observed decrease in enamel pore size and increase in the calcification of the enamel matrix over time) between mature and newly erupted human premolars by two different techniques. Stiefel and Binus suggested that the posteruptive maturation occurs for humans within 3–5 years and sometimes up to 8 years after eruption.

Although those findings tend to support the observations presented here, they do show some limitations. Those stud-

[3] D. M. Almy, Bonding properties of newly erupted and maturated human premolars. Ph.D. Thesis, Virginia Commonwealth University, Richmond, VA, 2004.

TABLE 2
Estimated End Age of the Mineralization Process for Different Tooth Positions

Tooth position	No. of samples	Parameters of posteruption tooth age dependence: Before subtraction of the corresponding native dose			After subtraction of the corresponding native dose			Estimated posteruption tooth age at MPE[a] (years)	Corresponding donor age at MPE (years)
		a	*b*	*c*	*a*	*b*	*c*		
First premolar	10	0.10 ± 0.03	1.11 ± 0.03	0.17 ± 0.02	0.00 ± 0.00	1.08 ± 0.01	0.20 ± 0.02	17.0 ± 1.0	27.8 ± 1.4
Second premolar	5	0.28 ± 0.20	1.16 ± 0.05	0.06 ± 0.02	0.02 ± 0.10	1.08 ± 0.11	0.09 ± 0.02	not defined	not defined
First molar	12	0.06 ± 0.03	0.99 ± 0.03	0.21 ± 0.04	0.00 ± 0.00	1.04 ± 0.003	0.15 ± 0.03	16.0 ± 2.4	22.5 ± 2.4
Second molar	4	0.39 ± 0.09	1.07 ± 0.03	0.05 ± 0.01	0.10 ± 0.04	1.09 ± 0.01	0.07 ± 0.02	not defined	not defined
Wisdom tooth	15	independent						0.0	19.0 ± 2.0

Notes. The deduced values are more realistic for tooth positions 4 and 6, while they cannot be defined for tooth positions 5 and 7. The signals for the wisdom teeth do not show any dependence on tooth age.

[a] End of mineralization process.

ies (1) did not identify the end of the remineralization process for each tooth position, (2) did not quantify the difference in the mineral concentration between the eruption and maturation processes, and (3) concentrated on animal data for certain tooth positions. Nevertheless, they all believed that odontogenesis, which represents the series of events taking place from the bud formation stage until the completion of calcification and maturation of the tooth, does not end as the tooth reaches the oral cavity. This means that the concentration of hydroxyapatite is not constant after tooth eruption, at least for a certain time depending on factors like fluoride sources. As a consequence, the number of induced free radicals after the action of ionizing radiation may depend on the stage of tooth maturation.

CONCLUSIONS

Up to now any EPR study on tooth dosimetry has been based on the assumption that odontogenesis finished as soon as the tooth erupted. However, the current results suggest that odontogenesis may not be finished with tooth eruption. This observation was made by means of EPR measurements on a large number of all types of molars that had been γ-irradiated with doses of 50 cGy, 1 Gy and 10 Gy. Our results are supported by some other studies in orthodontics[3] (*18–20*). The time between tooth eruption and the end of the maturation processes may be different for different tooth positions. The results presented here suggest that EPR measurements made on young teeth should be interpreted carefully unless further data are available that would allow one to determine quantitatively the effect of posteruptive enamel maturation on the EPR estimated dose. Based on our results, however, little or no correction is needed for older teeth. The current results also suggest that the tooth position could play an essential role in retrospective EPR dose assessment. This becomes evident if the data shown in Figs. 4 and 5 are compared to those shown in Fig. 6. This means that each tooth should be treated as an individual EPR dosimeter and that it should be corrected for different parameters, including posteruptive tooth age, before the resulting EPR estimated doses can be compared with those obtained from other teeth.

At present, it appears that at least positions 4 and 6 may show evidence for a change in the hydroxyapatite concentration with time after eruption. Before we can draw any final conclusions about the process of posteruptive enamel maturation, however, further work is required, because the overall uncertainties involved in EPR dosimetric measurements are of the order of 10%. To identify any effect showing a similar order of magnitude thus requires a large number of measurements. Clearly, although the number of samples we studied was quite large for an EPR study, we would have benefited from a larger sample number, in particular for samples with low posteruptive tooth ages. Therefore, further studies involving a larger number of tooth samples with different donor ages are needed to investigate the period of posteruptive enamel maturation for different tooth positions. It is recommended that a similar study of incisors and canines be done in the future.

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