EPR-Spectrometry on Calcified Tissue: Methodological Considerations Concerning Reliable Dose Reconstruction at Low Doses

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Abstract
Human and animal permanent and deciduous teeth as well as bone samples can be used as emergency dosimeters after exposure to ionizing radiation. Electron paramagnetic resonance (EPR) based retrospective dosimetry using calcified tissue can be useful for radiation victims and for subjects being occupationally or medically overexposed. It seems necessary to investigate the possibilities and the limitations of establishing the method of EPR on calcified tissue for the routine use in the field of retrospective dose estimation. Unfortunately the sensitivity of EPR on calcified tissue has been for decades several orders of magnitude less than that of thermoluminescence and could be reliably applied only to doses above 500 mGy. However, the recent development in EPR spectrometry has increased its sensitivity and reproducibility drastically and will possibly allow the detection of such small radiation exposures as several mGy. The
theoretical detection limit of modern EPR equipment is considered to be in the range of 10 mGy for tooth enamel and about 50 mGy for dentine and for the crystalline bone component. The practical limits are set by problems concerning preparation technique, measurement technique and methods to enhance sensitivity and signal to noise ratio without any loss in accuracy in the detection of the radiation induced signal.

1. Principles of electron paramagnetic resonance analysis of calcified tissue

1.1 Fundamentals of EPR-Spectroscopy

EPR spectroscopy is a nondestructive physical method and involves measuring the absorption of microwave energy by matter which contains unpaired spins. Paramagnetism is attributed to the circulation of charge on an atomic scale leading to permanent magnetic dipoles which can take up only discrete orientations in an applied magnetic field of strength B. This orientational difference is physically equivalent to a separation of energy levels; for instance E_1 and E_2 for a spin S=1/2 system. Resonant absorption occurs when the energy of a quantum of incident electromagnetic radiation (microwave energy) exactly matches the energy level separation.E_1-E_2 . At resonance the basic condition

$$\Delta E = h.\nu = g.ß.B$$

is fullfilled (Figure 1) (1)

ΔE denotes the energy difference E_1-E_2 at resonance, g is the spectroscopic splitting factor; ß is the Bohr magneton and h.ν is the energy of the absorbed microwave frequency.
Equation (1) shows that there are two possibilities of detecting resonant absorption of microwaves by a paramagnetic sample: First, the separation of the energy levels could be fixed by keeping the applied magnetic field constant and the microwave frequency is slowly varied until resonance occurs. The second and more convenient method for the experimental practise consists

in fixing the frequency and to vary the applied external magnetic field.
In principle, EPR analysis of calcified tissue can be conducted at different microwave frequencies ν for which the appropriate magnetic field strength B can be supplied. For practical and sensitivity reasons one usually uses frequencies in the microwave range between 9-10 GHz. This kind of EPR spectroscopy is called X-band spectroscopy.

1.2 Fundamentals of EPR-analysis of calcified tissue:

EPR on calcified tissue consists of observing resonance absorption due to radicals which are generated by ionizing radiation. It is well established that primarily the stable CO_2^- radical is produced as a consequence of the interaction of radiation with the inorganic tissue material and this radical essentially contributes to the measured EPR signal intensity.[2]
Bone as well as teeth consist of an inorganic material as the basic constituent of calcified tissue called hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ which is very sensitive to ionizing radiation. For teeth two main tissue materials are relevant for dose reconstruction, enamel and dentine. Enamel primarily records the external gamma radiation whereas bone and dentine are susceptible to circulating nuclides and so accumulate internal as well as external radiation.[4]
Calcified tissue also contains organic material called collagen which is troublesome for dose assessment since organic radicals produce a strong signal which masks the radiogenic signal at low doses. Enamel contains about 93% hydroxyapatite 3% water and only a few percent of organic material. Dentine and bone contain about 60 % inorganic and about 20-25 % organic material (collagene) and more than 12 % of water. To make reliable dose estimation at low doses possible the collagene has to be removed by preparative methods.
Radical dosimeters like calcified tissue obtain a simple equation according to the response to exposure: The intensity of the EPR signal is proportional to the applied radiation dose rate D times the irradiation time t. So the dose-response curve is simply described by the following equation:

$$\mathbf{I(t) = \int a.D.dt;} \qquad (2)$$

with a: material constant of the investigated dosimetric substance.
There are at least 5 signals observable after irradiation with very high doses but in the field of accident radiation dosimetry only the main signal at g = 2.0018 is used for retrospective dose determination. (See figure 2a)
The peak height of the signal is supposed to depend strict linearly on the absorbed dose from very low exposures up to above 100 KGy and thus serves as a good estimator of the absorbed dose. Conveniently the method of dose assessment is based on additive re-irradiation of the calcified tissue with subsequent EPR measurement. By the additive dose method the material specific dose response curve to irradiation can be checked and after several additional test doses the original dose D_0 may be evaluated by linear regression analysis. (See Figure 3)
This approach is called internal calibration. Usually about 4 to 5 additional test doses have to be applied to obtain reliable results by subsequent regression analysis.
Internal calibration is time intensive but essential if the material specific response to irradiation is not known and if the dose estimation must yield very accurate results. The applied radiation dose must be determined very precisely at any irradiation step since uncertainties in the applied dose will result in loss of accuracy in the estimation of the original dose D_0.

1.3 Dosimetric properties of EPR on calcified tissue

The following are some characteristics of the observed properties of calcified tissue with respect to reliable retrospective dosimetry.

1.) One requirement for a reliable dosimeter is a sufficent stable paramagnetic species in time. Thus the lifetime of defect centers must be longer than a few years. The radiation induced signal in calcified tissue has proven to be very stable in time (the decay constant is about 10^7 to 10^8 years); hence any radiation effects are integrated over the lifespan and even longer, making even the dating of fossiles possible.

2.) The radiation induced signal shows a strict linearity to the sample mass and renders easy readout and good repeatability possible.

3.) The main signal reveals no angular dependence if the sample is ground to small grains (with grain diameters less than 500 µm).

4.) The radiation induced signal does not strongly interfere with other EPR signals if the parameters of the measurement device are properly chosen and if the preparation of the material is optimized.

5.) The radical yield for a given absorbed dose is only slightly dependent on the incident energy (observed at low energy x-rays). Above 300 keV no significant dependence on the incident energy is measured [1,4]

6.) The yield of radicals produced per unit dose in the same dosimetric material is presumably independent of the grain size. For instance tooth enamel irradiated as a whole shows nearly the same radiogenic EPR-intensity than enamel powder gently pulverized before irradiation.

7.) The efficiency of radical production rate is relatively high. The efficiency is expressed by a so called „G-value". This value gives the medium number of radicals in the dosimetric material produced by 100 eV of absorbed radiation. For G = 1 (one paramagnetic radical produced per 100 eV) and an absorbed dose of 1 Gy the total number of radicals is about 6,3 x 10^{12}/100mg of sample mass.

The minimum detectable dose can be evaluated with the sensitivity of a commercial EPR spectrometer: For spectrometers of a newer typ it is about $7x10^9$ to 10^{10} radicals/Gauss. For the radiation induced signal in enamel the G-value is about 1 and the linewidth is approximately 10-12 Gauss. Thus the threshold dose for EPR analysis of enamel sample of 100 mg should be 10 to 20 mGy. The G-value for bone is dependent on the content of inorganic material and presumably on the age of the bone. Typically a value between 0,1 and 0,2 can be attached.

It is noteworthy that the detection limit for calcified tissue can be further reduced by a factor of at least three by adopting a sample of 300 mg, a cylindrical cavity and an improved sensitivity of a modern spectrometer.[6]

2. Materials and methods

2.1 Choice and preparation of the sample material

Whole human permanent and deciduous teeth as well as jaw-bone and teeth from pig and cow were used for analysis. All samples were stored in ethylenglycol for several weeks before preparation. The teeth were cut to remove all of the root and most of the dentin. The jaw-bone was also cut in slices of several cm length after careful washing and removal of bone marrow and surface organical material. The samples were put in saturated KOH for about two weeks at room temperature. To speed up the chemical process of dissolving the organic material the samples were placed in an ultrasonic bath for 20 minutes at 50 °C twice a week. The remaining coronal dentine in the enamel samples had become very soft and white and could now be easily

removed without mechanical trauma. After the chemical preparation the samples were washed for some hours in distilled water and gently crushed in icy water to finer grains of size 0,2 -0,4 mm. This procedure was not neccessary in many cases since the alkaline solution makes the calcified tissue very porous along the macrocrystalline border. So most of the samples broke up in smaller grains only by storage in saturated KOH-solution. Again the samples were placed in KOH for several days until nearly complete removal of the organic fraction was established. To eliminate surface radicals originating by the mechanical grinding the samples were treated with 0,1 m of HCl for one hour. Care was taken to avoid any local heating of the samples since it is well known that heating of the calcified tissue may result in unwanted additional EPR signals which might interfere with the radiogenic signal.[5,8] After washing with distilled water the samples were dried at 60°C for 30 to 40 hours until further heating did not result in additional loss of surface water. Enamel mass of 50-400 mg in weight was obtained from human permanent teeth depending on tooth type and soundness and about 20 to 60 mg from deciduous teeth. For animal tooth the mass yield was also dependent on the same parameters. Human dentine-mass differed from 5-50 mg dependent on tooth type, soundness and age and for jaw bone only 40% of the original bone mass remained for analytical EPR-investigation.

2.2 Irradiation equipment

The irradiation experiments were done with different samples of calcified tissue exposed to absorbed doses of 100, 250 and 500 mGy, 1Gy and 5Gy from a Co-60 source at a dose rate of approximately 0,1 Gy min^{-1}. Care was taken to satisfy the condition of secondary electron equilibrium. Twenty five samples were irradiated at one step by placing the probes of prepared and unprepared calcified tissue into small cavities drilled in a plexiglas plate of 40 cm diameter and 2 cm thickness. The error in irradiation dose (1%) was negligible in comparision with experimental errors due to EPR-spectrometry.

2.3 Measurement technique

All samples were analyzed with a Bruker ER-420-spectrometer equipped with a standard rectangular resonator operating at approximately 9.9 GHz and with modulation frequency of 100 kHz. The detection limit is 5 x 10^{10} spins/Gauss corresponding to less than 100 mGy/100 mg sample weight under ideal conditions. The spectrometer settings for calcified tissue were a modulation amplitude of 0.4 mT (4 Gauss), a time constant of 1 sec based on a line width of approximately 0.4 mT and a gain factor of $3x10^6$. Since the radiogenic signal does not strongly saturate at higher microwave power and the background signal saturates at significantly lower microwave power this difference in saturation behaviour provides a method for optimization of the measured EPR signal intensity. (See Figure 4) For our equipment the best microwave power level has shown to be near 25 mW.

All samples were weighted in mass and filled in commercial quarz tubes (inner diameter:3 mm). To ensure reproducible positioning the samples were gently shaked before measurement and the tubes were placed into a second quarz tube which was marked to guarantee equal positioning of all samples inside the resonator. All data were acquired as first derivatives of the absorption curve with respect to the applied magnetic field.

A new measurement procedure was established at our institute for the aim of reducing contributions from high and low frequency noise.[5] This procedure is called peak-height analysis routine and implements signal averaging at local field points

(the extremes of standard and radiogenic signal).
At first the MgO/Cr^{3+} standard is measured at fixed microwave-frequency. From a lorentzian fit and known g-factors of standard and radiation induced signal the magnetic field location of the signal extremes is calculated. Starting at the first of the four points of the spectrum about 1000 single measurements are carried out within 1 minute and the median is calculated. Than the next point is steered by the program routine. After a couple of minutes a measurement cycle has finished and the normalized signal intensity of the radiation induced signal is stored. After several „peak-height rounds" the median value and the 2-sigma standard deviation are obtained. Typical results of a sample of human tooth enamel irradiated with 1 Gy compared with the unirradiated tooth are shown in figure 6. The actual mean EPR peak-height of the radiation induced signal is calculated by subtraction of the mean peak height of the unirradiated sample from the apparent mean EPR peak height of the irradiated sample.

2.4 Evaluation of the tissue absorbed dose

The radiation induced EPR signal intensity of calcified tissue shows a high sensitivity to X-ray photons below 100 keV and reaches a constant value above 0,3 MeV if the EPR response is described per unit exposure. The response to X-rays between 20 to 40 keV is about 7 to 9 times higher than to high energy gamma radiation.[6] The inner shell ionization of median and heavy elements due to photoelectric interaction is the cause of the apparent energy dependence since at low energies (<100 keV) the photon response of a material is very dependent on the atomic number. So with dosimeter materials like Ca and P the response will differ by a large factor compared with tissue equivalent material like water. But one has to consider that the mass energy absorption coefficient at photon energy of about 50 to 90 keV is very high compared with high energy gamma radiation. If the efficiency for the production of radiation induced radicals considering the mass energy absorption coefficient of hydroxyapatite is taken into account, the relative EPR signal in tooth per unit absorbed dose is actually nearly constant for all gamma energies. Figure 7a illustrates the apparent energy dependence per unit exposure and Figure 7b shows the calculated EPR intensity per unit absorbed dose in dental enamel.
Since for retrospective dose assessment one is normally interested in the dose to soft tissue, the calculation of the tissue absorbed dose requires at least some basic knowledge of
a.) the EPR signal size with radiation energy in calcified tissue;
b.) the energy range of the incident radiation,
c.) the relation of quality factor Q between high energy and low energy gamma radiation, and
d.) knowledge of the ratio of mass energy absorption coefficients for calcified tissue and soft tissue for the radiation energy.[1]
To estimate the radiation dose to which subjects have been exposed the background radiation as well as the radiation dose which may have resulted from medical or dental radiological procedures have to be taken into account

$$I_{Total} = I_{Acc} + I_{Med} + I_{Nat} \qquad (2)$$

with: I_{Total} = total EPR-signal intensity;
I_{Acc} = accidental and/or occupational EPR-signal intensity;
I_{Med} = EPR-intensity due to medical or radiological treatment; and
I_{Nat} = EPR- signal caused by natural radioactivity.

The environmental annual background dose is about 1 mGy, so during 20 years this radiation adds up to about 20 mGy. In addition, dose from incorporated beta emitters also affects EPR intensity especially of bone and dentine. To separate the X-rays from high energy gamma rays it is necessary to cut the investigated material (for instance the tooth) in at least two parts.[1] High energy radiation essentially irradiates the tooth uniformly whereas low energy X-rays are attenuated very stronly by the passage through the tooth and bone. To evaluate the method of separating the tooth in a front and back side experiments were performed by Aldrich and Pass and the results demonstrated a strong attenuation of X-rays (dependent on tooth-type) between 40 - 80% while the attenuation of Co-60 photons was only about 10%. Therefore, by measuring the EPR signal of the two halves of a tooth it should be possible in principle to assess both the dose due to low energy and due to high energy radiation. In order to do this it is necessary to measure the Co-60 equivalent dose to both halves of the tooth and use the attenuation factor to calculate the equivalent dose received from both sources of radiation. The dose to soft tissue can then be calculated using the relevant ratios of mass energy absorption coefficients. Since dental radiological treatment is carried out frequently in industrialized countries due to the uncertainties of the above mentioned procedure at the moment it seems very difficult to evaluate small accidental doses below 50-100 mGy. This problem can be overcome in epidemiological research by the EPR analysis of milk-teeth and teeth from young persons which did not receive dental or medical diagnostic x-rays. Also the analysis of calcified tissue from animals living in the vicinity of a nuclear facility might be a helpful tool for assessing the environmental impacts after nuclear accidents since animals are usually grazing outdoors and do not receive confounding irradiation which usually confuses reliable dose determination for human beings at low doses.

3. Results

3.1 Variability of the radiation induced signal height per unit absorbed dose.

The radiation absorbed dose is determined from the internal calibration curve (the additive dose method) as long as the investigated samples differ considerably in production yield and as long as the dose estimation must yield very accurate results. Since this approach is time consuming it has shown necessary to investigate the variability of the dose response curve for the same type of calcified tissue. [7] By using the preparation technique mentioned above dental enamel has shown to be a fairly uniform dosimeter with relatively small variations in signal intensity for different teeth and for different subjects and animals. (See Figure 8)

The fact of relatively small differences in radiogenic sensitivity of enamel samples with respect to tooth type and subject gives an indication for the generalization of EPR-spectrometry on calcified tissue without application of individual additive dose procedures if only a rough estimation of the absorbed dose is required. First measurement results on eight irradiated dentine samples also show only a small variability in radiogenic sensitivity but the measured signal height per unit absorbed dose is about 4 to 5 times smaller than that of enamel. Thus the separation between enamel and dentine should be optimized for dose assessment since incomplete separation of the two in vivo dosimetric materials may lead to an under- respectively over-estimation of absorbed dose if the additive dose method is not applied.

3.2. Variability of the intrinsic background

To assess the uncertainty of the individual background signals of unirradiated teeth, 10 enamel samples from young persons, pigs and cows were analysed by EPR-spectrometry. Care was taken to obtain deciduous and permanent teeth from subjects which did not receive more than one confounding dental diagnostic roentgenogram.

Compared with the results of figure 8 the uncertainty in the background signal (2σ-error level) amounts to about 30 mGy which is comparable to the instrumental error due to fluctuations in electronic and instabilities in clystron power level. By knowing the uncertainty of the background signal the detection limit of the peak-height analysis method can be evaluated. Analysis of 3 samples of tooth enamel irradiated with 100 mGy from a Co-60 source proved the detectability of this radiation dose within a 2-sigma error level of 80%. So by this procedure it seems possible to obtain reliable dosimetric results at even low doses and with modern equippment the detectability of such small doses as 30 mGy should be possible in principle. Investigations of possible variations of the „intrinsic" background signal with a highly efficient EPR spectrometer will be necessary in the near future to validate the method of the peak-height analysis as a reliable method of dose reconstruction at even lower doses. Maybe the method of selective saturation [9] which is now also used in EPR-spectrometry will help to reduce the detection limit considerably.

3.3. Investigation of the influence of grain size on the measured EPR signal

As was mentioned before the credibility of a dosimetric material increases if there are no or only small observable changes in radiogenic sensitivity due to the grain size before irradiation. To estimate possible influences of grain size on the measured EPR-intensity three samples of unirradiated, but chemically prepared teeth were separated with respect to grain diameter. One sample was powdered to grains smaller than 100 µm, the second contained grains smaller than 500 µm and the last consisted of large grains with a diameter of approximately 2 mm. All samples were weighted to 100 mg and irradiated with 5 Gy from a Co-60 source. The measurement results indicate a slight attenuation in the strength of the hydroxyapatite signal with decreasing grain size. Comparison of the powdered sample and the sample containing large grains resulted in an observable difference of about 5% of the total signal intensity. At moment it seems not possible to give an unequivocal physical explanation for this apparent dependence of the radiation induced EPR-signal intensity on grain size and further investigation will be necessary to evaluate this effect more accurately.

3.4 First results of bone preparation and bone dosimetry:

EPR-analysis of chemically prepared bone samples disclosed some results which need further evaluation:

At first, by chemical extraction with saturated KOH and additional sample purification steps (see 2.1) the broad background signal which is reported in the literature could be significantly reduced.[4;9] It will be checked if the background reduction using this method of preparation leads to comparable results with the very time expensive method of soxlet extraction. Second the observed spectrum of unirradiated as well as irradiated bone did not remarkably differ from known spectra of dentine indicating the close affinity of both calcified tissue materials with respect to their potential usability as internal emergency dosimeters.

4. Conclusion

The consequences of refined preparation and measurement technique on the EPR dose assessment have been investigated and methodological problems arising at low dose estimation were reported. Differences in the EPR pattern of different teeth due to variabilities in radiogenic sensitivity, background and grain size were observed. All investigations support the applicability of calcified tissue as an in vivo detector material in retrospective dosimetry. The growing relevance of EPR-spectrometry on calcified tissue in view of retrospective dose assessment makes the standardization and optimization of this physical method necessary. A second international laboratory intercalibration effort on EPR dosimetry of tooth enamel will aim in exactly this direction. At moment it seems not possible to make an a priori decision of the best approach to solve the problems at low doses since there exist different techniques for optimization of preparation, instrumentation, computation as well as measurement routines which are refined at several laboratories in diverse countries. There also exist different sources of experimental error and different ways of their evaluation. Maybe the near future will bring progress in the development of this physical method to a standardized technology suitable for reliable dose estimation at very low doses.

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Figure 1:
Illustration of resonance for a paramagnetic sample with two orientations in the external magnetic field.

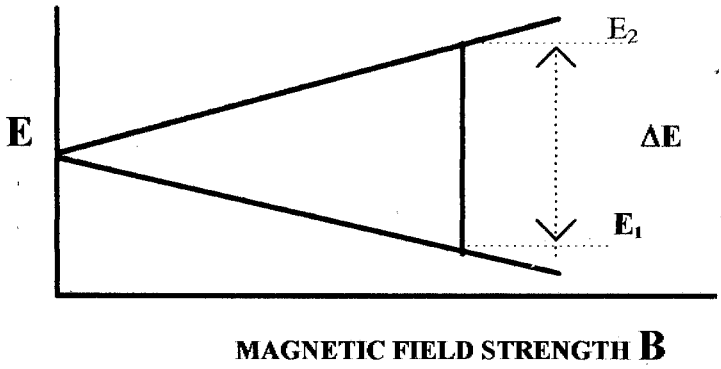
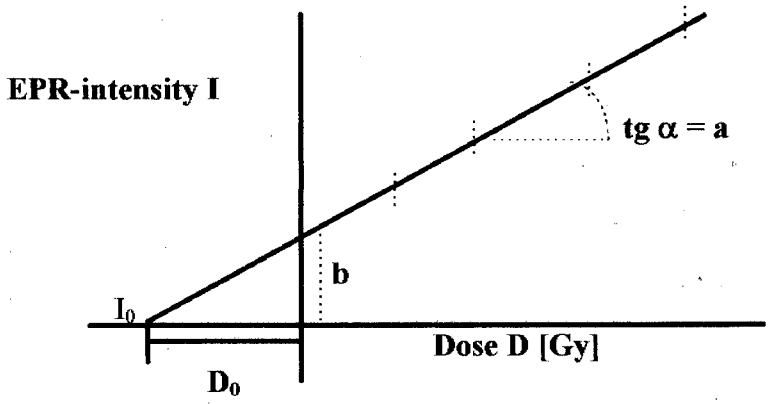


Figure 3:
Illustration of the additive dose method:



$$I = a \cdot D + b$$

$$\text{at } I_0 : I = 0 \text{ and } \quad b = -a \cdot D_0$$

$$\text{so } \quad D_0 = -b/a$$

Figure 2a:
Spectrum of tooth enamel unirradiated and irradiated with 2 Gy;

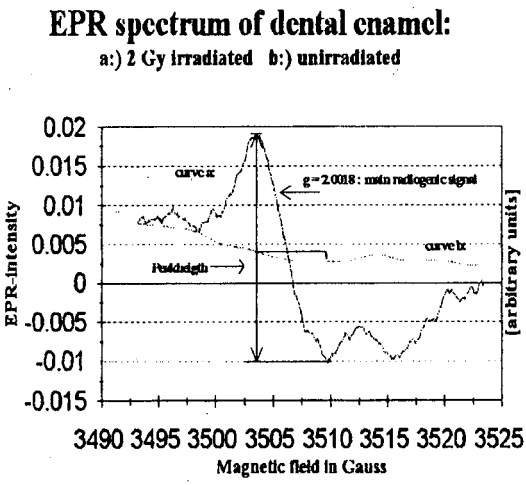


Figure 2b:
Simulation of an ideal powder spectrum:

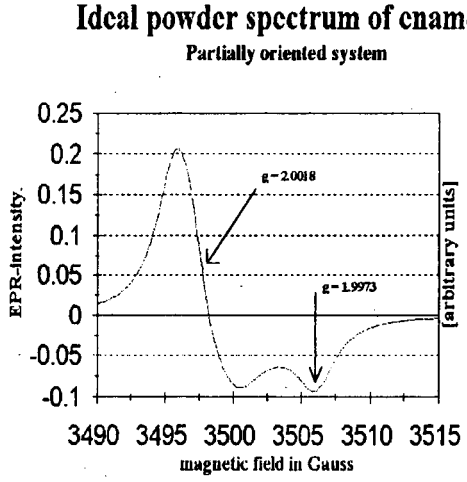


Figure 4:
Variation of the EPR-signal intensity with microwave power.

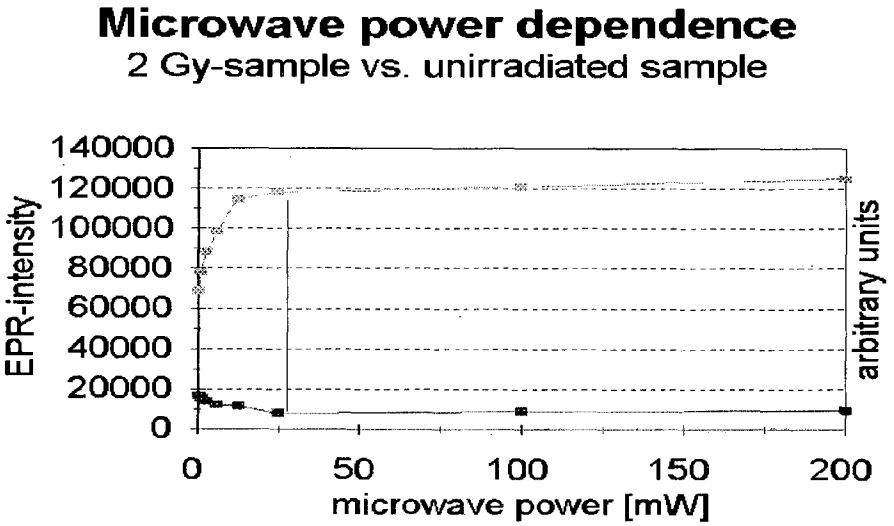


Figure 5:
Illustration of the EPR peak-height analysis routine:

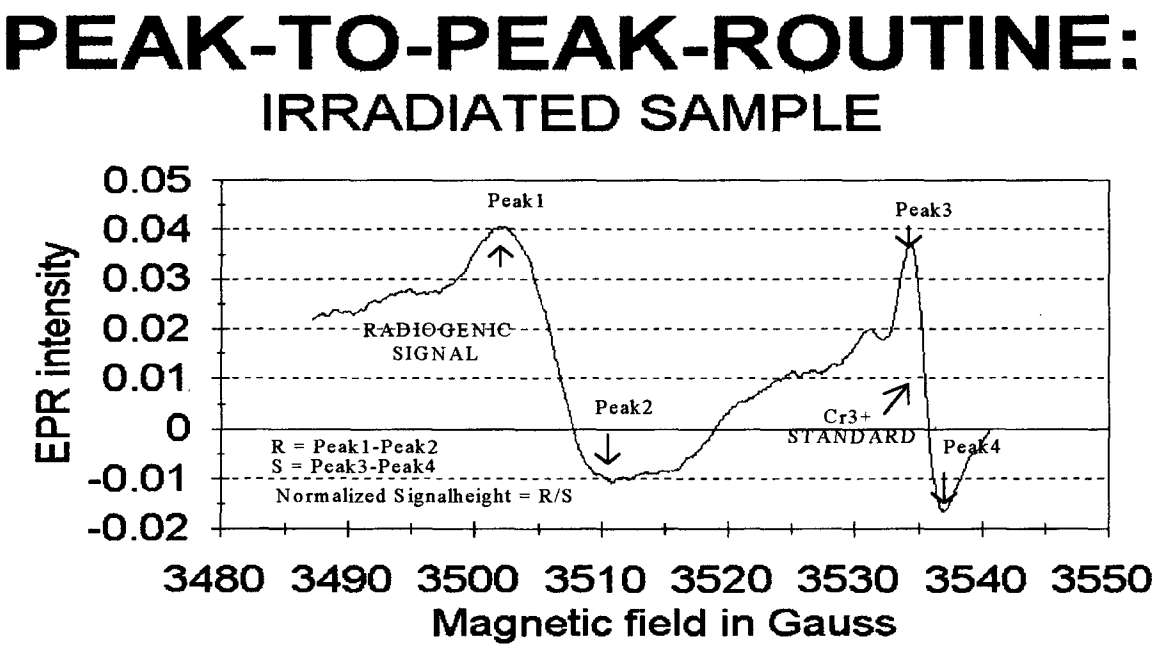


Figure 6:
Results of the EPR peak-height analysis for irradiated enamel (1 Gy) and unirradiated enamel.

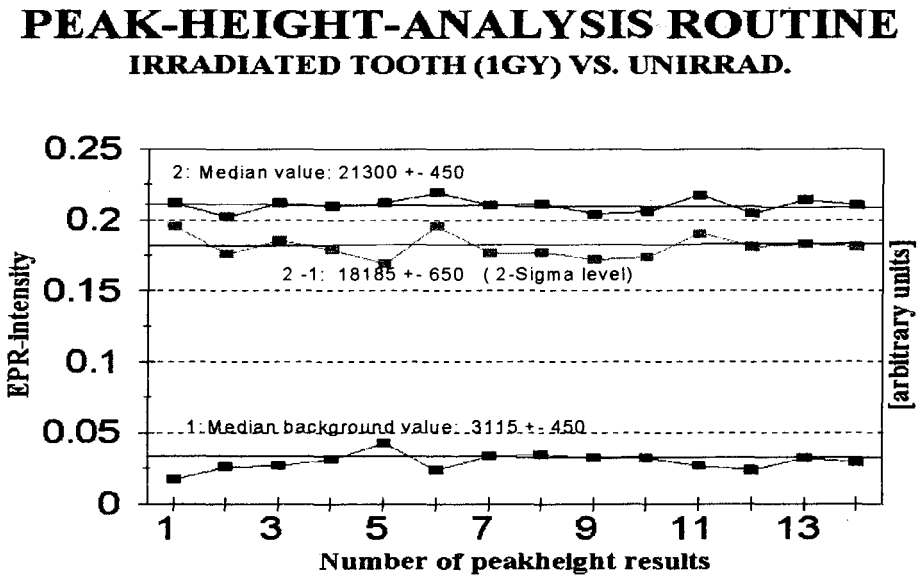


Figure 7a:
Apparent energy dependence per unit exposure;

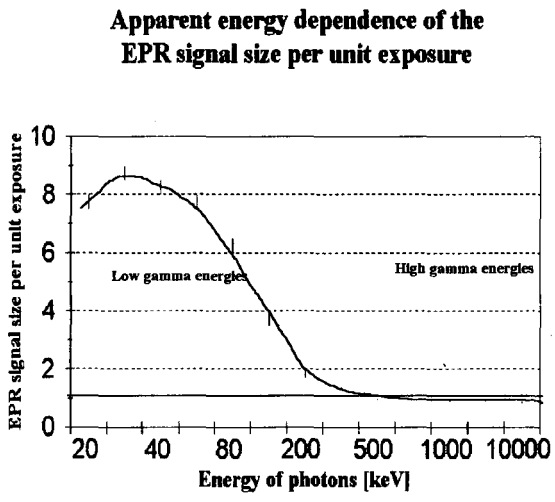


Figure 7b:
normalized EPR intensity per unit absorbed dose.

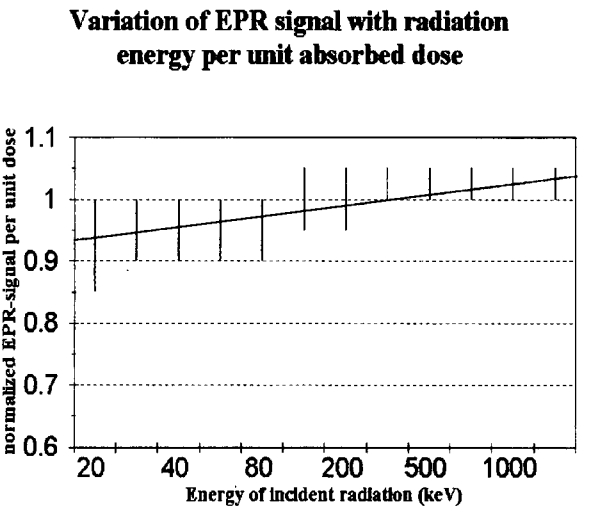


Figure 8:
Variability of enamel samples from human and animal permanent and deciduous teeth irradiated with 1Gy. (for all 26 measured samples the 2σ confidence interval due to the measurement error is drawn)

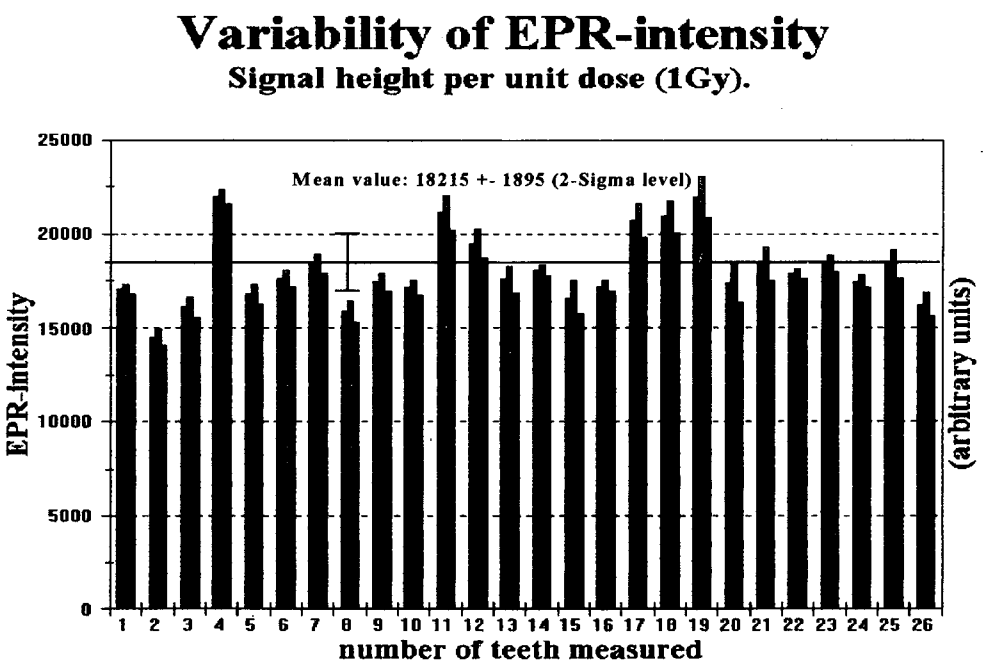


Figure 9:
Variability of the background signal of dental enamel.