FC08-89NV10805

APPENDIX 23.

EPR Techniques for Space Biodosimetry. (Presented at the Space Radiation Damage and Biodosimetry Workshop, Houston, Texas Sept. 9, 1996)

DISCLAIMER

CONF-9609294--1

EPR Techniques for Space Biodosimetry

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Abstract-Retrospective dosimetry of tooth enamel has become an increasingly complex and difficult discipline to undertake while still attaining excellent accuracy. This paper is an attempt at reviewing all the major obstacles, advances and pertinent phenominon accociated with low level retrospective dosimetry of human tooth enamel. Prospective directions for new approaches, methods and instrumentation are also reviewed.

Introduction

A recent international intercomparison of human tooth enamel dosimetry (ECP-10, Bailiff et. al 1996) put the accuracy levels at 25% for gamma irradiation doses between 500 and 1000 mGy. Likewise, samples having doses below 500 mGy could only be accurately reconstructed to within ±100 mGy's. Theoretically the minimum detectible dose should be on the order of 10's of mGy. That this level has not been achieved is indicative of the need to eliminate error inducing elements in the EPR dosimetry method. Recent leaps in accuracy (Haskell et. al 1996A) and new methodologies to be discussed give hope that this objective is within our grasp but final validation in an intercomparison has not been done for the real life dose reconstructive properties of such methods. This paper will cover the many sources of error in EPR dosimetry, their potential solutions and cover the different analysis and scanning techniques in use with their respective pros and cons.

Primary sources of errors and methods for addressing them

Mechanically induced effects

Originally reported by Marino and Becker (1968) for bone, there are signals superimposed on the dosmetry signal originating from crushing the sample (Desrosiers et. al 1989) or heating the enamel above 300° C (unpublished results from our labs, for lower temperatures see Room et. al 1994). Crushed enamel samples are known to have grain size dependant radiation sensitivities to irradiation (Iwasaki et. al 1993). These differing sensitivities of course give different dose estimates when the resultant sensitivity of the grains are different from the precrushing sensitivity inherent to the sample. It has also been shown that the intensity of the native nonradiogenic signal in enamel has a grain size dependancy (Polyakov et. al 1995, Sholom et. al 1996A). A short treatment in an Acetic acid solution however, can remove this grain size effect of the native signal (Albrecht Weiser, personal communication). The functional relationship between the sample grain size to both the radiogenic signals sensitivity and the native signals intensity are shown in figures 1a and 1b respectively. Corrections could then be made to a set of measurements due to these effects using data as shown in figure 1, this however is not a commonly used approach as the errors from other sources tend to dominate these.

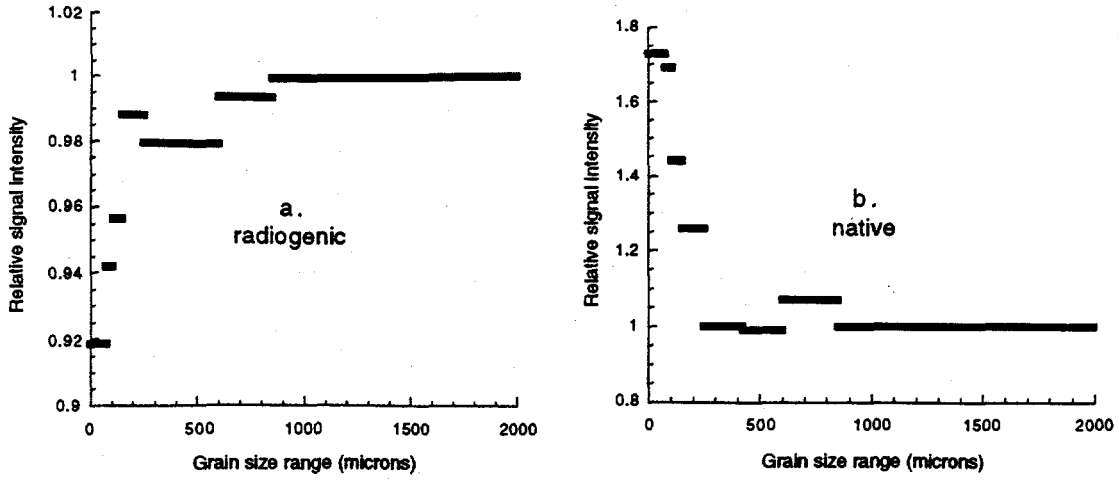

Figure 1. Grain size dependancies. Fig.1a shows the radiogenic dosimetry signals intensity dependancy on grain size. The dosimetry intentities shown are the normalized slopes from extensive dose responses done with the different grain size ranges. Fig.1b shows the dependancy of the native signals intensity on grain size.

Light induced effects

Ultraviolet light has been shown to contribute to the intensity of the dosimetry signal (Skvortzov et. al, 1995). This makes undesirable the use of incisor teeth or any others which may have been exposed to UV light through medical proceedures or natural sunlight. Teeth so exposed would give systematic overestimations of a gamma dose to be evaluated. Fortunately UV light also induces other signals at g=2.0052 and g=2.0083 which have distinctly different sensitivities to gamma and UV (Sholom et. al, 1996B). This offers a potential method by which correction can be made to the dose estimate based on the relative initial intensities of these signals. Sholom also demonstrated that the effect has a sufficiently small penetration depth which could therefore be removed through etching. The sensitivity of the dosimetry signal to 254 nm UV (Eprom Eraser, model DE-4) light at a distance of 4 cm is shown in figure 2.

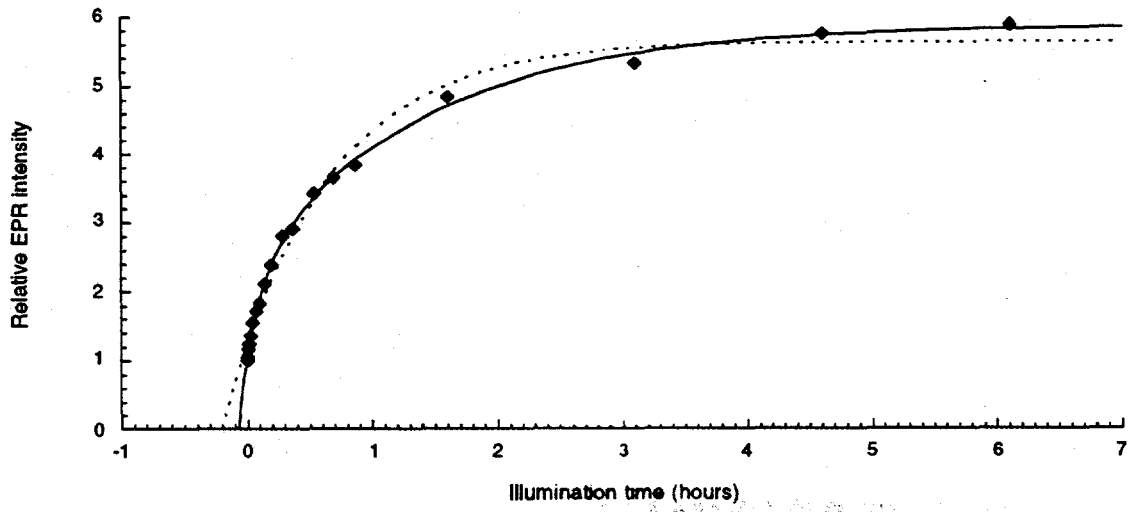

Figure 2. UV light dosimetry signal dependancy. The solid line is a least squares type fit for two saturating exponential functions and the dotted line is for a single saturating exponential. The fitting proceedure was done using the Marquardt modification of the Levenburg method (Press et. al 1994). The superiority of the

double saturating exponential fit clearly evidences that more than one ESR signal is being induced by UV. The two signals are presumably those with g-factors equivalent to 2.0018 and the 2.0052 as discussed by Sholom et. al (1996B). The 2.0052 signal is not one of the signals proposed to determine the total UV dose recieved by the sample.

Sample anisotropy

The full angular dependancy of the $g_{\perp}$ and $g_{\parallel}$ signals of tooth enamel were mapped out by Aoba et. al (1985). The intensity of these signals were shown to vary by as much as 50%. The impact of this effect is clear for retrospective dosimetry; eliminate the effects from this dependancy or the effect will eliminate the utility of your method.

The most common technique for overcoming this difficulty is crushing your sample to obtain a powder distribution (Ikeya 1994). With this comes all the difficulties described in the section on mechanically induced signals e.g., grain size intensity dependancies. In addition, there are limits on the minimum amount of sample required for a given grain size to achieve a true powder spectrum. As the grains become increasingly larger to minimize the deterent effects shown in figures 1a and 1b, the number of peices required for a powder distribution could not be fit into the maximum resonance region of a standard EPR cavivity. Alternatively by consistently using samples of a sufficiently large quantity such that the sample extends beyond the cavities detection region, a powder distribution can be consistently attained for grains of ca. 450 μ diameter. Alternatively, one can in theory just make corrections for the grain size dependancies shown in figures 1a and 1b although excellent results have been attained without this. By constructing a standard native signal spectrum (which will be discussed further in the standard native signal section), this standard spectrum can then be subtracted from that of an irradiated one. This was the technique that acheived the best results in the first intercomparison (Chumak et. al 1995A). That the signal region used has a radiation sensitivity (Polyakov et.al, 1995) is not a problem so long as it's increase is linear with dose as is of course the case for low doses. This method however does not in and of itself correct for the effects displayed in figures 1a. and 1b although it is partially compensatory. However, for the commonly attainable accuracies (described in the introduction) these do not appear at all consequential but will have to be reckoned with as accuracy increases.

A second method for addressing the anisotropy is to resort to soley using single peice specimens. This can be done by insuring that every scan of a specimen is done with the sample in the exact same position (Grun, 1996). In-vivo scanning with a portable spectrometer or EPR imaging have also been proposed (Ikeya 1993). The portable spectrometer can give valuable results where only a single spectrum is required to lift useful information (Ikeya 1994). In both of the latter two cases, sensitivity is orders of magnitude too small for use in low level retrospective dosimetry resulting in their lack of use in routine dosimetry. On the other hand, using Grun's method one would not expect a grain size dependancy of the dosimetry signal but rather an orientation one that would be unique to each specimen and orientation. It has not been established however that the native signal can be removed from the raw spectrum with this method. Furthermore, because a calibration curve could not be used for the sample, initial scan estimates of the samples dose could not be given. Unique to the single peice EPR dosimetry technique is the ability to "non-destructively" evaluate the dose. The additional irradiations would not undermine the integrity of the specimen and so could be carefully replaced in it's original host by a curator or dentist (if fake) depending on the samples source.

Alternatively, if the sample were rotated with a goniometry device, the anisotropy can be reproducably averaged over the angular range (Haskell et, al 1996A). In figure 3a we show the spectrum of an irradiated enamel sample having grain sizes of 0-75μ. The interfering signals in the lower field range of figure 3a are common for such small grains. In figure 3b however, we see the spectrum of the same sample scanned directly after the first one but this time using a constant rotation goniomter (Haskell et. al 1996A). With

this method, even deciduous teeth can be scanned whole with individual measurement errors approaching the theoretical limit (Haskell et. al 1996B). The advantages here are many. After a tooth is scanned, a quick density and volume measurement will give the dentine to enamel ratio of the sample such that a calibration curve can be used to give an initial estimate of the sample. If one is not evaluating deciduous teeth, then because of the relatively large enamel content, one can remove and use a large slice of enamel for which the sensitivity is the highest and characterized. Also one can take avantange of the higher sensitivity at higher microwave powers due to the increase in signal reproducibility. The only drawbacks to this approach are the high costs of complimentary equipment to optimize the method. Such items as FF-Locks, NMR gaussimeters and compatible goniometers can easily cost ten's of thosands of dollars for a neccisary package. These are needed because as the sample rotates, it will change the Q of the cavity which also alters its resonant frequency. The resulting spectrum then has a "smearing" of it's signals g-values which increases with the samples anisotropy. Real time corrections of this can be made with the mentioned instrumentation.

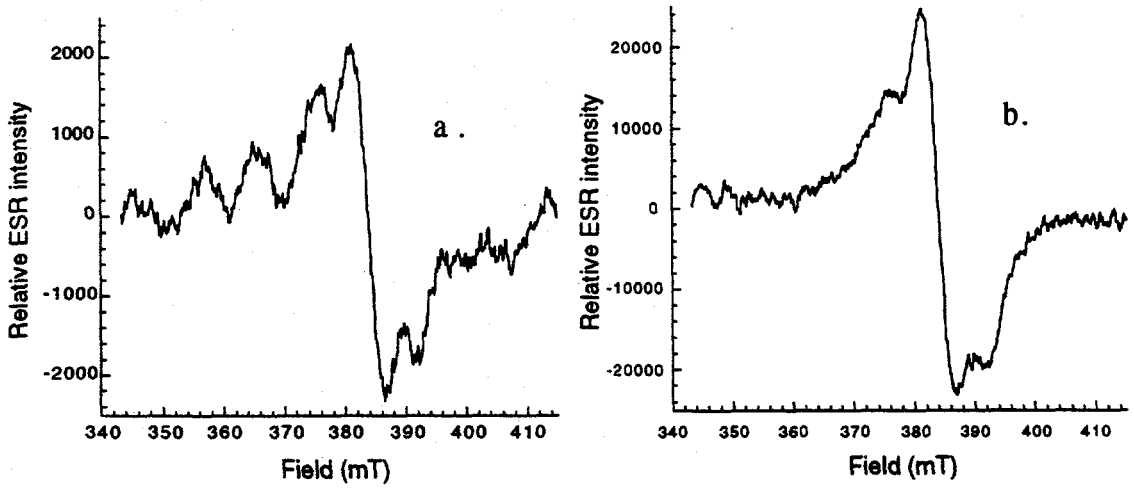

Figure 3. Comparison of spectra taken with and without a goniometer. These spectra came from an enamel sample having an 11.2 Gy gamma dose. The grain size is ≤ 75μ and the sample mass was ca. 29 mg. Figure 3a was taken while the sample was held still in the cavity. Figure 3b was taken while the sample was being slowly rotated and had 10x the number of sweeps than used for figure 3a. The EPR parameters were 20.5 ms Conversion time, 164 ms time constant, 5 Gauss modulation amplitude, 100 KHz modulation frequency and 1 mW microwave power.

Empty cavity signal subtraction

A standard EPR cavity has a signal of it's own even without any specimins inside. This signal is dynamic and varies more rapidly at higher powers. As an example, with EPR parameters of 10 mW microwave power and 5 Gpp field modulation, one or two background spectra will suffice for removal of this component from the total spectrum over 1 day. At 25 mW however, a new spectrum is required every few hours for proper corrections. This problem is compounded by the fact that when a sample is introduced into the cavity, the Q of the cavity is altered. The sample itself then changes the intensity and resonant frequency of the cavity as a function of the samples geometry and dielectric properties.

One method to correct for this was developed at the SCRM (Scientific Center for Radiation Medicine, Kiev, Ukraine). This method requires an in cavity standard whose position can be made to be fixed for any arbitrary length of time. The standard selected by the SCRM was Mn^{++} : CaO (National Scientific and Research Institute for Physical and Radiotechnical Measurements, Mendeleev, Russia). Here only the 3rd and 4th lines of the Mn^{++} are used. The Mn^{++} lines of the empty cavity spectrum can be manually aligned in

intensity and feild position with a samples spectrum for subtraction. By doing this, the empty cavity signal is removed at the correct feild and intensity values compensating for the cavity Q changes. An example demonstrating the neccesity of this method is shown in figure 4.

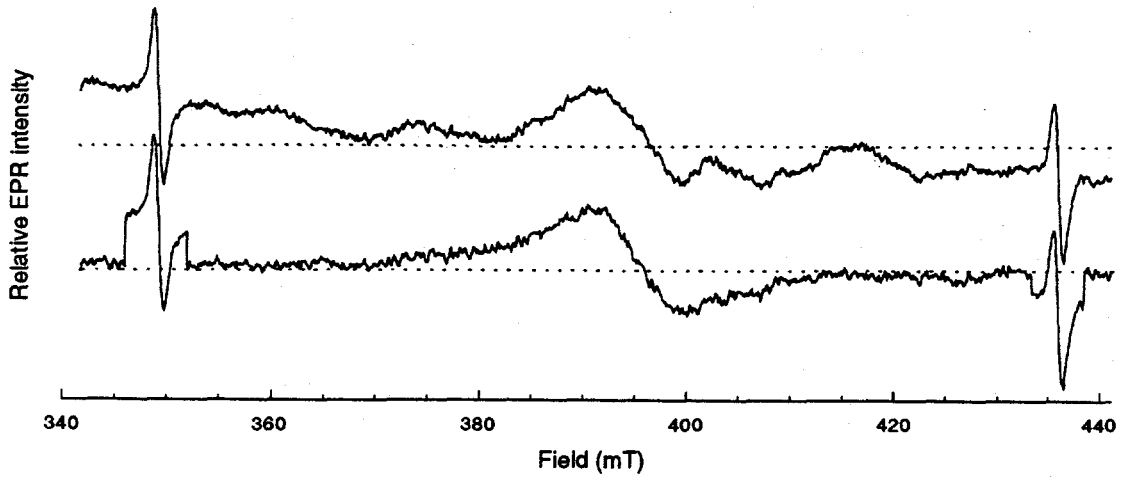

Figure 4. Result of subtracting out the empty resonator signal. The two spectra are the same with the exception that the bottom spectrum had the empty resonator signal subtracted from it. The sample came from enamel chips removed from a decidous tooth and was scanned using a constant rotation goniometer. The Mn^{++} lines from the in cavity standard are also shown, note that the subtraction of the empty resonator signal was done in such a way as to leave these Mn^{++} lines unaltered. The EPR parameters used were 82 ms conversion time and time constant, 5 Gauss modulation amplitude, 25 mW microwave power and 100 Gauss scan width.

An FF-lock or NMR Gaussimeter could also make the same corrections for the frequency adjustments as the in cavity standard. The advantages here are that smaller scan witdths can be used resulting in a larger proportion of the scan time occuring over your region of interest. This is because the 3rd and 4th Mn^{++} lines are ca. 86 Gauss apart whereas the entire enamel signal is within ca. 35 Gauss. Visual adjustment of the background signals intensity can still be done provided the scan width is made wide enough to contain regions where the sample has no signals.

This topic cannot be overstated and is likely one of the main source of inaccuracies. Errors equivalent to a few hundreds of mGy's can easily be introduced over time if one does not correct for the empty cavity signal.

Competing radiation sources

Generally, retrospective dosimetry is focused on some specific radiation exposure event. Background radiation and medical exposures then complicate the final dose estimate as they must first be quantified and then subtracted from the total dose. The possibility of injested radionuclides can further hinder accurate assesment unless corrected for. Finally, one must account for the energy dependancy and type of incedent quanta on the dosimetry signal. The most recent assesment of photon energy dependancy was done by Shauer et. al (1994).

The method for accounting for diagnostic x-rays first suggested for use by Pass and Aldrich (1986) uses the attenuation of the radiation field through the sample for differential analysis of the two sides of a tooth. This technique has proven to be effective. Examples of its accuracy are given by Shimano et. al (1989) and in the review by Haskell et, al (1996C).

Accounting for the cosmic ray contribution to the total dose is done by the conventional method of correllating the subjects mean lattitude and altitude or buriable depth with their appropriate coifficients.

Distinguishing between internal and external emmiters is also of concern when the possibility of ingested radionuclides exist. Wieser et. al (1996) were able to correlate the total ^{90}Sr body burden and the EPR tooth enamel dose estimate for the Techa river residents in Russia. By removing the organic component from the bone (Wieser et. al 1994), Haskell et. al (1995) showed that the increased accuracy there achieved has the potential for use in low level retrospective dosimetry of dentine. Comparing etched vs; nonetched peices of enamel that were in contact with the bone could give dosimetry information on incorporated alpha emmiters in the dentine as well.

Transient radiogenic signals

There are a number of radiogenic signals in enamel having mean lives of ca. 1 month (Oduwole and Sales 1991) and are shown in figure 5. These can effectively be annealed by heating at 95° C for 2 hours (Sholom et. al 1996B). A 15% overestimation in dose is incurred if these are not removed. A full characterization of these signals is given by Sholom et. al (1996) whereby it is made possible to numerically adjust your measurements if annealing is not applied. Of course to avoid the propagation of errors accrued in such corrections, appropriate annealing is reccomended. After these signals are removed, only those that are archeologically stable remain.

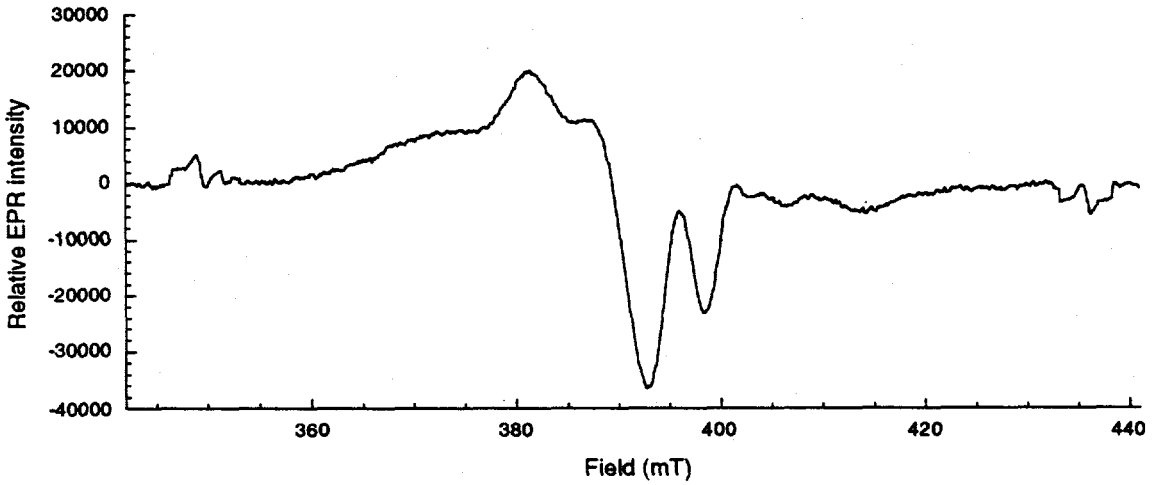

Figure 5. Transient radiogenic signals. This spectrum came from a single enamel chip of 12.5 mg. It was given ca. 2Kgy gamma irradiation over a period of 9 days then scanned directly after irradiation and then after a 2 hr. 90° C anneal. The spectrum shown is the difference spectrum of the two spectra discribed. The EPR parameters are the same as those used for fig. 4. Note the unaltered Mn++ lines still in the spectrum.

Sample purification

Caries, plaque and dentine are typically the primary elements removed from a sample prior to EPR evaluation. Dentine does give the same spectral shapes as enamel, it's deficient characteristic is a drastically lower sensitivity to irradiation and in this light is considered as an impurity in enamel. A direct purification method is to just cut off the dentine root and then remove the remaning dentine and plaque with a dental drill. Any remaining surface impurities could then be etched or disovled away. The SCRM technique (desribed by Pasalskaya et. al and Chumak et. al (1996B)) is to simply disovle away all the

caries and dentine using a concentrated base treatment at an elevated temperature in a hypersonic bath after an initial shattering of the tooth. This must be followed by a similar treatment with distilled water. Another technique which can be used in conjuntion with or instead of the previous method is heavy liquid separation.

True sample purity should only be considered acceptable after EPR scanning. With the empty cavity signal removed, there should be no remaning signals but that of the enamel. A well trained eye can generally make this determination visually. If using for example, a Mn^{++} in cavity standard, the 100 Gauss scan required to include the 3rd and 4th lines gives large enough regions of signal free spectra to attain a key indication if impurities exist in the sample. Another test which is useful comes by comparing a spectrum in question to those of appropriately constructed standard spectra. Painstakingly constructed standard spectra of both the native and radiogenic signals can be invaluable in this instance.

Analysis and measurement proceedures

Standard native and dosimetry signals

The standard native signal should only be obtained from a sample known to have had negligible or zero previous dose. It must of course have its empty cavity signal removed and could only be used as a reference for spectra taken with the same EPR parameters. The isotropic powder spectrum of large grained pure enamel specimens whose empty cavity signal has been removed can be thought of as a pure native signal. Such a sample is also ideal for creating a standard radiogenic signal.

Dentine has been used in the past for subtraction of the native component from the raw spectrum with excellent results (Toyoda et. al 1994, Haskell et. al 1996B). The subtraction of the native signal must be acompanied by appropriate frequency and intensity normalization. The frequency normalization can be accomplished through an in-cavity standard (such as Mn^{++}) or an NMR gaussimeter or FF-lock. Intensity normalization can be done visually by inspection of the differential spectrum. Although this introduces a slight degree of subjectivity, one can standardize on a resulting zero signal at the maximum of the native signal (ca. g=2.007).

A standard dosimetry signal would serve as the source of a calibration curve. By finding its sensitivity relative to the native signal in pure enamel, an initial estimate of the samples dose can be ascertained without knowledge of the samples mass. Normalizing the signal intensities to sample mass can then give another initial estimate of the samples dose. The correlation of these two quickly found estimates can then be used to characterize the sample as atypical or not and so judge the validity of any prescreening scan.

Spectrum aquisition

The standard method of signal aquisition is in the "add" mode where many sweeps are added together resulting in the final spectrum. The spectrum is also typically the first derivative of the actual absorbtion spectrum where intensity measurements are done peak to peak. For enamel, the radiogenic peak is ca. 2.0034 and the min ca. 2.0007 as indicated in figure 4.

An ingeneous modification to this predominant aquisition form came from Schabl (1996). Here, an in cavity standard (such as Mn^{++}) is quickly scanned to calibrate the entire field axis. An automation routine then goes to the field positions of the radiogenic signals extrema and scans only these two points for the next few minutes. A few thousand measurements are thus taken in this short time allowing one to have arbitrarily large signal to noise values compared to those of conventional methods.

Another recently proposed method (Galtsev et. al 1996) is to do a rapid scan of the second harmonic. The method both increases the signal to noise and the dosimetry signal relative to the native signal.

Doing conventional spectrum aquisition at two powers can allow one to isolate the radiogenic signal from the native (Serezhenkov et. al, 1995, Romanyukha et. al 1995). The native signal saturates well before the dosimetry signal and so by choosing 2 powers where the native signal is equal at the radiogenic signals extrema (2.0034, 2.0007), a differential spectrum of the two powers will eliminate the native signal component. Figures 6a and 6b show the saturation characteristics of the radiogenic and native signals respectively at these locations. This method has been tested in an actual intercomparison (see Haskell et. al 1996D) with impressive results.

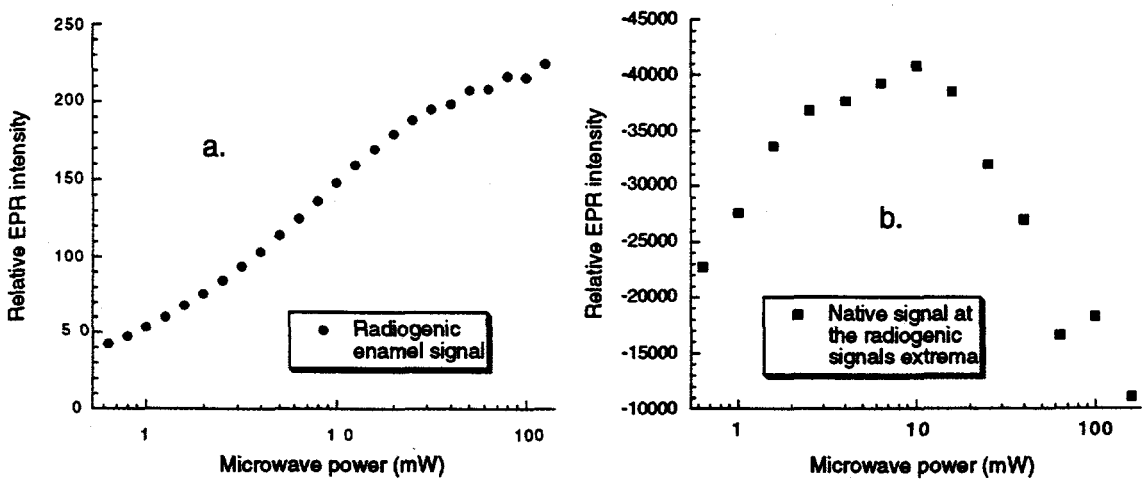

Figure 6. Microwave power dependancy of pertinent enamel signals for dosimetry. Figure 5a is the microwave power saturation curve for the standard dosimetry signal. Figure 5b is the microwave power response of the native signal evaluated at the location of the radiogenic signals extrema. These measurements did not have the corresponding empty resonator signals subtracted out and so are only approximations to the true responses. The apparent noise in the high power region of figure 5a was due to a fewer number of sweeps per spectra being taken there.

Spectral deconvolution has been used for archeological dating by Jonas (1995). Using the Levengerg- Marquardt method (Press et. al 1992), he deconvoluted his sample spectra after each succesive reirradiation. Although he did not model the native signal nor anneal subsequent to each irradiation, he still achieved useful results. An example of a deconvoluted spectrum which took these effects into account using the Levengerg-Marquardt method is shown in figure 7.

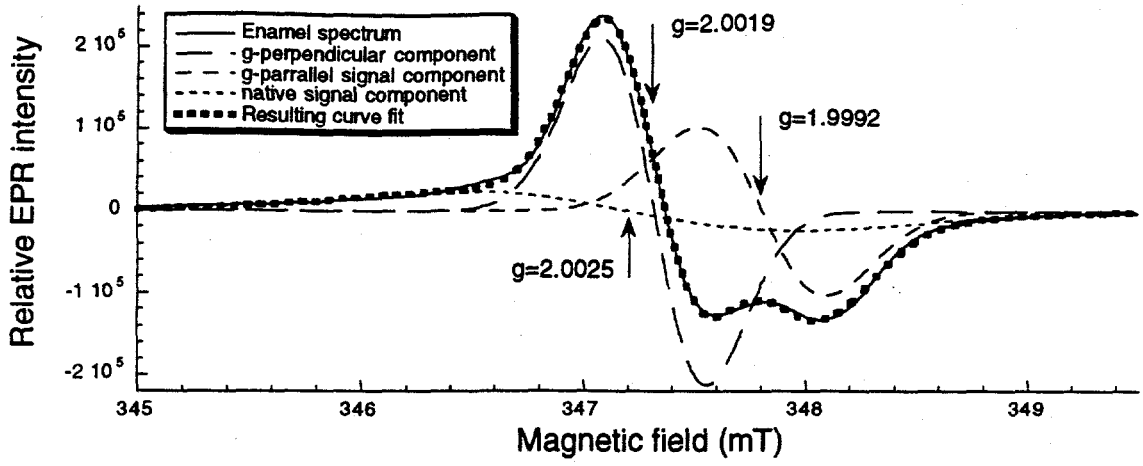

Figure 7. 100Gy irradiated enamel spectrum deconvolution. The g-values of the independant spectral components are shown, the g-value at the zero of the resultant fit is 2.0018. The g-value for the fit of the native signal component differs from that of an unirradiated sample (ca. 2.0045) due to the curve fit not including additional components for the signals located at 2.0083 and 2.0052.

Optimizing the dose distribution

If the region over which the doses to be applied is linear and each measurement can be characterized as having a normally distributed error with constant variance (standard deviation), then the optimum dose distribution is known (Hayes et.al 1996). For any line, there are only two degrees of freedom, a slope and an intercept. In a least squares fit, the error in the slope is minimized by placing all your points at the extrema of the dose distribution. The error in the intercept is minimized by concentrating your measurements at the zero of the abscissa. The only question remaining then is, "what is the optimum ratio of points at the zero to the total number of points used such that an extrapolation to obtain the dose estimate will have minimum error?" The details of the answer are given by Hayes et. al (1996).

A cautionary note, Grun (1996) showed that when the maximum dose applied is greater than 1% of the saturation dose, linear fitting can start to give worse results than fitting a saturating exponential to the data. In this context, the linear region is considered to extend to 10% of the saturation dose. Fortunatly, for solid bone Ostrowski et. al (1980) showed the saturation dose here was ca. 200 KGy. The saturation dose for human tooth enamel has not been presently determined but unpublished data from our lab put this above a few KGy. Finally, Rink and Schwarcz (1994) showed that for 19 different tooth enamel samples from various animals, saturation doses were ca. 4.3 ± 1.8 KGy when the 5 samples with saturation doses above 10 KGy are excluded (there were 24 samples total). This means that cumulative doses lower than ca. 200 Gy should always be fitted with a line for systematially better results. Also, Gruns conclusions were based on the response having soley 2% errors for the total measurement error. A follow up paper is in preperation where we look at these results as a funtion of the size of the fixed constant errors superimposed on the relative errors.

For saturating exponentials, some good rules of thumb for an acceptable dose distribution are given by Barabas et. al (1992).

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