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EPR study on biominerals as materials for retrospective dosimetry

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Abstract Stable paramagnetic species, induced by irradiation in biominerals, can be successfully used as an EPR dosimeter in numerous domains of scientific activity. In hydroxyapatite, the main mineral component of bones, the most stable signal is assigned to CO_2^- ion radical. The signal is dose dependent and allows to determine the absorbed dose in a wide range till 20 kGy, with detection limit for tooth enamel below 1 Gy. The additive dose method was applied to EPR dating of archeological objects and EPR dosimetry after accidental overexposing during radiotherapy. It was found that the age of remains excavated from the "tomb of giants" belonging to Nuraghic culture developed in Sardinia island is about 3160 years old (1160 years BC). The dose obtained by patients during the accident at the Białystok Oncological Center was evaluated at about 75 Gy. Searching for potential dosimeters among other biominerals shows that mollusc shells have very promising properties. The lowest dose detection limit was found for an *Arcidae* shell. Both fresh water and sea mollusc shells are useful to be used for dosimetry in the low dose range.

Key words dosimetry • EPR dating • accidental dosimetry • hydroxyapatite

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Introduction

In numerous biominerals exposed to ionizing radiation some stable paramagnetic species are induced. Their nature can differ depending on chemical composition of biominerals. The method of choice to study paramagnetic centers in biominerals is electron paramagnetic resonance (EPR) spectroscopy. When the relationship between EPR signal intensity of stable paramagnetic center and the dose has a linear or almost linear character, the material can be used as dosimeter of absorbed radiation.

Depending on sensitivity and range of linearity of intensity-dose dependence, the dosimeters can be used for different purposes as geological and archeological dating, standard and accidental dosimetry, detection of irradiated food [4]. The most important are low-dose dosimeters, with a detection limit of about 1 Gy or less. In the most frequently used biomineral dosimeters, the stable EPR signal is generated by ionizing radiation in hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, the main mineral component of bones and teeth. The natural hydroxyapatite, as well as synthetic apatites, exposed to ionizing radiation, have been investigated by EPR for more than 35 years [10]. The EPR spectrum detected in γ-irradiated apatites in the $g = 2$ region is complex. It consist of several signals due to paramagnetic species derived mainly from carbonate impurities, substituting hydroxy groups (so-called A site) or phosphate groups (B site) or those located on the surface of microcrystallites. The species differ in molecular structure and charge, for example, CO_3^{3-}, CO_3^-, CO_2^-, O^-, O_3^- have been identified. The most stable and intense signal in both synthetic and natural apatites represents CO_2^- radical located at surface position [1]. It is a singlet of the orthorhombic symmetry with $g_x = 2.0035$, $g_y = 1.9973$, $g_z = 2.0017$ and $\Delta H_{pp} = 0.85$ mT, which appears in mineralized tissues like bones, teeth, cartilages etc. Its intensity increases linearly with increasing dose up to 20 kGy [9]. Owing to that, this signal is presently used for EPR dosimetry and identification of irradiated meat containing bones.

The next group of biominerals potentially useful for dosimetry, are shells of mollusca. The shell consists mainly of calcium carbonates, calcite and aragonite, with varying amounts of impurities like calcium phosphate, magnesium carbonate, sulphates etc. It is proposed today that stable, paramagnetic species in irradiated shell are predominantly derived from CO_2^- and SO_2^- radical ions [4]. In contrast to bones and teeth, shells differ significantly in the composition and the structure. It is obvious that structural differences of this kind can effect stability, yields and the EPR spectra of paramagnetic centers produced by ionizing radiations in shells. For that reason, systematic studies on irradiated molluscan shell are necessary.

In this paper, recent studies on the application of stable EPR signals induced by ionizing radiation are presented. Two parts of the paper are dedicated to the application of CO_2^- signal in hydroxyapatite for archeological dating and accidental dosimetry. In the third one the EPR signals of different shells are analyzed in order to select the best one for EPR dosimetry.

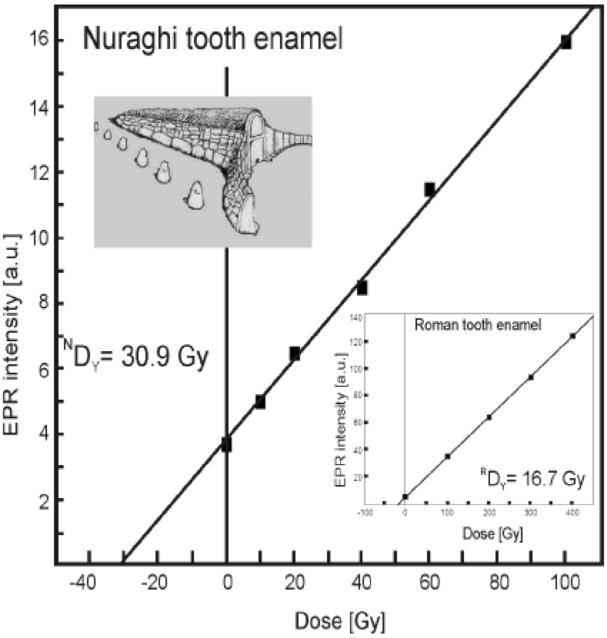


Fig. 1. Linear relationship between EPR signal intensities and adsorbed dose of ionizing radiation for the Nuraghi tooth enamel sample. Straight line extrapolation marks out the value of the cumulated dose of natural radiation absorbed in the sample. Upper insert: the outline of Tomb of Giants in La Testa (http://www.santateresagallura.com/hall.htm?tombag.shtml&2). Lower insert: relationship EPR signal intensities vs. dose for Roman tooth enamel.

Dating of nuragic skeletal tissue [II]

The human presence in the island of Sardinia is perhaps from the inferior Paleolithic period (about 100,000 years BC). From 1600 to 500 BC, the "nuragic" culture was settled there which is recognized by characteristic megalithic monuments, the so-called Nuraghi. Experts do not agree on their function. They propose that monuments served as royal palaces, tombs, temples or defensive works (the last one seems to be the most likely explanation). During the period 1500–1200 BC, in turn, the Tombs of Giants were developed. These are megalithic monuments for burial purposes, with sepulchre niches, closed in the front by flagstones fixed in the ground and flanked by two wings of masonry by which the boundaries of exedra were defined (see the upper insert in Fig. 1).

The remains of bones and teeth, the object of the study, were found in the archeological excavations of "the Tombs of the Giants", La Testa, S. Teresa di Gallura (Fig. 1). The tomb was brought to the light after the fire of surrounding bush [7]. Taking into account the age of ceramic and bronze sepulchral remains, the dating of the tomb was done roughly as ca. 1300–1200 years BC. Inside the tomb a large quantity of bones in several layers were found. Unfortunately, the remains were partially damaged because of root infiltration and wasting away through centuries.

Only a limited number of bone specimens was analyzed in order to date. These were: 6 fragments of tooth enamel, 4 fragments of tooth dentine, 4 fragments of cortical bone from phalangeal diaphyses, 4 fragments of bone from phalangeal epiphyses, 2 fragments of cortical bone from metacarpal diaphyses. Additionally, the samples of tooth enamel taken from a Roman skeleton from necropolis situated fortunately in the close vicinity of the Nuragic tomb and estimated by archeological methods as 1700 years old, were use for comparison. Because preliminary EPR examination of the nuragic sample mentioned above showed that good quality signals were obtained only for tooth enamel fragments, the quantitative measurements were carried out with two tooth enamel samples only. The samples were measured using an X-band Bruker ESP300 spectrometer and then they were irradiated using a ^{60}Co gamma source with doses of 10 Gy (two times), 20 Gy (two times) and 40 Gy (one time), respectively, in order to receive the most reliable results. Every irradiation step was followed by a successive EPR measurement.

The signal due to CO_2^- radical ion was carefully isolated from each of the EPR spectra in order to avoid the influence of the native signal ($g = 2.0055$). The peak heights (mean value of two measurements) of primary signal and the signals recorded after irradiation, were plotted vs. the doses applied. A linear relationship was found using the least squares method.

It was estimated that the cumulated dose from the natural radiation (cosmic rays and/or radiation from radionuclides in soil) absorbed in the Nuragic tooth enamel is equal to 30.9 ± 4.2 Gy.

Similarly to the Nuragic tooth enamel, the samples of Roman tooth enamel were irradiated step by step several times with additive doses of gamma irradiation. Following the procedure described for Nuragic enamel samples, the cumulated dose absorbed in the Roman enamel sample was found to be equal to 16.7 ± 2.0 Gy. Since the age of Roman skeleton was evaluated from earlier archeological data with acceptable accurancy (1700 years), it was possible to calculate the annual average dose of overall radiation delivered to Roman skeletons: 16.7 Gy/1700 years = 9.8 mGy/year. The value of annual dose is in good agreement with literature data; the level of annual dose absorbed by archeological samples as given by other authors is about 9.5 mGy per year [2, 4]. We did not expect a significant difference between the literature data and the values of annual dose ratio expected for nuragi teeth because the analysis of soils obtained from the vicinity of Nuragic tomb and Roman cementary shows that the concentration of radionuclides (^{226}Ra, ^{232}Th and ^{40}K) is comparable to the median value of the concentration of radioactive nuclides in the soils of the world.

Knowing the cumulated dose absorbed in Nuragic tooth enamel and annual dose ratio, it was possible to evaluate the age of Nuragic skeletons as: 30.9 Gy/9.8 mGy/year = 3160 years (1160 years BC). The total error of the EPR measurements was evaluated as about 15%.

The age of Nuragic skeletons based on EPR measurements, i.e. 3160 years is comparable with the age estimated from the dating of ceramic and bronze remains discovered there (1300–1200 years BC).

The radiological accident at Białystok Oncology Centre: dose estimation [12]

On February 27th 2001 a radiation accident occurred in the radiotherapy unit of Białystok Oncology Centre in Poland. Due to malfunction of the Neptun 10p linear accelerator, five breast-cancer patients received to the chest wall a single, high dose of 8 MeV electron beam generated by the accelerator. The accident was caused by the damage of the beam monitoring system leading to a large increase of the dose rate even though the display indicated the typical value. As a result, the limiter of the filament current of the electron gun was set to a high level so that the dose rate was practically unrestricted. The combination of these factors led to the substantially higher doses delivered to the patients.

Owing to various circumstances the exact doses received during the accident could not be reconstructed. However, based on early and late skin reactions and the measurements performed by medical physicists immediately after the accident, it was speculated that the dose was practically heterogeneous and could reached even 100 Gy.

The patients were at different stages of therapy and received different tumor doses prior to the accident. All patients experienced immediate pain and skin reddening, followed by moist desquamation and development of deep necroses by autumn 2001. In the spring/summer 2002, all the patients underwent surgical reconstruction the chest wall with a subsequent skin transplantation. In case of three patients, fragments of rib bone were removed. One of the patients (no. 5) was chosen for dose reconstruction using EPR spectrometry in the Institute of Nuclear Chemistry and Technology (INCT), Warsaw.

The sample delivered as a piece of the rib (total weight about 30 mg) submerged in formaldehyde solution was dried with a paper, rinsed two times with distilled water and defatted with an ethyl alcohol – chloroform solution. Each time the sample was kept submerged in the solution for 3 h. The sample was then dried under vacuum during 3 h.

Then, with the use of a surgery scalpel the soft tissue was removed from the sample which was subsequently divided with a small saw into two bits approximately of the same size. First of them was taken for dose measurement using reirradiation with a ^{60}Co source installed in the INCT, the second one was reirradiated using a Clinac 2300 CD medical accelerator.

The bone bits were placed in thin wall EPR tubes made of spectrosil glass, 4 mm in diameter and measured using the X-band Bruker ESP300 spectrometer. The heights of samples inside the tubes were about 10 mm, respectively. The basic measuring conditions were as follows: microwave power 20 mW, modulation amplitude 0.19 mT, time constant 20.48 ms. Each sample was always reproducibly centred in a resonant cavity of the EPR spectrometer. A 100-fold screening of the spectra was applied to attain a high signal to noise ratio that ensures desirable peak height estimations.

After the measurements, the first sample was reirradiated with the linear accelerator Clinac 2300 CD made by Varian in Oncology Centre, Warsaw. Nominal energy of electrons was 9 MeV, mean energy at the phantom surface E_0 = 8.2 MeV. The phantom was made by PMMA, the depth of irradiation was 18 mm PMMA, equivalent to 21 mm of water. The sample was re-irradiated with a total dose of 240 Gy, in four equal fractions. The second sample was irradiated in a ^{60}Co gamma source Mineyola installed in the INCT in Warsaw. The dose rate in irradiation position was 89 Gy/h. The ^{60}Co source was calibrated by the NIST experts (Report of Special Measurement, NIST, Gaitherburg, USA). The samples were re-irradiated step by step four times with a dose of 60 Gy, respectively. Re-irradiation in the gamma source has been done in small, thin-wall glass bottles.

After re-irradiation, the bits of samples were carefully transferred to the same measuring tubes in which the first EPR examination has been done. The measuring condition were adjusted at the same level as during the primary recording.

The peak-to-peak heights of EPR signals assigned to CO_2^- ion-radical were measured for preliminary signal and four times after each successive exposure to electron beam and gamma rays. The plot of peak heights vs. doses allows to calculate the point of intersection between the plotted line and the x-axis. The numerical value of this point corresponds to the unknown dose of radiation absorbed in the rib (see Fig. 2).

The dose was estimated with the accuracy of about 12%. This represents the summary error from the preparation procedure, re-irradiation, EPR measurement and extrapolation.

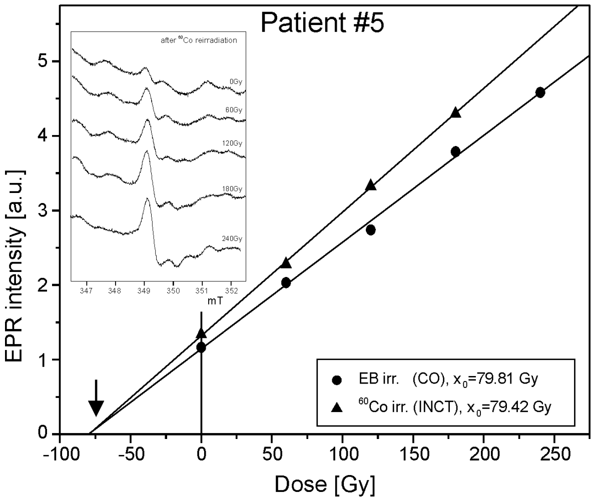


Fig. 2. Linear relationship between EPR signal intensities and absorbed dose for rib bone of patient no. 5. The arrow indicates the cumulated dose absorbed by the patient. Insert: EPR signals recorded in rib bone without additional irradiation and after irradiation with doses of 60, 120, 180 and 240 Gy.

The dose estimated on this way after exposure to 9 MeV electrons is equal to 79.8 Gy while the dose estimated after exposure to the ^{60}Co gamma rays was 79.4 Gy. The estimated dose expresses the cumulated dose absorbed in the patient rib, i.e. the sum of doses delivered during the earlier therapeutical exposures and the high dose of accidental irradiation. Because the initial dose, obtained by patient no. 5 prior to the accident was 5 Gy [3], the dose absorbed by the rib bone during the accidental overexposure was estimated as about 75 ± 9 Gy. This result is in excellent agreement with the comparative EPR measurements done in IRSN, France [3], where the value of total dose was assessed as 75 ± 3 Gy.

The search for EPR dosimeter among molluscan shells

For searching of dosimeters with the lowest detection limit, two groups of shells were chosen. The first group was collected in the Mazurian Lakes, Poland, and is representative for Middle and East Europe fresh waters. In addition, the shell of typical terrestrian mollusc – *Capaea homoralis* – has been also examined. As a second group, the shells of sea water molluscs were taken.

After cleaning, the shells were irradiated as a whole. For irradiation, the ^{60}Co gamma source Mineyola, INCT, dose rate 40 Gy/h, was used. Next, the shells were crushed to small pieces not exceeding 2 mm and placed into EPR quartz sample tubes with a diameter of 4 mm and subsequently measured using the X-band Bruker ESP300 spectrometer. The modulation amplitude was set to 0.2 mT, microwave power ranged from 1 to 40 mW in order to find the best detection sensitivity.

Generally, the shells of the first group gives lower EPR signal for the same dose in comparison to those of the second group. Among eight fresh water shells examined, only three were found to stabilise EPR signal after irradiation with a dose of 10 Gy. It was found that the lowest detection limit can be reached with the shells of *Dreissena polymorpha* (2 Gy) and *Viviparus contectus* (3 Gy). It is worthy of notice that detection limit of the shell of terrestrian mollusc *Capaea homoralis* is also at a 2 Gy level. Radiation induced EPR signals in the other shells of this group can be detected after their irradiation with a dose of about 10 Gy or higher. The list of molluscan shells and corresponding detection limits are collected in Table 1 [6, 8].

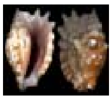
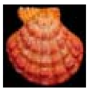
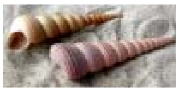
The second group of specimens is certainly more sensitive to radiation than the first one. The higher intensity of radiation induced EPR signals has probably its source in the higher crystallinity of shell mineral [5]. In general, the lower EPR detection limit was found in white marine shells. In coloured shells higher content of paramagnetic ions, especially Mn^{2+}, masks radiation induced signals. The shell of *Arcidae*, frequently found in sea and ocean waters, was found to be the most sensitive to radiation. It allows to detect the cumulated dose of 0.2 Gy. The next group of shells, which can be used for EPR dosimetry – *Tonna galea*, *Venus*, and

Table 1. Denomination of mollusca and the lowest levels of absorbed dose of ionizing radiation (^{60}Co) detected in fresh water shells

Species		Dose detection level [Gy]
Planobarium corneus		10.0
Viviparus contectus		3.0
Dreissena polymorpha		2.0
Anodonta complanata		no EPR signal
Anodonta anatina		no EPR signal
Anodonta cellensis		no EPR signal
Unio pictorum		no EPR signal
Unio tumidus		no EPR signal
Capaea homoralis		2.0

Corithium – has the detection limit of about 0.3 Gy. Slightly less sensitive to radiation are shells of *Cardium*, pearl oyster (*Meleagrina vulgaris*), *Lambis lambis* which allow to detect a dose above 0.5 Gy. Radiation detection levels of remaining shell specimens are found to lie between 1 and 5 Gy, similarly to the level found in fresh water molluscs. The results are collected in Table 2 [6, 8]. Several shell samples were irradiated with increasing doses (0.5, 1, 2, 5, 10, 20 Gy) in order to measure the dependence of the intensity of radiation induced EPR signal vs. dose. The relationship was found linear for the shells of sea water and fresh water molluscs.

Table 2. Denomination of mollusca and the lowest levels of absorbed dose of ionizing radiation (^{60}Co) detected in sea water molluscan shells

Species		Dose detection level [Gy]
Arcidae sp.		0.2
Tonna galea		0.3
Venus sp.		0.3
Corithium sp.	–	0.3
Cardium sp.		0.5
Meleagrina vulgaris		0.5
Lambis lambis		0.5
Tridacna squamosa		1.0
Lambis chiragra		2.0
Strombus sinuatus		2.0
Coralliophila sp.		2.0
Pecten sp.		3.0
Fusinus colus		3.0
Turitella terebra		5.0
White coral		0.3
Red starfish		2.0
Balanus (*barnacles*)		4.0

Conclusions

The presented examples clearly indicate that selected biominerals can be used as EPR dosimeters to measure therapeutic doses. The most commonly used biodosimeters are skeletal tissues – teeth and bones. The tooth enamel was found to be a very sensitive dosimeter, with a linearity within the range 0.5–20,000 Gy allowing to determine the absorbed dose with high precision when the additive dose method is applied. This enables to use EPR dosimetry for such problems as accidental dosimetry or dating, for example.

Various shells differ markedly in the sensitivity as detectors of ionizing radiation when measured by EPR technique. The lowest dose limit of 0.2 Gy was found with *Arcidae* shell. In general, the detection limit for sea molluscan shells is lower than for fresh water shells.

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