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## RADIATION-INDUCED RADICALS $\text{CO}_2^-$ IN BIOAPATITES

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We present the results of EPR studies of specimens of  $\gamma$ -irradiated biological apatites (tooth enamel, bone). It is established that, immediately after the irradiation, an additional signal is observed besides the known signal of  $\text{CO}_2^-$  radicals. This signal is unstable and disappears after a few weeks of storage at room temperature. Dynamic characteristics and radiospectroscopic parameters ( $g_x = 2.0044$ ,  $g_y = 2.0033$ ,  $g_z = 2.0019$ ) of this EPR signal point to  $\text{CO}_3^{3-}$  radicals as the origin of this signal. Based on the data obtained and literature data, different models of the radiation-induced formation of  $\text{CO}_2^-$  radicals in apatites are analyzed. The preference is given to the  $\text{CO}_3^{3-} \rightarrow \text{CO}_3^{2-} \rightarrow \text{CO}_2^-$  transformation mechanism.

has, in our opinion, the defining meaning for the applied usage of biological apatites. If radicals  $\text{CO}_2^-$  are formed from fragments of biological molecules or from molecules  $\text{CO}_2$  in organic components or on the surface of crystallites, then this calls into question the very bases of the retrospective EPR dosimetry and dating. Indeed, such precursors of radiation-induced defects are unstable, and their concentration can significantly vary in the course of time in living beings due to metabolism and on the storage of biological apatites due to the decay of organic components. In addition, the indicated factors will also affect the stability of already present radiation-induced radicals  $\text{CO}_2^-$ .

### 1. Introduction

Apatites of the biological origin, which compose the inorganic basis of highly mineralized biological tissues such as tooth enamel and bones, arouse a great interest of researchers due to their application in retrospective EPR dosimetry and EPR dating [1–5]. The well-known radiation-induced EPR spectrum (the so-called dosimetric signal) of irradiated biological apatites and their synthetic analogs is an asymmetric line (see, e.g., [6]) caused by radicals  $\text{CO}_2^-$  of various types [7]. Whereas the identification of main paramagnetic defects in biological apatites arouses no doubts, their localization and mechanisms of their formation are disputable.

There exist several models of the formation of radiation-induced radicals  $\text{CO}_2^-$  in biological apatites [8–10]. According to them, the precursors of  $\text{CO}_2^-$  can be neutral molecules  $\text{CO}_2$  in organic components and on the surface of crystallites, parts of organic molecules,  $-\text{CO}(\text{OH})$ , and molecular ions  $\text{CO}_3^{2-}$  in positions *A* (substitution of a hydroxyl group) and *B* (substitution of a phosphorus tetrahedron). The establishment of mechanisms of the formation and places of localization of radiation-induced radicals  $\text{CO}_2^-$

The determination of mechanisms of the formation of radicals  $\text{CO}_2^-$  can be favored by a comprehensive study of unstable paramagnetic defects arising in the process of irradiation of bioapatites. The EPR spectra of the latter (transient signals) were repeatedly registered in both enamel and bone tissue (see, e.g., [11–14]) and were identified as those of radicals  $\text{CO}_3^{3-}$ ,  $\text{CO}_3^-$ , and  $\text{O}^-$ . Since the most frequently registered transient signal EPR has  $g$ -factor  $\sim 2.003$ , it is overlapped with the dosimetric signal. Therefore, the main studies were directed to the minimization of its influence and the influence of other transient EPR signals on the dosimetric signal. In such a way, it was established that the above-mentioned EPR signal with  $g \sim 2.003$  disappears on the storage of irradiated enamel at room temperature for one month or on its annealing at a temperature of  $90^\circ\text{C}$  for 2 h [13]. In this case, the increase of the dosimetric signal by 15% was observed.

In the present work, the attention is focused on the analysis of lineshape changes of EPR spectra caused by the decay of unstable radiation-induced radicals in irradiated bioapatites with the purpose to clarify mechanisms of the formation of radicals  $\text{CO}_2^-$ .

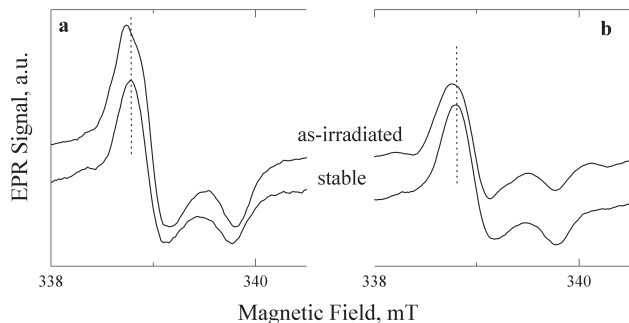


Fig. 1. EPR spectra of  $\gamma$ -irradiated powders of tooth enamel (a) and bone tissue (b) recorded immediately after the irradiation and in 2 months of the storage of specimens at room temperature. The vertical dotted line is drawn through the maximum of the stable spectrum for the clearer demonstration of a shift of the maximum caused by the presence of a transient signal

## 2. Materials and Methods

We used powders and plates of tooth enamel and bone tissue. Specimens were irradiated by  $\gamma$ -rays from a  $^{60}\text{Co}$  source at room temperature. The radiation power was 100 R/s ( $2.58 \times 10^{-2} \text{ C kg}^{-1} \text{ s}^{-1}$ ). The absorbed dose was not determined exactly. By estimates, it was several kGy. The EPR studies were carried out at room temperature on an EPR spectrometer of the X range ( $\sim 9.5 \text{ GHz}$ ). We used the high-frequency (100 kHz) modulation of the magnetic field with an amplitude of 0.05 mT. The magnetic field strength was controlled with a NMR gage, which ensures the accuracy of measurements of not worse than 0.01 mT. The EPR spectra were registered directly after the irradiation of specimens and after their storage for 2 months at room temperature.

## 3. Experimental Results and Discussion

In Fig. 1, we give the EPR spectra of  $\gamma$ -irradiated powders of biological apatites. The spectra are normalized by the maximum and were recorded at once after the irradiation and after the storage of specimens for 2 months at room temperature. The EPR spectra of the latter are typical dosimetric signals without any addition of transient signals [13]. On the contrary, the form of spectra recorded immediately after the irradiation of biological apatites differs significantly from that of the dosimetric signal. This is well seen by a shift of the maximum of the signal to the low-field region. In addition, there occurs a redistribution of the intensity

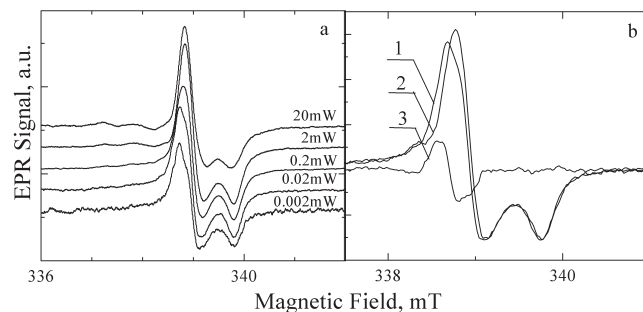


Fig. 2. EPR spectra of an enamel powder recorded at various microwave powers immediately after the irradiation of a specimen (a) and the separation of a transient EPR signal (b). 1 – spectrum immediately after the irradiation, 2 – stable spectrum, 3 – their difference

in the limits of the signal itself. Such a difference of the EPR spectra recorded at once after the irradiation of specimens and after their long-term storage at room temperature is observed both for specimens of enamel and for those of bone tissue. This fact indicates that the former EPR spectra contain a transient signal appearing from paramagnetic centers with short lifetime (metastable centers) which are formed in both materials under the action of ionizing radiation.

Let us consider some properties of this transient signal in more details by the example of the specimens of enamel. The studies of dynamical characteristics of the transient signal have shown that it is saturated at significantly less microwave powers than the signal from radicals  $\text{CO}_2^-$ . In Fig. 2, a, we present the EPR spectra of a powder of enamel which are recorded at various microwave powers immediately after the irradiation of a specimen. It is well seen that the microwave power optimal for the registration of the transient signal is equal to 0.02 mW, whereas that for the registration of the dosimetric EPR signal is 2 mW. In order to determine the spectral position of the transient signal and to estimate the interval of values of its  $g$ -tensor, it is possible to analyze the difference of the EPR spectra recorded at a microwave power of 0.02 mW immediately after the irradiation of the specimens and after their storage. It is seen from Fig. 2, b that the transient signal is observed at less intensive magnetic fields than the EPR signal which arises from radicals  $\text{CO}_2^-$ . In view of the fact that the parameters of the latter EPR spectra are well known (see, e.g., [7,15–18]), we can estimate the interval of  $g$  for the transient signal as  $2.0015 < g < 2.0040$ .

On the other hand, the line shape for the transient signal is similar to that of the EPR spectrum caused by

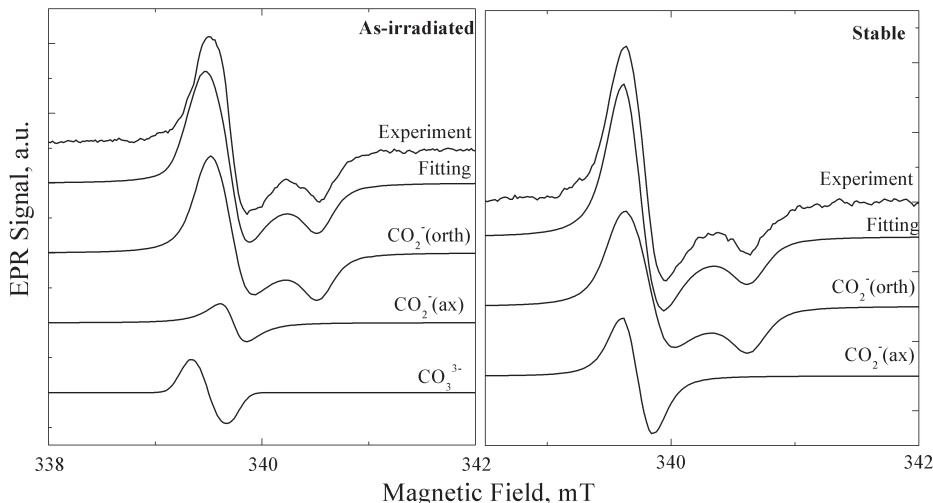


Fig. 3. Experimental EPR spectra recorded immediately after the irradiation of plates of tooth enamel and after their storage for 2 months and the model spectra, whose calculation is described in the text

radicals  $\text{CO}_3^{3-}$  at position  $B$ . The EPR spectrum of the latter is described by the parameters  $g_x = 2.0045$ ,  $g_y = 2.0034$ ,  $g_z = 2.0014$  [19], and the optimal conditions for its registration are lower microwave powers as compared with the spectrum caused by radicals  $\text{CO}_2^-$  ( $\approx 0.1$  mW relative to  $\approx 10$  mW for the spectrum of  $\text{CO}_2^-$ ) [7]. It is obvious that the direct comparison of the values of microwave powers optimal for the registration of EPR signals performed on different spectrometers is not correct. The difference of the absolute values of microwave powers can be related to specific features of the experimental equipment, in particular to different  $Q$ -factors of resonators. However, the fact that the optimal power for the observation of the EPR spectra of metastable centers and radicals  $\text{CO}_3^{3-}$  is by two orders lower than that for radicals  $\text{CO}_2^-$ , allows us to conclude that the metastable centers and radicals  $\text{CO}_3^{3-}$  have close or identical relaxation characteristics. In addition, radicals  $\text{CO}_3^{3-}$  are unstable and are transformed in the free state into radicals  $\text{CO}_2^-$  [18]. Thus, the properties of metastable centers are quite close to ones of radicals  $\text{CO}_3^{3-}$  at position  $B$ . Therefore, it is natural to consider that the transient EPR signal is caused by radicals  $\text{CO}_3^{3-}$ .

For the detailed analysis of lineshapes of the EPR spectra of irradiated bioapatites, it is necessary to bear in mind the following facts. It is known [20] that the dosimetric signal of EPR is mainly formed by contributions from two types of radicals  $\text{CO}_2^-$  – axial and orthorhombic ones. The spectra of powders of these radicals are similar, and it is difficult to separate them

for powder specimens. The completely other pattern is observed on specimens cut in the form of plates, where the EPR signals from axial  $\text{CO}_2^-$  demonstrate the angular behavior [20] determined by a certain ordering of radicals. Orthorhombic radicals are oriented chaotically and therefore give a EPR spectrum characteristic of powders. In what follows, we used plates.

For bioapatites, there exists such an orientation of a specimen in the external magnetic field, for which the axial radicals  $\text{CO}_2^-$  give contribution only near  $g = g_\perp$ . Therefore, the intensity of the experimental EPR spectrum at the point  $g = g_\parallel$  will be determined only by orthorhombic  $\text{CO}_2^-$ , which allows one to reconstruct the lineshapes of their EPR signals, by using their known radiospectroscopic parameters.

Figure 3 shows the experimental and model EPR spectra for as-irradiated specimens and the spectra of stable radicals in plates of enamel, as well as the separate components which used on the modeling; the experimental EPR spectra are given for the mentioned orientation of a specimen. The first component, which was used in the modeling, was an EPR spectrum characteristic of powders and reproduced the contribution of chaotic orthorhombic radicals  $\text{CO}_2^-$ . It was calculated with the use of the known radiospectroscopic parameters of these radicals:  $g_x = 2.0031$ ,  $g_y = 2.0017$ ,  $g_z = 1.9972$ . The intensity of this component is uniquely determined by that of the experimental EPR spectrum at the point  $g = g_\parallel$ . The second component reproduced the contribution of oriented axial radicals  $\text{CO}_2^-$ . To this end, we used

the experimental EPR line of the same plate after its annealing at a temperature of 300 °C for 1 h; the spectrum was recorded at the indicated orientation of the specimen [18]. The third component described the contribution of metastable radicals which was similar to that of powders. This component was used only for the modeling of the experimental EPR spectrum recorded immediately after the irradiation. It was found that a satisfactory description of the experimental spectrum is attained if, in the calculation of the third component, the parameters  $g_x = 2.0044$ ,  $g_y = 2.0033$ ,  $g_z = 2.0019$ , and the linewidth  $\Delta H_{1/2} = 0.2$  mT are taken. The EPR line calculated with these parameters is practically symmetric due to the fact that the  $g$ -tensor anisotropy is small relative to the linewidth. The obtained parameters are similar to those of radicals  $\text{CO}_3^{3-}$  at position  $B$  of the lattice of synthetic apatite [19, 14]. This additionally confirms the hypothesis on the identity of metastable centers with radicals  $\text{CO}_3^{3-}$ . The final intensity of each component was chosen in view of the condition to describe the experimental EPR spectra in the best way. It is seen from Fig. 3 that the use of all three above-mentioned components allows one to realize a good description of the experimental EPR spectra of stable radicals and the spectra including the transient signals. Unexpectedly, we obtained that the intensity of the line caused by axial radicals  $\text{CO}_2^-$  was significantly less than that of the EPR spectra recorded immediately after the irradiation relative to the dosimetric signal. A similar behavior was also observed for the EPR spectra of bone tissue.

In order to obtain the ratio of the contributions of axial and orthorhombic radicals  $\text{CO}_2^-$ , we integrated each component twice. The contribution of axial  $\text{CO}_2^-$  to the dosimetric EPR signal was 18%, which is in agreement in the limits of experimental errors with the published data [18]. At the same time, their contribution to the EPR spectrum recorded immediately after the irradiation was less than 5%. Thus, the relative contribution of axial  $\text{CO}_2^-$  to the total EPR spectrum grows significantly on the decay of metastable centers. In this case, the absolute intensity of the dosimetric EPR signal increases [13]. Hence, we can conclude that the formation of axial radicals  $\text{CO}_2^-$  in biological apatites happens via the decay of metastable centers.

It is known that the axial radical  $\text{CO}_2^-$  is a bulk paramagnetic center and occupies structural position  $B$  of the apatite lattice [16]. This radical is formed under the action of radiation from a molecular ion  $\text{CO}_3^{2-}$ . Most probably, this occurs via the metastable form, radical  $\text{CO}_3^{3-}$  [18]. Axial radicals  $\text{CO}_2^-$  can also be formed from

orthorhombic  $\text{CO}_2^-$  on thermal annealings of enamel [18]. In this case, both radical  $\text{CO}_2^-$  and its precursor are placed at the same structural position, which indicates the absence of the diffusion of charged ions in bioapatite. In the present work, we have registered the formation of axial radicals  $\text{CO}_2^-$  without any external action, which was accompanied by the decay of metastable radicals. To explain this phenomenon, it is natural to assume that metastable radicals are also placed at structural position  $B$  of the apatite lattice and are directly transformed into radicals  $\text{CO}_2^-$ , mainly those of the axial type. Since the latter are bulk radicals, their precursors are, most probably, bulk radicals. Thus, it is one more argument in favor of the assertion that metastable radicals in biological apatites are radicals  $\text{CO}_3^{3-}$  placed at position  $B$  of the apatite lattice.

Eventually, we consider the most probable, in our opinion, mechanism of the radiation-induced formation of radicals  $\text{CO}_2^-$ . The precursors for  $\text{CO}_2^-$  radicals are molecular ions  $\text{CO}_3^{2-}$  at position  $B$ . Below, we will use the following notations: we denote the molecular ions placed in a defectless domain of the crystallite as  $\text{CO}_3^{2-}$ (I) and the ions, near which the lattice defects are placed, as  $\text{CO}_3^{2-}$ (II). On the capture of a free electron which is generated by radiation, the ion  $\text{CO}_3^{2-}$ (I or II) is transformed into the metastable radical  $\text{CO}_3^{3-}$  of the corresponding type (I or II). In addition, the radiation creates new nonparamagnetic defects. This leads to the appearance of an additional channel of the formation of  $\text{CO}_3^{3-}$ (II), i.e.  $\text{CO}_3^{2-}$ (I)  $\rightarrow$   $\text{CO}_3^{3-}$ (II). The radicals  $\text{CO}_3^{3-}$ (I) and  $\text{CO}_3^{3-}$ (II) are unstable. They decay into  $\text{CO}_2^-$  of the corresponding type (I (axial) and II (orthorhombic)):  $\text{CO}_3^{3-} \rightarrow \text{CO}_2^-$ . The difference between orthorhombic and axial radicals is caused by the presence of a lattice defect in the environment of a radical, which lowers the symmetry of the center. The decay of  $\text{CO}_3^{3-}$ (I) is running more slowly than that of  $\text{CO}_3^{3-}$ (II). This is related to the fact that a defect adjacent to  $\text{CO}_3^{3-}$ (II) decreases the height of the potential barrier (decreases the energy of activation of the defect), which accelerates the process of decay. Therefore, we observed experimentally only  $\text{CO}_3^{3-}$ (I) which also decay completely in two months with the formation of  $\text{CO}_2^-$ (I). It is worth to note that the generation of  $\text{CO}_3^{3-}$ (II) by the mechanism  $\text{CO}_3^{2-}$ (I)  $\rightarrow$   $\text{CO}_3^{3-}$ (II) is more efficient on  $\gamma$ - than on UV-irradiation, because  $\gamma$ -radiation creates a greater number of additional defects and, respectively, orthorhombic centers. This should lead to the greater number of the latter on  $\gamma$ -radiation than that on UV-irradiation, which was observed in experiments [10].

#### 4. Conclusion

The EPR spectra of  $\gamma$ -irradiated bioapatites (tooth enamel and bone tissue) immediately after the irradiation have shown the presence of metastable paramagnetic centers, whose EPR spectrum can be described with the parameters  $g_x = 2.0044$ ,  $g_y = 2.0033$ ,  $g_z = 2.0019$ . On the basis of the study of their properties and the determination of the parameters of EPR spectra, we can identify them as radicals  $\text{CO}_3^{3-}$  located at position  $B$  of the apatite lattice. We have shown that the decay of these metastable centers is accompanied by an increase in the contribution of radicals  $\text{CO}_2^-$ , mainly of those of the axial type. This allows us to consider metastable radicals  $\text{CO}_3^{3-}$  to be the precursors of radicals  $\text{CO}_2^-$  and indicates the mechanism of the formation of the latter which can be schematically written as  $\text{CO}_3^{2-} \rightarrow \text{CO}_3^{3-} \rightarrow \text{CO}_2^-$ .

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#### РАДІАЦІЙНО-СТИМУЛЬОВАНЕ УТВОРЕННЯ РАДИКАЛІВ $\text{CO}_2^-$ В БІОЛОГІЧНИХ АПАТИТАХ

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#### Резюме

Методом електронного парамагнітного резонансу (ЕПР) проведено дослідження зразків біологічних апатитів (зубна емаль, кісткова тканина), опромінених  $\gamma$ -променями. Встановлено, що одразу після опромінення в спектрах ЕПР крім сигналу від радикалів  $\text{CO}_2^-$ , присутній також додатковий сигнал, який зникає після зберігання зразків при кімнатній температурі протягом декількох тижнів. Дослідження динамічних характеристик сигналу ЕПР та визначення компонент його  $g$ -тензора ( $g_x = 2,0044$ ,  $g_y = 2,0033$ ,  $g_z = 2,0019$ ) дозволили ідентифікувати його з радикалами  $\text{CO}_3^{3-}$ . На основі отриманих даних та літературних джерел запропоновано механізм радіаційного утворення радикалів  $\text{CO}_2^-$  в апатитах у вигляді  $\text{CO}_3^{2-} \rightarrow \text{CO}_3^{3-} \rightarrow \text{CO}_2^-$ .