Quantitative Evaluation of Irradiated Bone Grafts Resorption Rate by the Electron Spin Resonance Technique

In recent years some data concerning the appearance of stable paramagnetic species in irradiated bone tissue have been published[1-5]. It was found that in irradiated bone 2 types of paramagnetic species are involved (Figure 1). The first one, arising from bone collagen and showing a strong symmetric doublet in electron spin resonance (ESR), is unstable in the presence of air oxygen and disappears completely within 6 days after irradiation. The second one, arising from a radiation-induced structural defect in bone hydroxyapatite and showing an asymmetric singlet in the ESR spectrum, exhibits unusual stability in air whether the samples are wet or dried. Examination of irradiated bone samples after more than 600 days did not show any noticeable changes in the shape and intensity of this ESR spectrum[6]. It occurred to us that this signal (Figure 1, b) might be used to evaluate the rate of resorption of radiosterilized bone grafts in experimental systems. The unstable symmetric ESR doublet arising from bone collagen was not taken into account in the present study.

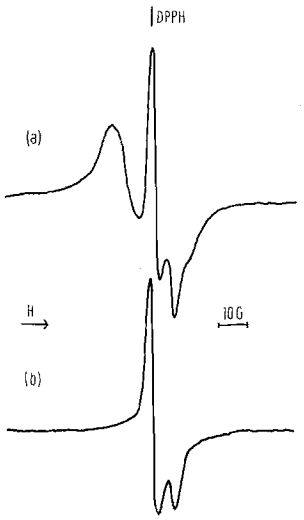

Fig. 1 First derivative ESR spectra of bone irradiated at room temperature with a dose of 3.3 Mrads: a) the ESR signal consists of an asymmetric singlet superimposed by a symmetric doublet derived from bone collagen, recorded immediately after irradiation; b) long-lived ESR singlet derived from defect structure of bone hydroxyapatite, recorded after 6 to 600 days of storage in air.

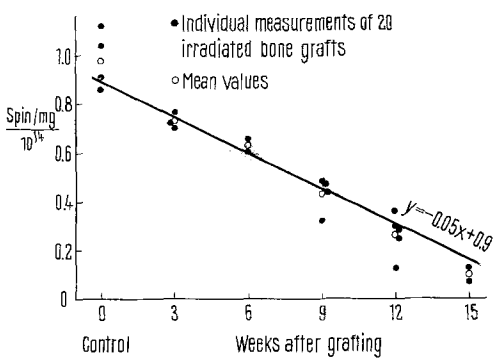

Fig. 2. Spin concentration in irradiated bone grafts removed at various time intervals after transplantation.

Material and method. Allogenic grafts of cancellous bone of calvaria were used in 16 adult rabbits. Rectangular (7 × 7 mm) lyophilized bone fragments were irradiated at room temperature in air atmosphere with a dose of 3.3 ± 6% Mrads in a Cobalt 60 source. The bone fragments were grafted orthotopically under Nembutal anaesthesia and the whole region of implanted bone was taken for the ESR measurements 3, 6, 9, 12 and 15 weeks after grafting. The samples were lyophilized, mechanically powdered (grain size about 200 μm) and the concentration of spins per mg was measured in the X-band (9500 MHz) with an EPR-2 spectrometer, under standard experimental conditions. The spin concentration was measured in excised and powdered fragments as well as in the corresponding samples which were not grafted.

Results and discussion. Figure 2 shows the results of the experiment. An almost linear decrement of spin concentration per mg of bone was obtained. Histological control of bone grafts resorption was done. Apart from the planimetric method used by CHALMERS[7], no other quantitative methods for evaluation of bone resorption exist. Since the ESR stable signal in irradiated bone is connected with defect hydroxyapatite structure, one may assume that decrement of spin concentration of samples taken from the regions where irradiated bone fragments were grafted, reveals the effect of bone resorption. The proposed method based on the use of the radiation-induced stable ESR signal in bone tissue as a new biological label might be useful in experimental as well as in clinical research. The detailed description of experiments performed on rabbits and dogs will be published elsewhere.

Zusammenfassung. Das durch die Bestrahlungssterilisation in Knochentransplantaten induzierte stabile ESR-Signal wurde als biologisches Merkmal für die quantitative Bestimmung des zeitlichen Verlaufs der Knochenresorption benützt. Allotransplantate am Kaninchen *Calvaria* ergaben einen linearen Abfall des Spins/mg im Zeitbereich von 0–15 Wochen.

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