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ELECTRON SPIN RESONANCE (ESR) AS A METHOD TO ESTIMATE THE TIME OF BLOOD EXTRAVASATION IN FORENSIC MEDICINE.

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Electron spin resonance (ESR) is known as a dating method and ESR dating was applied successfully in Quaternary geology, anthropology and archaeology. There have been proposed also an interesting attempt of application ESR dating of organic substances utilising paramagnetic degradation products by Miki et al. (Miki, Kai & Ikea, 1987). We have applied ESR spectroscopic method in order to estimate the time after a death or injury using extravasating blood. This method may be of importance as a practical test in forensic medicine. ESR spectrum of the coagulated blood consists of the three signals at g = 6, g = 4,3 and the strongest at g = 2,005. The sharp signal at g = 2,005 is due to ascorbyl radical in vitro and it corresponds to the level of vitamin C in vivo. The investigations were performed using the blood of six healthy donors. ESR spectra were recorded as a function of ageing time about 20, 60, and 90 hours after extravasation. The obtained results show a big individual variability among samples and ESR signal dependence on the condition in which the samples were stored (temperature, humidity) and small ESR signal differences in time. We can conclude, that the ESR spectroscopic method is not suitable for examination of blood extravasation.

INTRODUCTION

Electron spin resonance (ESR) was proposed as a dating method by Zeller as early as 1967, but its practical application began with the work of Ikeya in 1975. Since then, there have been proposed an interesting attempt of application ESR dating of organic substances utilising paramagnetic degradation products by Miki et al. (Miki, Kai & Ikeya, 1987; Miki , Kai & Ikeya 1988). ESR dating is a powerful tool to date the time of mineralization, sedimentation or last heating of minerals (Ikeya, 1993). The age of some organic materials can be deducted from the ESR intensity of the radicals and the radical formation rate, which is estimated from the day-by-day increase of the ESR intensity at various temperatures. For dating purposes, the unpaired electrons of interest are those created as a result of radiation damage. Any object is subject to radiation, but not all forms of radiation damage are sufficiently stable to serve as chronological markers. The ambiguity in the temperature assessment and variation in the environment are the major source of errors in this chemical dating as in all dating methods based on chemical reaction.

It has been shown (Ikeya & Miki, 1985) a correlation between the age and the ESR signal intensity of iron-complexes in organic materials which contain hemoproteins. Various physical and chemical processes may induce changes in the valency and/or the ligand fields of transition-metals. Thus, ESR dating may be used to identify the degradation products in dried blood and to estimate the time after the beginning of these processes.

We have applied ESR spectroscopic method in order to estimate the time of blood extravasation. This method may be of importance as a practical test in forensic medicine.

MATERIALS AND METHODS

The investigations were performed in vitro using the blood of six healthy donors (male, age=26-38 years). Blood was drawn from the arm vein, without adding an anticoagulant. Next, the specimens were spread on Petri dishe's and dried in the dark at room temperature. After the blood had dried, thin films of the dried blood were divided into three pieces (N1, N2, N3). Before the measurement, the material was prepared in the special way (care must be taken to secure the same stable conditions each time): pulverisation of the dried tissue in small porcelain evaporators with accessible air at room temperature, after 1 hour samples (20 mg of weight pulverised tissues in quartz tubes 5 mm diameter) were placed into the resonance chamber of ESR spectrometer, type SE/X 2544 Radiopan.

ESR spectra were recorded as a function of ageing time about 20(N1), 60(N2), and 90(N3) hours after extravasation. ESR measurements were carried out in the following conditions: 9.45 GHz frequency, 3 mW power, 100 kHz / 0.2 mT modulation, time constant 1s, scan 20 mT/4 min, room temperature. DPPH was employed as a standard to determine the g coefficient and the concentration of unpaired spins.

RESULTS

Figure 1 shows the ESR spectrum of coagulated human blood (Ikeya, 1993). ESR spectrum of the coagulated blood consists of the three signals at g = 6, g = 4,3 and the strongest at g = 2,005. The signal at g = 6 is associated with oxidized heme-iron (Fe^{3+}) of high spin states (S = 5/2) in hemoglobin. The signal at g = 4,3 is also associated with high spin Fe^{3+} (S = 5/2) in a strong tetrahedral field, but is due to non-heme iron, presumably formed by the degradation of hemoglobin. The two first signals are very weak and wide, therefore are not valuable for analysis. The sharp signal at g = 2,005, according us (Gonet, 1994) is due to ascorbyl

Data were analysed using Student t-test for comparison of the means of independent samples. The differences at the 5% level ($P<0,05$) were considered significant.

DISCUSSION

Miki et al. (Miki *et al.*, 1987; Miki *et al.*, 1988) has presented possible method of estimation the age of an organic material which contains hemoproteins using ESR signals at g = 6 and 4.3. According the mentioned paper the authors neglected the sharp signal at g = 2,005 explaining only that it may be attributed to peroxy radicals from the g value and the spectral shape or it is due to radicals. The intensity of signals they were investigated strongly depends on temperature. The biggest differences they were noted at temperature 70°C and 90° C. For a specimen with an age less than a few tens of days the "wait and see" method was proposed, because more accurate ages can be obtained. However, it should be noted that ambiguity of environmental conditions might cause large errors in determined ages of the specimen.

As to sharp (singlet) signal at g = 2,005 it was proved by Gonet (Gonet, 1994) - this signal is due to ascorbyl

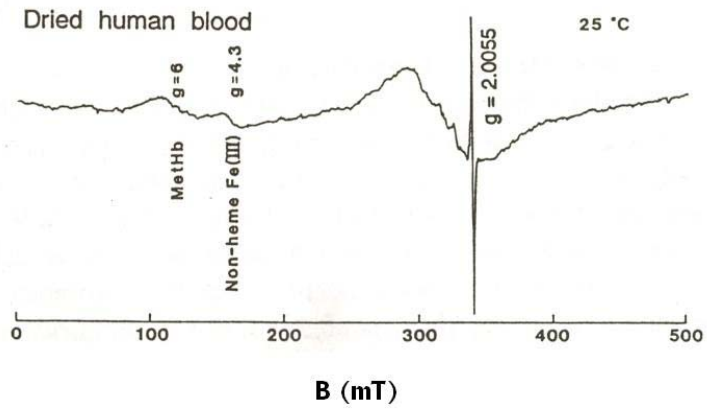

Fig. 1. The ESR spectrum of coagulated human blood (Ikeya, 1993).

radical $\dot{A}scH$ in vitro and it corresponds to the level of vitamin C in vivo. We have analysed this signal in our experiments. Figure 2 shows an example of recorded ESR signals for dried blood.

The intensities of the recorded ESR signals were varying in time. Changes of the mean ESR intensity (shape and ΔB_{pp} are the same for all signals) for the signals of radicals at g = 2,005 were described in the Table 1.

Comparisons between the obtained data are depicted in the Table 2.

radical $\dot{A}scH$ in vitro and it corresponds to the level of vitamin C in vivo. In this paper a new, free radical method of determining L-ascorbic acid content in tissues of laboratory animals has been presented. What is possible mechanism of this signal ? It has been shown in numerous studies (Cimbolaityte, Naktinis, Cerniauskiene, Vanin, 1982; Lohman, Holtz, 1984; Neubacher, 1984) that ESR signal in lyophilized tissues (blood, liver, lungs) measured with exposure to air in vitro originates from the ascorbyl free radical ($\dot{A}scH$).

It is produced during the interaction of $AscH^-$ ascorbic acid (ionized form) with the membrane matrices after lyophilization and exposure to ambient air. It is known that ESR signals (doublet) in vitro correspond to the $Asc^{\bullet -}$ ascorbate free radical (Buettner, Jurkiewicz, 1995). Regarding that the signals at g = 4,3 and g = 6 are of small amplitude comparing with the signal associated with ascorbyl radical at g = 2, 005 it is no clear why these signals were taken into account.

In our study, the recorded ESR signals of the blood after extravasation were varied in time but we did not notice the relationship presented by Miki et al. (Miki *et al.*, 1988). The differences between the samples are no significant statistically (P>0,05).

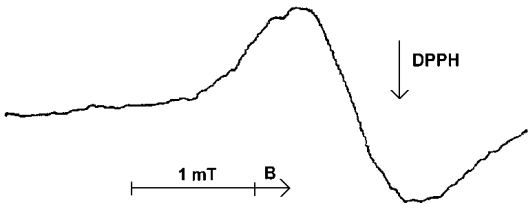

Fig.2. The example of ESR spectra recorded for dried blood.

Table 1. Mean value $\overline{X}$ (n=6) of ESR intensity signal (expressed in arbitrary units) of the dry blood as a function of time.

Time after extravasation		ESR signal intensity
20 hours (N1)	$\overline{X} \pm SD$	26,2 ± 2,9
60 hours (N2)	$\overline{X} \pm SD$	30,8 ± 5,6
90 hours (N3)	$\overline{X} \pm SD$	25,0 ± 2,8

Table 2. Comparison between the mean value of ESR signal intensity among samples of blood extravasating in time.

	Mean value of intensity for different times	Comparison of mean values of different times	t-test	Probability level
$\overline{X}_{20}$	26,2	$\overline{X}_{20 \text{ versus }} \overline{X}_{60}$	1,786	P>0,05
$\overline{X}_{60}$	30,8	$\overline{X}_{60 \text{ versus }} \overline{X}_{90}$	2,269	P>0,05
$\overline{X}_{90}$	25,0	$\overline{X}_{90 \text{ versus }} \overline{X}_{20}$	0,729	P>0,05

The obtained results also show a big individual variability among samples and ESR signal dependence on the condition in which the samples were stored (temperature, humidity) and small ESR signal differences in time.

CONCLUSION

Our present study shows that the investigating ESR spectroscopic method is not valuable to be used in forensic medicine application. Choosing the sharp signal of ascorbyl radical at g = 2,005 we were supposed of practical possibility of application this method in dating blood after extravasation. Additionally the proposed method was performed in room temperature making its more interesting from the practical point of view. Unfortunately, our experiments gave no confirmation of assumed concept.

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