LONG-LIVED RADIATION-INDUCED ELECTRON SPIN RESONANCES IN AN AQUEOUS BIOLOGICAL SYSTEM

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Radiation-induced unpaired electron species have been considered to be very reactive, reacting and becoming paired in microseconds (Bacq and Alexander, 1961) in active biological systems at physiological temperatures. Recently, long-lived unpaired electron species have been demonstrated after irradiation under physiological conditions in bones and hair of rats (Swartz, 1965). Unpublished studies in our laboratory indicate that similar long-lived resonances occur in irradiated bones and teeth of other species including humans and in irradiated insects. Several authors have noted similar phenomena in irradiated seeds (Lofroth, Ehrenberg and Ehrenberg, 1964; Cook, 1963). However, in bone, hair, teeth, insects and seeds there are "dry" areas where the unpaired resonances might be located. In this communication we report the existence of radiation-induced long-lived unapired electron species in a biological system without such potentially dry areas: normal human blood.

METHODS

The blood was collected in heparinized syringes and allowed to settle in the syringe at $5^{o}C$. The supernatant plasma and WBC layers were then removed leaving a concentrate of RBC's in a small amount of plasma, final volume being 50% of the whole blood volume. Irradiations were done in Pyrex test tubes in a rotating carrier in a Co^{60} gamma source at a dose rate of 1200 rads/min. Dose-response curves were obtained by removing aliquots periodically from a sample in the irradiator, with controls taken simultaneously

from the other half of the sample which was kept outside of the irradiator. The aliquots were placed into calibrated quartz electron spin resonance (ESR) tubes and cooled to 77°K. The decay of the ESR spectrum was observed by taking aliquots from a sample at intervals following its irradiation and also by following the same aliquot over a period of hours.

The unpaired electron species were measured with a Varian V-4500 ESR spectrometer as described previously (Swartz, 1965). All spectra were obtained at 77°K. Relative numbers of spins were obtained by measurements of derivative peak heights.

RESULTS

Figure 1 shows the spectra obtained from control and irradiated blood

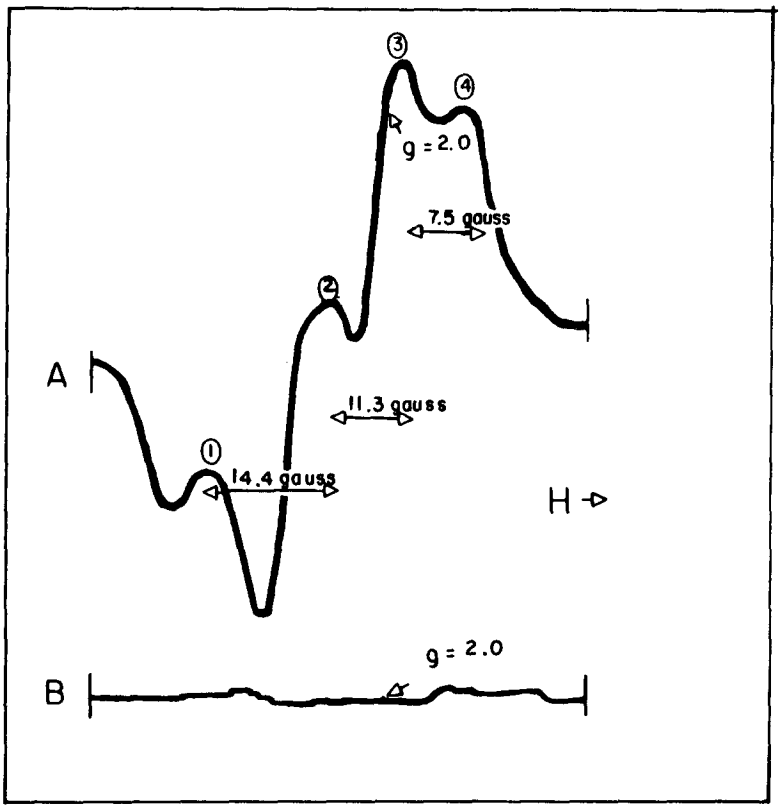

Figure 1. ESR spectra (1st derivative) of concentrated red blood cells A) irradiated at 293°K B) control, at the same spectrometer settings. The double headed arrows indicate the measured separations of peaks of the derivative curve. The position of the resonance of the free electron is noted as g = 2.0.

from the same donor. The hyperfine structure of the spectrum was quite reproducible from sample to sample and at doses from 30 to 480 kilorads (KR). The plasma was found to have no detectable ESR signal following doses as high as 2 megarads.

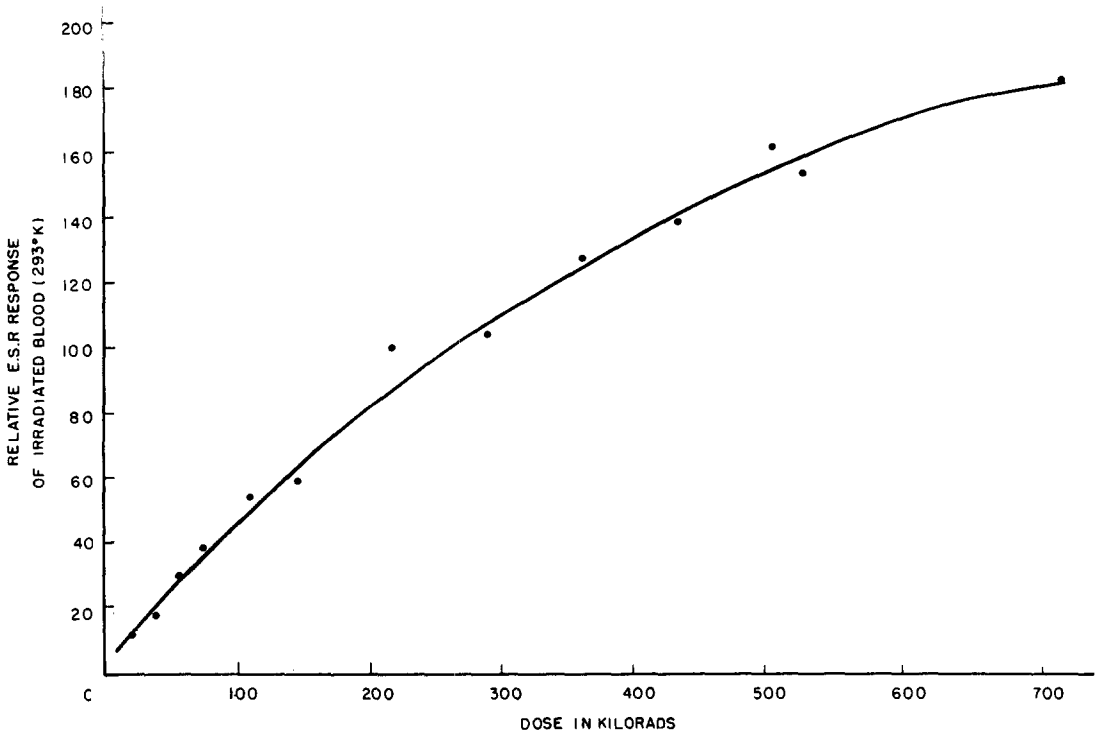

Figure 2. Dose response curve of concentrated red blood cells in plasma irradiated at 293°K.

Figure 2 demonstrates the dose response characteristics of the irradiated blood. An apparent saturation phenomena is noted at the higher radiation doses, similar to many published dose-response curves of radiation dose vs. ESR signal intensity.

No change was noted, qualitatively or quantitatively, in the ESR spectrum of irradiated blood kept at 293°K following irradiation for intervals from 14 seconds to 6 hours and then frozen to 77°K. (Table I)

TABLE I. Signal Decay in Irradiated Blood (72 KR) at 293°K

*Time	Relative Intensity	*Time	Relative Intensity
Unirradiated	2	30 Min.	60
14 Sec.	65	60 Min.	63
45 Sec.	64	6 Hrs.	65
10 Min.	66	**Unirradiated	2

*Interval between end of irradiation and freezing to 77°K
**Unirradiated sample held 6 hours at 293°K

DISCUSSION

While long-lived radiation-induced electron spin resonances in many dried biological materials, including hemoglobin, have been described previously (Gordy and Rexroad, 1961), the observation of persistent radiation-induced unpaired electrons at room temperature in an aqueous biological solution (blood) appears to be a new finding. This finding is of importance to basic radiobiological theory, because these resonances may represent significant energy available to cause damage well after the radiation period has ended. The existence of these unpaired species further suggests the possibility of shorter-lived species (but still existing much longer than the traditional microsecond lifetimes) which could not be determined by the methods employed in this study.

The site of the resonances appears to be in the red blood cells, inasmuch as the plasma fraction showed no ESR signals even after very high doses of radiation. The exact site within the red blood cell has not been determined. The presence of the fixed hyperfine structure indicates a specific site for the unpaired electrons. Small changes in the relative peak heights of peaks 3 and 4 with dose and microwave power as well as the asymmetrical separation of all of the peaks indicates that the observed hyperfine structure may be made up of at least two components. More evidence is needed to attempt to correlate the hyperfine splitting with a definite molecular structure.

Although the radiation doses used in this *in vitro* study were quite

high it is very possible that these ESR signals could be used as part of an *in vivo* dosimetry program for radiation accidents. We have observed similar resonances in *in vivo* irradiated blood in rats. The possibility of observing these resonances at lower radiation doses is based on three considerations.

1) A relatively high signal/noise ratio was observed at the doses used in this experiment (greater than 10/1 at 40 KR), so that the lower limit of detection with the present techniques has not been reached.

2) Using one of the "signal averaging" devices, a further increase of sensitivity of 10-100 times would be probable.

3) The molecules or the portion of the cell containing the unpaired electrons might be isolated and concentrated.

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