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Electron paramagnetic resonance imaging

Imágenes con resonancia paramagnética electrónica

LAWRENCE J. BERLINER

The Ohio State University, Department of Chemistry,
Columbus, Ohio 43210, USA

The history of *in vivo* ESR has been fraught with many problems involving artifacts, especially where tissues and organs were excised from the body for study. A second major problem has been the previous use of X-band (9.5 GHz) frequencies, where substantial degrees of dielectric loss and heating are experienced. In fact, when one analyzes the typical X-band microwave cavity for the magnetic H_1 field distribution, only a very thin sheet (*ca.* 1 mm) of pure H_1 field is available. The advent of the use of traveling wave helices by Feldman *et al.* (1978) was the first attempt at applying ESR to a living animal. In particular, this group implanted helices in the regenerated liver of a rat and then observed the ESR spectrum of nitroxides as they moved through the circulation into the liver. Unfortunately, the nitroxide signal decayed quite rapidly due to the many reductive/destructive reactions *in vivo*, particularly in the liver but also the animals suffered some form of discomfort due to the microwave heating at this high frequency.

The major advance in applying ESR to living systems was the (now obvious) choice of working at lower frequencies; in particular, lower than that of microwave ovens (2.45 GHz). The first reported *in vivo* application in this frequency L-band range (1-2 GHz) was from the group of James Hyde, Medical College of Wisconsin, Milwaukee, where the employment of loop-gap resonators (Froncisz and Hyde, 1982) was employed to observe melanin free radicals in a tumour in a live rat as well as nitroxides injected into an animal (Lukiewicz and Lukiewicz, 1984, 1985). A few years later, Nishikawa *et al.* (1985) introduced the flat-loop surface coil resonator in the L-band range. This simple device, which is depicted in Fig. 1, offered the advantage of having a very small surface area (*ca.* 8 mm diameter), easy accessibility to many parts of the body, and easily adapted to imaging experiments in the presence of magnetic field gradients. In the first reported biological application of *in vivo* ESR, Berliner and Fujii (1985) examined capillary flow in plant stems, such as *Apium graveolens*, where they observed the ESR spectra of two individual capillaries as aqueous nitroxides (*e.g.* 2,2,6,6-tetramethylpiperidine-oxyl) flowed through the capillaries (Fig. 2). They were also able to observe the eventual decay and damage of the cellular membrane structure between the capillaries as the nitroxide underwent lateral motion from one capillary area to the next (see Fig. 3). The system utilized gradient strengths of the order of 20-40 G/cm, which is about 10 times larger than that required for NMR imaging. A distinct difference between ESR and NMR is the natural linewidths of the free radical species. For example, a nitroxide tumbling rapidly has an intrinsic linewidth of 0.5-1.5 G, which is a larger field range than in a entire proton NMR spectrum. In terms of resolving separated lines in the presence of a field gradient, one

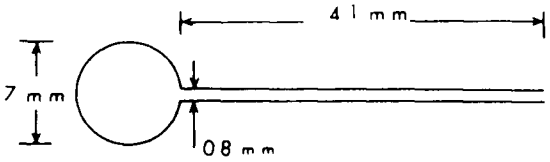

Fig. 1: The single turn, flat loop coil. Dimensions of the loop were 7 mm O.D., 41 mm length, and 0.8 mm gap. The loop was mad of 0.7 mm (O.D.) insulated copper wire. (From L.J. Berliner and H. Fujii (1985) with permission).

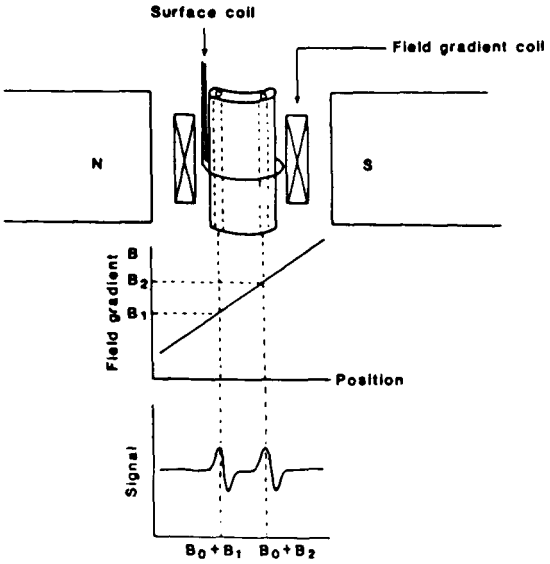

Fig. 2: Diagram of the one-dimensional EPR imaging experiment of a celery sample soaked in 1 mM TEMPOL. The two capillaries were aligned along the z-field gradient direction. B_0 is the applied magnetic field and B_1 and B_2 represent the static field contribution due to the field gradient at capillary positions 1 and 2, respectively. (From Berliner and Fujii, 1985, with permission).

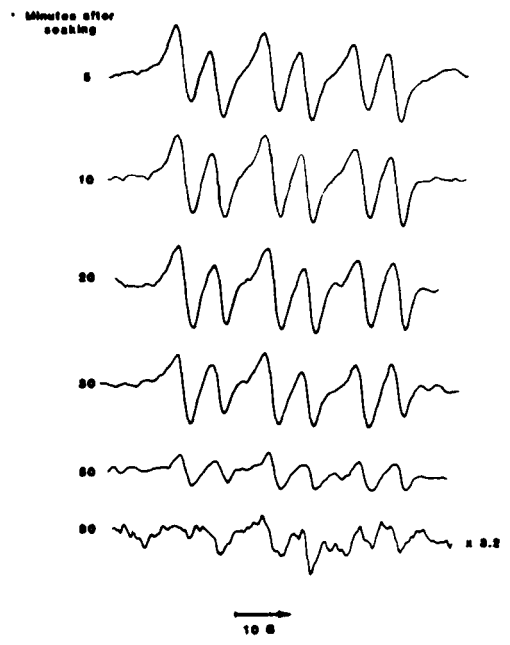

Fig. 3: Time dependence of the one-dimensional EPR spectrum of celery soaked in 1 mM TEMPOL. The z-gradient was 24.3 G/cm. Microwave frequency was 1.83 GHz; applied magnetic field, 0.0660 tesla; applied microwave power, 100 mW; and modulation frequency, 100 kHz. The two capillaries were 2.6 mm apart (center to center). After 50 minutes image resolution deteriorated with capillary breakdown and lateral diffusion of the nitroxide imaging agent. (From Berliner and Fujii, 1985, with permission).

must therefore resort to much larger field gradients than in the NMR case in order to resolve spectral bundles from individual points in an object. Recall that NMR lines are of the order of a few Hertz in linewidth, which corresponds to mG in magnetic field units.

Processes such as diffusion in biological systems are also of interest. Berliner and Fujii (1986) studied the diffusion of an aqueous nitroxide solution through a polyacrylamide rod as a model for biological tissue. They were able to image the nitroxide distribution with time as it diffused from the circumference towards the center (Fig. 4), or in another experiment from the center outwards (Fig. 5). The spectra showed that the nitroxide itself was not interacting specifically with the gel matrix, thus the spin concentration distribution reflected that of the solvent. The small molecule nitroxide diffusion constant was calculable from this data, giving a diffusion constant of $3.7 \pm 0.7 \times 10^{-6}$ cm^2/s which correlated exceptionally well with the known diffusion constants for molecules of this size. In another example of solvent diffusion into a matrix, Berliner *et al.* (1986) also examined the solvent induced swelling of synthetic organic polymers. Here the solvent interaction results in an expansion of the molded polymer matrix, which in some cases results in a stress or fracturing of the polymer matrix to yield a "crack", such as shown in Figs. 6A through D.

THE FUTURE

Some applications, particulary of relevance to biomedical problems are outlined below.

a) Biological radicals-these may encompass free radical centers in tumors, aging tissue and melanomas. Two limitations in EPR imaging are depth sensitivity and absolute sensitivity. Thus, the principal target will most likely be subdermal melanin containing tumors, such as found in skin cancer.

b) Extraneously introduced nitroxide spin labels - this has been the most promising

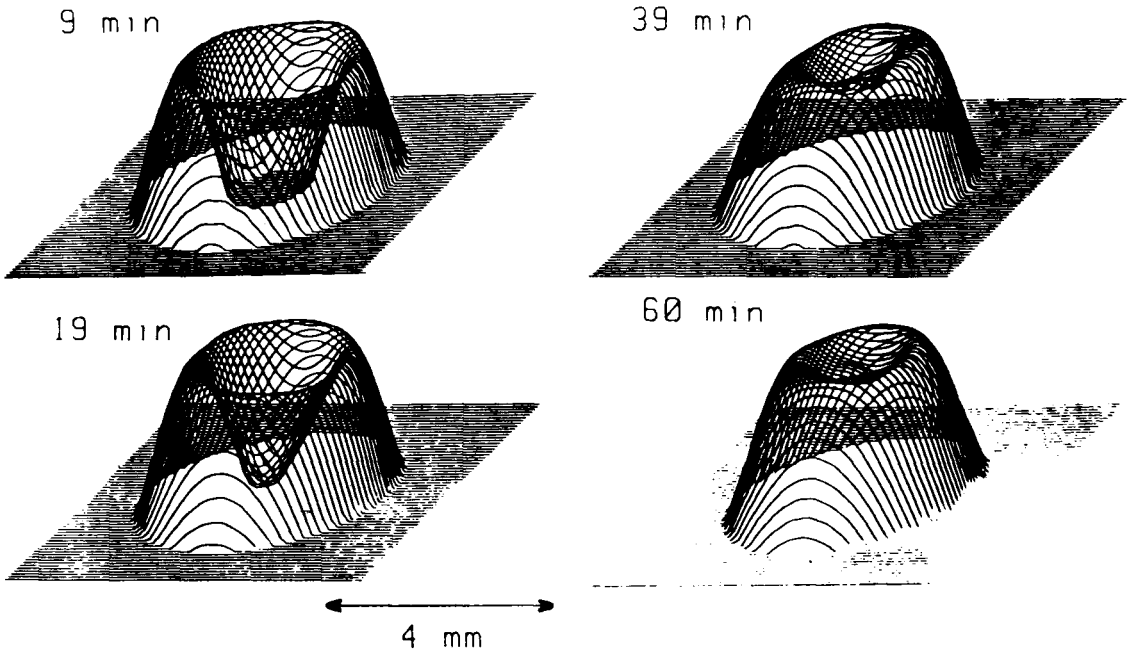

Fig. 4: Cross-sectional EPR contour map images (65 x 65) of 5 mM TEMPOL in Krebs solution diffusing radially into a 4.5 mm diameter 9% polyacrylamide gel rod. The gel was removed from the nitroxide solution after 5 min. and then EPR spectral projections measured at the total times indicated by rotating the sample in the z-field gradient. The contour levels represent spin density. The frequency was 1.5 GHz (580 G applied field), 0.05 to 0.1 G modulation amplitude. The field gradient was 2.0 G/mm. All other conditions were as in Fig. 3. From Berliner and Fujii, 1986, with permission.

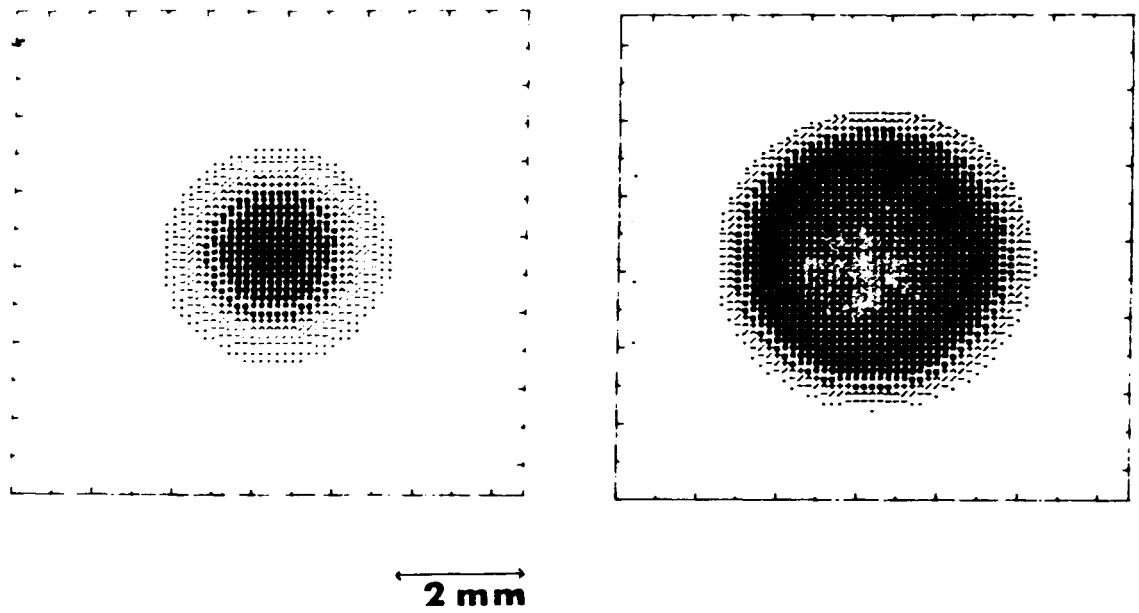

Fig. 5: Two dimensional cross sectional image of a 5 mM TEMPOL solution diffusing radially from the center of a polyacrylamide gel rod after 10 min. (left) and 40 min. (right). The experiment was started by placing a 5 mM TEMPOL solution into a 0.2 mm O.D. hole in the center of the gel rod. All other conditions were as in Fig. 4. From Berliner and Fujii, 1986, with permission.

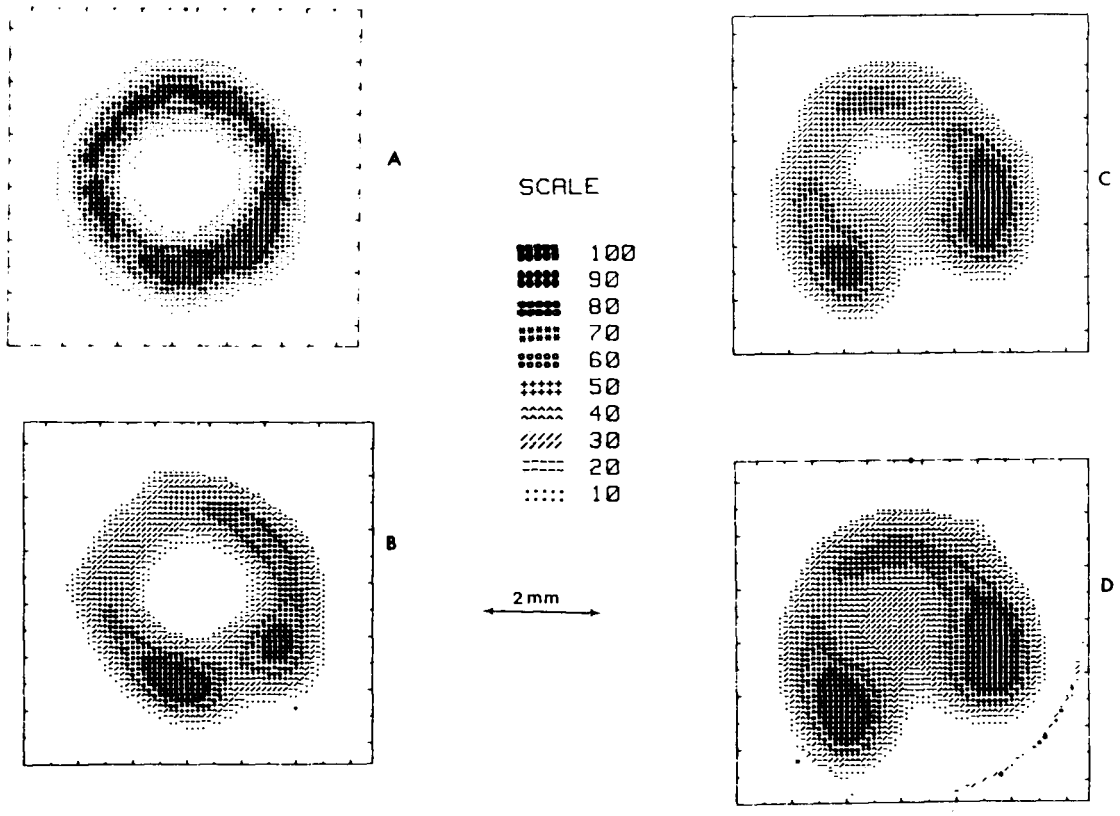

Fig. 6: Time dependent EPR images of polycarbonate (5.8 mm I.D. x 60 mm length rod) after solvent swelling in 5-10 mM TEMPOL (10% water/90% DMF) for the period shown: A, 12 hrs., B, 24 hrs., C, 72 hrs., D, 96 hrs. From Berliner, Wan and Fujii, 1986, with permission.

approach to date, as evidenced in the earlier examples in this paper. These range from the introduction of relatively non-specific spin labels into the circulation to specific spin labels or spin probes, which are targeted for particular proteins, membranes or cells (*i.e.* "spin labeled pharmaceuticals"). These latter approaches however, require substantial *in vitro* testing first to assess feasibility. On the other hand, water soluble labels, which reflect the solvent distribution have already been applied to imaging vascularization in a melanoma tumor in a mouse (Berliner *et al.*, 1987).

c) Spin traps - these are diamagnetic nitrones which form stable paramagnetic nitroxide adducts with other (biological) free radicals. These have the further advantage that "transient free radicals" products may be accumulated with time to yield detectable concentration of paramagnetic material.

While the breadth and versatility of NMR imaging cannot be dupplicated by EPR as a modality, the latter technique will have unique advantages for certain specific applications which NMR and other diagnostic techniques cannot meet.

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