JOURNAL OF MAGNETIC RESONANCE **84,** 431–437 (1989)

Field-Cycled Proton–Electron Double-Resonance Imaging of Free Radicals in Large Aqueous Samples

DAVID J. LURIE, JAMES M. S. HUTCHISON, LAWRENCE H. BELL,
IAN NICHOLSON, DAVID M. BUSSELL, AND JOHN R. MALLARD

*Department of Bio-Medical Physics and Bio-Engineering, University of Aberdeen,
Foresterhill, Aberdeen AB9 2ZD, United Kingdom*

Received May 3, 1989

We have recently published a new method of imaging free radicals in aqueous solutions called *p*roton–*e*lectron *d*ouble-*r*esonance *i*maging (PEDRI) (*1*). In this technique a conventional proton NMR image is collected while the EPR resonance of a free radical solute is irradiated. If the EPR irradiation has sufficient power, the NMR signal from those protons being relaxed by the paramagnetic solute is enhanced, and the parts of the sample containing free radical exhibit greater intensity in the final image. Unlike EPR imaging (*2*) the sample size in PEDRI is not constrained by magnetic field gradient requirements. In this Communication we present the first results of an extension of PEDRI which uses magnetic field cycling, allowing larger samples to be imaged with lower levels of applied radiofrequency power.

PEDRI is an imaging version of a dynamic nuclear polarization experiment (*3*–*5*). The enhancement of the NMR signal upon irradiation of the EPR of the solute may be written empirically as

$$E = \frac{A_z}{A_0}, \qquad [1]$$

where A_z and A_0 are the NMR signals with and without EPR irradiation. Assuming that the main relaxation mechanism for protons is a dipolar interaction with the free radical's unpaired electron, the enhancement is given by the relationship

$$\frac{1}{1-E} = \frac{2\gamma_P}{|\gamma_S|}\left\{1 + \frac{1}{\gamma_S^2 B_2^2 \tau_1 \tau_2}\right\}, \qquad [2]$$

where γ_S and γ_P are the electron and proton gyromagnetic ratios, B_2 is the EPR irradiation RF magnetic field in the rotating frame, and τ_1 and τ_2 are the electron relaxation times. E is frequently negative, indicating that the NMR signal changes phase by 180° upon irradiation of the EPR resonance, while its magnitude changes by the factor $|E|$.

With a given sample, a particular RF magnetic field strength B_2 must be applied to achieve a certain enhancement. We may further write

0022-2364/89 $3.00

$$B_2^2 \propto P, \tag{3}$$

where P is the power of the applied EPR irradiation. Assuming a constant sample conductivity, the power is related to the EPR frequency ν_2 and the volume of the EPR resonator V as

$$P \propto \nu_2^2 V \tag{4}$$

or

$$P \propto B_0^2 V, \tag{5}$$

where B_0 is the static magnetic field strength.

If we impose a requirement for a certain level of enhancement with a particular type of sample, the maximum volume of the sample is constrained by the power available for the EPR irradiation (provided RF penetration into the sample is not a limiting factor). If biological samples are to be imaged, or if PEDRI is to be used *in vivo*, it is desirable to keep the power per unit volume as low as possible to avoid excessive sample heating. Initial PEDRI experiments were performed using a static magnetic field of 0.04 T, giving an EPR frequency of 1123 MHz (*1*). Using a sample of 2 m*M* TEMPOL free radical solution (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) (Aldrich Chemical Co.) an enhancement of $E = -8$ was obtained with a resonator volume of 1.5 ml by applying a power of 0.9 W. Assuming a good filling factor, the applied power per unit mass of the sample was approximately 600 W/kg, too high for experiments on biological samples.

Equations [2] to [5] indicate that a large decrease in applied power per unit volume can be achieved by reducing B_0. A 4-fold reduction in B_0, for example, will yield a 16-fold decrease in P/V. Provided B_2 remains constant, the enhancement level will be maintained at the lower field strength. If the irradiation power is limited by the available hardware, the volume of the sample can thus be increased by a factor of 16 in this case.

The price which must inevitably be paid for decreasing B_0 is a degradation of the image signal-to-noise ratio. Assuming a $B_0^{3/2}$ dependence, a fourfold reduction in B_0 would decrease the SNR by a factor of eight. While signal averaging can be used to recover SNR, scan times may become unacceptably long if B_0 is reduced too far. Taking the figure of 600 W/kg from our 0.04 T PEDRI experiments, Eq. [5] indicates that a reduction of the static field to 0.0025 T should bring the applied power down to less than 2.5 W/kg, an acceptable level for biological experiments. The SNR, however, would be reduced by a factor of approximately 64, requiring 4096 averages to restore image quality. Fortunately the conflicting requirements on B_0, namely the need for a low value to reduce the applied RF power and a high value to maintain SNR, are not mutually exclusive if the powerful technique of magnetic field-cycling is used in conjunction with PEDRI.

Field-cycling has been used for a number of years to study relaxation phenomena at very low magnetic field strengths (*6*). A field-cycling (FC) experiment includes three distinct periods during each of which the static field B_0 has a different value: polarization at B_0^P (high field), evolution at B_0^E (low field), and detection at B_0^D (intermediate field). The nuclear magnetization is allowed to build up during the polarization period which usually lasts longer than the NMR T_1 of the sample. Relaxation occurs during the evolution period which lasts approximately as long as T_1. Finally B_0 is switched to an intermediate level and the NMR signal is detected by applying a 90° pulse in the usual way. The field is switched between levels in a time much less than T_1. The gain in sensitivity of an FC experiment over a conventional NMR experiment performed at a constant field of B_0^E ranges from $B_0^P\, B_0^D / B_0^{E^2}$, if the evolution period t_E is short compared with T_1, to B_0^D / B_0^E, if t_E is long compared with T_1 (*6*).

In field-cycled PEDRI (FC-PEDRI) the polarization and detection magnetic fields B_0^P and B_0^D are chosen to give an acceptable SNR. The EPR irradiation takes place during the evolution period with B_0^E depending on the desired EPR irradiation frequency, which can be chosen independently of B_0^P and B_0^D in order to achieve an acceptable power per unit volume figure.

We have implemented FC-PEDRI on our homebuilt whole-body proton NMR imager. This instrument has a four-coil, vertical-field, side-access, resistive magnet which normally operates at 0.04 T, giving an NMR frequency of 1.7 MHz (*7*). For this work we have modified the apparatus to operate at 0.01 T; this field was used for both polarization and detection, giving an NMR frequency of 425 kHz. Circuitry was constructed to down-convert the RF pulses from 1.7 MHz to 425 kHz and to up-convert the 425 kHz NMR signals to 1.7 MHz. This approach allowed us to use the original transmit and receive electronics without readjustment when the imager was used at the lower field strength. A miniature split-solenoid RF coil (diameter 85 mm) was used for transmission and reception at 425 kHz. It was connected to the RF transmitter and receiver via a passive transmit/receive switch.

Field-cycling was achieved using a field compensation technique (*6*). We did not attempt to switch the current in the imager's magnet since this would have placed unacceptable demands on the magnetic power supply and coil insulation due to the large inductance of the whole-body magnet. Instead, the field from the main magnet was held constant at 0.01 T and the current in a much smaller secondary magnet coil situated inside the imager's gradient coil tube was switched on and off. The field produced by the secondary coil was arranged to be in opposition to that from the main magnet so that when the secondary coil was driven the net field at the center of the coils was reduced. The secondary coil was an air-cored, water-cooled, two-coil Helmholtz design with an internal diameter of 22 cm and an inductance of 24 mH. Each coil of the Helmholtz pair had 188 turns of 2.5 mm diameter copper wire and the two coils were connected in series, requiring a current of 3.67 A to produce a field of 0.005 T at the center of the magnet. A constant-current power supply (Hewlett–Packard 6269B) was used for the secondary coil, and the current was switched under control from the imager's pulse programmer using power MOSFET transistors. The switching time of the secondary coil was less than 10 ms.

The field compensation approach to field-cycling has the advantage that only the main magnet is driven during the detection period, when the greatest demands are placed on the spatial homogeneity and temporal stability of the magnetic field at the sample. In FC-PEDRI the homogeneity of the magnetic field during the evolution period need only be good enough to irradiate the EPR line of interest throughout the sample: in these experiments the linewidth was more than 4 MHz at an EPR fre-

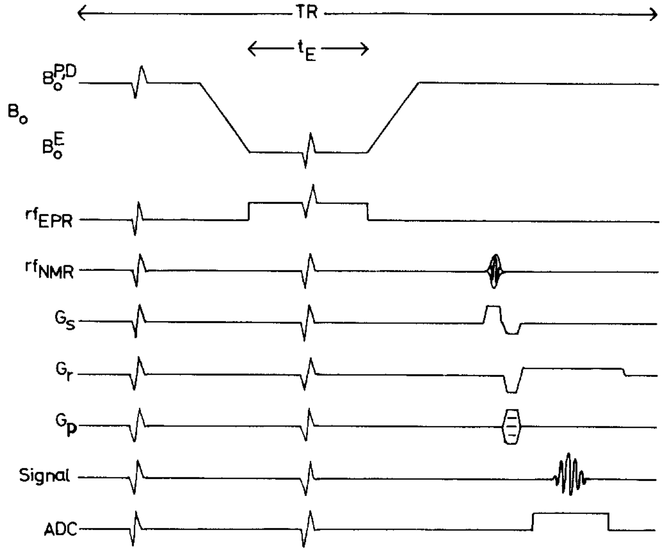

FIG. 1. FC-PEDRI pulse sequence.

quency of 160 MHz so that a variation of B_0^E of more than ±1% over the sample volume could be tolerated, while the calculated homogeneity of the Helmholtz pair was better than ±1000 ppm over the sample volume. The disadvantage of field compensation is the inevitable interaction between the primary and the secondary magnet coils caused by their close proximity: in our apparatus this gave rise to an instability of the primary magnetic field when the current in the secondary coil was switched. The effect became more serious as the primary and secondary field strengths were increased, so that we were restricted to an upper limit of 0.01 T for B_0^P and B_0^D.

A synthesized frequency generator (Farnell Instruments, UK) provided the EPR excitation signal which was amplified by a 1 W broadband amplifier (Farnell Instruments) before being applied to the sample by a single-turn loop resonator situated inside the NMR coil. FC-PEDRI experiments were performed on a phantom containing a 2 m*M* aqueous solution of the nitroxide free radical TEMPOL at room temperature. The EPR irradiation frequency was fixed at 160 MHz and FC-PEDRI experiments were carried out by irradiating one of the characteristic EPR lines of the nitroxide triplet which were observed at B_0^E values of 0.0037, 0.0051, and 0.0072 T; the intermediate resonance was used for most experiments. Non-field-cycled PEDRI experiments were also performed at a constant field of 0.01 T, in which case the EPR irradiation frequency of the intermediate resonance was 288 MHz; some of these experiments used a homebuilt 4 W RF amplifier for the EPR irradiation.

Figure 1 shows a typical FC-PEDRI pulse sequence. For simplicity, we have made the polarization and detection magnetic field strengths equal in our experiments ($B_0^P = B_0^D = 0.01$ T). Table 1 summarizes the observed enhancements obtained with 2 m*M* TEMPOL samples in the PEDRI experiments at 0.04 and 0.01 T and in the FC-PEDRI experiments with $B_0^E = 0.0051$ T. Also listed are the power per unit mass figures for enhancements of $E = -5$ with aqueous samples. The variation of the applied power with EPR frequency agrees reasonably well with Eq. [4].

TABLE 1

Summary of PEDRI and FC-PEDRI Results

Field strength (T)	EPR frequency (MHz)	Applied power (W)	Observed enhancement E	Power required for $E = -5$ (W)	Volume of resonator (ml)	Power per unit mass of sample for $E = -5$[a] (W/kg)
0.04	1123	0.87	−7.6	0.60	1.5[b]	400
0.01	288	1.0	−8.9	0.58	36.0[c]	16.1
0.01	288	4.0	−7.1	2.91	129.0[d]	22.6
0.0051[e]	160	1.0	−7.1	0.72	129.0[d]	5.6

[a] Assuming 100% filling factor.

[b] Single-turn loop resonator; diameter 10 mm, length 20 mm.

[c] Single-turn loop resonator; diameter 42 mm, length 26 mm.

[d] Single-turn loop resonator; diameter 63 mm, length 40 mm.

[e] Field-cycled, with $t_E \gg T_1$.

Figure 2 shows FC-PEDRI images of a resolution phantom; a field-cycled non-PEDRI image is also shown for comparison. The center of the phantom consisted of five tubes of internal diameters 15, 9, 5, 4, and 3 mm filled with 2 mM TEMPOL solution. These were enclosed in a cylindrical container of diameter 4 cm which was filled with water doped with copper sulfate to give the same T_1 as that of the free radical solution (650 ms at 2.5 MHz). Around the outside of the cylinder were attached 14 sample tubes with internal diameters of 8 mm, alternate tubes being filled with 2 mM TEMPOL solution or copper sulfate-doped water. The overall diameter of the phantom was 6 cm, about the size of a small rat. The three FC-PEDRI images were obtained using t_E values of 750, 1000, and 1500 ms with a T_R of 2000 ms, and the average observed enhancement factors were −4.0, −5.3, and −7.1, respectively. The instantaneous power level in the EPR irradiation was approximately 7 W/kg, while the average applied power ranged from 2.7 to 5.3 W/kg depending on the pulse sequence timing.

In FC-PEDRI the enhanced versus unenhanced image intensity ratio depends not only on the power of the EPR irradiation but also on the relative timing of the polarization and evolution intervals, and the values of B_0^P and B_0^D. At the beginning of the evolution period the size of the magnetization depends on the length of the polarization period compared with the sample's T_1 at B_0^P. During the evolution period the magnetization decays at a rate determined by the sample's T_1 at B_0^E. Meanwhile the magnetization in regions of the sample containing free radical increases, again at a rate depending on T_1, toward an equilibrium value which depends on the EPR irradiation power. The sequence timing parameters will therefore influence the detectability of the free radical and must be optimized for each FC-PEDRI experiment, particularly if very low concentrations of free radicals are being sought.

In conclusion we have shown that the sample volume in PEDRI is constrained by the power available for the EPR irradiation. Lowering the magnetic field strength reduces the power required to achieve a given enhancement level. FC-PEDRI allows the applied power to be reduced to levels which are acceptable for biological experi-

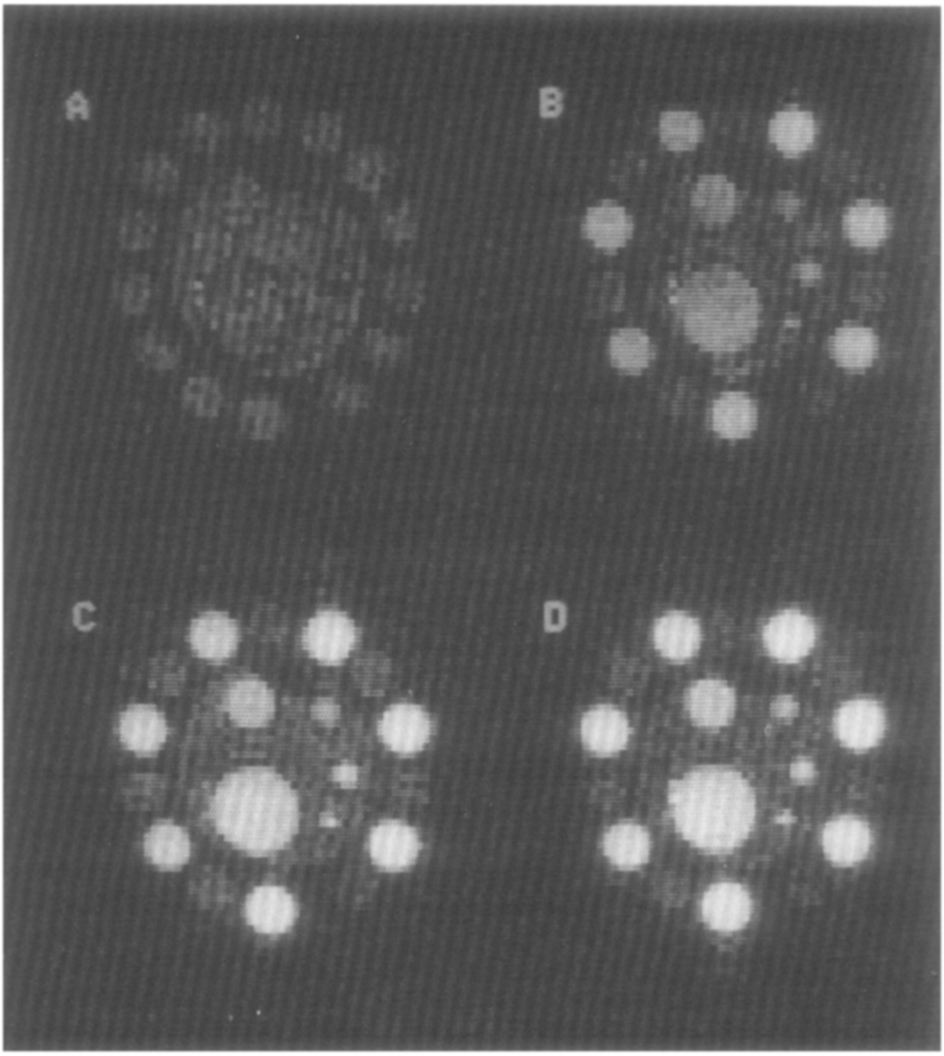

FIG. 2. Images of phantom containing 2 mM TEMPOL solution and copper sulfate-doped water. Images are 64 × 64 sections of original 128 × 128 images, which had 15 cm field of view, 15 mm slice thickness. All images are field-cycled, with $B_0^P = B_0^D = 0.01$ T, $B_0^E = 0.0051$ T; saturation-recovery NMR sequence with $T_R = 2000$ ms, four averages. Image A has $t_E = 1000$ ms, no EPR irradiation. Images B–D have EPR irradiation at 160 MHz, power 1 W. Image B has $t_E = 750$ ms; observed enhancement factor $E = -4.0$. Image C has $t_E = 1000$ ms; $E = -5.3$. Image D has $t_E = 1500$ ms; $E = -7.1$.

ments without compromising the SNR of the image. By scaling up the field-cycling apparatus it should be possible to image free radicals in larger experimental animals, providing a useful tool for biological and medical research. We believe that this is the first time that field-cycling has been used in an imaging experiment above the Earth's field strength. Field-cycled NMR imaging is itself likely to be of use in studying relaxation *in vivo* at extremely low magnetic fields.

ACKNOWLEDGMENTS

We thank Mr. E. Stevenson and Mr. D. Jack for constructing the secondary magnet coil. This work was funded by Aberdeen University and the Medical Research Council, which supported two of the authors (D.M.B. and L.H.B.). One of us (I.N.) was a recipient of an M.R.C. Research Studentship.

REFERENCES

1. D. J. LURIE, D. M. BUSSELL, L. H. BELL, AND J. R. MALLARD, *J. Magn. Reson.* **76,** 366 (1988).
2. K. OHNO, *Appl. Spectrosc. Rev.* **22,** 1 (1986).
3. K. H. HAUSSER AND D. STEHLIK, *in* "Advances in Magnetic Resonance" (J. S. Waugh, Ed.), Vol. 3, p. 79, Academic Press, New York, 1968.
4. R. A. DWEK, R. E. RICHARDS, AND D. TAYLOR, *Annu. Rev. NMR Spectrosc.* **2,** 293 (1969).
5. W. MULLER-WARMUTH AND K. MEISE-GRESCH, *in* "Advances in Magnetic Resonance" (J. S. Waugh, Ed.), Vol. 11, p.1, Academic Press, New York, 1983.
6. F. NOACK, *Prog. NMR Spectrosc.* **18,** 171 (1986).
7. J. M. S. HUTCHISON, W. A. EDELSTEIN, AND G. JOHNSON, *J. Phys. E: Sci. Instrum.* **13,** 947 (1980).