MAGNETIC RESONANCE IMAGING

Second Edition

Volume I

Clinical Principles

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A Survey of MRI Techniques

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$x = x_0$ (Fig. 3–8). This gradient modulates the resonance frequencies of all volume elements lying outside the nodal plane. The modulation inhibits the formation of a steady-state transverse magnetization and leads to destruction of the magnetization outside the plane $x = x_0$. Two additional time-varying gradients $g_y(t)$ and $g_z(t)$ are applied simultaneously along the other two axes with nodal planes at $y = y_0$ and $z = z_0$, respectively. When three incommensurate modulation frequencies are used, only the steady-state transverse magnetization of the sensitive point remains and determines the observed signal.

By moving the three nodal planes, it is possible to move the sensitive point systematically through the object. A point-by-point image is then obtained. This simple technique can produce good image quality. However, it is slow. In particular, it should be noticed that after each measured point the magnetization purposely destroyed must be allowed to recover before the next point can be measured. The data acquisition rate is limited to about 1 point per *T1* relaxation time.

Field Focusing NMR (FONAR) and Topical NMR

Another scheme for selecting a "sensitive point" has been suggested by Damadian.[17] In this technique, called FONAR, the static magnetic field is shaped in such a way that good homogeneity is obtained only in one single small volume. The homogeneous region is normally near a saddle point of the field (Fig. 3–9). Most of the object will give rise to a broad background signal, while the region around the saddle point gives a dominant contribution at one frequency. Additional contributions to the same frequency coming from the extended but narrow regions for $\Delta B_0 = 0$ may be negligible under suitable circumstances. The sensitive point is fixed in space, and it is necessary to move the object to record a full image.

Also part of original Damadian Patent

This scheme often does not lead to adequate spatial resolution for a high-resolution image, and it may be necessary to enhance selectivity by suitably shaped radio frequency fields. It is a useful method, however, for in vivo spectroscopy to acquire spectral information (chemical shifts) of a localized area within an object. This approach, known as topical NMR,[53] was the first technique for recording resolved NMR

(see Doc. 20a)

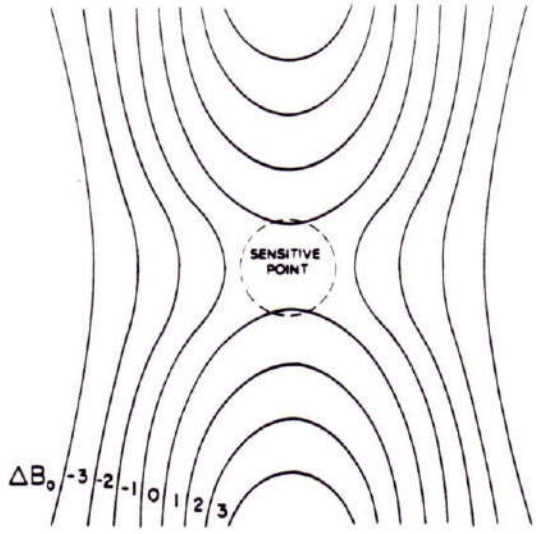

Figure 3–9. Shaping of the static magnetic field used in FONAR and topical NMR. Only the region near the saddle point is sufficiently homogeneous to give rise to strong narrow signals; the remainder of the object contributes only weak broad resonances. (Adapted from Ernst RR et al: Principles of Nuclear Magnetic Resonance in One and Two Dimensions. London, Oxford University Press, 1986.)

spectra of a localized organ in a living being. It allows the study of physiologic processes in a nondestructive and noninvasive manner. Particularly, ^{31}P NMR proved revealing for the measurement of metabolite concentrations and pH in living tissue.[53–56]

Although the requirements for spatial resolution are less severe for in vivo spectroscopy than for imaging, such a simple field-focusing technique is often inadequate for investigating smaller organs, and alternative approaches have been proposed for better localizing the area of observation.

Volume-Selective Pulse Sequences

The selective excitation of a specific volume element within an object is highly desirable in the context of in vivo spectroscopy. Although it is easy to selectively excite a single plane, the selection of a single volume element is more elaborate. It normally involves a three-step process whereby a point in the object is defined by the crossing of three orthogonal planes. This requires the intelligent combination of three "plane-selective" pulses.

Aue et al.[57,58] have described a clever technique for "volume-selective excitation" (Fig. 3–10). By the simultaneous application of a selective $-\pi/2$ and a nonselective $\pi/$

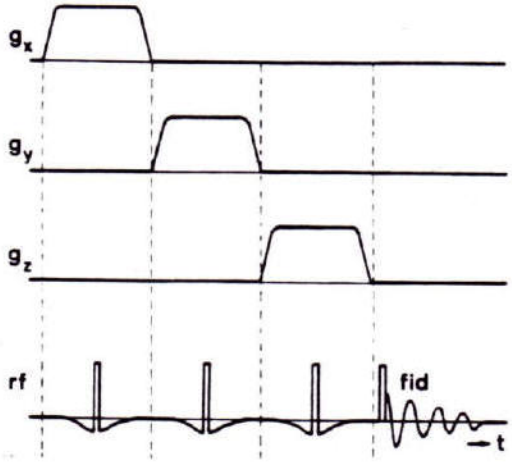

Figure 3–10. Sequence for volume-selective excitation.[57] Each of the three phases destroys the magnetization except within a single plane. In effect, all magnetization, except for the selected plane, is rotated into the *xy* plane by the combination of a selective and a nonselective $\pi/2$ pulse. *T2* processes lead to the decay of transverse magnetization. At the end, magnetization in a single volume element remains exclusively. It can be used for local spectroscopy by applying a last pulse.

could terminate activity even in the presence of large amounts of ambient but unbound GTP. Thus, the deactivator role of GTPase is not mediated via the cytoplasmic GTP depletion implied by previous work[1,2].

It is worthwhile to compare the mechanism proposed here with that of hormonally activated adenylate cyclase, also known to require GTP cofactor[11]. Both systems activate effectively in GMP-PNP[12] and neither can deactivate without the action of a membrane-bound GTPase[13]. As in RDM, however, it should not be possible for GTPase to deactivate the cyclase mechanism alone. The hormone–receptor complex needs to be deactivated as well. Reduction of blood hormone concentration alone might in some cases lead to adequate dissociation of the hormone–receptor complex to complete the deactivation on a time scale appropriate to needs for control. However, to achieve more rapid regulation, a hormone–receptor might be quenched by phosphorylation in a process similar to that which quenches the capacity of rhodopsin to activate RDM phosphodiesterase.

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Localization of metabolites in animals using ^{31}P topical magnetic resonance

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High-resolution phosphorous (^{31}P)-NMR spectra of biological molecules provide detailed information about the metabolism of living systems[1–4]. Although the NMR method is non-destructive, all studies so far, with two exceptions[5,6], have been carried out on excised, perfused organs and tissues or have required some form of surgery[2] for *in situ* measurements. The use of 'surface' radiofrequency coils[5] does not require surgery but is best suited for tissues close to the surface of the animals. We describe here 'topical magnetic resonance'—a new, non-surgical method for acquiring ^{31}P-NMR spectra from a selected, localized place deep within an animal by modifying the main magnetic field, B_0, using only static-field gradients. The method is conceptually similar to one spin-imaging method[7] but primarily provides biochemical rather than spatial information. This new technique can be used in fundamental investigations into living systems, clinical diagnosis and the estimation of the efficacy of drug therapy.

To limit the volume from which the high-resolution ^{31}P-NMR signals are received, the effective homogeneous volume of B_0 must be reduced and centred on the region of interest. The topical magnetic resonance (TMR) technique consists of superimposing high-order, static magnetic field gradients on to B_0 via room-temperature magnetic field profiling coils mounted coaxially inside the bore of the magnet.

The magnetic field, $\beta(r, \theta)$, generated by a profile-coil system with azimuthal symmetry, can be described in terms of a Taylor expansion about the origin[8],

$$\beta(r, \theta) = \sum_{n=0}^{\infty} \beta_n r^n P_n(\cos\theta) \qquad (1)$$

where $\beta(r, \theta)$ is the magnetic field at (r, θ), $P_n(\cos\theta)$ are the Legendre polynomials of order n, and the field derivatives, β_n, are defined by the coil geometry and the d.c. current flowing through the coil system. A sensitive volume of homogeneous field can be delineated, centred on the origin and surrounded by inhomogeneous field gradients. A detailed discussion of the rationale of coil design will be given elsewhere.

The description of the magnetic field profile is simplified if only fields along the z-axis ($r = z$, $\theta = 0$) are considered. In a coil system of maximum order $n = 4$ where $\beta_0 = 0$ and β_4 is negative, the resultant profile along the z-axis produced by the sum of B_0 and $\beta(z)$ is shown in Fig. 1.

The inhomogeneity, ΔB, across the central region of axial extent, $2a$, is given by:

$$\Delta B = \frac{\beta_2^2}{2\beta_4} \qquad (2)$$

where

$$2a = 3.1\left(\frac{\beta_2}{\beta_4}\right)^{1/2} \qquad (3)$$

By varying β_2 and β_4 the sensitive volume can be adjusted to match that of the centrally placed region within any given object.

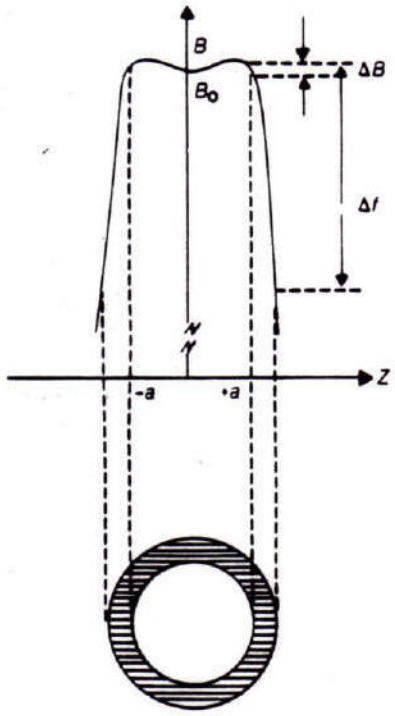

Fig. 1 The profile of the magnetic field along the z-axis in TMR. The inhomogeneity, ΔB, within the central region shown schematically in the projection below, is constrained to remain less than the typical ^{31}P linewidths. In the shaded region the ^{31}P lines will be broadened by the field gradients.

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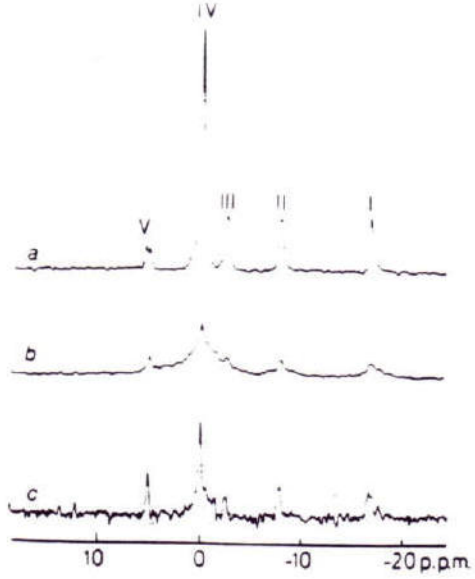

Fig. 2 ^{31}P-NMR spectrum of a two-compartment phantom containing ATP (signals I, II and III), phosphocreatine (IV) and inorganic phosphate (V). A single-turn saddle-shaped radio-frequency coil operating at 73.8 MHz was used in a standard wide-bore (89 mm) superconducting magnet. Profiling was obtained with a coil system designed to provide spatial localization across an internal volume ($2a \sim 15$–45 mm) whilst maintaining a homogeneity of ~ 0.1 p.p.m. at an operating B_0 field of 4.3T. The working bore of the system was 45 mm. The spherical compartment contained 4.65 mM ATP, 9.30 mM PCr and 3.72 mM P_i, and the cylindrical compartment contained 4.65 mM ATP, 18.60 mM PCr and 1.86 mM P_i. *a*, Spectrum (300 scans) in the absence of localizing fields. A 6-Hz line broadening exponential multiplication was used to enhance the signal-to-noise ratio. *b*, Spectrum (600 scans) with the axial extent reduced to 20 mm. The same line broadening was used as in *a*. *c*, Spectrum obtained on removing the broad component of spectrum *b* by convolution difference[9]. Line broadenings of 6 and 60 Hz were used and the vertical scale in the spectrum was multiplied by 4 for clarity of presentation. Chemical shifts are defined as positive in the high-frequency direction with phosphocreatine taken as an internal reference.

Adjacent to the sensitive volume lie regions where the magnetic field changes vary rapidly with position, causing broadening of the spectral lines. The radiofrequency coil will receive signals from the whole sample so the total signal will contain both the narrow, high-resolution lines and the inhomogeneously broadened lines which must be separated out to recover the high-resolution spectrum of the sensitive volume.

Experiments were carried out on a two-compartment test phantom consisting of a cylindrical compartment of diameter 30 mm surrounding a spherical compartment of diameter 20 mm, each containing ATP, phosphocreatine (PCr) and inorganic phosphate (P_i) (for concentrations, see Fig. 2 legend), dissolved in 150 mm KCl to simulate the electrical conductivity of tissue. The internal *p*H was adjusted to be 0.35 units less than that of the external *p*H, thereby producing a chemical shift difference between the P_i resonant frequencies of the two samples.

The spectrum of the sample was obtained with the axial extent set to 45 mm (Fig. 2*a*). The three ATP peaks and the PCr peak are unaffected by the difference in *p*H, whereas two clearly distinguishable P_i peaks appear. Using the PCr peak as a reference, the internal and external P_i peaks correspond to *p*H values of 7.10 and 7.45 respectively. The P_i concentration ratio measured from the peak areas is in good agreement with the expected value.

When the axial extent is reduced to 20 mm, a broad component at the base of each peak arises from the metabolites in the external compartment, which now lie in a region of inhomogeneous magnetic field (Fig. 2*b*). This broad signal is eliminated using the convolution difference technique[9] (Fig. 2*c*). The narrow peaks correspond to the metabolites present only in the spherical compartment, and the *p*H of the P_i peak in this spectrum is 7.10, in agreement with the position measured previously.

To demonstrate the potential of the method in animal experiments, we have studied the liver of a live rat. In the spectrum obtained from the rat in the absence of any localizing fields, the ATP and PCr peaks are readily identifiable, but the P_i and the sugar-phosphate region of the spectrum is less readily assigned because the 2,3-diphosphoglycerate from blood contributes two signals at ~ 5.0 and 6.5 p.p.m. and the first of these overlaps with the P_i signal (Fig. 3*a*). Blood contributes a negligible amount to the ATP peaks. Therefore the ATP signals arise primarily from liver and muscle tissue, the PCr arises exclusively from muscle, whereas the signals in the P_i and sugar-phosphate region may have contributions from liver, muscle and blood.

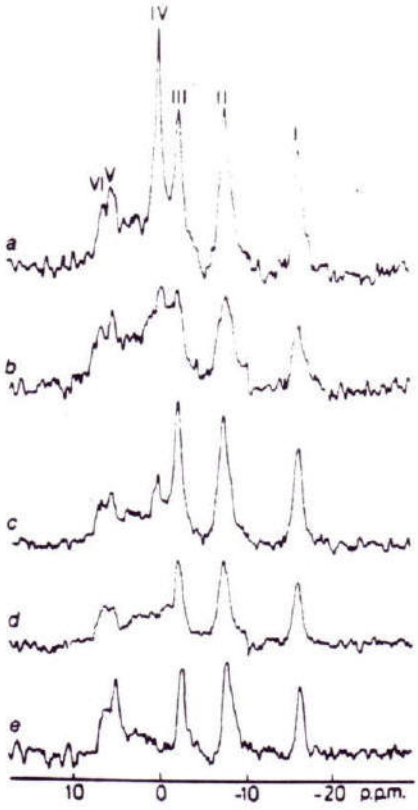

Fig. 3 Spectra *a*–*d* were obtained from an intact rat anaesthetized with pentabarbitol. 150-g male Wistar rats, starved for 24 h, were used for all experiments. Signal assignments are: I, II and III, the β-, α- and γ-phosphate, respectively, of ATP; IV, phosphocreatine; V, inorganic phosphate; VI, sugar phosphate, AMP and IMP. Peaks V and VI may also contain contributions from 2,3-diphosphoglycerate in the blood. Peak II contains a small contribution from ADP and peak III has contributions from ADP, NAD and NADH. *a*, Spectrum in the absence of localizing fields ($128 \times 90°$ pulses at 2-s intervals). *b*, The same as *a* with localization (axial extent 20 mm). *c*, Spectrum in the absence of localizing fields ($1,024 \times 90°$ pulses at 220-ms intervals). *d*, The same as *c* with localization (axial extent 20 mm). *e*, Spectrum of a perfused rat liver ($480 \times 90°$ pulses at 220-ms intervals). For clarity of presentation the spectra in *c* and *d* were divided by 4. The broad components were eliminated using the convolution difference technique[9] with line broadenings of 16 and 233 Hz. Chemical shifts are defined as positive in the high-frequency direction, and phosphocreatine is taken as an internal reference. Measurement of T_1, using progressive saturation technique[11], on the β-phosphate resonance of ATP gave a value of 0.14 s in the presence of localizing fields. This is characteristic of ATP in liver. The remaining small PCr signal in *b* is likely to arise from the diaphragm.

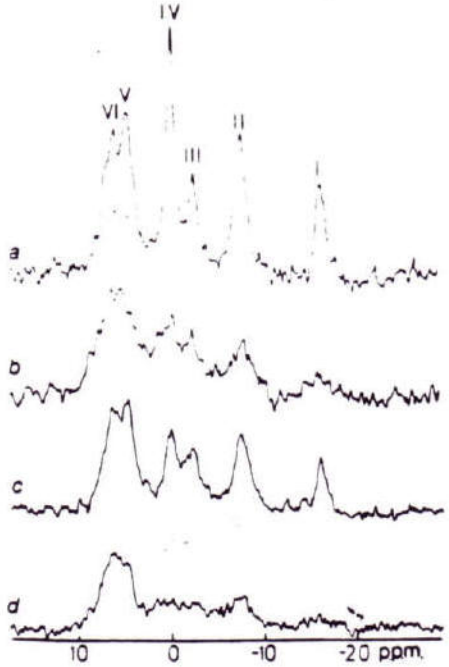

Fig. 4 ^{31}P-NMR spectra of a rat after surgery to cut off the hepatic blood supply. The spectrometer conditions were the same as in Fig. 3 legend. Spectra *a*–*d* are analogous to spectra *a*–*d* of Fig. 3.

The sensitive volume was reduced ($2a = 20$ mm) and the resulting TMR spectrum is shown in Fig. 3*b*. The smaller peak intensities indicate that now high-resolution signal is being acquired from a smaller volume, and as the spectra of Fig. 3*a* and *b* were obtained with the same spectrometer conditions, there will be an inevitable reduction in signal-to-noise ratio. Nevertheless, a change in the relative proportions of the metabolite resonances is apparent.

The spectra of Fig. 3*a* and *b* were obtained using a radio-frequency pulse interval of 2 s, which is typical for ^{31}P-NMR studies of whole tissue. Figure 3*c* shows a spectrum obtained with a pulse interval of 220 ms, in the absence of localizing fields. The PCr peak intensity is now significantly reduced in comparison with its intensity in Fig. 3*a*, because its spin–lattice relaxation time, T_1, is long (~3 s). The reduction in the ATP peak intensities is far less, as the ATP in tissues such as muscle has a rather shorter relaxation time (~1–2 s), and also because a significant percentage of the ATP is in the liver, and liver ATP has very short T_1 values (~100 ms)[10]. Figure 3*d* shows the spectrum obtained using the short pulse interval with the reduced sensitive volume ($2a = 20$ mm). This spectrum contains no PCr signal and is very similar to the spectrum of perfused liver shown in Fig. 3*e*, which was obtained using the same pulse interval of 220 ms. This suggests that the signals of Fig. 3*d* are predominantly from the liver. The improvement shown in Fig. 3*d* can only obtained using the localizing fields.

The P_i/ATP ratio is lower in the liver of the whole animal (Fig. 3*d*) than in the perfused liver (Fig. 3*e*). Previous NMR studies of the brain and skeletal muscle of whole rats also show remarkably low P_i levels[6].

By cutting off the hepatic blood supply of the animal using surgery, similar measurements were made on the same animal (Fig. 4). The marked reduction of ATP and increased levels of P_i after ligation (Fig. 4*b* and *d*) indicate unhealthy ischaemic liver, verifying the origin and interpretation of the previous TMR spectra. In contrast, the PCr signal intensity in Fig. 4*a* is very similar to that in Fig. 3*a* indicating that the metabolic state of the muscle is little affected by the operation.

Thus, we have identified *in vivo* liver tissue by the ^{31}P spectral characteristics and the relaxation time of the β-ATP resonance Global liver ischaemia has been diagnosed in a non-invasive manner. The method is easily extended for the investigation of other organs of laboratory animals.

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Excretion, isolation and structure of a new phenolic constituent of female urine

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The regular occurrence of a peak due to an unidentified substance (X) in the gas chromatographic traces obtained from phenolic extracts of urine from human pregnant and non-pregnant females has been reported[1]. The biphasic excretion of X with maxima in the luteal phase of the ovulatory cycle and relatively high levels in the first trimester of pregnancy were noteworthy and suggested that the substance may have a biological significance. Close similarities between the excretory pattern, the chemical and chromatographic properties of X and of those of the known phenolic steroids suggested initially that this compound was steroidal in nature. The same, or a similar, substance seems to be excreted in the vervet monkey (*Cercopithecus aethiops pygerythrus*)[2]. We now report the excretory pattern of X in more detail, the isolation of the pure compound from pooled pregnancy urine and the chemical structure. The structure determined by mass spectrometry, IR spectroscopy and NMR spectrometry is: *trans*-(±)-3,4-bis[(3-hydroxy phenyl)methyl]dihydro-2-(3H)-furanone (HPMF) and was confirmed by synthesis.

Sequential analyses were carried out on urine collected daily: (1) by volunteers throughout the normal ovulatory cycle; (2) when monitoring oestrogen excretion in patients who were being treated for anovulatory infertility following treatment with postmenopausal gonadotropin; and (3) when following the urinary excretion of oestrogens and of X from conception to term[3]. After acid hydrolysis of the urinary steroid and other conjugates, phenolic fractions were prepared, the extracts acetylated and analysed by gas liquid chromatography (GLC) using a Pye 104 gas chromatographic system[3]. Figure 1 shows

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