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# NMR IMAGING.
# $T_1$ MEASUREMENTS AND CALIBRATION IN RELATION TO SO-CALLED $T_1$ IMAGES

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Summary. — *Relaxation phenomena play a fundamental role in NMR. This fact is directly related to the low frequency range which characterizes this spectroscopy.*

*Two different relaxation processes can be distinguished: the first one, called longitudinal relaxation corresponds to an energy relaxation while the secon one, called transverse relaxation is a phase relaxation. These relaxation processes can be frequently described by a simple exponential law but it is important to point out that non-exponential behaviours are far from exceptional.*

*Even in the case of a pure exponential time dependance of the longitudinal (or transverse) component of the magnetisation, the measurement of the so-called relaxation time and the corresponding images require to take care of different experimental and methodological problems. Computer and experimental simulations are very helpful to illustrate the difficulties in obtaining significant $T_1$ images. These simulations lead also to some requirements which must be taken into account to define phantoms in NMR imaging.*

Riassunto. — *I fenomeni di rilassamento giocano un ruolo fondamentale in NMR. Questo fatto è correlato direttamente con l'intervallo di basse frequenze che caratterizza questa spettroscopia.*

*Esistono due processi distinti di rilassamento: il primo, detto di rilassamento longitudinale, corrisponde ad un rilassamento di energia, mentre il secondo, detto rilassamento trasversale, è un rilassamento di fase.*

*Questi processi di rilassamento possono spesso essere descritti da una semplice legge esponenziale, ma è importante rimarcare che comportamenti non-esponenziali non sono affatto eccezionali.*

*Anche nel caso in cui la componente longitudinale (o trasversale) della magnetizzazione mostra una pura dipendenza esponenziale dal tempo, la misura del cosiddetto tempo di rilassamento e le immagini corrispondenti richiedono la considerazione di diversi problemi sperimentali e metodologici.*

*Simulazioni di calcolo e di esperimento sono molto utili per illustrare le difficoltà di ottenere immagini $T_1$ significative. Queste simulazioni consentono anche di identificare alcuni requisiti importanti nella definizione di fantocci per l'*imaging *NMR.*

## Introduction

At the present moment, essentially three NMR parameters i.e. the nuclei concentration, $\rho$, the longitudinal relaxation time $T_1$ and the transverse relaxation time $T_2$ are being used to define images in NMR imaging techniques. $\rho$, $T_1$ and $T_2$ values are associated with each pixel (picture element) and this is the basis of the imaging technique. At the beginning of this paper, it seems important to point out that one $\rho$ value effectively characterizes each pixel. On the contrary, in many cases, and due to pixel inhomogeneity, it is impossible to characterize a pixel by one $T_1$ or by one $T_2$ value. This problem is not always sufficiently emphasized in the literature. It will be discussed later.

## Relaxation processes

The NMR specialist, be he a physicist, a chemist or a physician, is concerned with two different relaxation processes, namely energy relaxation (called longitudinal relaxation) and entropy or phase relaxation (called transverse relaxation). By relaxation we mean the irreversible process which corresponds to the return to equilibrium of a spin system perturbed by a radio-frequency pulse. This process is not necessarily exponential even in a homogeneous sample. In the case of a two-level spin system (like $^1H$ or $^{31}P$ in a magnetic filed $B_0$) involving longitudinal relaxation, exponential behaviour is observed if, but only if, cross-relaxation and cross-relaxation phenomena are negligible [1]. A nice example of cross-relaxation leading to a non-exponential relaxation function for water in biological samples was described in 1978 by Edzes and Samulski [2]. For nuclei characterized by $I > 1/2$, multi-exponential behaviour is far from

exceptional [3]. In the case of protons (included in water molecules), longitudinal relaxation is clearly mono-exponential as far as pure water is concerned. The same mono-exponential (or at least aproximately mono-exponential behaviour) is observed in most aqueous solutions containing macromolecules, but exceptions are known [2]. This observation seems to prove that a fast exchange generally occurs between the various kind of water which can be defined in such a medium [4]. However, it can also be argued that the precision and accuracy which characterize an inversion-recovery experiment (in the case of $T_1$ measurement) do not easily allow the detection of multi-exponential behaviour.

Using the well-known and oversimplified two-phase model, the results obtained for homogeneous solutions of macromolecules lead to the conclusion that the observed longitudinal relaxation of water is essentially characteristic of the low percentage of so-called bound water [5].

If we consider as a rough approximation that the relaxation mechanism is purely dipolar and intra molecular in this kind of medium, $R_1 = 1/T_1$ ; is given by the following relationship [6]

$$R_1 = \frac{2}{5} \frac{\gamma^4 h^2 I(I+1)}{r^6} \left[ \frac{\tau_c}{1+\omega_0^2 \tau_c^2} + \frac{4\tau_c}{1+4\omega_0^2 \tau_c^2} \right] \quad (1)$$

where $\gamma$ is the magnetogyric ration, I the spin quantum number, r the proton-proton distance in the water molecule, $\tau_c$ the correlation time and $\omega_0$ the Larmor frequency (in radian $sec^{-1}$). $T_1$ is frequency independent only in extreme narrowing conditions. These conditions are not fulfilled in biological samples [7]. The clear-cut proof of this lies in the fact that $T_2$ is always smaller than $T_1$. The $T_1$ frequency-dependency is well illustrated in the graph given in reference [5]. The consequences of this $T_1$ frequency-dependency are not always correctly understood in biological and medical NMR literature, where it is possible to find tables of $T_1$ values without any indications of $B_0$ value. Since the relaxation mechanisms of water in biological samples is certainly not purely intramolecular, it is hopeless to describe quantitatively the difference between $T_1$ and $T_2$ using equation (1) and the corresponding equation giving $R_2$ [6]. However, these equations can be used at a qualitative level to justify either the variation of $T_1$ with the radio-frequency, or the difference between $T_1$ and $T_2$. They can even be used to select the type of materials to employ as phantoms in NMR imaging. More precisely, the great difference between $T_1$ and $T_2$ (with $T_2 \ll T_1$) which characterizes biological samples implies that phantoms must be such that the zero frequency term plays a role at the level of $T_2$. As we shall see, this constraint directly affects the choice of materials adequate to simulate biological tissues.

Our aim in this paper will be to point out some aspects of $T_1$ measurements in relation with NMR imaging even if, as we shall see, the so-called $T_1$ (and $T_2$) images are probably very different from what a true $T_1$ (or $T_2$) image should be.

## Precision and accuracy of pure $T_1$ images

In NMR imaging it is claimed that an adequate choice of pulse sequences leads to images which are determined by $T_1$ values without any direct influence of $\rho$ (proton concentration) or $T_2$ values. If we first consider what is known in the field of high resolution NMR, we can say that the Inversion-Recovery Fourier transform experiment (IRFT) or its fast variant (FIRFT) are the most common procedures for obtaining $T_1$ values [9-12]. The corresponding time dependency of $M_z$ are given by equation (2) for IRFT and (3) for FIRFT.

$$M_z(\tau) = M_0 (1 - 2 e^{-\tau/T_1}) \quad (2)$$

$$M_z(\tau) = M_0 (1 - 2 e^{-t_1/T_1}) e^{-\tau/T_1} \quad (3)$$

IRFT is a time-consuming method because it is necessary to wait for a period $t_1$ (with $t_1 > 5\ T_1$) before each $180° - \tau - 90°$ pulse sequence. Considering that accurate $T_1$ determination necessitates at least 10 samplings corresponding to different $\tau$ values, it becomes obvious that such a method is inapplicable in NMR imaging. Even the FIRFT method suffers from a similar defect. In this case, the best waiting time $t_1$ is of the order of $T_1$ and the first signal (which is not $T_1$ dependent) must be eliminated in the accumulation procedure in order to avoid systematic errors. Of course these two methods can be used and, indeed, have been used in *in vitro* measurement on samples of different tissues obtained by biopsy. The majority of the vast amount of published results have been obtained by IRFT or FIRFT methods [8].

The only criticism which can be made concerning the *in vitro* $T_1$ measurements concern the treatment of data. Many authors determine $T_1$ values by a semilogarithmic regression based on the following equation which is derived from equations (2) and (3)

$$\ln(A_0 - A_i) = \ln(\alpha - A_0) - \tau_i/T_1 \quad (4)$$

with $\alpha = 2$ for IRFT and $\alpha = 2 - e^{-t_1/T_1}$ for FIRFT, and where $A_0$ corresponds to the equilibrium value of the signal intensity ($A_0$ is proportional to $M_0$). $A_i$ is proportional to $M_z$ ($\tau_i$). It can be shown that this procedure may lead to inaccurate results for reasons which are purely mathematical [13, 14]. The only valid procedure which gives an unbiased estimate of $T_1$ is an exponential regression based on the true variables $A_i$ and $\tau_i$, with $A_0$ and $T_1$ becoming adjustable parameters. This can easily be performed using the Newton-Raphson method [13, 14]. Using a set of simulated data, we were able to show the extent of the risk associated with the use of equation (4) in $T_1$ measurements [14]. This conclusion is only relevant for *in vitro* measurements. We are emphasising this point here because *in vitro* $T_1$

values are frequently used as absolute reference data in NMR imaging [8].

To return to the so-called pure $T_1$ image, it must therefore be clear that the $T_1$ values which are used to build these images are not obtained by methods that have been well tested in high resolution NMR. Different kinds of constraints and especially the time constraint which is of fundamental importance in NMR imaging, preclude the use of IRFT or FIRFT sequences as they are used in non-medical NMR. Other methods have therefore been introduced which are characterized by a considerable time saving. The question which has to be asked is obvious: is it certain that these methods give significant $T_1$ values and therefore significant $T_1$ images? The term "significant" necessitates some comment. In imaging technique, the problem is not to know if, in this particular pixel, $T_1$ is equal to 0.35 s or to 0.52 s. The real problem is to know if two pixels, vicinal or not, are characterized by identical, similar, or different $T_1$ values. The real problem is to know if any two sub-domains in the image characterized by two different shades of grey, or at least giving two distinct visual impressions, are really different on a $T_1$ scale. This question can be solved essentially by two techniques: the first is based on computer simulation and can be described as a computer experiment. The second is wholly experimental. The experimental technique is based on the use of phantoms defined by Bottomley as "a fabricated sample which incorporates a specific spatial structure designed to test the image device" [15]. The construction of an efficient phantom is not trivial because it requires materials characterized by $T_1$ and $T_2$ values of the order of magnitude of those measured on living tissues.

## The simulation of Fourier transform NMR imaging

### *1. The measurement of $T_1$*

Compared with other techniques, the time required to produce an NMR image is slow. This fact is not the result of a technical problem but of an intrinsic restriction in the sense that the basic signal-to-noise ratio of an NMR spectrometer is low. To reduce the effect of noise, it is possible to average the result of a series of measurements at the risk of increasing the time required to produce an image. This averaging process is necessarily slow in order to avoid the loss of the signal by saturation: repetition time may not be much shorter than $T_1$. As in high resolution NMR [12, 16], several techniques have been developed in NMR imaging to obtain the best signal-to-noise ratio in a definite period of time. As a consequence, the signal intensity is often a function of several of the three basic NMR parameters $\rho$, $T_1$ and $T_2$, even for methods which are reputed to be selective with respect to these parameters, i.e. spin echo for $T_2$ or inversion recovery for $T_1$ [17, 18].

The spin-echo technique is widely used in NMR imaging because it has the advantage of allowing the easy switching of the various gradients which are necessary for spatial coding. When different spin-echo sequences are produced successively every $t_1$, the signal strength of the spin echo is

$$I_{SE} \propto \rho[1 - \exp(-\frac{t_1}{T_1})] \exp(-\frac{2t_2}{T_1}) \qquad (5)$$

where $\rho$ is the proton concentration and $t_2$ is the time between the 90° and 180° pulses.

In practice, if $t_1 < 5T_1$, the relaxation delay between two spin-echo sequences is not long enough to obtain a full relaxation of the spin system. Here this sequence will be called "a fast spin echo".

In the same way as with a fast inversion recovery sequence [12], spin-echo amplitude is reduced by a saturation parameter, $[1 - \exp(-\frac{t_1}{T_1})]$, which contains the information $T_1$. Therefore, the right choice of $t_1$ and $t_2$ is critical for observing a contrast between tissues of different $T_1$ and $T_2$ [19]. Figure 1 shows computed simulated curves for two tissues characterized by $T_1$ = 0.5 s and $T_2$ = 0.04 s for the former, and $T_1$ = 1 s, $T_2$ = 0.08 s for the latter. It is obvious that the curves

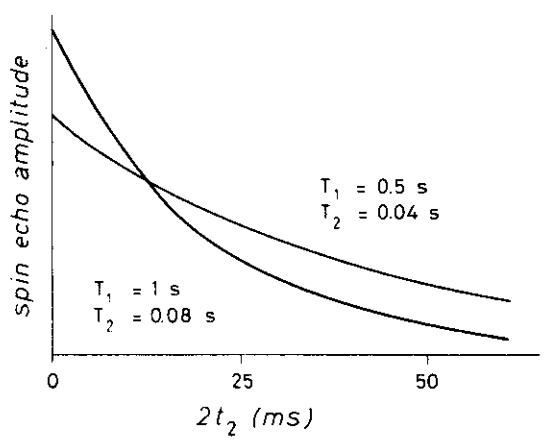


Fig. 1. Simulation of the spin echo amplitude of two different tissues measured by a "fast spin echo" technique, with a fixed delay $t_1$ = 1 s between two successive spin echo sequences. The choice of the time $t_2$ between the 90° pulse and the 180° pulse is critical for observing a contrast between the two tissues.

shown are of great significance. Despite the 100% difference in the relaxation characteristics of the two tissues, they can only be distinguished if $2t_2$ is larger than 25 or 30 ms. On the other hand, if $t_2$ is too long, transverse relaxation may reduce NMR signals to background levels.

Several methods have been developed to enhance the effect of $T_1$ on the intensities of NMR signals. We will describe here the results of three of these methods.

a) Two different "fast spin-echoes" can be acquired with the same $t_2$, but with different $t_1$. From equation (5), we can compute the ratio $R_1$

$$R_1 = \frac{I}{I'} = \frac{1 - \exp(-\frac{t_1}{T_1})}{1 - \exp(-\frac{t_1'}{T_1})} \qquad (6)$$

$T_1$ can then be obtained by Newton's method.

In Fig. 2, curve *a* shows the computed relationship between $T_1$ and the ratio $R_1$ for $t_1 = 0.2$ and $t_1 = 2$ s. The curve (which can be easily assimilated to a straight line) shows that a 100% contrast in $T_1$ (*e.g.* $T_1 = 0.3$ and 0.6 s) is measured by a difference in R of about 66%($R_1 = 2.05$ and 3.4 in this case).

$$\text{The slope of the line } \frac{\Delta T_1}{\Delta R_1} \quad \text{is } 0.21$$

b) Two FID can be acquired after the two 90° pulses of the sequence 90°, $t_1$, 90°, $t_2$, where $t_1 < 5T_1$ and $t_2 > 5T_1$.

The value of $T_1$ can be easily computed from the ratio $R_2$

$$R_2 = \frac{I_1}{I_1 - I_2} \quad \text{then,} \quad T_1 = \frac{t_1}{\ln\left(\frac{I_1}{I_1 - I_2}\right)} \qquad (7)$$

where $I_1$ and $I_2$ are the amplitude of the NMR signals, respectively acquired after the first and the second 90° pulse.

In curve *b* of Fig. 2 where $t_1 = 0.2$ s, $T_1 = f(R_2)$ is also very close to a straight line and has a slope

$$\left|\frac{\Delta T_1}{\Delta R_2}\right| = 0.55.$$

c) The first signal is acquired with a repeated free induction decay sequence 90°, $t_1$, 90°, $t_1$ ..... The second signal is acquired with an independent fast inversion recovery technique 180°, $\tau$, 90°, $t_3$, 180°, .....

The ratio $R_3$ of the two signals is given by

$$R_3 = \frac{1 - 2\exp\left(-\frac{\tau}{T_1}\right) + \exp\left(-\frac{t_1}{T_1}\right)}{1 - \exp\left(-\frac{t_1}{T_1}\right)} \qquad (8)$$

if $t_1$ is chosen as being equal to $\tau + t_3$.

Curve *c* of Fig. 2 has been calculated with $\tau = 0.4$ s and $t_1 = 1$ s. The slope $\left|\frac{\Delta T_1}{\Delta R_3}\right| = 0.86$ if the curve is approximated by a straight line. From Fig. 2, it is difficult to conclude about the optimum choice of a $T_1$ measurement method. If the different ratios $R_n$ ($n = 1$ to 3) undergo a certain level of error due to noise, it is clear that the pure $T_1$ information as well as the errors will be equally transmitted by the three curves which characterises the three different relations between $R_n$ and $T_1$, indipendently of the slope of the curves. Therefore, the choice of the optimum method will be the result of a compromise taking into account both the intrinsic sensitivity of the method to $T_1$ and the best signal-to-noise ratio for a definite period of time.

In Fig. 2 the experimental parameters have been arbitrarily chosen for a good sensitivity of the three methods to a value of $T_1$ around 0.5 s.

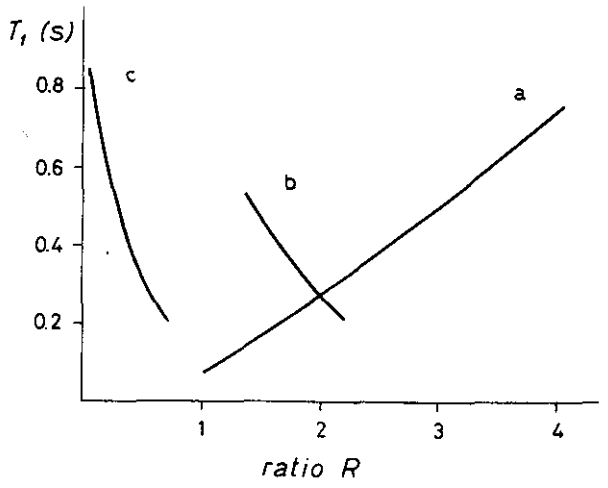


Fig. 2. – Simulation of the measurement of $T_1$ by three different "$T_1$ enhanced" methods.

*2. Bi-exponential relaxation*

The problem associated with the detection of multi-exponential relaxation behaviour is illustrated in Fig. 3. We have simulated the case of two reservoirs of water, differently associated with macromolecules in such a way that their longitudinal relaxation times $T_1$ are 0.5 and 1 s.

Curves A and B illustrate the cases of an 80-20%and a 20-80% mixture respectively, and computed if a steady state free precession technique (SSFP) is used.

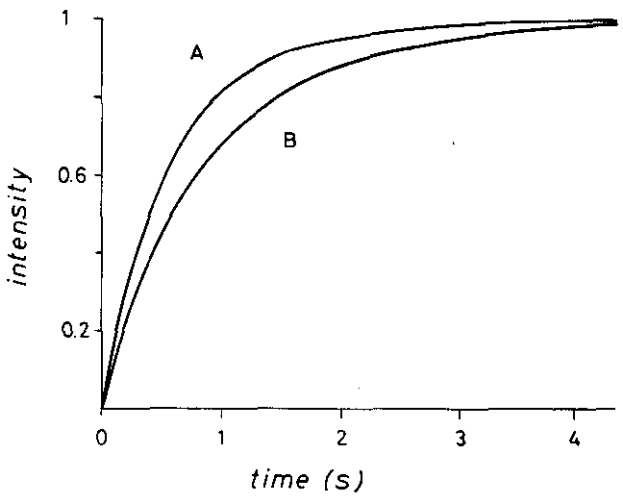


Fig. 3. Simulation of the longitudinal relaxation curves (SSFP) of 80-20%(A) and 20-80%(B) mixtures of two different reservoirs of water characterized by longitudinal relaxation times equal to 0,5 and 1 s respectively. The four zones of "local" $T_1$ measurement are accentuated on the abscissa scale.

Table I gives the apparent $T_1$ as they should be measured by method a) described above. The values for the couple $t_1$ and $t_1$ have been chosen rather close to each other in order to estimate the local value of the apparent $T_1$. If, on the contrary, two widely spread $t_1$ values are used, an average $T_1$ is computed for the whole relaxation curve. It must be emphasized that the two averages

do not differ by more than 10% from the "local" values. Therefore, during an imaging experiment with a certain level of noise, it can be considered that the choice of $t_1$ is not critical in the evaluation of the apparent $T_1$.

Table 1. – *Computer simulation of the apparent $T_1$ "measured" by a "fast spin echo" technique, using the delays $t_1$ and $t'_1$ which are underlined on the time scale of Fig. 4*

| $t_i$ (s) | $t_1$ (s) | apparent $T_1$ | |
|---|---|---|---|
| | | A | B |
| .1 | .3 | .534 | .799 |
| .5 | .7 | .541 | .796 |
| 1 | 1.2 | .558 | .828 |
| 2 | 2.2 | .597 | .887 |
| .2 | 2.0 | .551 | .820 |

**The significance of the $T_1$ image in a non-exponential case**

It is clear that if the pixel is inhomogeneous, relaxation is not mono-exponential but multi-exponential. The number of exponentials is equal to the number of sub-domains in the pixel. As we pointed out at the beginning of this paper, this problem is important because, in many real cases, multi-exponential behaviour must be the rule rather than the exception. Of course, the techniques which are used to derive a $T_1$ image are quite unable to give any information about the multi-exponential time dependency of the magnetization vector after a 180° pulse. The so-called $T_1$ value obtained by the two point technique has not any physical meaning. As is usual in imaging techniques, the real problem is to know if the meaning of the "apparent $T_1$" remains sufficient to give any credit to an image based on this quantity. The simple case just discussed seems to prove that such an image is in fact significant.

**The choice of phantoms in NMR imaging**

All NMR specialists interested in imaging are convinced that the use of phantoms is of prime importance to test the image device. A phantom is characterized by geometrical features but this problem will not be considered here. A phantom is also characterized by the materials used to build it and this aspect is the only one we will be concerned with. As we have already pointed out, NMR imaging based on proton NMR implies the observation of water protons. These protons are such that the relaxation processes and, more specifically, longitudinal relaxation is generally not mono-exponential. Nevertheless, even if mono-exponentiality is taken as a rough approximation, it is clear that $T_2 \ll T_1$.

This observation introduces the first constraint in the choice of correct phantom material: the correlation time(s) of water (or part of it, if conditions of fast exchange prevail) must be sufficiently long to preclude conditions of extreme narrowing in the system. After a lot of attempts, we were able to select a gel (agar-water) slightly doped with $CuSO_4$ as the best material to build phantoms. A typical example of such a material is obtained by putting 60 mg of agar in 1 ml of water in which $10^{-6}$ M of $CuSO_4$ is dissolved. Typical values: $T_1$ = 1 s, $T_2$ = 40 ms are obtained for such a medium, but these values are rather dependent on the preparation procedure of the gel. This last remark appears as a limitation on the use of this kind of material to build phantoms. We could add that the life-time of such a medium is certainly not infinite and this fact appears as a second limitation. Nevertheless, it must be emphasized that as far as possible, the phantoms must simulate the biological samples and in our experience, this goal can only be attained with gels and not with true solutions. As far as gels are concerned the dependency of their characteristics with respect to preparation procedure or their thermodynamic (in)stability are facts which have to be taken into account.

The possibility of slightly modulating the $T_1$ and $T_2$ values of our agar-water system by adding $CuSO_4$ endows that system with a versatility which renders it possible to place different reservoirs with slightly different $T_1$ and $T_2$ values in the same phantoms. Even though it is not determinant, another advantage of the agar-water system lies in the fact that the relaxation characteristics of water in this system has been extensively studied [20]. Finally, we would like to point out that the $T_1$ and $T_2$ measurements were carried out on a 80 MHz high resolution spectrometer. The frequency dependency of $T_1$ and $T_2$ both on our ager system and on biological samples is under study.

**REFERENCES**


1. REISSE J., 1983. Relaxation processes in nuclear magnetic resonance. In: "*The Multinuclear Approach to NMR Spectroscopy*". J.B. LAMBERT & F.G. RIDDELL (Ed.). D. Reidel Publishing Company, Dordrecht.

2. EDZES H.T., & SAMULSKI E.T. 1978. The measurement of cross-relaxation effects in the proton NMR spinlattice relaxation of water in biological systems: hydrated Collagen and muscle. *J. Magn. Res.* **31**: 207-229.

3. LURCAT F., 1955. Relation between the relaxation times and the transition probabilities in nuclear magnetic resonance (magnetic

and quadrupole). *C.R. Acad. Sci, (Paris)* **240**: 2402-2403.

4. de CERTAINES J., 1981. Variations of spin-lattice and spin-spin relaxation time of biological tissues in proton nuclear magnetic resonance. *J. Biophys. et Méd. Nucl.* **5**: 107-116.

5. MANSFIELD P. & MORRIS P.G., 1982. *Imaging in Biomedicine*. Advances in Magnetic Resonance. Suppl. 2. Academic Press, New York, pp. 17-18.

6. ABRAGAM A. 1961. Les principes du magnétisme nucléaire. Institut National des Sciences et Techniques Nucléaires et Presses Universitaires de France (Paris), p. 291.

7. BLOCK R.E. & MAXWELL G.P., 1974. Proton magnetic resonance studies of water in normal and tumor rat tissues. *J. Magn. Res.* **14**: 329-334.

8. DAMADIAN R., (ed.) 1981. *NMR in Medicine*, Springer Verlag.

9. FARRAR T.C. & DECKER E.D. 1971. *Pulse and Fourier Transform NMR*. Academic Press., New York.

10. FREEMAN R. & HILL H.D.W., 1970. Spin-lattice relaxation in high resolution NMR spectra of $^{13}C$. J. Chem. Phys. **53**: 4103-105.

11. LEVY G.C. 1973. Carbon-13 spin-lattice relaxation studies and their application to organic chemical problems. *Accounts Chem. Res.* **6**: 161-169.

12. CANET D., LEVY G.C. & PEAT J.R., 1975. Time saving in $^{13}$ C spin-lattice relaxation measurements by inversion-recovery. *J. Magn. Res.* **18**: 199-204.

13. VANDENBOSCH J.C. 1978. Détermination de barrières entravant la rotation interne par la mesure de temps de relaxation longitudinale en résonance magnétique nucléaire. Thesis (Bruxelles).

14. VANDENBOSCH J.C., ZIMMERMANN D. & REISSE J. Unpublished results.

15. BOTTOMLEY P.A., 1982. NMR imaging techniques and applications: a review. *Rev. Sci. Instrum.* **53**: 1319-1337.

16. ERNST R.R., ANDERSON W., 1966. Application of Fourier transform spectroscopy to magnetic resonance. *Rev. Sci. Instrum.* **37**: 93-102.

17. GORE J.C., DOYLE F.H., DENNOCK J.M., 1983. Relaxation rate enhancement observed in vivo by NMR imaging. In: NMR Imaging. *C.L. Partain, A.E. James, F.D. Rollo & R.R. Price. (Eds).* Chap 7. Saunders Company, Philadelphia.

18. KAUFMAN L., CROOKS L. & MARGULIS A. 1981. *NMR Imaging in Medicine*. Chpa. 3. Igaku-Shoin. New York.

19. CROOKS L.E., MILLS C.M., DAVIS P.L., BRANT-ZAWADZKI M., HOENNINGER J., ARAKAWA M., WATTS J. & KAUFMAN L. 1982. Visualization of cerebral and vascular abnormalities by NMR imaging. The Effects of Imaging Parameters on Contrast. *Radiology* **144**: 843-852.

20. WOESSNER D.E. & SNOWDEN B.S., Jr. 1970. Pulsed NMR Study of Water in Agar Gels. *J. Colloid and Interface Sc.* **34**: 290-299.

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**Discussion on the effects of differences between various techniques on measurements**
*Moderator:* B. Maraviglia

MARAVIGLIA – I would like to give a brief summary of the discussion and to invite people to further debate. Essentially, many aspects have been proposed and I think that we all reasonably agree on the problems. The main differences are these: I pointed out that pure parameter images are difficult to obtain in a short time, but they can be produced if times longer than the usual ones are avaible. Then Mr. Luiten showed how things become complicated with repeated sequences, and he attempted to find some sulutions within these repeated sequences to possibly extract the information that one wants to have. Similarly Dr. Taylor proposed steady state free precession sort of sequence which is actually bound to the ratio of $T_2$ over $T_1$ as information. Finally Prof. Reisse emphasized the non–exponential aspect besides the repeated sequences problem which must not be forgotten, of course, and which is difficult to untangle. Now I'd like to point out that my position is somewhat different in the sense that tentative repeated sequences are necessary for clinical imaging because there a short time is really required. For tissue characterization instead, it may be more convenient to use longer times and to obtain eventually distributions of pure parameters. This can be done by different people who are not really interested in diagnostics but rather in establishing correlation of pathology with a certain distribution of $T_1$ parameters, by working eventually on animals and then deciding, at least for certain pathologies, whether these NMR parameters are reliable. Then possibly in the near future, if this is reasonably established, it can also be applied to man. At that point, for medical diagnosis, doctors can get first the usual image in a short time, and then eventually for further insight they could get in a longer time a pure parameter image for more definite analysis. But I have the feeling that first a period of research is necessary to establish if this is really convenient in terms of correlation between pathology and NMR parameters. Now the discussion is open.

TAYLOR – Just to answer that last point you made, I think that what Mr. Luiten and I were both saying was that, providing that you were in the regime where your repeat time was much longer than $T_2$ and yet still going rapidly, you could in principle uniquely extract pure $T_1$ and $T_2$ values. There's no problem with that, apart from perhaps Prof. Reisse's point that the accuracy might go. A second comment that I just wanted to make was that I think that what Mr. Luiten and I also showed was that Prof. Reisse's equation for fast spin–echo was an approximation - the approximation is that the waiting time before the next pulse sequence starts is much longer than the $\tau$ value - that was just a small point really.

ORR – The first speakers all appeared to have reasonable agreement on the theoretical equations on relationship of the pulse sequences. But we are all talking about ideal bits of equipment, and the equipment with which tissue characterization will be made will be far from ideal because they will be wide bore with all the problems. I'd like to ask if we feel that if we were given a bottle of the same material, would we all come up with exactly the same answers, for spin–lattice relaxation and spin–spin relaxation, and if we were given two bottles of different materials, would they have the same relative value in practice?

MARAVIGLIA – My answer would be yes within 10%, if it is a bottle of water, because in pure substances $T_1$ is normally obtained within 10% . I don't think that the procedure should affect it unless the experimentalist is doing something wrong.

LUITEN – I think the question is on the accuracy again here. I mean, whether a statement of 10% is important or not is of course very relevant when the differences which are decisive for decision are of that order of magnitude. I must freely admit that in the short time length you talk about in an ideal situation, there are quite a number of situations which are not ideal. The first thing is of course that your $B_0$ field is not homogeneous so you are not always at a Larmor frequency, and Dr. Taylor pointed out very carefully that that's true. I haven't gone through all the mathematics as he has already done and shown that you get the same values even if you are just off–resonance somewhat. I am pleased to hear that the problem is more serious than the solution. I think another point in homogeneity is of course the $B_1$ field - the r.f. field - and that is inhomogeneous for a number of reasons: the excitation field is not homogeneous so a 90° pulse is not a 90° pulse everywhere. It depends where you are, and the object may easily have variations of about 20% of the $B_1$ field. Fortunately the signals from 80° , 100° and 90° are almost the same. But whether the relaxation behaviour is the same, I don't immediately have an opinion about this. Another fact left out of the discussion is the question of penetration, because all we assume is 100% penetration, but once we go into frequencies beyond 10 MHz then I think this point too will come to influence the signal strengths. The only thing you finally have is a reconstructed image with a number of values - it's an area of a matrix of numbers - that's the only thing you've got after measurement. But how reliable are these numbers? Another point which is really related to imaging is namely, if you do a slice imaging we all know that the selective excitation across the slice thickness is not just a square cross section that has at the edges low excitation. Now in some cases you might be satisfied to have this kind of Gaussian shapes in excitation density distribution, or some of the more square shapes, but certainly from the middle of the slices the excitation of 90° drops off on both sides. One of the points is, of course, when you do the measurements, how do you find out the 90° signal? The way we usually do it is to make the signal maximum, but then you aver-

age over a number of situations so the variable will be that the centre is beyond 90° and 180° . How do we make 180°? - we just double the signal. So what happens then is again a source of error. Then of course there is a great difference if you do slice imaging or volume imaging. It may be that in volume imaging when you excite a whole volume and take slices in three dimensions, the effect of excitation is less serious than if you use single slice imaging, and there may be a difference when you do multiple slicing or use the three–dimensional Fourier transform technique. The question is whether these effects do really influence the value you finally obtain, so if you put it all together, then what accuracy you can get up to is difficult to state. Fortunately some of these effects are systematic and some are random, so they might cancel out. In conclusion it may be that the situation is less severe than *a priori* foreseen. What I forgot is the partial volume effect of course - that's also an effect which affects the image whether you've got a small slice or a thicker slice. If you take a thicker slice, you have got less noise but if you go to a thick slice then you have more partial volume effects on the accuracy. So it's a great problem to find out whether we really can approach errors of 5 or 10% in $T_1$.

BOVEE – I would like to repeat Prof. Orr's question in a slightly different way. If you have one apparatus and you get one sample, not a bottle of water but a piece of tissue or perhaps still better a very carefully prepared protein solution, and you take three different operators, would they get the same answer for the relaxation rates?

MARAVIGLIA – I don't think so, but my feeling is that if they use the same procedure, the main difference would arise from the exponential behaviour according to what they think would be closer to their ideal, their mental idea of exponential.

STYLES – I'm in rather an unenviable position chairing, or partially chairing this session because I've done no imaging at all. But it does seem to me that this meeting is about the characterization of tissue. All the medical practitioners have been given various machines from various manufactures with techniques which are designed, presumably, to produce the prettiest pictures. If we stand any chance of characterizing tissue, is it possible to make comparisons between the machines or the methods of the various manufacturers, or is our only hope to go back to the sort of fundamental experiments that you've talked about, and then perhaps try and see how these extrapolate within a given method?

TAYLOR – Perhaps I could say just a little bit on that. I think with care - and that's quite a large statement because a lot of manufacturers will not spend the money in taking the care - you can calibrate out a lot of the unknowns in any given machine - $B_1$ homogeneity, $B_0$ homogeneity and so on. At the same frequency you ought to be able to get $T_1$s within 10%, but the question is whether all these different systems round the world will be calibrated to that degree and have the same frequency and so on, that's what I mean by "with care" - it's whether people will take the time to actually do that work.

BOVEE – I would like to comment on this but I'm going to give a contribution later on, concerning this particular point. It is not only a question of getting a good calibrated apparatus and defining how much the non–exponentiality is or how it should be handled. It is also very important what kind of delays you use, the distances between the $\pi$ pulses, pulse widths, homogeneity of the r.f. field, the static magnetic field, they can all influence your relaxation rates.

LUITEN – I think that the differences will be less than you might expect and I agree with Dr. Bovée - you just have to define your measurement conditions. Something which is still being often neglected is that for any picture and for any data you should state what the delay times are, as well as the field strength, the pulse sequence and so on. If that is reproduceable, of course you still need calibration - the settings of the NMR equipment might be inaccurate but in principle I think there should be a very good agreement between the various methods and the main differences measured now have not much to do with the equipment but more to do with the varying conditions, both of the sample and of the measurement technique.

ORR – May I ask Dr. Taylor to perhaps explain more fully what he means by "calibrating out" - how exactly would you calibrate out these variables in such a way that Dr. Luiten could use exactly the same calibrating methods?

TAYLOR – That's a very difficult question. I think that what I'd do is to listen to Dr. Bovée's talk first and then answer the question. I'm sorry, I don't know if it's possible to answer that question. There are well–known sequences, I believe, that will monitor the $B_1$, you can map the $B_0$ quite accurately, and so on. What I haven't done at this point in time is to follow such methods through as correction procedures.

REISSE – To answer this question, the only convincing procedure would imply to have different apparatuses in the same building or in the same city, and to put in each machine the same phantom, the same scientist working on each machine. I am personally convinced that we need good phantoms. I am a little surprised to hear that the accuracy is not a problem which is related to machines but only related to a correct choice of pulse sequences. I am sure that the choice of sequences is very important, but it remains a fact that, as far as I know, the NMR machines including the medical NMR apparatus, are far from perfect. As physical chemist working on $T_1$ measurements since many years, I know that all the machines are not equally good (I speak about high resolution spectrometers). I remember very well that five years ago we had the chance to have two machines in the same lab, and even the same experimentalist working on the same sample was not able to obtain the same $T_1$ values. We were forced to work for months in order to understand what happened and what was the most accurate value. It is very important to realize that NMR spectroscopists, and especially physicists, have been working in NMR spectroscopy for more than forty years, and it is a risk now to reinvent in medical NMR

things which are known since many years in other NMR fields. The problem of $T_1$ or $T_2$ imaging is, from the experimental point of view, a big problem, and I am quite sure that many false values, many artifacts are published in the literature. It would be a miracle if that were not the case. It was the same situation twenty years ago - 80 or 90% of the $T_1$ values published in the literature for homogeneous samples were not accurate. The difficulties are much more important in the medical field, where we are constrained to estimate $T_1$ on the basis of a very small number of data. The errors can be very important, much more than 10% - as in high resolution NMR, perhaps 100% or even 200%.

DERBYSHIRE – I think I'm going to be a little more optimistic than Prof. Reisse, in that my feeling is that the problems of $B_0$ and $B_1$ in homogeneity, while not absolutely solvable, are probably solvable in real terms, because for looking at the small samples, people have evolved techniques for measurements of $T_1$ and $T_2$ that largely cancel out the effects of $B_0$ and $B_1$ in homogeneities. One development, for example, was reached on going from Carr–Purcell to Gill–Meiboom sequences to cancel out at least the effect of $B_1$ inhomogeneities. We might look for similar developments in imaging.

LUITEN – Of course if the errors are due to the techniques or to different interpretations or to mathematics, it is possible to come to an agreement. In particular, even if the $T_1$ being measured is a kind of pseudo–$T_1$, the important question is whether the experiment is repeatable. That is one point. Another point is of course whether a number of inaccuracies are of a systematic nature - i.e. the homogeneities stay the same. However the point may be the inaccuracies due to the random errors, and that's where the noise comes out. Once you have got accuracy, that means you have to make reproduceable data to be measured, then you are in an area in which the quality of detectors and amplifiers and the number of averages are of importance. That is, I think, one of the most limiting parameters because in many cases you cannot take hours to perform the measurements - you have to do a limited number of points, you can not average too much. This influences your accuracy probably more to my mind than the systematic differences between the various equipment.

ERMANS – In the commercial devices, which are now available for NMR imaging, it seems that simple procedures (*proton density*, $T_1$ *and* $T_2$, *etc.*) *can be easily* used routinely. My question is does this mean that these commercial firms have taken account for all the problems you mentioned in your talk? In other words, can we accept, at the present time that measurements of $T_1$ and $T_2$ carried out on these available machines have an acceptable accuracy?

MARAVIGLIA – Of course these machines have $T_1$ and $T_2$ images and they mean $T_1$ and $T_2$ contrasted images, not pure parameter images. This is clearly meant by everyone I think. I'd like to come up again with a proposal I've already made. Everyone seems to agree that $T_1$ is a complicated parameter due to the procedures and to the fact that man is not consistent: he can eat different stuff, he can be excited, he can be feverish, he can be unhappy, he can have so many things which can alter his $T_1$s. What probably is not altered much though is the relative change from the pathological to the normal sections of the same organ. So if one has to characterize these tissues, I propose that we give emphasis to this within the framework of clearly defined pathological situations. Presumably the relative variation of $T_1$ in the same sort of disease from the sick part to the normal part is more reproduceable than the absolute values. If this is not the case, I'am afraid $T_1$ won't be useful at any time.

TAYLOR – *I'd like to make a couple of general observations on that. Certainly from in vitro studies, and a little bit from in vivo,* there's a lot of evidence for a wide spread of values - 25% , 30% - across human beings and so on. I think we really have to get away from absolute values. Then we're looking to normalize our measurements at one point in the body, and then there is also evidence that a diseased state will alter the $T_1$s across several organs, not just the one localized, and so although I agree in principle with your basic point, and I think that is the way we'll have to go, I don't think it's quite as clear–cut as that.

LUITEN – That reminds me of a remark Prof. Mallard made years ago when he collected data on $T_1$ values. There seemed to be a lot of differences between the values of the same tissues of different persons, but he told me that the relation between the relative $T_1$s and *the ratio of the* $T_1$ *between the various tissues in the* organs in the same subject was far more consistent than the individual values. So you might normalize the relaxation properties of various organs to those of liver, for instance. The relation to some kind of tissue which you take as a standard might be interesting from a practical point of view. If you can calibrate on every patient - that would be very fine.

FOSTER – This is certainly true in the imaging situation, but one tends to look at the disease in relation to the organs round about. The only situation where this isn't covered is where you have very strong systemic effects from any particular disease. One of the things that I'm very interested in, as is anyone who's working in the field, is the possibility that these systemic effects will affect the overall picture of the body because this takes away our simplest possible calibration. If I want *to look at liver diseases, I will check that the colour of* the spleen next to it is normal and this will then tell me how far my liver has deviated. As you say, you use the body itself as its own calibration. But if we have some disease which affects the liver and the spleen, then you get the problems. The overall effect is changed.

TAYLOR – We looked at some early whole body images and from those images there is quite a lot of evidence for a wide spread of values across a given organ, to the extent, in these images (I should stress they were early images) that the spread across an organ was almost as great as the difference between different human beings. I don't think we should just assume that there is a single $T_1$ in an organ. Then you get the partial volume effects

and also another point which we should have been considering is repositioning for re–examination. Why start dotting the 'i's and crossing the 't's on our technique for $T_1$ dependence and throw it all away by the way we line the patient up?

LUITEN – This would mean that, if the $T_1$ is varying over the organ, then imaging the spatial distribution, is vital in the whole determination because a single point doesn't say very much. The way the $T_1$ varies across a certain organ may be the information you need to see whether you're getting the right $T_1$ value. In this case I say measurement and imaging are very intimately correlated.

REISSE – Let me come back to multi–exponential behaviour. I would like to be sure that all of you agree that the multi–exponential behaviour is the rule and not the exception. Do you agree with this comment? If you agree let me ask, what is the signification of $T_1$?

MARAVIGLIA – That's a very good question. In fact, one should also give a definition of what fraction of the magnetization recovery one should consider. However, the non–exponentiality is not that large, in my experience.

REISSE – I agree with you. I think that we will probably continue to use expressions like $T_1$ images and so on, but the non–exponentiality or the multi–exponentiality have a direct consequence on the problem of the good choice of delays. It is always an important problem in NMR spectroscopy, but it is also in imaging, especially when the relaxation behaviour is not exponential. In this case it would be even necessary to oblige people to always use the same kind of delay or, at least, to specify their experimental conditions. If you look at the literature, you can find images with good contrasts, but too frequently the information which is given is completely insufficient to repeat the experience. In the biological literature you find sometimes $T_1$ values without any indication of the $B_0$ value.

RADDA – Listening to this discussion as an outsider, we seem to be going round in circles. You don't know how to measure it, you don't know what you are measuring, you don't know how to interpret what you have measured, and you don't know how to use it in medicine. I think you are talking about all four of them at the same time. I think we ought to try and focus down and say "we are now going to talk about those aspects of $T_1$ that relate to the scientific, biophysical basis of understanding what we can measure, assuming we can measure it". Then let's talk about the various ways in which we can measure it, then the sort of samples that we want to have. But we are sort of jumping from putting the patient back in the same position, taking a little piece of tissue out, putting it in an NMR machine which is different from somebody else's and it is four times the frequency. I really feel that we are just going round in circles - we somehow ought to separate these different stages.

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# TECHNICAL ASPECTS OF HUMAN METABOLIC STUDIES BY NMR

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Summary. – *This talk will introduce some of the techniques which have been used in the study of metabolic events by NMR. These are broadly two areas that require special attention, namely the localisation of the region of interest and optimisation of sensitivity. These overall requirements will be discussed in relation to receiver coil design, and exploitation of field profiling. Surface coils have proved to be of great value in achieving some degree of localization together with reasonable signal–to–noise. However, the inherent inhomogeneity of the field that they produce presents certain difficulties and this will be considered. Possibilities for overcoming these problems will be mentioned.*

Riassunto. – *Verranno introdotte alcune delle tecniche usate nello studio NMR di eventi metabolici. Due problemi principali richiedono particolare attenzione, cioè la localizzazione della regione di interesse e l'ottimizzazione della sensibilità. I requisiti fondamentali saranno discussi in relazione alla progettazione della bobina ricevente e all'utilizzazione di tecniche di sagomatura del campo. Le bobine di superficie rappresentano uno strumento molto valido per ottenere un certo grado di localizzazione e valori accettabili del rapporto segnale–rumore. Alcune difficoltà sono tuttavia associate alla inomogeneità del campo prodotto da tali bobine. Saranno menzionate e discusse alcune possibilità di superare questi problemi.*

In a meeting that is focussing attention on both the technical aspects of obtaining relaxation time enhanced proton images, and the interpretation of the biological information contained in such data, it may seem irrelevant to spend time on high resolution human NMR. To take this view, however, would be to ignore the complementary nature of the two experiments from a biological standpoint, and understimate the relevance that technical developments in one field have on the other. This presentation will introduce a few of the experimental details of the high resolution "metabolic" experiment, with particular emphasis on the problems of signal detection and localisation.

The fundamental problem of metabolic NMR is sensitivity. The most promising nuclei for study are $^{13}C$ and $^{31}P$ and, when one considers the product of inherent NMR sensitivity, metabolic concentration, and, in the case of $^{13}C$, isotopic abundance, it is immediately apparent that the optimisation of signal–to–noise ratio is of utmost importance. Unlike the imaging experiment, it is not feasible to obtain recognisable signal from a few cubic millimetres of tissue within a human. Instead, one requires several cubic centimetres of sample, and even then, close magnetic coupling between sample and receveir coil is a prerequisite. In the majority of applications, this coupling is optimised by using a surface coil [1], which is simply one or more turns of wire, tuned in the conventional manner, and placed over the region of interest.

To understand both the advantages and disadvantages of this system, consider Fig. 1 which illustrates the magnetic flux pattern around a circular coil which has current flowing in it. The strength of the magnetic field at some point in space is determined by the closeness of the lines of flux. This field strength determines, not only the magnitude of the $B_1$ r.f. field when the coil is being used as a transmitter, but also the sensitivity

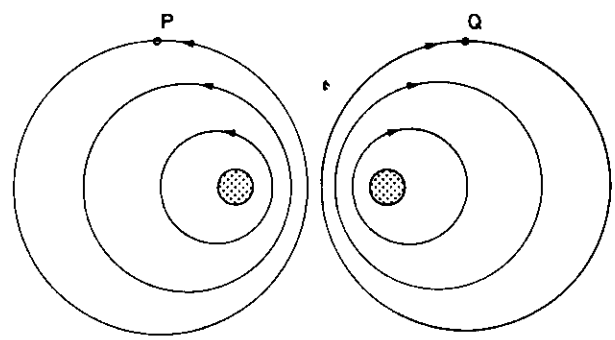


**Fig. 1 – The lines of magnetic flux generated by current flowing in a surface coil.**

of the coil when it is used to receive the NMR signal. It will be seen that a surface coil is most sensitive to signal coming from positions close to the plane of the coil, but will also detect appreciable signal from regions some distance from the coil. This is the feature which makes the surface coil so useful for examining tissue that is situated on, or near to the surface of a much larger sample.

The NMR experiment, however, is a complicated event, and it is necessary to draw attention to a few significant points which can be ignored when dealing with the more conventional saddle–shaped and solenoidal coils, but which have a profound effect on the operation of the surface coil.

If the same coil is used to transmit the $B_1$ r.f. irradiation, then the strength and direction of this field will vary throughout the sample. The strength variation can be used to advantage. If one is examining tissue that is situated below the surface of the subject, then the pulse length can be chosen so that the sample close to the coil experiences a 180°pulse, and thus contributes little or no signal to the resulting free–induction decay.

The effect of field orientation can be understood by considering points P and Q in Fig. 1. If this picture represents the plane at right angles to the static magnetic field $B_0$, then signals will be obtained from these points. (The opposite direction of the lines of flux at these two points is of no consequence since the same coil is being used for both transmit and receive functions). If, however, we look at the equivalent points to P and Q in the plane parallel to $B_0$ the situation is now different because the transmitter field has no component which is orthogonal to the $B_0$ field, and thus no free induction decay (FID) signal is generated at these points.

To summarise, the *sensitive region* of a coil depends on the $B_1$ field strength, its orientation with respect to the $B_0$ field, and the pulsing conditions used. In the case of a simple surface coil, this region extends into the sample to a depth of about 1.3 $r$ ($r$ being the radius of the coil), and is approximately oval in shape when viewed from above the coil.

Although the single surface coil is routinely used for "pulse and collect" experiments, the inherent inhomogeneity of the associated $B_1$ field can prove problematic when measuring relaxation times. $T_1$ measurements are possible with such a coil, but spin–echo experiments for the determination of $T_2$ are hopelessly inaccurate. In this case it is usual to use a surface coil to optimise the received signal, but employ a separate coil for the transmitter. Under these conditions, a further variable has to be considered, namely the relative orientation of receiver and transmitter flux lines throughout the sensitive region. This can be appreciated by again considering the points P and Q in Fig. 1. If the transmitter field is unidirectional and the surface coil is used only as a receiver, the spins at P and Q will be in phase after the transmitter pulse, but generate out–of–phase signals in the receiver coil due to the direction of the receiver coil flux lines. Thus the sensitive volume of a surface coil has a different shape when the coil is used with a separate transmitter coil. Having discussed some of the features and idiosyncracies of the surface coil, let us turn our attention to spatial localisation. As the tissue of interest is usually surrounded by dissimilar tissue, one needs to collect unambiguous spectra from a well defined volume within a larger heterogeneous sample. If the tissue is situated very close to the surface, as, for example, is skeletal muscle and brain, then the surface coil itself may offer the necessary localisation. This method is routinely used in both animal and human studies, but a more sophisticated approach is required for the investigation of most other organs. There are three possible approaches to this problem of localisation.

The first is the "field profiling" method developed by Oxford Research Systems under the name of Topical Magnetic Resonance [2]. Axial shim coils are incorporated in the bore of the magnet, and produce a $B_0$ field profile consisting of a homogeneous region close to the magnet centre which is surrounded by a region of very poor homogeneity. Sample situated in the inhomogeneous $B_0$ field will give very broad spectral lines, and these can be separated from the narrow lines which originate from the homogeneous region.

The second possible method of localisation exploits the inherent inhomogeneity of the $B_1$ field generated by the surface coil. Pulse sequences can be employed which select a region on the basis of the tip angle experienced by the sample. One example has beeen proposed by Waterton *et al.* [3]. This experiment employs a repeated r.f. pulse plus homospoil sequence which saturates spins at all locations except where the pulse is 180°. By terminating the sequence with a 90°pulse, signal is received only from this region. More recently, Bendall and Gordon [4] have proposed a "depth pulse" in which a sequence of nominal 180°pulses in a modified spin–echo experiment select a region for examination.

Both the field profiling and selective pulse approaches are wasteful because only part of the available signal is used. Although the lost signal may not be of prime interest, it could well contain information helpful in recognising the region of interest. For this reason, spectrally resolved imaging techniques may well prove to be the best methods of localisation. As mentioned earlier, it is not feasible to obtain picture quality images of, say, phosphorus–containing metabolites, but very crude spatial localisation of high resolution spectra is possible using most of the imaging techniques. Several example appear in the literature including image reconstruction [5], sensitive point [6,7], 2DFT [8,9], and rotating frame [10] methods. Although this approach, may, at first sight, seem to solve all the problems of localisation, there is a premium to be paid in terms of signal–to–noise ratio which will vary according to the method chosen. A further consideration concerns the geometry of the selected volume elements, and here the choice may be dictated by the local anatomy.

Most of the experiments in this category have only been performed on phantoms, and perhaps the most exciting technical development in metabolic NMR will be the refinements of such methods to enable their use for routine human examination.

## REFERENCES


1. ACKERMAN, J. J. H., GROVE, T. H., WONG, G. G., GADIAN, D. & RADDA, G. K. 1980. Mapping of metabolites in whole animals by $^{31}$P NMR using surface coils. *Nature (London).* **283**: 167-170.

2. GORDON, R. E., HANLEY, P. E., SHAW, D., GADIAN, D. G., RADDA, G. K., STYLES, P., BORE, P. & CHAN, L. 1980. Localization of metabolites in animals using $^{31}$P Topical Magnetic Resonance. *Nature (London).* **287**: 736-738.

3. WATERTON, J. C., GADIAN, D. G. & RADDA, G. K. 1982. UK patent No. 8225330.

4. BENDALL, M. R. & GORDON, R. E. 1983. Depth and refocusing pulses designed for multipulse NMR with surface coils. *J. Magn. Reson.* **53** : 365-368.

5. BENDEL, P., LAI, C. & LAUTERBUR, P. 1980. $^{31}$P spectroscopic zeugmatography of phosphorus metabolites. *J. Magn. Reson.* **38**: 343-356.

6. BOTTOMLEY, P. A., 1982. Localized NMR. Spectroscopy by the sensitive point method. *J. Magn. Reson.* **50**: 335-338.

7. SCOTT, K. N., BROOKER, H. R., FITZSIMMONS, J. R., BENNET, H. F. & MICK, R. C. 1982. Spatial localization of $^{31}$P Nuclear Magnetic Resonance signal by the sensitive point method. *J. Magn. Reson.* **50**: 339-344.

8. BROWN, T. R., KINCAID, B. M. & UGURBIL, K. 1982. NMR chemical shift imaging in three dimensions. *Proc. Natl. Acad. Sci. USA.* **79**: 3523-3526.

9. MAUDSLEY, A. A., HILAL, S. K., PERMAN, W. H. & SIMON, H. E. 1983. Spatially resolved high resolution spectroscopy by "four-dimensional" NMR. *J. Magn. Reson.* **51**: 147-152.

10. COX, S. J. & STYLES, P. 1980. Towards biochemical imaging. *J. Magn. Reson.* **40**: 209-212.

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# SPATIAL LOCALIZATION OF HIGH RESOLUTION SPECTRA AND RELAXATION TIMES USING A ROTATING FRAME IMAGING TECHNIQUE

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Summary. – *A method for obtaining one–dimensional distributions of high resolution $^{31}P$ spectra in spatially inhomogeneous samples is presented. The method uses the $B_1$ field gradient of a single surface coil for performing a rotating frame imaging experiment. Advantages and limits of this method are discussed and further improvements for obtaining relaxation times are described.*

Riassunto. – *Viene descritto un metodo per ottenere distribuzioni monodimensionali di spettri $^{31}P$ NMR in alta risoluzione in campioni spazialmente disomogenei. Il metodo utilizza il gradiente di campo generato da una singola bobina superficiale, per eseguire un esperimento di* imaging *nel riferimento ruotante. Vengono discussi i vantaggi ed i limiti di questo metodo e vengono inoltre descritti gli sviluppi ulteriori necessari per ottenere misure di tempi di rilassamento magnetici nucleari.*

## Introduction

For NMR-imaging experiments utilizing nuclei, such as $^{31}P$ or $^{13}C$, it is essential to retain chemical shift information. That is the reason why only a few imaging methods described for protons are useful for those nuclei, as well, for example, the sensitive point method [1], projection-reconstruction [2, 3] for $^{31}P$– or $^{13}C$–imaging and Fourier transform methods using rapidly switched magnetic field gradients [4,5]. Although the surface coil and the topical magnetic resonance provide spatially resolved high resolution spectra, they are not imaging methods [6,7]. It has been demonstrated that rotating frame imaging [8] provides spatially resolved high resolution spectra [9]. Recently we demonstrated that one–dimensional rotating frame imaging can be performed using a single surface coil [10]. The aim of this report is to discuss the properties and limits of rotating frame imaging using surface coils and to show how relaxation times data can be obtained.

## Theoretical description

In rotating frame imaging experiments one applies a radiofrequency field ($B_1$) gradient for spatial localization and a homogeneous static magnetic field ($B_0$) for preserving chemical shift information. A set of high resolution spectra, each acquired with a different pulse length $t_x$ of the $B_1$ field gradient, is obtained. The intensity of each spectral component varies with the pulse duration $t_x$. At a point x with the $B_1$ field strength $B_{1x}$ the variation is sinusoidal with the frequency:

$$F_{1x} = \gamma B_{1x} \qquad (1)$$

where $\gamma$ is the gyromagnetic ratio of the observed nucleus. If the set of spectra is Fourier–transformed at each chemical shift value with respect to $t_x$, a two–dimensional plot will be obtained with one–dimensional spatial resolution for each chemical shift value.

Rotating frame imaging with surface coils employs the natural $B_1$ gradient of this coil for performing the experiment. The $B_1$ gradient of a circular surface coil can be calculated using the generated magnetic field distribution by unit current flowing in a circular loop [11]. We have calculated the $B_1$ field distribution in a x,y–plane orthogonal to the z–axis for a coil plane oriented parallel to the $B_0$ field (z–axis). Fig. 1 shows the $B_1$ field strength in the x,y–plane through the surface coil center. The strong $B_1$ field close to the coil wires falls off rapidly within distances of about 0.4 $a$ around the wire ($a$ = coil radius). In the region covered by the coil, at distances between 0.2 $a$ and 1.5 $a$ there is a nearly linear $B_1$ gradient orthogonal to the coil plane. Therefore in this region it is possible to perform the one–dimensional rotating frame imaging experiment. The $B_1$ field plot clearly shows that strong $B_1$ gradients parallel to the coil plane are also present in regions not covered by the coil. Such gradients can cause difficulties to the application of this method in animals [12], because different points in a sample with the same

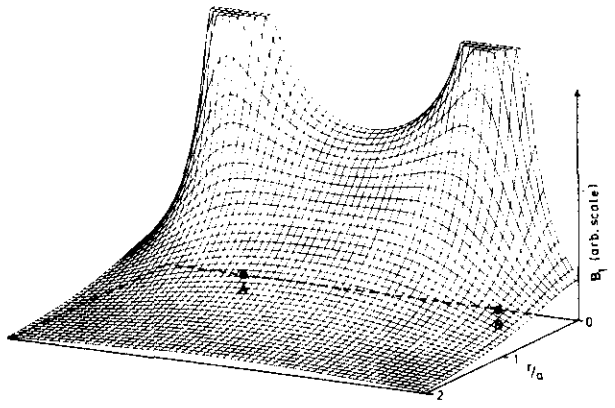
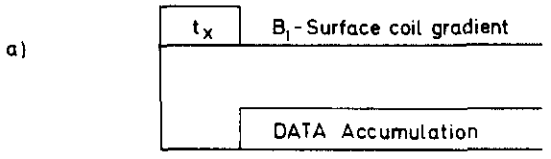



Fig. 1 – Plot of $B_1$ field in a x,y–plane orthogonal to the coil plane and $B_0$ through the center of the coil. The coil wire is indicated at points A, B. The coil radius is $a$. The calculations are only performed at distances more than $0.2\,a$ from the coil wires.

$B_1$ field strenght but with different $B_1$ gradient directions, cannot be distinguished by methods using a single surface coil.

## Experimental methods

The experiments were performed with a 4.2 T magnet, using a spectrometer interfaced to a Nicolet 1180 293A computer with two dimensional Fourier transform routines. A 2.2 cm diameter surface coil (two turns of 0.5 mm insulated copper wire) was tuned to the $^{31}P$ frequency (73.836 MHz). For the relaxation time measurements an additional Helmholtz coil is required, which is oriented orthogonal to the surface coil. The r.f. pulses transmitted by the surface coil were incremented by 20 $\mu$s in 32 steps from 0 to 620 $\mu$s. The 90°pulse length from the Helmholtz coil was 140 $\mu$s. The




b) DAT.File($t_1$,$t_2$) —FT,TD→ DAT.File($F_2$,$t_1$) —FT,TD→ DAT.File($F_1$,$F_2$)

Fig. 2 – a) Timing of a one–dimensional rotating frame imaging experiment. $t_X$ is the pulse lenght of the transmitted $B_1$ field gradient. b) Data processing, $t_2$ = unit time of the FID; $F_1$, $F_2$ = frequencies after Fourier transform; FT = Fourier transform; TD = transposition of two–dimensional data files; DAT. File = two–dimensional datafile.

sweep width was ± 500 Hz with quadrature detection and automatic baseline correction. The delay between each scan was 5 $T_1$, except for the relaxation time measurements. The series of 32 free induction decay signals (FID) was Fourier- tranformed and phase corrected using the spectrum with the shortest nonzero pulse duration for determination of phasing parameters. The spectra were transposed, zero filled and Fourier–transformed with respect to the pulse length $t_X$. The timing of the experiments and data processing are summarized in Fig. 2.

The phantoms were composed of glass bulbs (4 mm diameter) filled with specified solutions at concentrations of about 200 mM. The bulbs were immersed in physiologic saline (150 mM NaCl). Standard reagents of analytical grade were obtained from Fisions Chemical Co. Ltd. ATP and phosphocreatine (PC) were obtained from Sigma Chemical Co.

## Results

The initial experiment was designed to measure the actual $B_1$ gradient of the surface coil in a direction orthogonal to the coil plane. A single glass bulb filled with inorganic phosphate was positioned on different points on the axis of the coil. The frequency $F_{1X}$ was measured after performing the experiment with the described rotating frame imaging procedure. $B_{1X}$ was obtained from (1). The results are summarized in Fig. 3 and are compared with the theoretical $B_{1X}$ variation in dependance on the distance to the coil. As the results indicate, the $B_1$ gradient can be predicted by the theory.

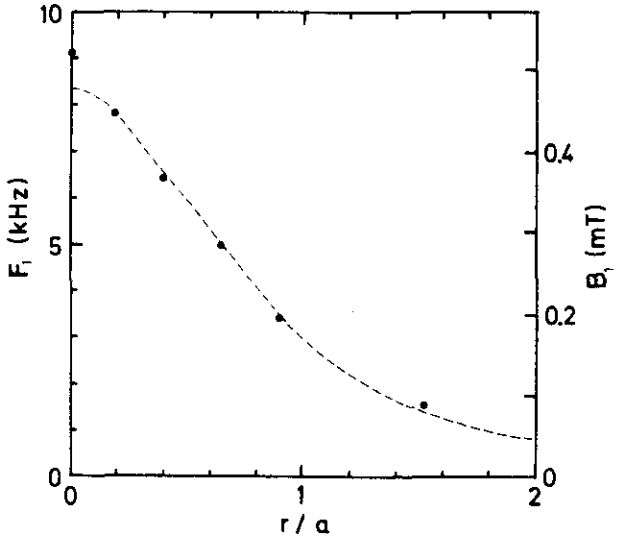



Fig. 3 – Theoretical and experimental plot of $B_1$ as a function of distance $r$ on axis from the plane of the coil.
$a$ = coil radius.

The second experiment was designed to test the spatial resolution on the axis of the $B_1$ field and the preser-

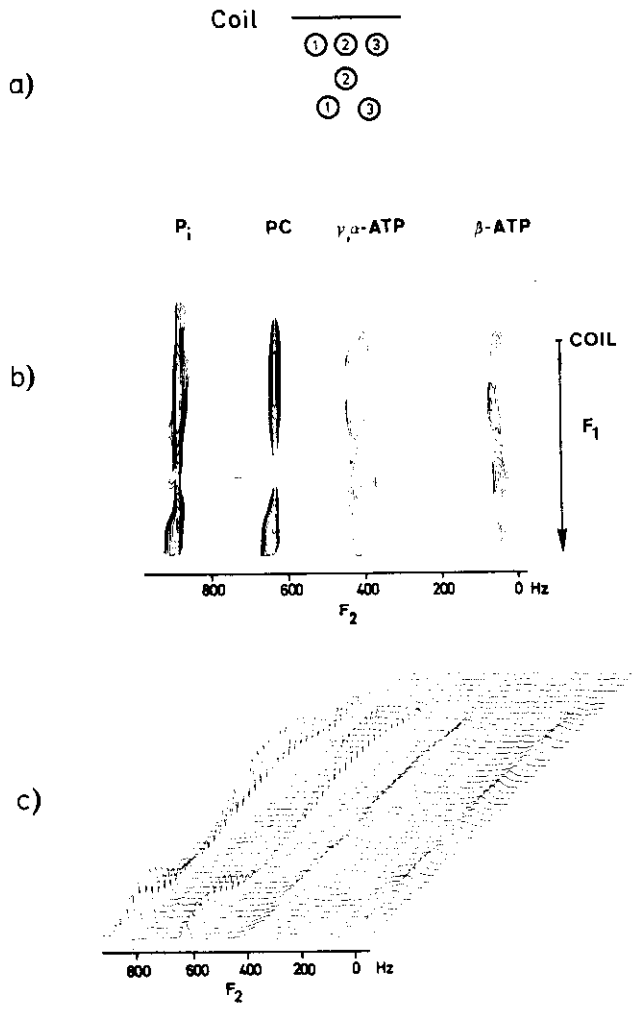



Fig. 4 – a) Diagram describing the phantom. The bulbs are separated by 1.5 mm between each other. The first row of bulbs is situated at distances of 2 mm from the coil plane. 1 = phosphocreatine (PC); 2 = ATP; 3 = inorganic phosphate ($P_i$). b) Intensity contour plot of the imaging experiment. Abscissa is the chemical shift axis $F_2$, ordinate is the $F_1$ axis. This axis is not scaled due to the problems associated with the used computer software. c) Stacked plot of the imaging experiment.

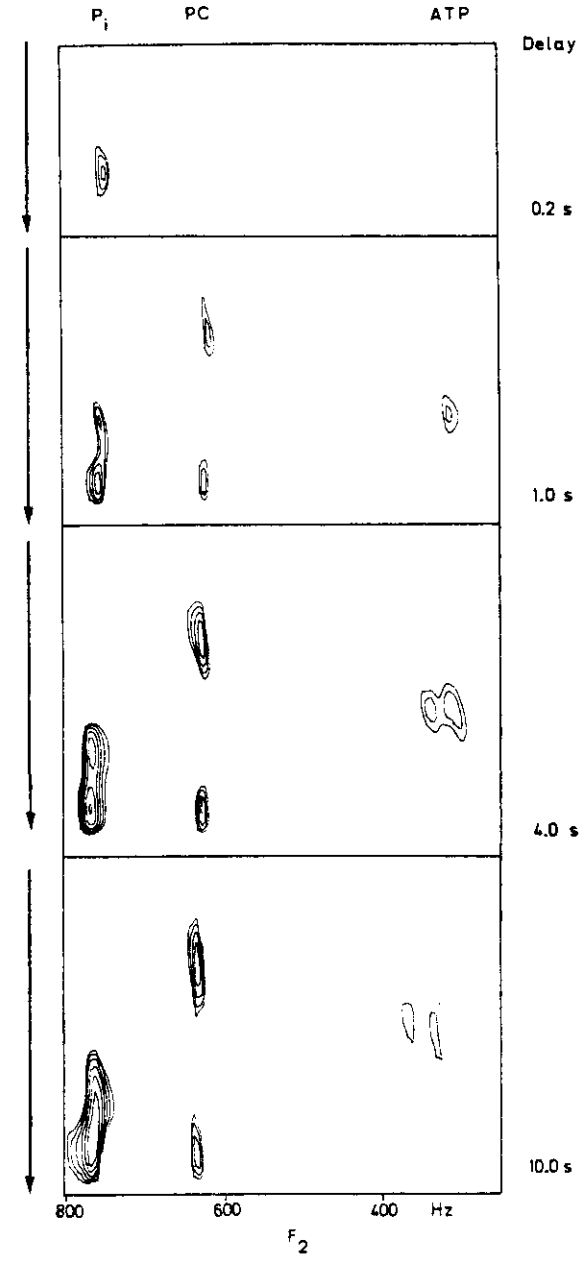



Fig. 5 – Saturation recovery experiment on a phantom composed of 3 bulbs with bulb 1 (closed to the coil) containing PC; bulb 2 containing ATP (shown are only γ–, α–ATP) and bulb 3 containing $P_i$. The delays DE between the pulses are indicated in the figure.

vation of the chemical shift information in complex solutions. The phantom was composed of 6 bulbs containing ATP, phosphocreatine and inorganic phosphate ($P_i$), as indicated in Fig. 4. The whole phantom was covered by the coil. The results in Fig. 4 show that the first 3 bulbs in one line produce spectra which are located in two-dimensional plots at nearly the same frequency $F_{1X}$. This demonstrates that in a region covered by the coil, a one dimensional image can be obtained. The chemical shift information is preserved and the spectral lines are not broadened. The signal intensity declines with distance from the coil, as the intensity is dependant on $B_1$. The third experiment was designed to test the possibility of relaxation time measurements by this method. The phantom was composed of 3 bulbes each containing pure solution of $P_i$, ATP and PC. The $T_1$ saturation recovery experiment with this method requires the following pulse program:

$$(t_x{}^{0°} - 90^{90°} - \text{Acq} - \text{DE} - t_x{}^{0°} - 90^{270°} - \text{Acq} - \text{DE})_n$$

where DE is the delay time between the experiments. The intensity of the sum of the 2 FID in the pulse train is dependant on $t_x$. The effect of the 90° pulse is that the magnetization lies in the "x', y'-plane" of the rotating frame, while the z-component is zero. The 90° pulse is produced by the Helmholtz coil. The results are shown as intensity contour plots in Fig. 5. The different species show different relaxation behaviours, with the longest saturation recovery for $P_i$.

## Discussion

The experiments prove that 1) the imaging procedure preserves high resolution spectra and chemical shift information. 2) the one–dimensional spatial resolution is at least 0.15 $a$ ($a$ = coil radius), 3) only small one–dimensional image distortions are obtained in the region covered by the coil, 4) the $B_1$ gradient of the surface coil can be theoretically predicted and therefore 5) the one–dimensional image can be correctly scaled. The rotating frame imaging procedure has an excellent S/N property per measuring time of Fourier transform methods, as for each scan the signal is obtained from the whole volume. The surface coil has the advantage of selecting a region of interest in a whole body and also provides an extremely good filling factor. The rotating frame imaging using surface coils combines the advantages of both methods for producing one–dimensional spatially resolved high resolution spectra.

There are however limitations of the method when a single surface coil is used for biological applications. As shown in Fig. 1, the coil produces $B_1$ gradients orthogonal to the coil plane in regions covered by the coil and $B_1$ gradients parallel to the coil plane in regions lateral next to the coil. By performing the imaging procedure, one obtains one–dimensional images from different regions and those images are mixed together. With one surface coil there is no way to separate those images. One possibility could be that of selecting one region by a homogeneous magnetic field and destroy the images from the other regions by inhomogeneous fields.Another possibility is to build a probe with 2 orthogonal coils and perform two–dimensional rotating frame imaging experiment. The third experiment shows that saturation recovery $T_1$ experiments using the rotating frame imaging procedure, can be performed. No image distorsions with different delays between the pulses are obtained. This means that the imaging time can be dramatically reduced in some cases, because it is not necessary as in the first two experiments, to wait $5T_1$ between the pulses. The problem here is, and it was not entirely solved in those experiments, that the 90° pulse must be transmitted to the whole sample. This requires a homogeneous Helmholtz coil. If this problem can be solved, then it will be possible to perform Carr- Purcell pulse train with 180° pulses transmitted by the Helmholtz coil.

The conclusion is that one–dimensional spatially resolved high resolution spectra can be obtained using a single surface coil from regions covered by the coil, presuming that the other regions are located in inhomogeneous magnetic fields. This report is preliminary and we are working on a two–dimensional application of this method on perfused organs of laboratory animals.

*Acknowledgements*

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## REFERENCES

1. BOTTOMLEY, P. 1982. Localized NMR spectroscopy by the sensitive point method. *J. Magn. Reson.* 50: 335-338.

2. BENDEL, P., LAI, C.M. & LAUTEBUR, P.C. 1980. $^{31}$P spectroscopic zeugmatography of phosphorus metabolites. *J. Magn. Reson.* 38: 343-356.

3. HALL, L. D. & SUKUMAR, S. 1982. Chemical microscopy using a high resolution NMR spectrometer. A combination of tomography/spectroscopy using either $^{1}$H or $^{13}$C. *J. Magn. Reson.* 50: 161-164.

4. BROWN, T. R., KINCAID, B. M. & UGURBIL, K. 1982. NMR chemical shift imaging in three dimensions. *Proc. Natl. Acad. Sci. USA.* 79: 3523-3526.

5. MAUDSLEY, A. A., HILAL, S. K., PERMAN, W. H. & SIMON, H. E. 1983. Spatially resolved high resolution spectroscopy by "Four Dimensional NMR". *J. Magn. Reson.* 51:147-152.

6. ACKERMAN, J, J. H., GROVE, T. H., WONG, G. G., GADIAN, D. G. & RADDA, G. K. 1980. Mapping of metabolites in whole animals by $^{31}$P NMR using surface coils. *Nature (London).* 283: 167-170.

7. GORDON, R. E., HANLEY, P., SHAW, D., GADIAN, D. G., RADDA, G. K., STYLES, P., BORE, P. I. & CHAN, L. 1980. Localization of metabolites in animals using $^{31}$P topical magnetic resonance. *Nature (London).* 287: 736-738.

8. HOULT, D. 1979. Rotating frame zeugmatography. *J. Magn. Reson.* **33**: 183-197.

9. COX, S. & STYLES, P. 1980. Towards biochemical imaging. *J. Magn. Reson.* **40**: 209-212.

10. HAASE, A., MALLOY, C. & RADDA, G. K. 1983. One dimensional spatial localization of high resolution spectra with a surface coil. ENC Meeting Asilomar.

11. SMYTHE, W. R. 1968. *Static and dynamic electricity*. 3rd edn. McGraw-Hill, New York, p. 291.

12. MALLOY, C., HAASE, A. Unpublished results.

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**Discussion on the interaction of measurements with localization**
*Moderator:* P. Styles

STYLES – To summarize, the purpose of this session was to try and explore areas of common ground between the imagers, and those who are trying to do high resolution NMR. It could be argued that what we've been talking about has gone off at a bit of a tangent from the main direction of this meeting, but hopefully some of the things that we've talked about will generate cross-disciplinary interest. Has anyone got anything they'd like to say about that?

SAUZADE – I tried to have a more sophisticated form of coil to have a more uniform radio-frequency field, because you can use, for example, two opposite coils or something like that.

STYLES – Are we talking now about the two-dimensional imaging experiments? I think the philosophy is that, because sensitivity is such a problem, you are almost bound to use a surface coil. This seems to give the best filling factor as Dr. Haase was saying. You are stuck with the gradients with that coil, but as the two-dimensional experiment that Dr. Haase has described relies on gradients, it's a way of getting round the problem. This method then becomes particularly appropriate for that coil structure.

PODO – Dr. Styles, would you please comment on the future possibilities of using imaging techniques for obtaining metabolic information on tissues? What kind of experimental restrictions do we encounter today in the attempt of using NMR imaging approaches for obtaining spectroscopic information *in vivo?*

STYLES – The fundamental problem, I think, is that by NMR imaging you can only get very, very crude spatial resolution. If you plug in the numbers for the sensitivity, as I think David Taylor was saying yesterday, the resolution you can expect is very crude. The advantages of doing an imaging experiment is that you get signals from the whole of the sample at once, and therefore you are not throwing anything away. That can usefully help you in identifying exactly where your tissue is. But there's bound to be some cost to pay in signal–to–noise by doing this experiment, and I think it's a question of finding the method which has the smallest premium to pay in terms of sensitivity. I think Dr. Haase might have something to say about this, because I think he's done some calculations about the signal–to–noise in his experiment.

HAASE – The signal-to-noise of one volume element in a rotating – frame imaging experiment is the same as for a high resolution spectrum of the whole tissue divided by the number of volume elements.

STYLES – The only other thing to be said in answer to your question, Dr. Podo, is that, because we haven't got any sensitivity to play with we are not going to produce real pictures from the high resolution image . We've got just to take very crude blocks of tissue and try and localize them by, perhaps, using an imaging technique. The question that we've got to address ourselves to is what sort of geometrical resolution is going to give us the most useful biochemical information? The surface coil is good for taking slices through the sample, and that might be the best approach - it might give us the most useful biological information or physiological information. But of course if other methods are used, then perhaps rectangular blocks or concentric spheres might be more appropriate, or other shapes might be optimal for certain methods. So it is open to a lot of discussion at the moment as to which is the best way to localise, not only in terms of doing an NMR experiment, but also in terms of getting something useful to enable you to recognize which part of your sample you are actually looking at.

DERBYSHIRE – These are quite early days, so do you have any feeling for how close your coil designs are to the optimum? Can we look for, say, an order of magnitude improvement?

STYLES – You can certainly look for it. But other things have been tried, different geometries, and as yet nothing has worked as well as the surface coil.

DERBYSHIRE – This was almost the first design that was tried, wasn't it?

STYLES – That's right. But certainly if anybody has got any ideas, we'll be very pleased to hear about them. For now I can't see any dramatic improvements, although I'm open to any suggestions.

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*PROBLEMS OF CHARACTERIZATION OF TISSUES AND BIOLOGICAL MATERIALS, USING STANDARD TECHNIQUES*

# MECHANISMS OF RELAXATION FOR PROTONS IN BIOLOGICAL SYSTEMS

M. VILLA

*Dipartimento di Fisica "A. Volta", Università degli Studi, Pavia, Italy*

Summary. – *The proton NMR response of a tissue has been for a long time identified with the NMR response of its water molecules. Experimental evidence is presented which points to the shortcomings of this assumption and to the importance of the water protons/macromolecular protons interactions. The contributions of different mechanisms to proton relaxation in the muscolar tissue are estimated. It is here argued that it is very difficult to reach unambiguous and quantitative conclusions about the dynamic of water in biological systems on the basis of NMR data alone. The NMR response of water in tissues is usually determined by the "interactions" with the macromolecules and the relaxation rates of a water proton will be higher the higher is its probability of being at a macromolecular surface. Diffusive processes average out the differences in relaxation rates of protons in many cases. In other cases, such an averaging process cannot be completed during the measuring time; a non-exponential relaxation is observed and NMR provides a rather indirect information on the "macroscopic" structure of the biological system.*

Riassunto. – *La risposta* $^1$*H NMR di un tessuto è stata per lungo tempo identificata con la risposta NMR delle sue molecole d'acqua. Vengono qui presentate evidenze sperimentali che indicano le limitazioni di questa assunzione e sottolineano l'importanza delle interazioni tra protoni dell'acqua e protoni delle macromolecole. Vengono altresì stimati i contributi di diversi meccanismi ai processi di rilassamento protonico in tessuti molecolari. Viene sostenuta la conclusione che è assai difficile raggiungere valutazioni non ambigue e quantitative sullo stato dinamico dell'acqua in sistemi biologici, sulla base dei soli dati NMR. La risposta NMR dell'acqua in tessuti è generalmente determinata dalle sue "interazioni" con le macromolecole e le* rates *di rilassamento protonico dell'acqua sono tanto più elevate quanto più elevata è la probabilità che essa si trovi a contatto con le superficie macromolecolari. In molti casi i processi di diffusione mediano a zero le differenze nelle* rates *di rilassamento protonico. In altri casi i processi che mediano tali differenze non sono completi nell'intervallo di tempo della misura. Allora il rilassamento risulta non esponenziale e la risonanza magnetica nucleare fornisce informazioni indirette sulla struttura "macroscopica" del sistema biologico.*

## Introduction

For nearly twenty years, it was believed that a simple relationship existed between microscopic parameters of water mobility and the NMR response of protons in biological systems. Models have been proposed which envisage two or more aqueous fractions that exchange rapidly on the time scale of the NMR relaxation times. However, the feeling that these models are essentially a parameterization of the data is supported by the observation that they can accomodate a fraction of *bound* water ranging from less than 0.1% [1] to 10% [2]. On the other hand, a number of papers have called attention to the role of cross-relaxation and spin diffusion in determining the NMR response of protons in biological systems [3–6]. This contrasts with the assumptions made by most phase models. Furthermore, it has been suggested [7,8] that proton exchange processes between water and macromolecules, rather than the existence of different phases, may account for the non-exponentiality of the spin-spin relaxation processes. Here, we would like to convey our belief that phase models have not helped our basic understanding of water dynamics and its interactions with the macromolecules and that we need new and methodologically different approaches. In the following, an overview is given of the experimental work, which clarifies the role of physically different phenomena (such as cross-relaxation, diffusion, chemical exchange) and of the theoretical efforts which attempt to avoid the shortcomings of the phase models.

## Experimental observations

It was demonstrated in 1970 [9] that the major spin-lattice relaxation mechanism of protons in water/protein

mixtures is the intermolecular dipolar interaction between the protons of the macromolecules and the water protons. Several years later, this fact was rediscovered and shown to be rather general [3–6]. Two problems arise, namely i) the characterization of the chemical nature of the magnetic "sinks", i.e. the fast relaxing protons and ii) the mechanisms of transfer of the magnetization between the sinks and the majority of protons.

Concerning the first question, it seems likely that the magnetic sinks belong to mobile portions of the macromolecules, such as methyl groups [6]. It can be ruled out that sinks belong to groups with an appreciable proton exchange rate. In this case, it would be difficult to explain the insensitivity to deuteration of the proton spin–lattice relaxation process. Although some guess can be made, we have no direct information about density and efficiency of these sinks in natural tissues.

Concerning the second question, it is likely that nuclear magnetization is predominantly transferred between proteins and neighboring water molecules *via* spin exchange and then conveyed to the "bulk" water mostly through a self–diffusion mechanism. However, Eisenstadt [6] has presented some evidence that spin diffusion within the water phase is not entirely negligible in the process of equilibration of the magnetization due to the macromolecular protons.

It has been assumed for long time that the proton signal of the macromolecular component of most tissues has a free induction decay (FID) which vanishes in the microsecond region, while the remaining FID is entirely due to water protons. Accordingly, failure of obtaining the complete disappearence of the narrow proton signal after careful deuteration has been interpreted in terms of a "non–exchangeable water fraction" [1]. However, Fung [7] has estimated that 6–8% of the narrow signal is due to protons in mobile organic molecules or segments. By accurately calibrating the spectrometer, we have found that, after subtraction of the microsecond fraction, the intensity of the proton signal in about 50 human skeletal muscles equals that of a water sample with a weight of 90 ± 5% the muscle weight.

A possible cause of the markedly non–exponential spin–spin recovery of protons in deuterated muscles [9–10] is the increased relative weight of the mobile organic segments. Interesting discussions about the role of the protein signal and proton exchange processes in determining the non–exponentiality of the spin–spin recovery have been given by Fung [7, 8]. While the complexity of this matter is certainly great, the question of the non–exponentiality of the spin–spin recovery is likely to play a central role in the field of diagnostic applications of proton NMR. In fact, it is a very common observation that details of the spin–spin relaxation process are affected by physiopathological conditions and sample handling procedures. For this reason it appears desiderable to determine, with the aid of suitable model systems, the extent to which the non–exponentiality is controlled by slow exchange processes of water molecules in different compartments and by the dynamics of a fraction of the macromolecular protons.

## Theoretical models

While most phase models assign to each water molecules a characteristic correlation time, it is conceivable that a single molecule undergoes motions taking place in different time scales. For example, water molecules close to a substrate may undergo a weakly anisotropic reorientation [11–13]. A complete averaging of the intramolecular dipolar interaction requires that the water molecule diffuses between regions where the substrates have different orientations with respect to the magnetic field. In this case, the long correlation time does not signal the existence of a "strong" interaction with the substrate but reflects the "size" of the macromolecular segments. A rough evaluation of this correlation time ($\tau$) is made through the equation $\tau = l^2/D$, where the water self–diffusion coefficient, D, is of the order of $10^{-5}$ cm$^2$/s for most tissues and the "size" of the regions over which averaging is incomplete, l, depends upon geometrical details and is likely to be $l \simeq 10^{-7} - 10^{-5}$ cm. This simple reasoning predicts that the spectrum of correlation times for protons (and deuterons) in tissues has a broad component around $10^{-7}$ s or that $T_1$ shows a dispersion around the MHz region.

Another situation where geometrical effects play an important role in the NMR response is that of diffusion in a heterogeneous system with magnetic sinks [14]. Recently, Cohen and Mendelson [15] have shown how the non–exponential recovery of nuclear magnetization contains information about geometrical structures which are "macroscopic" with respect to the range of the NMR perturbing Hamiltonian. A multiple–phase type of behaviour may arise even in the absence of a distribution of correlation times. If the magnetic sink is identified with a small (i. e., "invisible") fraction of fast relaxing protons, the Cohen and Mendelson's model becomes a typical two–phase description [1] for the state of water, in which an estimate for the exchange times between phases is provided. However, the discussion of the previous Section indicates that magnetic sinks for protons exist which cannot be identified with a fast–relaxing water fraction.

The previously discussed models apply to proton relaxation as well as to relaxation of quadrupole–perturbed nuclei. Recently, it has been pointed out that intermolecular nuclear interactions may produce "low–dimensionality effects" in the NMR response of heterogeneous systems [13]. These effects arise from the fact that in confined spaces molecules keep "memory" of their relative positions over times much longer than their correlation times. It is believed that these effects enhance the role of the interaction between macromolecular and water protons and accounts for the fact that protons and deuterons relaxation rates in biological systems often do not scale with the square of the perturbing Hamiltonians [1,10,13].

## Conclusion

By its own nature, the NMR response of water in biological systems represent an "average" over the measuring time, t, or, if one prefers, over domains of size of the order of $(Dt)^{1/2}$. The efforts of describing such an average in terms of well–characterized phases proved inconclusive or downright misleading. While the interactions responsible for the relaxation times have a range comparable with that of chemical bonds, many features of the NMR response reflect the way in which the "average" takes place, which in turn depends essentially upon "macroscopic" geometric features. It is possible to modify, to a certain extent, the "measuring time" and perform the average over domains of different size. In some casese, it is possible to make an estimate of the extent of the macroscopic inhomogeneities [16].

Some experimental evidence [7,8,10] that the proton NMR response of biological system is sensitive to the interaction between aqueous and macromolecular protons put into a new perspective the problem of relating $^1H$ relaxation rates and the physicochemical state of the macromolecules. In other words, it appears more correct, and more general, to say that a change in the state of the macromolecules modifies the distribution of the magnetic sinks rather than using a description in terms of "structured water". I hope that analyses of relatively simple model system will attain a microscopic description of the magnetic sinks and of the mechanisms through which their distribution is modified.

In summary, while we lack any detailed understanding of the relationship between state of a tissue and its proton NMR response, we are slowly learning how to avoid the pitfalls of early interpretations and how to ask questions to which a unambiguous experimental response can be found.

## REFERENCES


1. FOSTER, K. R., RESING, H. A. & GARROWAY, A. N. 1976. Bounds in "bonded water": transverse nuclear magnetic resonance relaxation in barnacle muscle. *Science.* **194**: 324-326.

2. CHANG, D. C. & HAZLEWOOD, C. F. 1975. Comments on PMR studies of tissue water. *J. Magn. Reson.* **18**: 550-554.

3. FUNG, B. M. & McGAUGHY, T. W. 1980. Cross relaxation in hydrated collagen. *J. Magn. Reson.* **39** : 413-420.

4. EDZES, H. T. & SAMULSKI, E. T. 1977. Cross relaxation and spin diffusion in the proton NMR of hydrated collagen. *Nature (London).* **265** : 521-523.

5. FUNG, B. M. 1977. Proton and deuteron relaxation of muscle water over wide ranges of resonance frequencies. *Biophys. J.* **18**: 235-239.

6. EISENSTADT, M. 1981. NMR relaxation of protein and water protons in methemoglobin solutions. *Biophys. J.* **33**: 469-474.

7. FUNG, B. M. 1977. Carbon-13 and proton magnetic resonance of mouse muscle. *Biophys. J.* **19**: 315-319.

8. FUNG, B. M. & PUON, P. S. 1981. Nuclear magnetic resonance transverse relaxation in muscle water. *Biophys. J.* **33**: 27-38.

9. NOACK, F. & KIMMICH, R. 1970. Zur Deutung der kernmagnetischen Relaxation in Proteinloosungen. *Z. Naturforsch.* **25a**: 1680-1684.

10. BORGHI, L., DE AMBROSIS, A., ALDROVANDI, S. & VILLA, M. 1983. NMR of water in biological systems. II: comparison with the experiments. *Nuovo Cimento D* (in press).

11. WOESSNER, D. E. 1977. Nuclear magnetic relaxation and structure in aqueous heterogeneous systems. *Molec. Phys.* **34** : 899-920.

12. WALMSLEY, R. H. & SHPORER, M. 1978. Surface–induced NMR line splittings and augmented relaxation rates in water. *J. Chem. Phys.* **68** : 2584-2590.

13. VILLA, M., BORGHI, L., DE AMBROSIS, A. & ALDROVANDI, S. 1983. NMR of water in biological systems. I: theoretical considerations. *Nuovo Cimento D* (in press).

14. BROWNSTEIN, K. R. 1980. Diffusion as an explanation of observed NMR behavior of water absorbed on wood. *J. Magn. Reson.* **40** : 505-510.

15. COHEN, M. H. & MENDELSON, K. S. 1982. Nuclear magnetic resonance and the internal geometry of sedimentary rocks. *J. Appl. Phys.* **53**: 1127-1134.

16. BORGHI, L., SAVOLDI, F., SCELSI, R. & VILLA, M. 1983. Nuclear magnetic resonance response of protons in normal and pathological human muscles. *J. Appl. Neurol.* (in press).

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# FACTORS INFLUENCING THE VALUES OF THE EXPERIMENTALLY OBTAINED RELAXATION RATES OF TISSUES

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Summary. – *The $T_1$ and $T_2$ magnetisation recovery c.q. decay curves are never in principle single exponential. The following causes of multi-exponential relaxation behaviour are discussed: 1) cross-correlation, cross-relaxation and exchange effects; 2) several fractions with different relaxation rates contribute to the NMR signal. Their influence on the observed relaxation rates is indicated. It is shown that the amplitude of the r.f. field, delay times, distances between the π pulses in the Carr-Purcell (CP) or Carr-Purcell-Gill-Meiboom (CPGM) $T_2$ pulse train, and inhomogeneities in the r.f. and static magnetic field effect the observed relaxation rates.*

*A suggestion is given about the way in which relaxation data should be determined and reported, in order to make comparisons between data from different laboratories more meaningful.*

Riassunto. – *Le misure di rilassamento longitudinale ($T_1$) e trasversale ($T_2$) non sono, in linea di principio, degli esponenziali singoli. Vengono qui discusse le seguenti cause di rilassamento multi-esponenziale: 1) cross-correlazione, cross-rilassamento ed effetti di scambio; 2) diverse frazioni con diverso tempo di rilassamento contribuiscono al segnale NMR. L'influenza di questi fattori sulle "rates" di rilassamento viene qui indicata e discussa. Si dimostra che le "rates" di rilassamento osservate dipendono dai seguenti parametri: intensità del campo r.f., tempi di ritardo tra gli impulsi, distanze tra gli impulsi π nelle sequenze di Carr-Purcell e Carr-Purcell - Gill-Meiboom, disomogeneità nel campo magnetico statico e a radiofrequenza.*

*Vengono proposti metodi e criteri per determinare e riportare i dati di rilassamento, al fine di poter stabilire confronti significativi tra i dati ottenuti in diversi laboratori.*

## Introduction

Damadian [1] originally showed that normal and neoplastic tissue may be discriminated by the fact that the latter has larger proton magnetic relaxation times. This result was later on affirmed [2] by many investigators by means of *in vitro* and *in vivo* NMR experiments on tissue. The relaxation times $T_1$ and $T_2$ can give information about the dynamic behaviour and the structure of tissue water, and about interactions between water and cellular macromolecules. Changes in these phenomena were the basis for the proposed explanations of the differences in relaxation rates between normal and malignant tissues [2]. The basic cause(s) for these differences is still unknown and debatable. To get more insight into this problem and (still more important) into the potential of NMR relaxation times to discriminate between normal and malignant tissues, it is important to have a lot of experimental relaxation rates available which can be compared with each other. This can hardly be done with the data available from the literature, because different experimental conditions were used. The experimental relaxation rates strongly depend on these conditions and the way in which $T_1$ and $T_2$ are defined, as will be discussed in this paper. Some standard procedure for measuring relaxation rates in biological tissue should therefore be introduced.

## I. Some causes of non exponential relaxation and their influence on the experimental relaxation rates

The $T_1$ and $T_2$ magnetisation recovery, c.q. decay curves of biological tissue in principle never are single exponential, so a $T_1$ or $T_2$ value is not defined in this case. Unambiguous parameters characterising the relaxation have therefore to be defined. Suggestions concerning this point will be given in section III. Some causes of multi-exponential relaxation behaviour are:

### *1) Different submagnetisations*

Several isolated submagnetisations $M_i$, with relaxation times $T_i$ contribute to the NMR signal. The relaxation of the sum magnetisation is given by

$$M(t) = \sum_i M_i(t) \sim \sum_i C_i \, e^{-t/T_i} \qquad (1)$$

$T_i = T_{1i}, T_{2i}, M(t)$ may be the longitudinal or transverse magnetisation

*2) Cross relaxation*

If two or more magnetically inequivalent spins relax each other, their magnetisations are coupled and a multi-exponential relaxation results in general. For two spins A and B follows

$$\dot{M}_{zA} = \frac{(M_o - M_z)_A}{T_{1A}} + \frac{(M_o - M_z)_B}{T_{AB}} \quad (2)$$

$M_o$ is the equilibrium magnetisation, $T_{AB}$ is the cross relaxation time constant. An analogous equation for $\dot{M}_{zB}$ holds.

The solution of equation (2) is given by

$$M_{zA}(t) = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} \quad (3)$$

$C_1$ and $C_2$ depend on the preparation of the spin system at time $t = 0$. Moreover, $C_1$, $C_2$, $\lambda_1$ and $\lambda_2$ depend on $M_{oA}$, $M_{oB}$, $T_{1A}$, $T_{1B}$, $T_{AB}$ and $T_{BA}$. Depending on the experimental conditions, the relative values of $M_{oA}$ and $M_{oB}$, and the relative values of $T_{1A}$, $T_{1B}$, $T_{AB}$ and $T_{BA}$ the relaxation behaviour is more or less double-exponential; in special cases it is purely single-exponential. If more than two inequivalent spins are involved, the relaxation curve will be a superposition of more than two exponential functions of time.

*3) Cross-correlation*

If two equivalent spin $\frac{1}{2}$ nuclei are mainly relaxed by mutual dipolar interactions, their relaxation is single-exponential. If more than two (in)equivalent spins are involved the relaxation may become multi-exponential, because the correlated motions of the relaxation vectors prevent the interactions between the spins to be added as independent pairwise interactions.

*4) Chemical exchange*

This problem is very analogous to the cross-relaxation problem. Exchange between two regions A and B (longitudinal relaxation times in the absence of exchange $T_{1A}$ and $T_{1B}$, exchange rate from A to B and B to A $\tau_A^{-1}$, $\tau_B^{-1}$ resp.) can be described by the equation

$$\dot{M}_{zA} = \frac{(M_o - M_z)_A}{T_{1A}} - \frac{M_{zA}}{\tau_A} + \frac{M_{zB}}{\tau_B} \quad (4)$$

and an analogous equation for $\dot{M}_{zB}$. As in the case of eq. (3) the solution is double-exponential. The degree of double-exponentiality depends, besides the factors mentioned after eq. (3), also on the relative values of $T_{1A}$, $T_{1B}$, $\tau_A$ and $\tau_B$. In the equations for the transverse relaxation corresponding with eq. (4) terms enter depending on the chemical shift difference, $\Delta\omega_{AB}$, between sites A and B. From the solution of these equations it appears that the double-exponential character of the relaxation depends on the factors mentioned in relation with the longitudinal relaxation, on $\Delta\omega_{AB}$, and in a Carr-Purcell-Gill-Meiboom (CPGM) $T_2$ spin-echo experiment also on the ratio of the exchange rate and the separation between the $\pi$ pulses. When exchange takes place between more than two sites, more than two exponentials are needed to describe the relaxation.

The theory concerning the subjects mentioned in 2), 3) and 4) can be found in standard NMR text books [3, 4]. It can be estimated that for proton relaxation in biological tissue non-exponential relaxation due to cross-correlation (item 3) above) can be neglected. Items 1), 2) and 4) (in particular item 4 in the slow exchange region) give rise to multi-exponential relaxation. In the way in which the relaxation times are usually measured (see section II) one gets a kind of average relaxation time "$T_1$", which depends on the amplitudes $C_i$ and rate constants $\lambda_i$ of the exponential functions of time involved. As the $C_i$ are determined by the initial conditions (the preparation of the spin system by a perturbing pulse at time zero), "$T_1$" strongly depends on the way in which the experiment is performed. To give an example which might occur in pratice: the "$T_1$" value is determined with the initial rate approximation (IRA) method of section II, exchange takes place between sites A and B: $M_{oA} = M_{oB}$; $T_{1A} = T_{1B} = 1$ s, $\tau_A = \tau_B = 0.1$s. If in the 180-$\tau$-90 $T_1$ program spins A are selectively inverted by the $\pi$ pulse a "$T_{1A}$" value of 0.09 s is found, while if A and B are both inverted one finds "$T_{1A}$" = "$T_{1B}$" = 1 s. The initial conditions are determined by r.f. inhomogeneities, r.f. offset, and r.f. field strength in relaxation to the spectral width. The latter might seem not to be a problem in practice, as the tissue proton spectrum seldom exceeds a width of 1 kHz, so the usual r.f. field strengths would be sufficient to excite the whole spectrum uniformly. In the presence of a strong field gradient (spin imaging), however, a non uniform excitation occurs, resulting in different "$T_1$" values for different parts of the sample, that ought to have the same "$T_1$" value. Moreover, 10 to 40% of the protons, which are normally not observed in the high resolution NMR spectrum, and a part of which contributes very efficiently to the relaxation (protons in macromolecules and tightly bound water) give a solid-state-like NMR spectrum with a width of 10 to 50 kHz, resulting in a non uniform excitation. R.f. amplitudes therefore may effect the experimental "$T_1$" value, and should therefore be reported together with the relaxation data.

## II. Dependence of the observed relaxation rates on the esperimental method

Before the consequences of multi-exponential relaxation on the experimental relaxation rates will be considered, some ways in which the relaxation rates are usually determined will be discussed. We confine ourselves to $T_1$ values determined by a $\pi$-$\tau$-$\pi/2$ pulse program. In cases where one number, a kind of effective $T_1$ value, is determined from the experiment, notwithstanding the fact that because of multi-exponential relaxation more time constants and relative amplitudes would have to be reported, this $T_1$ value will be denoted by "$T_1$":

a) the most simple way is the null method: "$T_1$" is determined from the time interval between the inverting $\pi$-pulse and the moment the longitudinal magnetisation $M_z$ is zero. This method is known to be very unreliable and sensitive to all kinds of errors;

b) the time "$T_1$" is determined in which the deviation of $M_z$ from its equilibrium value immediately after the disturbance decreases with a factor e, the 1/e method. This method is less sensitive to errors than the null method;

c) the initial rate approximation, IRA; the first part of the magnetisation recovery curve is approximated by one rate constant, which e.g. in the case of eq. (3) is given by $(C_1\lambda_1 + C_2\lambda_2) / (C_1 + C_2)$. This method gives only information about the first part of the magnetisation recovery curve.

d) In practice often the following method is used; a "relaxation time", "$T_1$", is determined by fitting all the experimental data points on the magnetisation recovery curve to a single exponential function of time. Such a "$T_1$" value depends on the $C_i$ and $\lambda_i$ of all the exponential terms involved. Moreover it strongly depends on the fitting method used [5] (linear or exponential, two or three parameter fit), which should therefore be mentioned too when reporting the relaxation data.

The methods a) - d) give no insight into the degree of multiexponentiality of the relaxation;

e) the best method would be to determine all the $C_i$ and $\lambda_i$ from the magnetisation recovery curve. Taking into account the (in)accuracy of NMR signal intensity measurements this is impossible in practice, unless there are only two $\lambda_i$'s differing a factor 4 or more, while $C_1 \sim C_2$. Therefore in general one of the methods c) or d) is used. It is stressed that in all the methods mentioned above (except e)) an experimental "$T_1$" value is obtained which depends on the $C_i$ and $\lambda_i$ of the several exponentials describing the relaxation.

Even if the same method (a), or b), or c), or d)), the same sample, temperature and field strength are used, different "$T_1$" values may be found on different apparatus. This is due to differences in r.f. field strength and offset effects resulting in a non uniform excitation of the different magnetisations in eqs. 1), 2) and 4), and hence in different $C_i$ values, as argued in section I. Therefore, in order to be able to compare experimental relaxation rates from different laboratories, besides temperature and field strength also the $T_1$ method, r.f. field strength, homogeneity of the r.f. field, offsets, strengths of gradients, pulse delays etc. should be reported together with the relaxation rates.

Some remarks concerning the transverse relaxation still have to be made. The interpretation of $T_2$ values of tissues is still much more uncertain and debatable than that of $T_1$ values. Moreover, the measurements are very sensitive to disturbances and experimental artifacts. Nevertheless some kind of effective $T_2$ value is needed in practice. It is in general determined by the CPGM spin-echo program. In addition to the factors discussed above in relation with $T_1$, the separation between the $\pi$ pulses in this program, $t_{cp}$, should also be reported together with the experimental $T_2$ values, because $T_2$ is very dependent on $t_{cp}$, especially in the region where $t_{cp}^{-1}$ equals about the exchange rate. Moreover, reporting the value of $t_{cp}$, the homogeneity of the static magnetic field, and the strength of field gradients is important, because diffusion effects decrease the observed $T_2$ value drastically for increasing $\tau_{cp}$ and inhomogeneities. One should be aware of these facts in the production of spin images using refocussing pulses and echos.

## III. A practical way of measuring and reporting relaxation data of biological tissue.

From sections I and II it will be clear that the best method to characterize the $T_1$ relaxation in biological tissue is method e) of section II. It is however an elaborate procedure and one does not obtain a number, some kind of effective $T_1$ value, which is a measure for the time in which $M_z$ reaches its equilibration value after a disturbance. A more practical method will be a combination of b) and c); the IRA gives a good number for the first part of the magnetisation recovery curve, where a considerable part of the magnetisation returns to equilibrium; the 1/e value gives insight in the degree of non -exponentiality, if it is compared with the IRA value.

Reporting both data, together with the experimental conditions as described in section II gives a better possibility to compare relaxation rates from different laboratories.

Analogous remarks can be made about the transverse relaxation. The best way to characterize it is by means of a CPGM $T_2$ program. From the experimental points on the magnetisation decay curve an IRA and 1/e value should be abstracted and reported together with the experimental conditions as in the case of $T_1$. The separation between the $\pi$ pulses, the inhomogeneity of the static field and the strengths of applied field gradients should also be reported.

## REFERENCES

1. DAMADIAN, R. 1971. Tumor detection by nuclear magnetic resonance. *Science.* 171: 1151-1153.

2. MATHUR-DE VRÉ, R. 1979. NMR studies of water in biological systems. *Prog. Biophys. Mol. Biol.* 35: 103-158.

3. ABRAGAM, A. 1961. *The principles of nuclear magnetism.* At the Clarendon Press, Oxford, Chapter VIII.

4. REEVES, L.W. 1975. In: *Dynamic Nuclear Magnetic Resonance Spectroscopy.* L.M. Jackman, F.A. Cotton (Eds.) Academic Press, New York, Chapter IV.

5. LEVY, G.C. & PEAT, I.R. 1975. The experimental approach to accurate carbon-13 spin-lattice relaxation measurements. *J. Magn. Reson.* 18: 500-521.

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# CAN MEASUREMENTS OF PROTON RELAXATIONS OF TISSUES TELL US ABOUT THEIR BIOENERGETICS ?

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Summary. – *It is often stated that, unlike CT scans, proton NMR images contain "chemical information". At present such statements express the hope that this is so rather than the results of detailed studies. Our understanding of the factors which affect proton $T_1$ and $T_2$ relaxation times is very limited. At low magnetic fields, such as used in the majority of imaging studies, the observed proton signal originates largely from tissue water and fats. As the two signals are not resolved the measured relaxation time will be a composite number. Thus variations in fat to water ratios in the tissue or within a given cell will influence the value derived. Cellular viscosity, metal ions, pH, metabolic state, $O_2$ constant are some of the often possible parameters that are expected to alter relaxation times. Their influence on $T_1$ and $T_2$ might be expected to be different and be field dependent. This brief outline shows that we have a long way to go before the "chemical content" of proton $T_1$ and $T_2$ images becomes extractable. Several problems are likely to be solved if we are able to do measurements at higher fields. Comparison of proton spectra and relaxation times with metabolic information obtained from high resolution spectra ($^1H$, $^{31}P$ and $^{13}C$) would provide some of the necessary insights. Measurements of $^{31}P$ NMR spectra from animals and man do give the required metabolic information. Examples on human muscle will be given to illustrate the type of information one may obtain.*

Riassunto. – *Si afferma spesso che, a differenza delle immagini TAC, le immagini ottenute mediante risonanza magnetica nucleare contengono informazioni chimiche. Al momento attuale queste affermazioni esprimono una speranza più che una certezza basata su risultati di studi dettagliati. La nostra comprensione dei fattori che influenzano i tempi di rilassamento $T_1$ e $T_2$ è in realtà assai limitata. A campi magnetici bassi, quali quelli usati nella maggioranza degli studi di tomografia NMR, il segnale protonico osservato è dovuto soprattutto all'acqua e ai grassi contenuti nel tessuto. Poiché i due segnali non sono risolti, il tempo di rilassamento misurato deriva dalla combinazione dei loro contributi rispettivi. Pertanto, variazioni nei rapporti tra contenuti di acqua e di grasso nel tessuto o all'interno di una data cellula, influenzano il valore del tempo di rilassamento misurato. Viscosità cellulare, contenuto di ioni metallici, pH, stato metabolico, e stato di ossigenazione sono solo alcuni dei parametri che possono prevedibilmente alterare i tempi di rilassamento. La loro influenza sui valori di $T_1$ e $T_2$ può essere diversa e dipendente dal campo. Queste considerazioni indicano che siamo ancora lontani dalla possibilità di estrarre informazioni chimiche dalle immagini protoniche di tipo $T_1$ o $T_2$. Molti di questi problemi saranno probabilmente risolti se si potrà lavorare a campi più alti. Il confronto tra spettri protonici, tempi di rilassamenti e informazioni metaboliche ottenute da spettri in alta risoluzione ($^1H$, $^{31}P$ e $^{13}C$) sarebbe in grado di fornire alcune interessanti delucidazioni in questo campo. Misure effettuate su spettri $^{31}P$ NMR ottenuti da animali e dall'uomo sono adatte a fornire le informazioni metaboliche richieste. Verranno presentati esempi su muscolo umano, che illustreranno il tipo di informazione che si può ottenere.*

Within the context and organisation of this meeting my contribution may be regarded by some as peripheral. The topic of this symposium is "Identification and characterisation of biological tissues by NMR"; yet almost all the papers are concerned only with measurements of proton relaxation times in tissues. Is this the appropriate way to identify and characterise biological tissues by NMR ? It is of course not, but before this is taken as a criticism of this interesting workshop, let me qualify what I have to say. Clearly the aim of this group is to evaluate to what extent proton NMR *for reasons of its clinical future as an imaging technique*, can be used to "identify and characterise biological tissues". This proposition is different from the original statement and highlights the need to define the premise on which my presentation is based.

It is often stated that, unlike CT scans, proton NMR

images contain "chemical information". I take this statement as one aspect of the present workshop and the one I wish to discuss. Indeed, tissue proton NMR contains chemical information but the crucial question is different. It must be: "Can we extract the appropriate chemical information from the tissue proton NMR ?". At low fields we measure only *one* composite proton signal, dominated by the contribution from tissue water. $T_1$ and $T_2$ relaxation times are the two parameters of this signal that we can measure. Our knowledge of how these parameters depend on tissue chemistry is very limited and in my view it is doubtful if they can be regarded as indicators of "specific" tissue chemistry in view of their multiparameter dependence. But this does not mean that proton NMR measurements cannot be used to "identify or characterise biological tissues". Identification means discrimination and is likely to follow from a combination of approaches: i) statistical evaluation of relaxation parameters; ii) comparison with other imaging techniques and iii) the judicious application of contrast techniques and chemical contrast media. Characterisation on the other hand implies "understanding". At low fields the observed proton signal originates largely from tissue water and fats. As the two signals are not resolved, the measured relaxation time will be a composite number. The two signals are easily resolved at higher fields e.g. 1 Tesla or above, provided field homogeneity is optimised. Thus some understanding can be provided by studies at higher fields and as we have done on human muscle [1]. Given that one can resolve signals from tissue water (largely intracellular in origin) it is essential to relate relaxation parameters to tissue biochemistry. The later can be studied by high resolution NMR spectroscopy of $^{1}H$, $^{31}P$ and $^{13}C$ possibly, even at the same magnetic field strength, as used for obtaining the proton image.

One biochemical parameter that affects $T_2$ is tissues oxygen resulting from the effect of the oxygenation state of haemoglobin on the $T_2$ of water protons in blood [2]. This effect is observed at higher and medium magnetic fields (80 - 470 MHz for protons). $T_2^{-1}$ depends quadratically on the field strenght and on the proportion of haemoglobin that is deoxygenated. Deoxygenation increases the volume of magnetic susceptibility within the erythrocytes and thus creates local field gradients around these cells. It was shown that the increase in $T_2^{-1}$ with increasing blood deoxygenation arises from diffusion of water through these field gradients. In contrast, at 182 MHz, $T_1$ was found to be independent of oxygenation. $T_2$ experiments can be done with spatial resolution and this way blood oxygenation of the tissue can be studied [3]. Even in the absence of a three–dimensional image, it is of interest to know that $T_2$ values of whole tissue may also reflect oxygenation levels. For example, the proton linewidth obtained from a rat kidney *in vivo* increased from 41 to 110 MHz (at 4.2 Tesla) on induction of ischaemia. Since the vascular volume of the kidney is large (~ 15 %) this change probably reflects the blood oxygenation effect.

There are many other possible "metabolic" parameters that will influence the water relaxation rates. Among these we note: ion concentrations, viscosity (related to intracellular protein concentration), intracellular pH, changes in charged metabolites that bind divalent cations and redox–states.

$^{31}P$ NMR can be used to measure some of these variable (see for example reference [4]). Although it is inappropriate to review here the results obtained by this technique it is worthwhile to mention the limits of variation in some of these metabolic parameters. For example, intracellular pH is normally around 7.0 – 7.2 but could decrease to as low as 6.0 (e. g. in skeletal muscle) in normal individuals and possibly to even lower in pathological states. Whether pH has any effect on $T_1$ and $T_2$ would be a simple study in human muscle. Ionic composition in general is fairly stable but as a large proportion of intracellular $Mg^{++}$ is complexed to ATP, degradation of the latter compound would lead to significant increases in free $Mg^{++}$ ions and possibly in some paramagnetic ions such as $Mn^{++}$. Defined studies on the time course of changes in phosphate–containing metabolites and water relaxation parameters during ischaemia and reflow could easily be done using isolated perfused organs.

Tissue redox states may well influence proton relaxation rates as they alter mitochondrial redox equilibration involving paramagnetic centres. This problem might well be investigated in a variety of hypoxic states using isolated tissues or animals. $^{31}P$ NMR can readily be used to describe the extent of hypoxia. We should also remember that in any study on animals were oxygen delivery is altered or energy demand is increased, significant changes in blood flow take place. This also effects some "relaxation images".

In conclusion, I believe that the scope for combining the information derived from NMR spectroscopy with that obtained from proton imaging has important implications for the interpretation of proton relaxation measurements in human tissue. It remains an open question whether both measurements should be done at the same single field, or the information content is enhanced by comparing low field images with high field spectroscopy.

## REFERENCES

1. TAYLOR, D. J., BORE, P. J., GADIAN, D. G. & RADDA, G. K. 1983. Bioenergetics of intact human muscle: a $^{31}P$ Nuclear Magnetic Resonance study. *Mol. Biol. Med.* (in press).

2. THULBORN, K. L., WATERTON, J. C., MATTHEWS, P. M. & RADDA, G. K. 1982. Oxygenation dependence of the transverse relaxation time of water protons in whole blood at high field. *Biochem. Biophys. Acta.* 719: 265-270.

THULBORN, K. R., WATERTON, J. C., STYLES, P. & RADDA, G. K. 1981. Rapid measurement of blood oxygenation and flow by high-field $^1$H NMR. *Biochem. Soc. Trans.* **9**: 233-234.

GADIAN, D. G. & RADDA, G. K. 1981. NMR studies of tissue metabolism. *Ann. Rev. Biochem.* **50**: 315-318.

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**Discussion on biophysical factors determining relaxation behaviour and other NMR properties in tissues**
*Moderators*: J. - M. Lhoste and W. M. M. J. Bovée

LHOSTE – Just to summarize I think we have quite a large view of the biophysical problems deriving from the heterogeneity of the living systems, as explained by Dr. Villa. Then we have learnt that, even in a homogeneous phase, relaxation is not always an exponential phenomenon.

RADDA – I thought I'd start by trying to address myself to what this meeting is about: identification and characterization of biological tissues by NMR. What does that mean to me? I'm going to do that on a specific example that we have a lot of data about: that is, skeletal muscle. First of all, identification. We've already said that identification is the same as discrimination. Now we can all take a piece of fish muscle and a piece of steak, and we can very readily identify the two. We don't actually need $T_1$ measurements to do that, but you could probably do it by $T_1$. That's simple discrimination, and in biology and in medicine quite often that's sufficient. Now characterization: when I look at that piece of fish muscle or steak, we then begin to have a rather complex looking structure, and I need to say why they are different, what it is basically that makes one red and the other one white, and how does that relate to their function? That's what I mean by characterization. So when I talk about the character of the muscle or of a piece of tissue, I consider three separate points. First of all, I need to know its composition. Without knowing composition, we don't know the character of the object that I'm looking at. Secondly I want to say something about its structure. And thirdly, in biological systems, unlike in physics, we need to consider the dynamics of the system because we are dealing with highly dynamic situations. I think one ought to consider how NMR can contribute to each of these separate problems. Let me first say something about structure, because structure is where we are worst off with respect to NMR when it comes to molecular structure, but best off when we talk about structure in terms of anatomy. The imaging people talk about anatomy, and there is no doubt that there are enormously good structural features in the NMR information at the level of anatomy, microscopic anatomy, that are well worth having in relation to medicine. Molecular structure – if you look at a muscle tissue, it has fibres, it has mitochondria, it has little deposits of glycogen, and it has enzymes, and other small molecules, and that is the sort of structure we can get from electron-microscopy. NMR isn't going to approach that in any way. It might tell us about bits of that structure – you can look at water, which is what you do with protons, and say something about water distribution, perhaps. You can look at some molecules, as we do, which are metabolically involved and then perhaps you can say something about their interactions with some components, but you are not going to approach that structure. So we have to accept that, when we talk about structure, we are only going to talk about aspects of the structure that relate to the nature of the measurement that you are going to make. I think the strongest part of NMR relates to dynamics. You can look at changes in structure, composition, flow of material and molecular motion, and all those are within our reach. Even though I may appear to make derogatory remarks (which I don't intend to) about $T_1$ and $T_2$ measurements, they are enormously valuable in telling us something about dynamics, motion, and interactions of that sort. I feel that this is where NMR has unique advantages compared to some of the other static techniques that tell us structure.

Now going on to dynamics I would just like to remind you that some of the biochemical processes that we are considering in relation to how tissues might be characterized relate to the handling of energy. Obviously that is what I'm primarily interested in. This is done by the well-known pathways of oxidative phosphorylation making ATP or the back-up reaction from phosphocreatine or by the breakdown of glycogen by anaerobic glycogenolysis producing lactate. I think that, without going through the details, we can say that NMR, and certainly phosphorus high resolution NMR, allows us to measure the concentrations of phosphocreatine, ATP, inorganic phosphate, and indirectly of lactate. It allows us to measure the fluxes in these pathways, because we can look at enzyme catalysis reaction rates. It allows us to measure how you switch from one form of metabolism to another, it allows you to measure the flux to glycogenolysis which in the end produces hydrogen ions, protons, and we know about pH, and how that pH often relates to movement of calcium, and how the whole thing is then controlled. So we begin to look at, not only composition, but flow and control, and control is a unique feature of biology where, again, if we have a non-invasive technique, we have something to say. I believe that the imagers have not given perhaps enough attention to considering some of the underlying processes that they might be able to follow, even though indirectly, and that one can study more directly by high resolution NMR methods. Let me now give you a few examples. The first thing is, if you look at proton spectra at high fields, you can see that you can well resolve the spectra from tissue water and from the fat composition. You see that a male subject will have much less fat than a female subject. You can in fact show that this is statistically significant. Yet you wouldn't dream of using that as a method of determining the sex of your subject. There's 80% certainty that you can use that method to tell whether it is a female or a male subject. But the question is, why should one want to do that with NMR when there are better ways of doing it? So what we also have to address ourselves to is, what are the types of information and the sorts of measurements we

want to do with NMR that gives us something unique that you can't do in more simple ways? In a high resolution phosphorus NMR spectrum of skeletal muscle which we can obtain in a scan time of somewhere between 10 seconds to 1 min, you can determine the chemical composition of your muscle with respect to ATP, phosphocreatine, inorganic phosphate and the pH. The variability is very, very small over a very large population in this static baseline composition of the high energy phosphates – intracellular pH is 7.03 ± 0.03 over I don't know how many hundred subjects. So in one sense this is constant, and the biological variation is little. But in fact it is not terribly useful because it doesn't depend on disease states, it's insensitive to clinical conditions, except in a few cases. For example, if we look at distrophic muscle compared to normal muscle on the proton spectra, we can see that in normal muscle we have perhaps a little fat in the muscle fibers,whereas the distrophy muscle shows an enormously high fat contribution compared to water. It is well-known that in dystrophic muscle you have fat infiltration. I think it would be extremely interesting to image these and look at the fat distribution because it is not just fat, subcutaneous fat, but it's in between fibres and I think a comparison of the high resolution information, with an image, over a wide range of conditions, would be helpful. Now if we look at the phosphorus composition, this is one condition where in fact the baseline composition is quite different. In the dystrophic muscle there is an enormous decrease in phosphocreatine. There is a new characteristic peak – we don't know what it is, but it's there. We can also see from the inorganic phosphate signal that the pH in the dystrophic muscle is very high. So if pH has any effect at all, here is a case where I think you might want to do imaging on proton relaxation time and compare it with the pH measurement that is very readily obtainable. But basically we should come back to the question of how one can increase the sensitivity of our medical diagnosis by looking at the biochemistry and the main information in NMR comes from looking at changes and dynamics of changes. Already I think there have been references to, for example, looking at changes in imaging during muscle contraction, recovery and afterwards. Here we have a technique that can tell us precisely what happens in each individual during that sort of process because during, for example, aerobic exercise of the arm, you decrease the phosphocreatine content, increase the phosphate content and decrease the intracellular pH. That occurs over a very wide range of subjects. Now we come to the problem, however, that this is a sensitive method, but it is also very sensitive to large biological variations. If we take different individuals doing the same amount of work, they will do that same amount of work at different steady state levels of phosphocreatine. They use more or less phosphocreatine to do the same work or worse still, they are going to utilize glycogen to more or less extent for the same work. Some people can go on working at practically no change in intracellular pH while other people drop their intracellular pH down to 5.9 and go on working at that pH. So you see the variability here. If I'm going to use that as a diagnostic tool, I either have to get around that problem or start understanding what causes it. Both avenues are open. It is not surprising, therefore, that your $T_1$s are variable if a simple parameter like the pH is variable from one individual to the next. What is, as far as I can see, constant yet sensitive is control. In spite of how much work people do and how their breakdown of glycogen is, I find that the amount of phosphocreatine breakdown in an exercise regime and the pH change, the onset of the pH change, fall on a very narrow range. After about 60-70% of the phosphocreatine has been broken down in an exercise - then, and only then, do normal people switch on glycogenolysis to produce lactic acid which shows up as an intracellular acidification. That is control, and that switch in normal individuals is constant. In abnormal cases you can see that switch being mucked up. So that's one of the dynamic relationships one can look at. Further, if you try and look at the rate of recovery from an exercise period, most people will fall on a relatively narrow range of rate of resynthesis of phosphocreatine with a half time of just under a minute, and that represents the rate of oxydative phosphorylation. You can use that as a sensitive index in pathological conditions for the biochemical well-being of the oxydative pathway. In the same way inorganic phosphate disappears at twice the rate by which it is incorporated into phosphocreatine and that's an interesting problem because it relates to intracellular handling of phosphate. In this simple protocol of doing exercise, looking at the response and recovery, we have studied over 200 patients in the last year and a half and can pick out very sensitively and specifically individual defects and individual enzymes in people with muscle disorders, people with mitochondria problems, those who cannot use oxygen very effectively because their electron transport chain is blocked, or those who don't use it effectively because their mitochondria are slightly uncoupled – all of those show up in a characteristic way on the NMR response, in the same way as dystrophies. The muscle is a very simple window on systemic disorders that affect all the different organs in some way and we have looked at people with renal failure, diabetes, high and low levels of thyroid hormones, etc. In some cases you can see major abnormalities, in other cases you see nothing. From other conditions we have looked at, I just want to consider cases of people with unknown muscle weaknesses, to show how one can pick out deficiencies in control. Most individuals during an exercise regime show a relationship between phosphocreatine breakdown and hydrogen ion generation that falls on a standard sort of line. There is, however, a group of patients who, during exercise, acidify enormously early and drop their intracellular pH well before they need to have done so. We have seen many patients like that with post-viral infection. The experiments are fairly simple and I think there is plenty of scope to try and look at metabolic composition, metabolic changes, and pH changes in relation to possible images and measurements of $T_1$ and $T_2$ in relatively simple systems which can be mimicked on animal models.

DERBYSHIRE – I'd like to ask Dr. Radda a question. In the other work on topical NMR there has been fair amount of work done on $^{13}C$, but you've never mentioned $^{13}C$ today. It is because it's falling into disrespect?
RADDA – No, I haven't mentioned $^{13}C$ first of all because I only had 15 minutes, and secondly because we haven't ourselves done a great deal, but there is plenty of scope to do similar types of experiments with $^{13}C$. It's well within reach.
DERBYSHIRE – What sort of information has been derived from $^{13}C$?
RADDA – Can I have another half hour? One of the most interesting aspects of $^{13}C$ as far as I can see is that you can feed the system with selected and enriched $^{13}C$ compounds, and that way you can select out and follow particular pathways. Let me take a specific example. If you take acetate which you label with $^{13}C$ on carbon 2, that goes into the pathway, partly to the citric acid cycle and partly made into amino acids. Now you can actually see from $^{13}C$ how many times a carbon atom goes around the cycle before it comes out on a sidepath of producing glutamic acid. That allows you to measure relative fluxes of different pathways, and I think that sort of thing is probably the most useful aspect of $^{13}C$.
TAYLOR – I've got two questions. One is for Dr. Bovée - whether he could indicate the relative significance of those many factors which would cause non–exponential relaxation in biological tissue. I can see that there are possibilities, but it's not obvious that all of them are significant in real tissue. The second question is, that while the suggestion of a way of characterizing relaxation behaviour sounds very nice, in practice how many images are you going to have to take to define that characterization, and is that feasible in the real world?
BOVEE – As to your first question, I thought that if you had several different submagnetizations with different $T_1$ values, they all contribute to multi–exponential relaxation, and it depends on the relative amounts of $T_1$ values how much. The cross–correlation problem will not contribute very much, and neither probably will the cross–relaxation problem. Exchange may contribute rather a lot, but it depends again on the kind of system and on the relative quantities. You can make an estimate of its contribution. You need two different magnetisations - if you have one it is no problem. You don't see these different magnetisations always in the spectrum, most times you only see one peak, but protons of macromolecules and immobilized water molecules on the one hand and protons in free water molecules on the other hand, may be considered as two different magnetizations. The relative value of the former, as I heard from Dr. Villa is 5 - 10 % and sometimes 15 - 20 % of the total proton signal. This may really influence the non–exponentiality of the relaxation in the case of slow exchange, changing the apparent relaxation rate by a factor 2, 3 or 4, depending on the r.f. field strength. Then your last question about how many separate images you need, if you are going to extract relaxation data from your spin images and you want to compare them with other people, you should have a good way of characterizing the relaxation curve. One way would be to give the initial relaxation rates and some values for longer times on the relaxation curve. In addition to that you should give all your experimental conditions: off–set, homogeneity of the r.f. field, and strengths of the r.f. field. Things like that should be mentioned in the literature. Perhaps after this conference the first step (a small step) would be if all those who were publishing relaxation data would mention these things in the future. This would be a small step, but still better it would be if everyone were to do these experiments in exactly the same way.
TAYLOR – Could I just answer that? If you deal with live subjects - I mean, to pin down that initial decay, you are going to have to do 6, 7 or 8 images and that is a real practical problem.
BOVEE – Yes, it is. But if you want to compare these data with other people and if you want to draw conclusions from them, and if I may use the word, if you want to characterize something by these data, you need standard data.
VILLA – My opinion about the problem of non–exponentiality in imaging is this: realistically the entire recovery process cannot be imaged, but the experiment *in vitro* should be performed as carefully as possible to find out in which condition, for example, there is a long tail in the relaxation process, and in which condition this tail correlates to a pathological condition. At this point a $T_2$ experiment made with a very long time can be the answer if we know from the *in vitro* experiments that a certain pathological condition creates a region in which $T_2$ is very long and the fraction of the relative amplitude of this slow relaxing portion is interesting. At this point we have to do, for example, a $T_2$ experiment with a very long interval between the exciting and refocusing pulses. So I feel that we still have to do $T_2$ maps or $T_1$ maps regularly, just as we do now. The non–exponentiality just helps to know that non– exponentiality is significant, and to choose the experimental conditions to increase contrast, but realistically one cannot now make maps of non–exponentiality factors.
LUITEN – Going to the question of how many measurements you should do to make that bi–exponentiality visible, I think you just need 2 measurements to measure the initial slope. Anything else you do is an improvement of accuracy. You might also evaluate the deviation from the exponential decay, by measuring the delay times corrisponding to 10-20 % and to 1/e of the decay, respectively. That means that three measurements are necessary, and if you do more it improves your accuracy. There's one problem which makes it more complicated because, if you do imaging, you have a lot of very different $T_1$ to be measured simultaneously and so you have to focus on what is the part of the image of interest. Is it the white or grey matter, or is it the membrane or is it the fluid? If you want to make a universal image of $T_1$, you end up with a number of measurements less appropriate to the tissue of interest and that will mainly determine your difficulties. So general $T_1$ measurements in this way will be hardly possible to perform.

BOVEE – Perhaps I may make a small comment on this. If you are willing to get good relaxation data which can be compared with literature data and you are trying to extract from two points a slope, you are getting the kind of relaxation data we have enough now and which cannot be compared. All people who are doing practical relaxation work will affirm this.

LUITEN – Is that due to, say, the inaccuracy of the measurements?

BOVEE – Yes.

LUITEN – Then that is a noise problem. That is the same problem as in imaging - what we do right now is that we just take two measurements. As a matter of fact you actually do more than two measurements because if you are imaging, you are looking at a number of points. So although you do only one measurement at each point you effectively do more because you do a correlation over an area. That improves your signal–to–noise ratio. So in one way or another you might get within the limits of the required accuracy, and here again the problem is that we do not know at the moment what accuracy of these $T_1$s you need in order to come to a diagnostic conclusion. That is of course always the vital question.

ERMANS – I would like to ask Dr. Radda if besides his biochemical studies, he has the opportunity to analyse the dynamics of blood vessels? This is a very interesting approach and I would like to know if he believes that this particular application could be important in the development of the characterization of some diseases.

RADDA – We have studied blood flow by NMR in a number of instances, and in fact the remaining seven slides that I did not show are related to that particular problem. Obviously the imaging people are able to measure blood flow as well. So there are different methods of doing that although they haven't yet all been worked out, but there are possibilities of looking at flow in relation to the rest of the biochemistry. Obviously diseases with abnormal blood vessels such as diabetes could be studied.

LHOSTE – May I just ask a question? Maybe it is possible to reconcile the spectroscopy and the $T_1$ measurements using $^{13}C$. I think $^{13}C$ is a very good nucleus if you do measurements of molecular dynamics *in vivo.*

RADDA – $^{13}C$ is a particularly difficult nucleus in many ways to cope with without spinning etc. But yes, I'm sure you could do those sorts of measurements. I think one has to take these things one step at a time. I can see $^{13}C$ as something that will catch on in the next five or so years in a bigger way. But at the moment I think that, for high resolution spectroscopy, I'd put my money on phosphorus. So do other people who are supporting me.

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# TISSUE DISCRIMINATION *IN VIVO* BY NMR IMAGING

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Summary. — *NMR imaging is a useful technique in anatomical studies but can also yield additional information on the physiological and pathological state of the tissues. Interpretation of the proton relaxation characteristics in terms of physiology depends on a full understanding of the various factors which can cause variation in relaxation time, especially where these are "operator controlled" as opposed to directly associated with the disease state. Such operator controlled, or extrinsic factors include:*

*– measurement frequency: it is widely considered that the higher the measurement frequency the better the image. There is, however, both experimental and theoretical evidence to suggest that, at least for abdominal tissues, this is not the case. Ideal operating frequencies need to be sought;*

*– measurement temperature: this is relatively unimportant in most NMR imaging studies but the temperature dependence of $T_1$ should be born in mind in imaging areas with reduced blood flow;*

*– machine variability: day to day fluctuations in the instrument can lead to erroneous values for relaxation times in NMR images as well as spectrometers. Adequate use of well designed phantoms should be used for imager quality control;*

*– pulse sequence: different pure sequences or even different timings of the same sequence can yield very different images. This fact can be used to enhance tissue contrast but care must be taken that the use of inappropriate sequences or timing does not disguise essential information.*

*Factors intrinsic to the biological subject can also affect the relaxation characteristics. These include:*

*– linearity and tissue fat content: when $T_1$ values are taken from NMR images they are often the result of single-point calculating and may ignore the possibility that the relationship between signal and tau is multi-exponential. This makes comparison between different instruments very difficult;*

*– paramagnetic effects: paramagnetic metal ions accumulating in certain tissues may decrease their normal $T_1$ values. This can be used as a sign of abonormality. It is also possible to introduce paramagnetic agents into the body to enhance contrast of certain organs or to study function of organs such as the kidney;*

*– water content: in general increasing water content increases tissue $T_1$ but the relationship is not always direct. In certain cases, e.g. fluid - filled regions such as the amniotic sac, a false result for water proton density can be obtained when certain pulse sequence timings are used;*

*– pathological state: in general a pathological change in a tissue is associated with an increase in $T_1$ value but this is not the case in those diseases where there is metal ion accumulation, e.g. spleen in haemolytic anaemia;*

*– physiological state: certain physiological states, e.g. fatigue, affect the $T_1$ value of tissues. These may cause apparent abnormalities unless fully investigated and catalogued beforehand.*

Riassunto. — *La tomografia NMR si presenta come tecnica utile per studi anatomici, ma può anche fornire informazioni addizionali sullo stato fisiologico e patologico di tessuti biologici. L'interpretazione delle caratteristiche di rilassamento protonico in termini di informazione fisiologica dipende da una piena comprensione dei vari fattori che possono causare variazioni dei tempi di rilassamento, specialmente nei casi in cui questi fattori sono "estrinseci" al sistema cioè sono "sotto il controllo dell'operatore" piuttosto che associati allo stato patologico.*

*Tra questi fattori "estrinseci" considereremo i seguenti:*

*– frequenza: generalmente si ritiene che la qualità dell'immagine sia tanto migliore quanto maggiore è la frequenza di lavoro. Tuttavia numerose evidenze sperimentali e teoriche indicano che questo non è vero, almeno nel caso dei tessuti addominali. Le condizioni ideali di frequenza debbono ancora essere individuate;*

*– temperatura: questo parametro è relativamente poco rilevante nella maggior parte degli studi di NMR imaging ma si deve tener conto della dipendenza del $T_1$ dalla temperatura nelle aree dell'immagine associate ad una riduzione della circolazione sanguigna;*

*– variabilità strumentale: fluttuazioni giornaliere dello strumento possono causare valutazioni erronee dei tempi di rilassamento sia nell'impiego di tomografi che in quello di spettrometri analitici. Un uso appropriato di oggetti campione consentirebbe un controllo della qualità della strumentazione;*

*– sequenza di impulsi: diverse sequenze di impulso e persino l'uso di intervalli temporali diversi nella stessa sequenza possono dare luogo ad immagini molto diverse. Questo fatto può essere utilizzato per aumentare il contrasto tissutale ma è necessario adottare particolari cautele onde evitare che l'uso di sequenze inappropriate possa condurre a risultati erronei;*

*Anche fattori intrinseci al soggetto biologico possono influenzare le caratteristiche di rilassamento. Tra questi fattori considereremo:*

*– la linearità del tempo $T_1$ e il contenuto di grasso nel tessuto: quando i valori di $T_1$ vengono ottenuti da immagini NMR, sono spesso il risultato di calcoli ottenuti da punti singoli e possono prescindere dalla possibilità che la relazione tra il segnale ed il tempo tau sia multiesponenziale. Ciò rende assai difficile il confronto tra strumenti diversi;*

*– effetti paramagnetici: l'accumulo di ioni metallici paramagnetici in certi tessuti può indurre un decremento nei valori normali di $T_1$. Tale decremento può essere utilizzato come segno di anormalità del tessuto. E' anche possibile introdurre agenti paramagnetici nel corpo al fine di aumentare il contrasto di alcuni organi o di studiare la funzionalità di organi come il rene;*

*– contenuto di acqua: in generale un aumento nel contenuto di acqua fa aumentare il $T_1$ del tessuto, ma la relazione tra questi due parametri non è sempre diretta. In alcuni casi, ad esempio in regioni ricche di fluidi come il sacco amniotico, si possono ottenere risultati erronei per la densità protonica, se si utilizzano valori inappropriati di intervalli temporali tra le sequenze di impulsi;*

*– stato patologico: cambiamenti patologici di un tessuto sono generalmente associati ad un aumento di $T_1$, ma ciò non si verifica nel caso di malattie in cui c'è accumulo di ioni metallici, come ad esempio nella milza nell'anemia emolitica;*

*– stato fisiologico: alcuni stati fisiologici, come la fatica, alterano il valore di $T_1$ nei tessuti. Essi possono essere causa di anomalie apparenti ed è pertanto necessario procedere ad una ricerca accurata e ad una preventiva catalogazione di questi casi.*

## Introduction

NMR proton imaging is now widely accepted as a medical imaging technique. It becomes increasingly important, therefore, to understand what is being seen, what are the limitations of the method and what it can offer which is different to the capabilities of other imaging techniques such as X-ray computed tomography (CT), isotope or ultrasonic imaging.

Like most imaging techniques, NMR proton imaging can be used solely for the purpose of visualising the internal anatomy of the body. Tissues vary in their water content and a water proton density (PD) image reflects this variation by delineating the various organs. So, in a section of the head (Fig. 1), we can see the skull quite clearly as a low PD region. The shape of the brain is visible and a ventricle within it. The general outline of structures in the nose and throat regions are seen, along with the vertebral column. We should, however, note that all of these structures are identified by two things, firstly the different proton density which produces the image and secondly our own previous knowledge of the anatomy of the head region. It is worth noting that, if presented with a single pixel from somewhere in this image, we would be fairly unlikely to be able to identify the tissue being examined.

In other parts of the body we combine our knowledge of anatomy with an image of variations in $T_1$ value. PD images of the abdomen show little difference between soft tissues but the proton relaxation times of tissues such as spleen and liver are sufficiently different to enable us to visualise the organs (Fig. 2a). Anatomical studies of this kind can be useful, particularly when looking for pathologies such as tumours which can affect the shape or size of an organ. Fig. 2b shows an NMR $T_1$ section through the abdomen of a patient with non-Hodgkin's lymphoma in which there is a grossly enlarged spleen, which is clearly visible on the image. There are, however, other imaging techniques which can demonstrate anatomical abnormalities of this type. Indeed ordinary palpation would be the simplest way of identifying a spleen as grossly enlarged as this.

What, then, can NMR imaging offer beyond this? NMR proton imaging looks at the relaxation characteristics of the protons of small, very mobile molecules, mainly water and free lipids. The amount and proportion of water and lipid varies between different tissues, as does the way in which these small molecules are associated with the larger molecules or structures of the cells. There may also be differences in content of paramagnetic materials or other factors which can affect the relaxation of the protons. The total effect is to give each tissue its own NMR proton relaxation characteristics. However, because so many factors affect proton relaxation, and because so many changes in state of the body can affect the factors we have, in NMR imaging, a very sensitive monitor of variation (both normal and pathological) in bodily state.

The sensitivity of this system can be of great medical use, but it can also, to some extent, be a disadvantage in that it may be possible to accept as abnormal a change which is merely a small variation in the normal state of the body. It is, therefore, essential that we come to understand the possibilities for normal variation in the NMR relaxation characteristics of the tissues so that we can not only discriminate between the different normal tissues but also be able to state unequivocally that a certain change is within or outside the normal variation of the body.

What I would like to do, therefore, is to examine some of the factors which can affect the $T_1$ of a normal

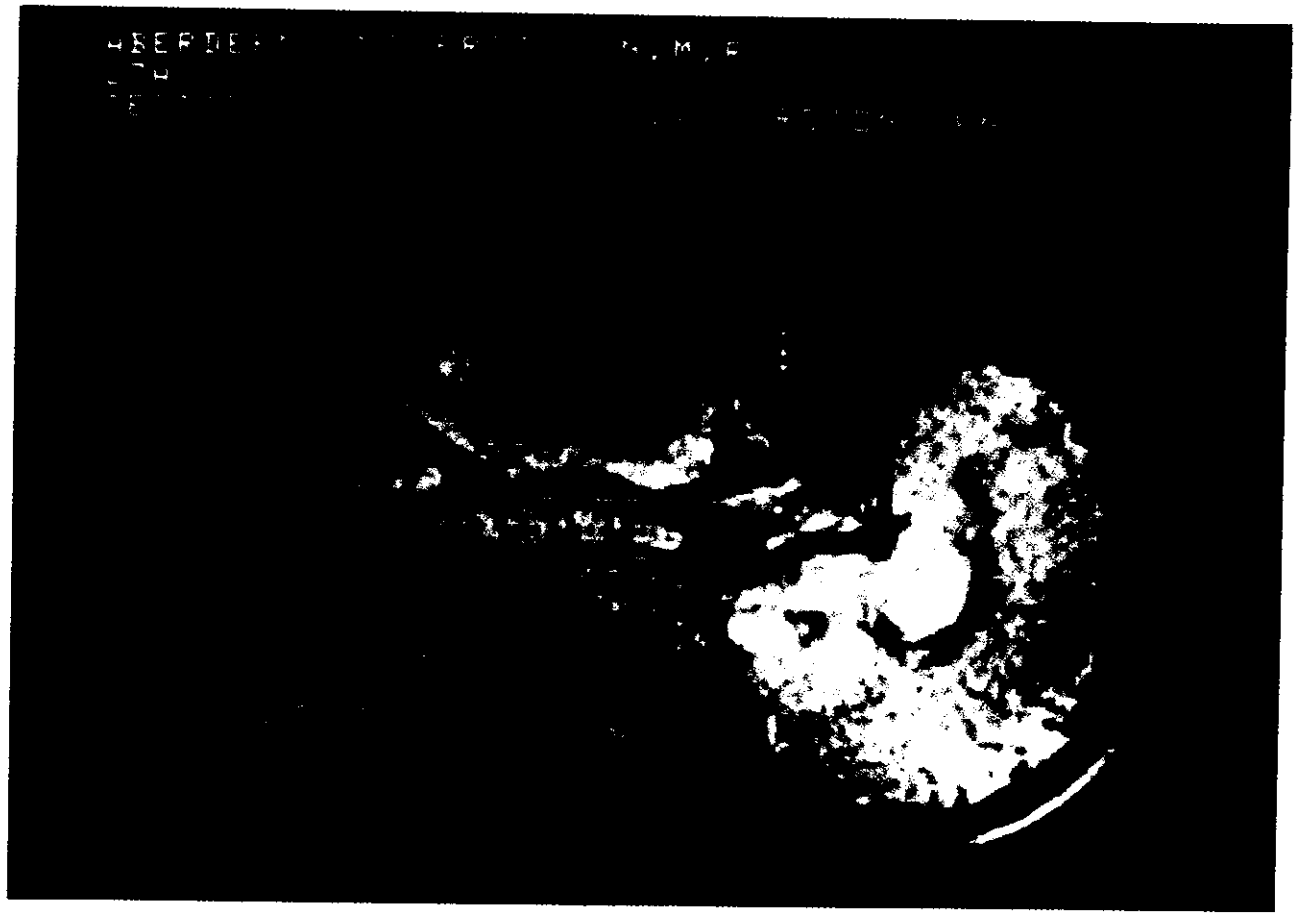
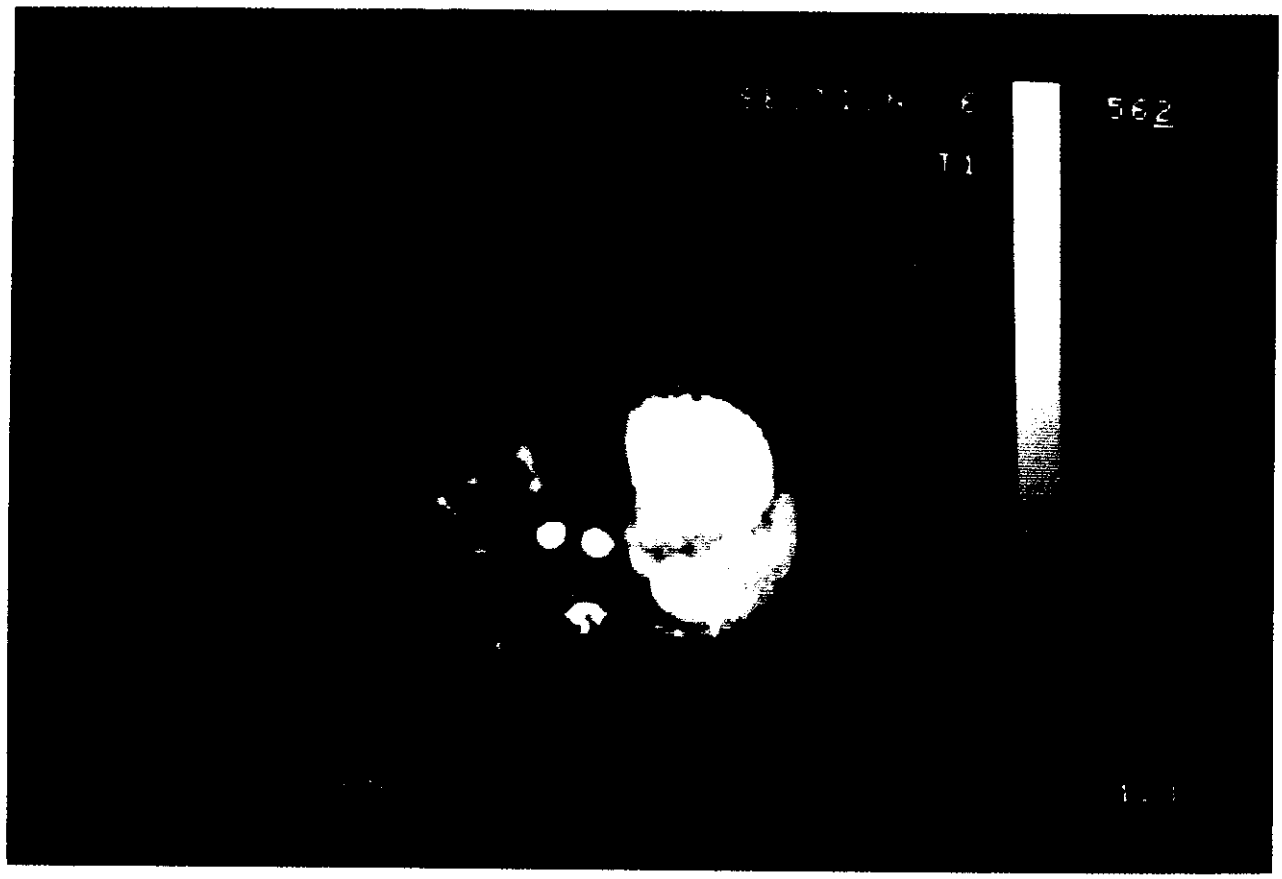
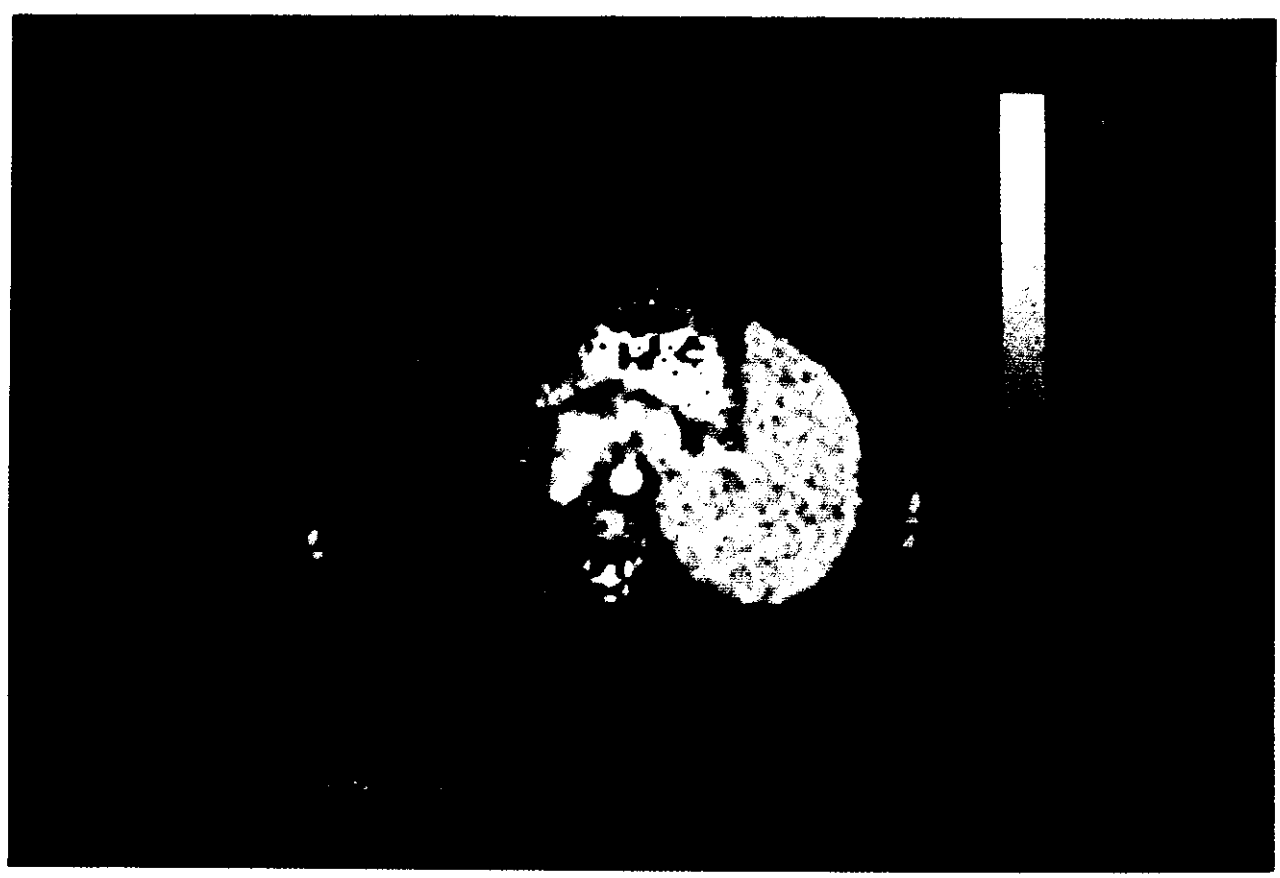


Fig. 1. — NMR proton density image through human head taken on Aberdeen Mk II imager operating at 3.4 MHz. Bony structure and outline of organs are clearly demonstrated.




(a)



(b)

Fig. 2. – Anatomy of the upper abdomen. NMR $T_1$ images of (a) normal and (b) patient with enlarged spleen associated with non-Hodgkin's lyphoma.

tissue seen *in vivo*. Some of these factors are already being covered in detail by other speakers and I will concentrate on those which generally receive less attention. It is not my intention to present an exhaustive revue, but to discuss one or two points of particular interest.

## Factors affecting measured $T_1$

Table 1 lists some of the factors which affect the $T_1$ value obtained from a particular tissue examined either *in vivo* or *in vitro*. Some of these, such as the handling technique or the time after death are essentially associated with *in vitro* examinations, whereas others, e.g. physiological state are more related to *in vivo* studies. Table

Table 1. – *Some factors affecting $T_1$ value*

| | |
|---|---|
| Extrinsic | |
| frequency | |
| temperature | *in vitro* |
| machine variability | and |
| pulse sequence | *in vivo* |
| tissue handling | *in vivo* |
| Intrinsic | |
| linearity of plot | |
| paramagnetic content | *in vitro* |
| water content | and |
| pathological state | *in vivo* |
| animal maturity | |
| temperature | *in vivo* |
| physiological state | |
| time after death | *in vitro* |

## Extrinsic factors

### *a) Measurement frequency*

The effect of measurement frequency has been mentioned by other contributors but deserves a brief mention here.

At the moment most imaging systems are operated by workers who know the importance of reporting their operating frequency. Unfortunately, however, there exist in the literature quite a number of *in vitro* NMR studies in which there is no mention of spectrometer frequency. This reduces the value of the studies almost to zero since there is no possibility of comparison of the results with those obtained by other groups. In general it is true to say "the higher the frequency, the longer the $T_1$". This is certainly true up to about 40 MHz but there may be a levelling off after that. Frequency effects, however, are not constant. When rabbit tissues are observed *in vitro* at 24 and 2.5 MHz (Table 2) it is seen

Table 2. – *In vitro $T_1$ values (rabbit)*

| | $T_1$ *(ms)* | | *Ratio* |
|---|---|---|---|
| *Tissue* | *24 MHz* | *2.5 MHz* | *24:2.5* |
| grey brain | 644 | 332 | 2.00 |
| liver | 311 | 141 | 2.22 |
| spleen | 509 | 258 | 1.98 |
| heart ventricle | 637 | 243 | 2.62 |
| thigh muscle | 554 | 182 | 3.05 |
| white brain | 469 | 264 | 1.77 |
| spinal cord | 464 | 325 | 1.43 |

that for most tissues the ratio of the $T_1$ values at these frequencies is about 2:1. For tissues high in membrane, especially the heavily myelinated white brain and spinal cord, the ratio is closer to 1.5:1, whereas for muscle it is about 3:1. This rather suggests that the ability to differentiate between certain tissues is frequency-related and the question "should we image at higher frequency" may be dependent on what we hope to see.

Although the rabbit tissues were observed *in vitro*, it is possible to see *in vivo* that tissues behave differently at different frequencies. Fig. 3 graphs some $T_1$ values obtained from NMR images of a normal individual on the two Aberdeen proton imagers, Mk I operating at 1.7 MHz and Mk II at 3.4 MHz. Fat and fatty tissue like marrow changes very little with frequency whereas tissues like kidney medulla and blood show a much greater effect. Differences seen between *in vitro* and *in vivo* effects may be related to different temperatures of the tissue as well as to the greater frequency range *in vitro*.

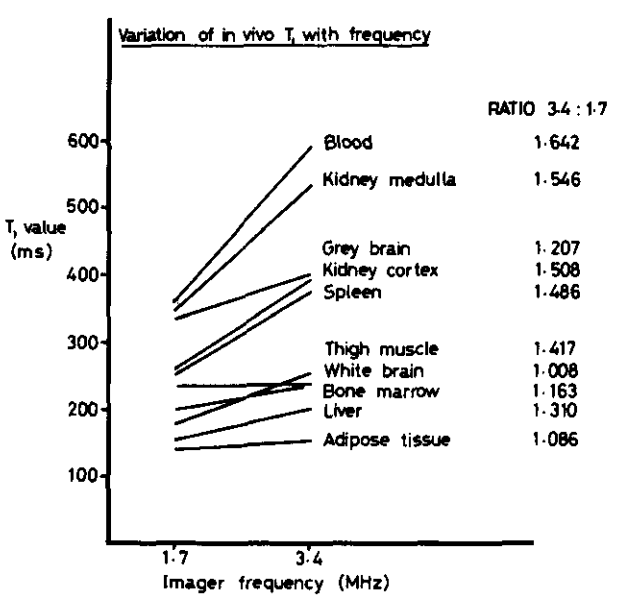



Fig. 3. – Comparison of $T_1$ relaxation time values obtained *in vivo* at 1.7 and 3.4 MHz (information obtained from Dr. L. Eastwood).

There is a much greater spread of values at the higher frequencies and it is possible, therefore, that there will be an increased ability to discriminate between tissues at this higher frequency. Comparable sections at the moment, however, look very similar in their ability to resolve abdomen structure.

One very interesting observation is the very slight frequency-dependence of the $T_1$ of white brain tissue. If this holds true over an even greater frequency range it suggests that high fields (within safety limits) would improve any aspect of brain imaging which involves white and grey tissue differentiation. On the other hand there may be little, if anything, to be gained by the use of high fields in general body imaging.

*b) Measurement temperature*

Temperature affects viscosity which in turn affects the correlation time. The correlation time of the water protons governs their NMR relaxation rate. Temperature, therefore, is a very important factor in determining the $T_1$ value obtained from a particular sample. Fig. 4 shows a graph of $T_1$ relaxation time of a solution of manganese chloride measured at 1.7 MHz at different temperatures. There is a fairly linear trend over the range observed, with a rise of approximately 2% in $T_1$ per degree centigrade. Samples of muscle or fat cooled to different temperatures show very different relaxation characteristics in the imager.

Temperature effects are, obviously, of major importance to anyone undertaking relaxation studies *in vitro*, and one of the things most commonly omitted from the description of method. It is, perhaps, of less importance to workers *in vivo* since the human body has remarkable homeostatic mechanisms and a very narrow range of operating temperature. There exists however, the possibility of examining hypothermic patients and particularly patients with circulatory defects which affect temperature in a region of the body such as a limb. $T_1$ measurement in such a region could be deceptive.

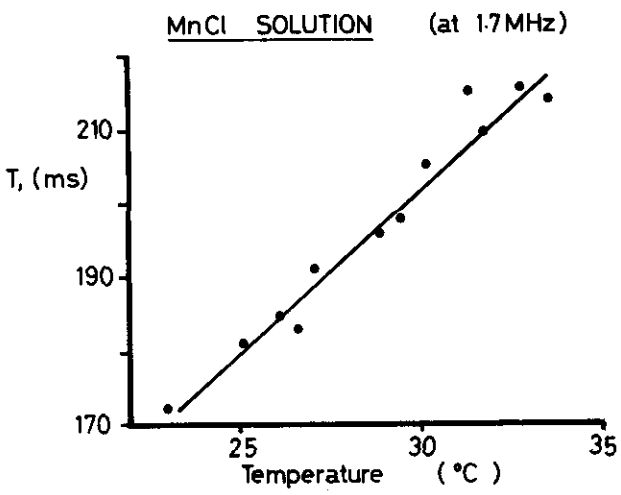



Fig. 4. - Variation in $T_1$ relaxation time with temperature of a solution of manganese chloride in water (information from Dr. McRobbie).

*c) Machine variability*

This is really a problem which has to be faced individually by each imaging and *in vitro* study group since the problems will be slightly different for each machine. Day-to-day variations will have to be checked, presumably by use of samples of material which is much simpler than a body tissue. The absolute accuracy of the relaxation times must also be checked from time to time since all machines are capable of drift from the initial tuning.

One of the major sources of error in the measurement of $T_1$ values, especially where single point measurement is being made, is related to the accuracy of the r.f. pulses. In $T_1$ measurement, for example, any slight inaccuracy in setting up the 180° pulse will give a shorter $T_1$ relaxation time since the inversion will always be less than the 180°. This is not important for a full plot $T_1$ if the error is constant for each measurement taken to make the plot but it does affect $T_{null}$ values considerably. There are many problems which can lead to variation in the pulse angle other than the simplest one of an inaccuracy in frequency or power of the r.f. pulse itself. Inhomogeneity in the magnet, if large, can mean that different parts of the sample have different Larmor frequencies, hence the inversion angle can differ over the sample volume - this is very important in imaging. Any disturbance in the field during measurement, e.g. due to movement of metallic objects in the vicinity of the magnet, will have a considerable effect on the field and hence the precession frequency. Changes can also occur due to field drift associated with, for example, heating of the magnet.

Drift effects will be reflected even in full plot $T_1$ measurement, although they can be reduced by collecting the values in a "nonlogical" order, e.g. tau intervals of 10 ms then 180 ms, then 70 ms, etc. If there is drift it will be much closer to the "true" value than if the points had been taken consecutively. Another way of reducing this error, the way in use in the Aberdeen imager, is by means of adiabatic fast passage to produce the spin inversion. A normal 180° pulse is delivered at the Larmor frequency of the protons, in a very short time and at right angles to the spin orientation. It inverts the spin orientation round its own axis. The adiabatic fast passage pulse is delivered more slowly and in the same plane as the initial spin orientation. It sweeps from below to just above the Larmor frequency and as it does so it effectively picks up the spin population and pulls it over to 180°. It is a longer pulse of lower power and hence is much more controllable, and also it covers a range of frequencies and so automatically compensates for small field inhomogeneities or drifts. It is, therefore, a much more "forgiving" pulse than the standard 180° pulse.

In discussing machine variability and performance it is also important to consider phantom design for imagers. This, however, is the subject of other papers in these Proceedings, so I will not cover it here.

*d) Pulse sequence*

Several other papers in these Proceedings discuss the effects of pulse sequence on the measured relaxation times so I will not attempt to enter this field in any depth. There is, however, one factor which I would like to mention, which is the pulse repetition frequency.

The $T_1$ range of normal tissues at 1.7 MHz is from 150 to 350 ms and hence the majority of the protons will have several times $T_1$ to relax back before the next 90° pulse is applied. In certain cases, however, $T_1$ is considerably longer, as for example the fluid of a fluid-filled cyst or the amniotic fluid in the pregnant uterus. In these cases, when the $T_1$ of the fluid is over 500 ms, the signals obtained from pulses delivered at one second intervals will be reduced in size because of incomplete relaxation between the pulses. The Aberdeen imaging system, for example, uses a two-part pulse sequence to obtain $T_1$ information. At first a 90° pulse is applied with appropriate gradients and gradient changes. This provides mainly proton density information. The pulse sequence is then repeated but in this instance it is preceded by an adiabatic fast passage r.f. pulse which turns the spins through 180°. The 90° pulse sequence follows after a 200 ms relaxation period, so providing $T_1$ information when the signal from the sequences with or without the 180° inversion are compared. The interval between successive 90° pulses in one second, hence the interval for relaxation between a 90° pulse and the AFP pulse of the next sequence is only 800 ms.

If, however, there is reduction in signal due to incomplete relaxation in the second between the pulse sequences then there is considerable abnormality in the apparent results. For example there may or may not be sufficient signal to allow calculation of the $T_1$ value of a particular pixel. Where sufficient signal does exist the long $T_1$ value of the fluid will give a white pixel but if the signal is small it will show, effectively, as no $T_1$, i.e. a black pixel, so the fluid region will appear as a mixed-up black and white area where image interpretation will be very difficult (Fig. 5a). This same phenomenon can, however, be very useful in certain cases. For example, if a proton density image is obtained for the section shown in Fig. 5a, because of incomplete relaxation, the apparent proton density is very low so the fluid appears black (Fig. 5). Foetal tissue, however, has a high water content and long $T_1$ (but not long enough to upset its measurement) so we see the foetus extremely clearly floating in a pool of "black" fluid. The apparent effect is, therefore, to increase the contrast and make the foetal structure much more obvious.

*e) Tissue handling*

This is mainly a problem for *in vitro* studies, but it should always be born in mind that it may be possible to affect the water content of a tissue by excessive blotting after excision, or to alter the size or type of water compartments by excessively harsh handling of tissue pieces. The ideal would always be to handle the tissues as little as possible prior to *in vitro* relaxation measurement. When examining very soft tissues such as parts of the brain or bone marrow our own technique is to use a sample tube which is open at both ends, one end being just cut glass which has not been flamed smooth. This end is drilled into the tissue in the same way as a cork borer, so removing a core of the tissue with relatively little damage. The tube is then "drilled" into a thin piece of paraffin wax which seals the end, preventing water loss or tissue dropping out during measurement. The $T_1$ of the wax protons is too short to interfere with the tissue $T_1$ measurement.

Many experiments in Aberdeen have shown that tissue is remarkably hardy. Even a tissue as soft as liver can withstand a small amount of mechanical pressure during insertion into a sample tube, and tissue $T_1$ remains constant for a considerable period after the death of the animal. Most tissues show little effect over a period of 8 hours if the sample is kept on ice (but not frozen) and is prevented from drying out. Comparison of $T_1$ values obtained *in vivo* and *in vitro* are sufficiently similar to suggest that the majority of values obtained *in vitro* are a true reflection of the living state (with the possible exception of very dynamic tissues such as the kidney) (Table 3).

Table 3. — *Comparison of* in vitro *and* in vivo $T_1$ *relaxation times (ms) of various tissues.* In vitro *values obtained from rabbit tissue measured at 2.5 MHz and room temperature,* in vivo *from living human subjects at 1.7 MHz.*

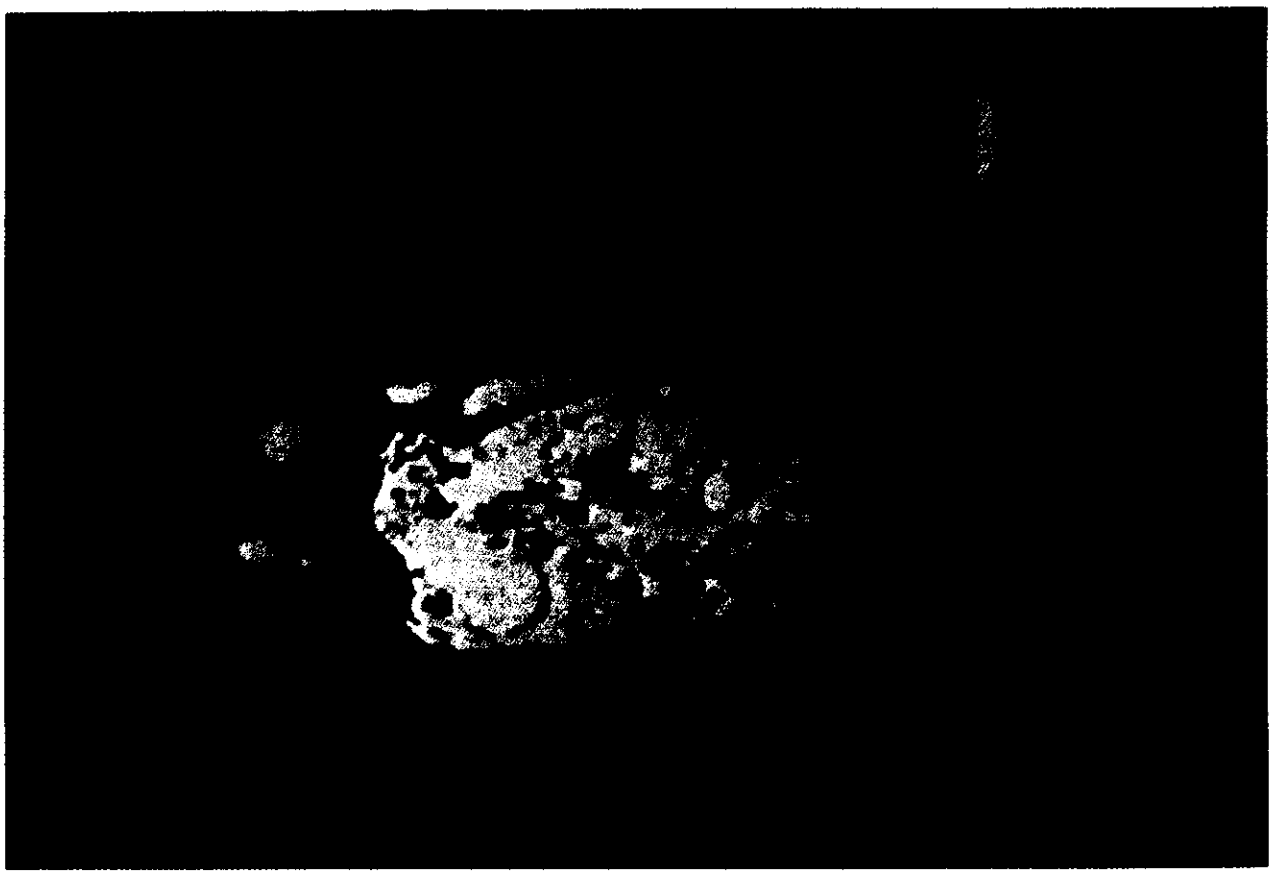
| *Tissue* | *in vivo* | *in vitro* |
|---|---|---|
| liver | 157 | 141 |
| spleen | 276 | 258 |
| thigh muscle | 182 | 182 |
| heart ventricle | 247 | 243 |
| grey brain | 294 | 332 |
| white brain | 232 | 264 |
| kidney cortex | 332 | 206 |
| kidney medulla | 320 | 426 |

**Intrinsic factors**

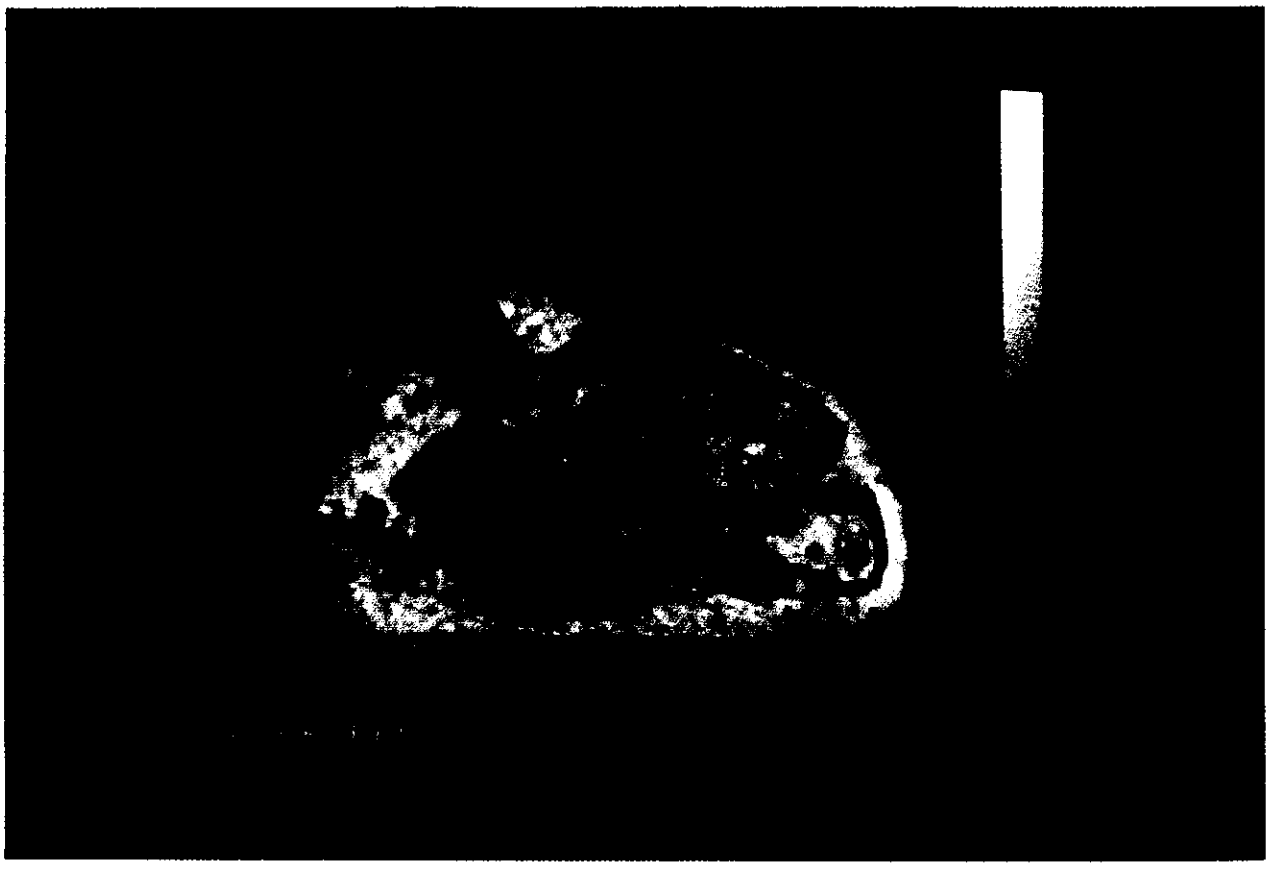
Let us now turn to some of the intrinsic (i.e. tissue-related) factors which affect the recorded $T_1$ value (Table 1). Here I have included those factors which are inherent properties of the spin system of the tissue and which, although they must be correctly recorded by the operator, can rarely be controlled directly by him.

*a) Linearity and tissue fat content*

One of the most frequently mentioned of these intrinsic factors is the "linearity" of the relaxation, ie. whether the relaxation graph can be expressed by means



(a)



(b)

Fig. 5. – NMR proton images of five-month pregnant goat. (a) $T_1$ image showing abnormality due to the long relaxation time of the amniotic fluid, (b) proton density image showing the apparent enhancement of the foetuses due to the black region of amniotic fluid.

of a simple exponential. This is of major importance, especially when single point values are reported as, for example, when the $T_{null}$ value is taken to obtain $T_1$. If signal size is plotted against relaxation interval (Fig. 6) a simple system gives a single exponential curve for spin lattice relaxation. The null point, at which the system is half relaxed, is constant on the signal size axis

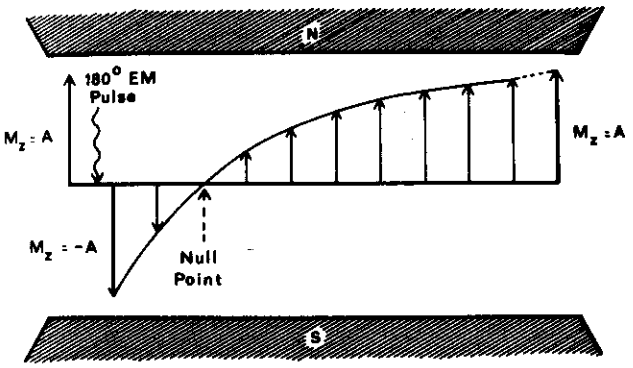



Fig. 6. - Diagrammatic representation of exponential increase in size of NMR signal from a spin population after delivering a 180° pulse to the system. The null point, at which the spins are half relaxed is shown.

but varies along the time axis with variation in $T_1$. Hence $T_1$ can be calculated for this system. Another approach, used in the Aberdeen imager, is to keep the time interval constant (in our case at 200 ms) and to estimate $T_1$ from the variation along the signal size axis. These are both accurate ways of calculating $T_1$ if the relaxation process is simple but neither can give an accurate value if there are several proton populations present and hence the relaxation curve is multi-exponential. Also both are very susceptible to field inhomogeneity or drift or to badly set-up 180° pulses (hence the use of adiabatic fast passage at Aberdeen).

For a mixed sample, such as a body tissue, we would always expect to get a non-linear plot for the $T_1$. For many tissues, however, the plot is linear - for example liver or spleen. This is presumably due to the fact that most pulse sequences, especially those employing spin-echoes, are so arranged that the very fast relaxation times from protons or organic molecules such as proteins, are not "visible" in the relaxation curve (the interval between the 90° and 180° pulses of a spin-echo sequence, even though very short - 8 ms in my *in vitro* machine - allows sufficient transverse relaxation to hide the microsecond relaxation times of these tightly bound protons). A second factor tending towards linearity is that the relaxation time of the water proton is long compared to its rate of movement through the various water compartments. During its relaxation it can sample the tightly bound compartment round the protein and the free water further away from it so the value reflected by the $T_1$ plot is a weighted average of the relaxation times of these various compartments and will not reflect them as individuals. This will not happen, however, if there is some impedence to the movement of the water molecule - i.e. a physical separation of the compartments, or if molecules other than water have a sufficiently long relaxation time to contribute to the $T_1$ plot - as for example free lipids which are the commonest molecules other than water to be observed in proton $T_1$ studies.

Single point $T_1$ measurements give a value which combines parts of the $T_1$ curves from all the compartments or proton groups making up the total relaxation characteristic of the system. This is not necessarily an average of all the $T_1$s, indeed it will be very far from an average if the individual relaxations are extremely different, such as those from lipids or loosely-bound water. The single value will, however, reflect some proportion of the different compartments so that, for example, when we look at adipose tissue we obtain a very short $T_1$ value reflecting the high proportion of lipid in the system. Fig. 7 shows $T_1$ and PD images obtained from a pig. This was obtained as part of a study to assess the ability of NMR to discriminate and quantify the amount of fat on the animal, so that its potential carcase value could be assessed. The short $T_1$ relaxation time of adipose tissue shows very well, but the proton density image does not distinguish the fat so clearly from the muscle.

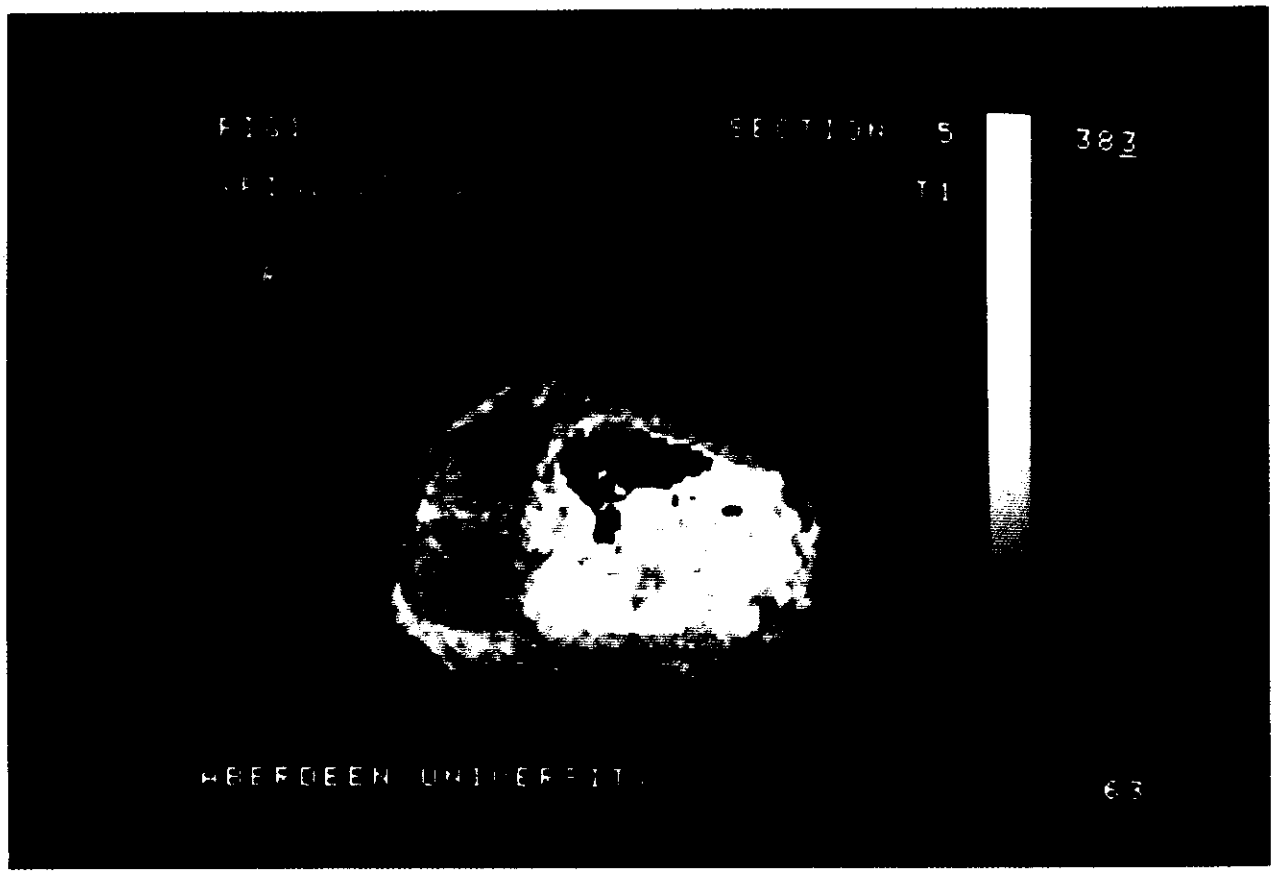
Fat in the breast can help in displaying the mammary tissue. This can be of use in examining breast tumours or in looking at the extent of spread of mammary tissue in the lactating breast. The presence of milk in the breast causes a considerable increase in the $T_1$ of the mammary tissue.

*b) Paramagnetic effects*

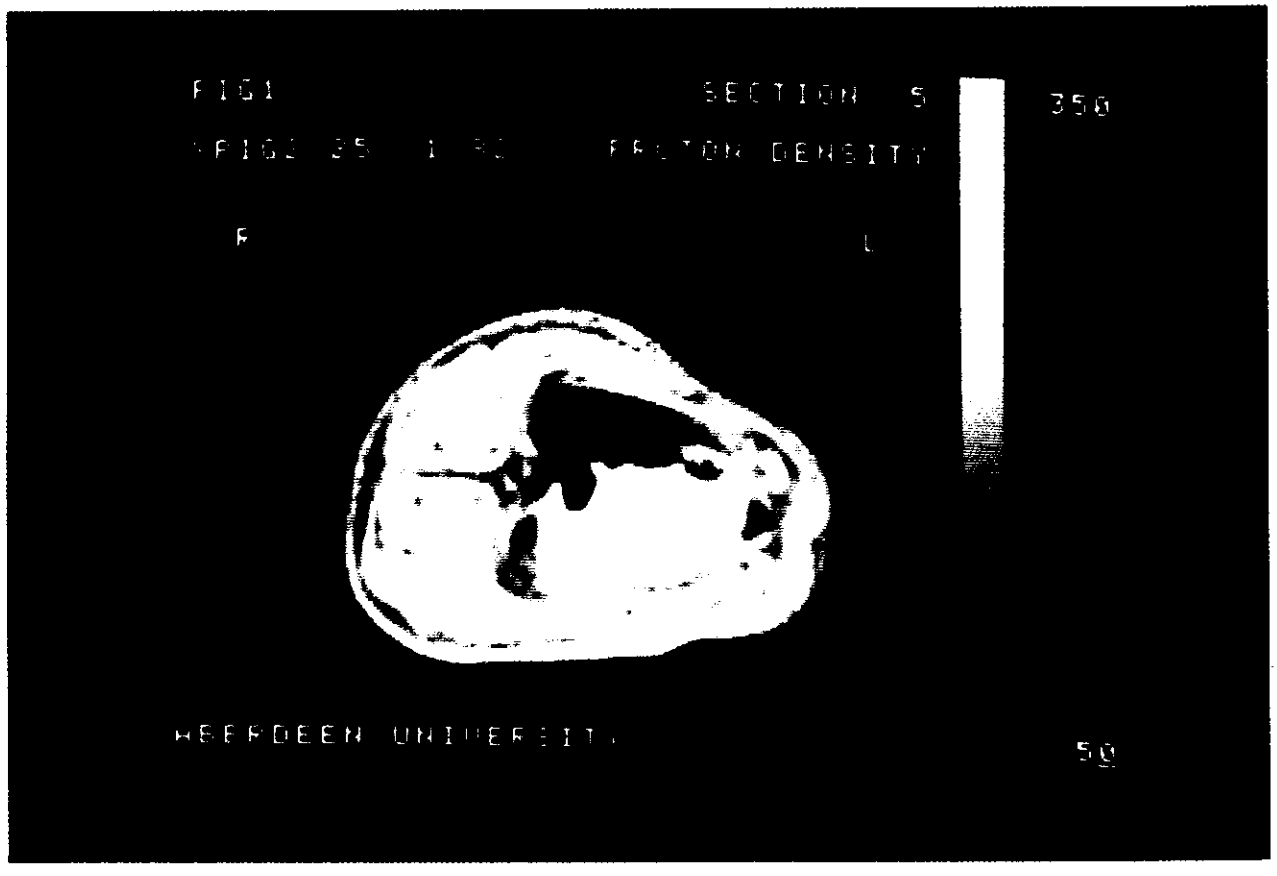
The presence of paramagnetic materials in a NMR sample of any kind increases the rate of relaxation of the water protons. This phenomenon is to be discussed separately but it is worth mentioning here that the paramagnetic effect can be useful in imaging. For example it has been suggested that certain diseases such as cirrhosis of the liver can be associated with an increase in paramagnetic metal ion (in this case copper) content and hence the organ will exhibit a reduced $T_1$ value. This may also be true of spleen tissue packed with red cell debris, including haem iron, in patients with haemolytic anaemia. Paramagnetic agents could also be used for contrast enhancement or study of organ function in certain circumstances. For example chromium EDTA, injected into a rabbit, reduces the $T_1$ value of the kidney, the effect disappearing as the agent is cleared from the blood system.

*c) Water content*

The effect of water content and aspects of structuring of the water is another factor which is being discussed by other contributors. In general one finds that, for tissues showing a simple exponential relaxation, a plot of relaxation rate against tissue water content yields a straight line. This suggests that the relationship is a fairly direct one and is rather surprising when one considers

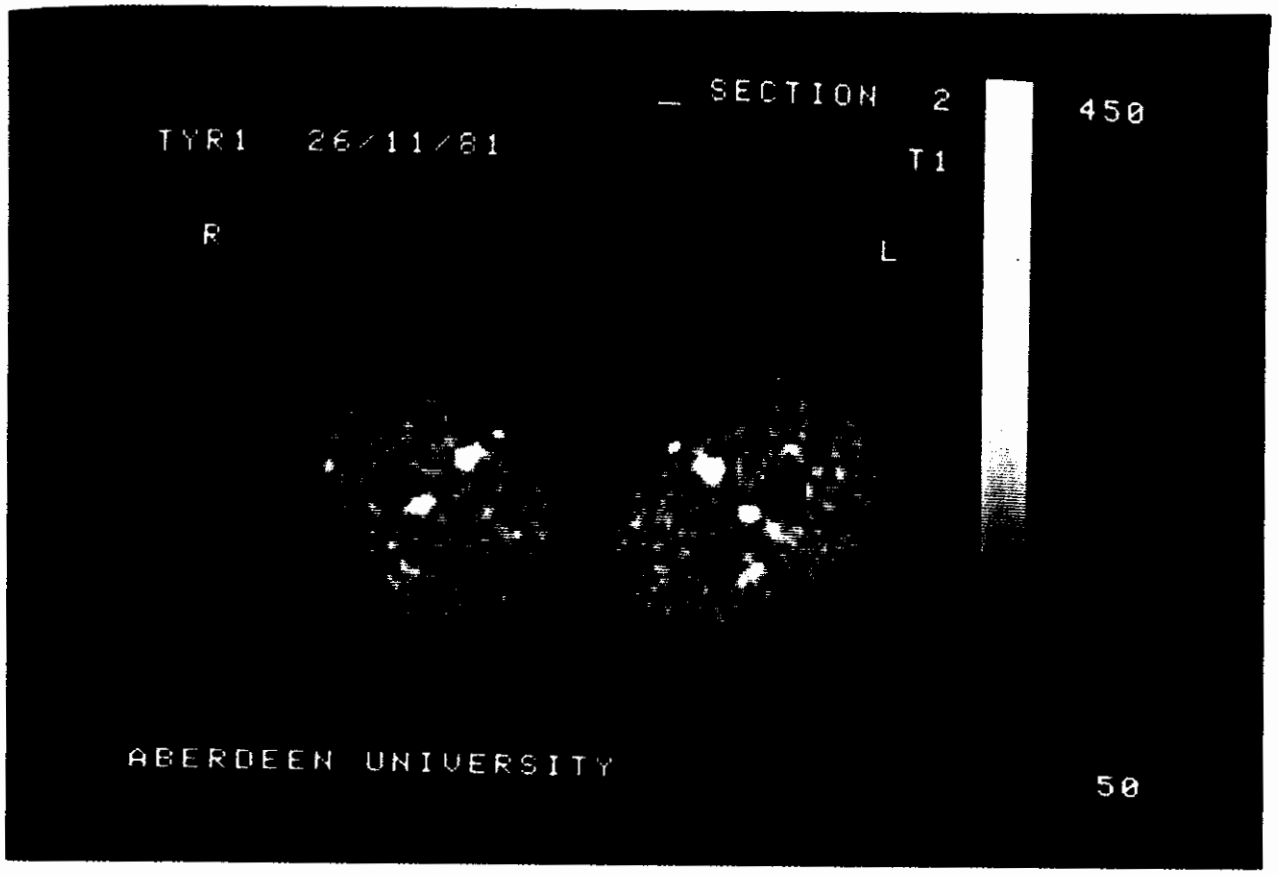



(a)

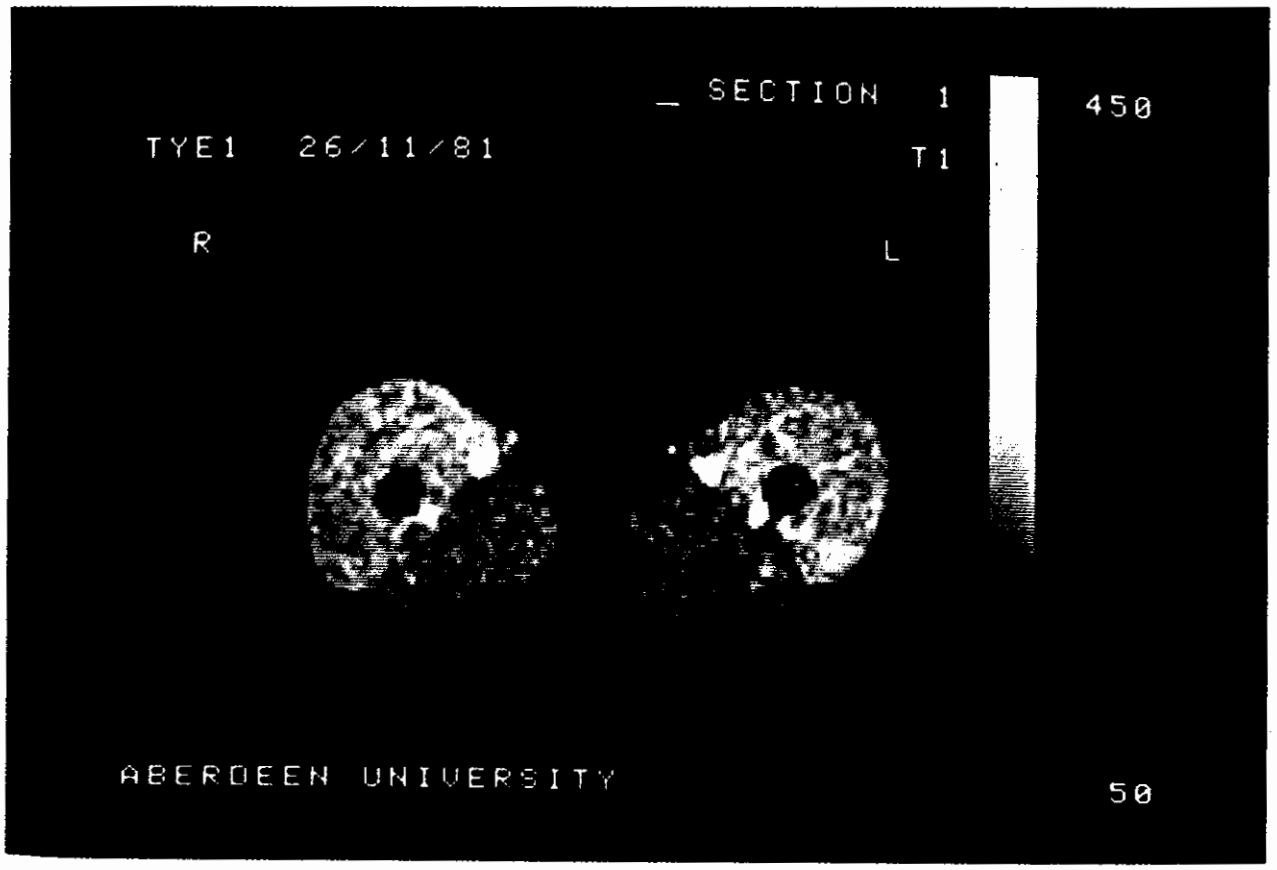



(b)

Fig. 7. — NMR images throgh shoulder region of a young pig, demonstrating fat distribution. (a) $T_1$ image and (b) PD image (1.7 MHz).




(a)




(b)

Fig. 9. — NMR $T_1$ relaxation image through legs of volunteer (a) before and (b) after exercise by walking up steps for 10 minutes. Measured at 1.7 MHz.

the enormous variety in chemistry and structure of different tissues. These are, however, noticeable variations from this general pattern (Fig. 8), for example adipose tissue or mammary gland, both of which have a very high free lipid content. The values shown here are all obtained from full plot, *in vitro* examinations of tissue *from several species.*

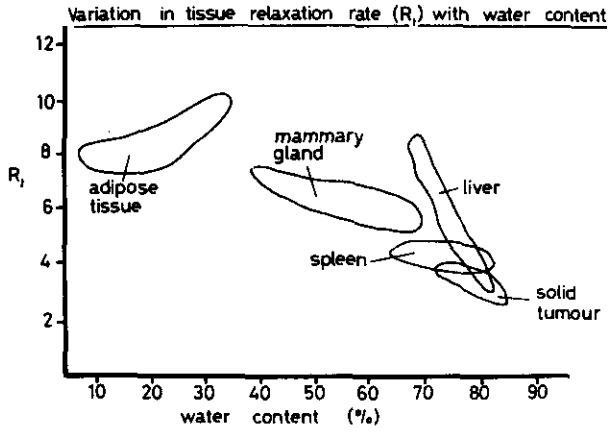



Fig. 8. - Spin lattice relaxation rate ($R_1$) of various tissues compared with water content. Samples examined by full plot method at 1.7 MHz and room temperature (information from *Dr. E. Rimmington*).

A point of interest in NMR imaging is the difference in value of the images of proton density (reflecting water content for most tissues) and $T_1$ relaxation time. The proton density images, such as Fig. 1, are excellent for bony structures but of little use in the abdomen. Here the $T_1$ image is much superior for tissue discrimination (Fig. 2). Providing that the imaging system can demonstrate levels of difference in PD and $T_1$ equally well, one might expect to see the abdominal organs equally well on the PD image if the proton density is a simple reflection of tissue water content which, in turn, is the controlling factor for the $T_1$ value. Abdominal images clearly demonstrate that this is not the case and would appear to imply that either the $T_1$ is not solely dependent on the water content of the organ, or that the proton density image is being affected by something other than water.

*d) Pathological state*

A wide variety of pathological conditions can lead to changes in both water content and NMR relaxation characteristics of tissues. Tumours are being dealt with in another paper of these Proceedings, so I will not detail malignant changes other than to say that it was really the possibility of early cancer detection by NMR relaxation methods which gave the impetus to the whole of the proton imaging field. It is still to tumours we turn for the most dramatic illustrations of disease demonstration by NMR.

Many other conditions are visible on $T_1$ images, ranging from renal failure to arthritis in the knees, since they cause either oedema due to local inflammatory reactions, or accumulation of fluid pools in or around the abnormal organ or joint. Similarly conditions which cause disturbances in the structure or in blood flow can be imaged by NMR. This topic is much too large to attempt to cover in one section of a talk.

*e) Physiological state*

As well as changes in relaxation characteristics related to pathological changes, it is worth noting that some changes can be associated with normal variations of the individual. NMR imaging can be used to monitor such variations as regression and then re-growth of mammary tissue in a goat which passes through lactation into a second pregnancy, or the changes in leg muscle subjected to exercise (Fig. 9). In this latter experiment volunteers were imaged through the thigh region after a 30 minute rest period. They were then asked to undertake a bout of fairly heavy exercise either continuously walking up and down stairs or operating an exercise bicycle for 10 minutes, followed immediately by imaging the thighs again. Exercising on the stairs involves a lot of work for the muscle at the front of the thigh, clearly shown on the NMR image. The exercise bicycle, however, fatigues all the muscle and when the image is analysed the total shift in $T_1$ value of the pixels over the muscle area can be seen to displace the histogram after exercise (Fig. 10). The fat, however, remains unchanged demonstrating that this is unlikely to be a temperature effect.

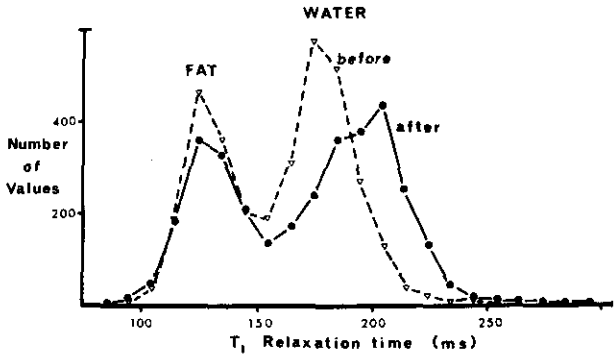



Fig. 10. Histogram of number of pixels of a particular $T_1$ value in images of legs before and after exercise.

There are many sources of possible normal variation which should be investigated. For example much of the body's chemistry exhibits diurnal variation - is this reflected in tissue $T_1$? The female cycle is known to be associated with changes in water retention - are these detectable by NMR imaging? These and other possibilities are under examination at Aberdeen.

**Conclusion**

The NMR relaxation characteristics of a tissue can be varied by a great many internal and external factors. Too

much of the existing literature is inadequate as far as experimental detail is concerned, which leads to difficulties in deciding how much we do actually know already about the field in which we work. Many aspects of the range of normal variation have been ignored in favour of the, perhaps more glamorous, study of pathologies.

To realise the full potential of this versatile medical and experimental technique it is necessary to approach the studies in an orderly and informed manner. This involves a full knowledge of the limitations, which can only be gained by exhaustive study of the techniques and instrumentation. Adequate clinical trials, especially cross-comparisons with other techniques, should be used to establish the medical role. The fullest use should be made of the types of information which are unique products of NMR studies rather than limiting its applications to fields such as anatomical studies where it overlaps with other available imaging techniques.

NMR imaging is almost certainly the road ahead in medical imaging. It is the role of research workers in this field to ensure that all users are fully aware of the true width and direction of this road.