Ann. Ist. Super. Sanità
Vol. 19, N. 1 (1983) pp. 7-8

INTRODUCTION TO THE AIMS OF THE WORKSHOP

F. PODO

Laboratorio di Biologia Cellulare, Istituto Superiore di Sanità, Rome, Italy.

This is the second NMR Workshop held in Europe on behalf of the EEC Standing Working Group on Biomedical Engineering (SWG-BME). The first one, organized by Professor Morucci, was held in Toulouse, in December 1981. A survey on the most recent developments of medical applications of NMR in our Countries already suggested at that time that the definition of an appropriate standardization of materials and methods in these fields would represent an attractive area of common interest for possible future joint cooperations at European level. The BME Group agreed on this view and decided that a second Workshop should take place this year on the subject of identification and characterization of biological tissues by NMR.

A critical review of NMR studies on tissue characterization clearly suggests that a correct interpretation of the experimental results and a useful development of their diagnostic and clinical applications definitely require at this stage: 1) a more clear understanding of the relationships between NMR parameters and biological and physiological properties of tissues; 2) the definition of an appropriate standardization of the experimental procedures, including protocols for preparing and using substances suitable for calibration and test objects useful for performance assessment. This Workshop will mainly concentrate on these aspects, with the principal objective of identifying research lines of common interest, to be undertaken together on a cooperative basis.

Let me try to summarize in a few words the principal aspects of the scientific background to the field of interest of this Workshop.

Discovered in 1946, the nuclear magnetic resonance phenomenon has been essentially utilised for more than thirty years for characterizing molecular structures and dynamic behaviour of chemical and biochemical compounds in solution or in homogeneous suspensions. This phenomenon is based upon the property of some nuclear species of possessing an intrinsic spin and therefore an associated magnetic moment. When placed in a magnetic field, these nuclei orient themselves along discrete directions with respect to the field. In these conditions a nuclear spin system is able to absorb energy from electromagnetic waves possessing appropriate frequencies (typically in the radio frequency domain), an event which is causing a change in the spatial orientation of spins. Nuclei in different chemical environments have different resonance conditions. When the trasmission of radiofrequency is interrupted, nuclear spins tend to resume their original orientation state, by first–order relaxation processes, characterised by exponential time constants, i.e. the spin–lattice (T_1) and spin –spin (T_2) relaxation times.

Analyses of NMR spectra in terms of frequency (chemical shift), multiplicity and peak areas of the resonance signals have been at the basis of extensive applications of NMR in organic chemistry and *in vitro* biochemistry.

Thanks to the significant technological advances which have taken place around the seventies, NMR spectroscopy and NMR tomography have more recently emerged as non invasive approaches for studying physiological and pathological properties of tissues and organs.

In particular, the possibility of monitoring biochemical reactions as they occur in living tissues, without altering their natural function, represents one of the most relevant advantages of the use of NMR spectroscopy in the field of tissue characterization.

On the other hand, NMR offers, in the complex relaxation properties of biological tissues a unique and potentially highly powerful tool which may play a very important role in the identification and characterization of tissues and organs. Different relaxation behaviour is in fact generally exhibited by different tissues of the body and significant alterations may be induced on relaxation rates by pathological conditions.

Early *in vitro* measurements indicated that proton NMR relaxation times are often significantly altered in neoplastic tissues. These results stimulated a number of further studies, aimed at evaluating the possibi-

lity of utilizing NMR for diagnosing and monitoring malignancy. However results published in the literature are not always fully consistent and the exact meaning of these observations is still rather unclear from a theoretical point of view. Other studies also indicate that alterations of NMR relaxation properties may not be specific of a cancerous condition, since other pathological states are also able to produce similar effects on NMR relaxation properties as those induced by malignant processes. Correlations between NMR relaxation times and various physical parameters such as tissue hydration, fat content and pH have been suggested and are still investigated.

A critical review of *in vitro* studies generally leads to the following conclusions: 1) in most cases NMR relaxation times are meaningful parameters for detecting and monitoring the existence of pathological conditions in tissues; 2) too many analyses have been however performed so far without or with only a few appropriate controls; 3) care must be taken in discriminating between effects caused by uncontrolled changes in the physical and/or biological conditions and those related to malignant growth or to other pathological conditions.

These considerations suggest the interest of directing common efforts in various laboratories towards the definition of suitable protocols and standardised experimental procedures, in order to assure reproducibility and a direct comparison of the results.

It should also be kept in mind that the nature of the tissues in which a pathological state occurs is likely to be variable and heterogeneous. Different portions of the tissues may in these conditions have different T_1 and T_2 values, whose distribution can hardly be studied by *in vitro* measurements. The possibility of measuring T_1 and T_2 in tissues *in vivo*, which is now offered in principle by NMR imaging methods, opens new perspectives to the identification and characterization of biological tissues by NMR. It is in fact well known that NMR images depend upon the combination of various physical parameters, notably density of hydrogen atoms and their relaxation times T_1 and T_2. In particular relaxation times are widely responsible for the high soft tissue specificity, so peculiar to this imaging modality. A significant example is represented by the well known possibility of differentiating between grey and white matter, two tissues in which significant differences in relaxation times are confronted with only slight differences in hydrogen spin density. Differences in relaxation times between adjacent tissues are therefore commonly utilized in NMR imaging to improve the anatomical information and to discriminate lesions like tumours, abscesses, hematomas etc. from the surrounding tissues.

However, NMR imaging uses these differences almost exclusively for enhancing the soft tissue contrast, generally neglecting the physiological and pathological information potentially available in the real values of NMR relaxation in tissues. These limitations are due to a number of problems encountered today in the clinical measurements of relaxation properties of tissues *in vivo*. These problems are mainly related to the complexity of the system, as well as to the large number of the imaging procedures in use. Because of our insufficient technological knowledge, the optimal approach which would permit the comparison and the evaluation of the different procedures and results has not been defined to a satisfactory level.

Once the main technical problems of these measurements are hopefully solved, the significance of the NMR relaxation behaviour might be further investigated, in order to assess to which extent the specificity of the diagnostic information can be enhanced and become of real clinical use.

The programme of this Workshop comprises Sessions and Working Panels respectively devoted to the following subjects:

1) a critical review of the physical and physiological background to the field of identification and characterization of biological tissues by NMR;
2) technical aspects of the measurements of relaxation behaviour and their relationship with tissue properties, with a particular emphasis given to: the effects of the differences between various techniques on measurements; the problems of different technical approaches; the interaction of measurements with localization; the problems of characterization of tissues and biological materials, using standard techniques; the biophysical factors determining relaxation behaviour in tissues; various aspects of the experimental measurements in real tissues; protocols for the preparation of biological samples for characterizing tissues; possibilities of reviewing and categorizing the measured values; identification and assessment of suitable contrast agents;
3) standardization and calibration methodologies, with particular attention to: specification of relaxation properties required in substances for calibration; identification of such substances and protocols for preparation; specification of design of phantoms and protocols of use for performance assessment with respect to spatial and contrast discrimination; possibility of designing a standard instrument for safety assurance by measuring electromagnetic effects.

The last session of the workshop is devoted to the elaboration of proposals for the possible activation of a common research programme at European level, in the field of the characterization of biological tissues by NMR.

Ann. Ist. Super. Sanità
Vol. 19, N. 1 (1983) pp. 9-12

PRESENT TRENDS OF NMR TECHNOLOGY IN MEDICINE

F. DE LUCA, B.C. DE SIMONE and B. MARAVIGLIA

Dipartimento di Fisica, Università di Roma "La Sapienza", 00185 Rome, Italy

Summary. – *In the last decade the application of NMR spectroscopy to medicine has observed a continuously increasing expansion. In particular the area of NMR imaging which is now booming has caused a renewal of interest on more traditional* in vitro *studies like tissue NMR characterization and classical chemical shift and relaxation time measurements.*

With this contribution the large existing number of imaging methods are reviewed and analyzed in particular for the more promising ones.

Riassunto. – *Nell'ultimo decennio la spettroscopia NMR ha trovato una continua e crescente applicazione in medicina. In particolare* l'imaging *NMR ha risvegliato l'interesse per i tradizionali studi* in vitro, *come la caratterizzazione NMR dei tessuti, il* chemical shift *tradizionale e le misure dei tempi di rilassamento.*

In questo articolo sono rivisti e analizzati i più promettenti tra i metodi di scansione sinora realizzati.

Introduction

In the last decade the potentiality of NMR spectroscopy applied to medicine has greatly widened. Due to the versatility of this coherent spectroscopy, many tentatives have been made to find out convenient procedures either to obtain the classic spectroscopic informations (spectra, relaxation times, etc.) within a small volume of a living organism, or to map a space distribution of NMR parameters functions. The latter objective, namely the formation of NMR images, has been achieved through several different approaches, some of which are at the moment prevailing over the others. The whole field (NMR imaging) is now living a very fast evolution and excellent results have become more and more common. The development of the *in vivo* NMR spectroscopy has arisen, in a way, as a consequence of some of the methods proposed for imaging. Spectroscopy of a small volume element in a living organism, which can at the same time be imaged, is of course the ultimate goal. High field imaging can in fact satisfy this combined relevant aspects (Fig. 1).

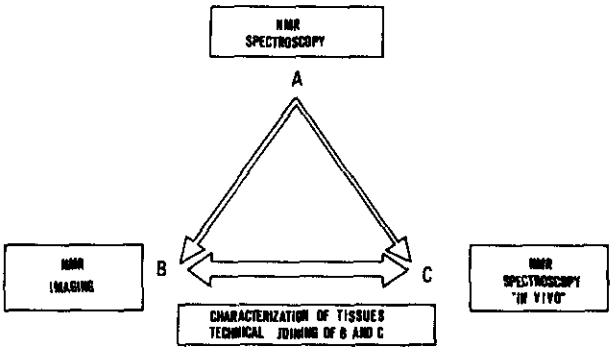


Fig. 1. – From classic NMR spectroscopy two very promising research areas have been generated: NMR imaging (B) and *in vivo* NMR spectroscopy (C). Although the physics, which is the basis of these two fields, is the same, the interpretation and correlation of B and C will be a hard task. The "closing of the triangle" with interpretation of the relations between B and C will necessarily go through studies for the characterization of tissues and through technical tentatives of creating high field instruments capable of investigating both B and C at the same time.

Methods

Let us consider first the imaging techniques which represent a rather broad area, although only a few seem at the moment the really promising ones. All imaging methods are based on high power pulsed NMR and have many features in common like of course a wide bore magnet, magnetic field gradients and a computer. Still some basic differences make most of the approaches unlikely to become interesting for clinical imaging.

Among the imaging techniques used is the projection-reconstruction (PR) method [1], originally proposed by P. Lauterbur (with the name zeugmatography) as a very first NMR imaging procedure. The NMR signal, after the exciting r.f. pulse sequence, is detected

with a costant magnetic field gradient applied in a certain direction. By Fourier transforming the detected free induction decay signal (FID) one obtaines the projection of the density distribution of protons along the gradient. The measurement is repeated for different directions of the field gradient so to get a series of projections, which, by means of reconstruction algorithms similar to the ones used in X-ray computer-assisted topography (CAT), can produce the wanted image. The proper choice of the excitation sequence (saturation-recovery, inversion-recovery, spin-echo) can generate NMR images commonly called spin density, spin-lattice relaxation time (T_1), spin-spin relaxation time (T_2) images [2]. None of these is anyway a single parameter image but rather a function of all of them. Different sequences simply give more emphasis to the chosen parameter rather than to the others. In Fig. 2 an inversion-recovery sequence is shown as an example of PR measuring method. In this sequence, like all the other PR sequences, the x and y gradients amplitudes are varied for each projection. In the time period 3, the 90° pulse is a selective one. In fact, after the 180 degree wide spectrum pulse, which inverts the orientation of the magnetization, the selective 90° pulse associated with a z-gradient, during the time interval 3, selects a x, y slice of the body with a chosen thickness. Normally the selective pulse results from the mixing of the r. f. with a sin x/x or a gaussian function produced from the computer and a digital to analog converter. In some cases the PR method has been implemented with a steady state free precession (SSFP) sequence [3]. In a way this is the melting of the PR method with part of the so called sensitive point method [4], which at the moment seems to have little chance of providing good images in a short time and for this has recently been neglected.

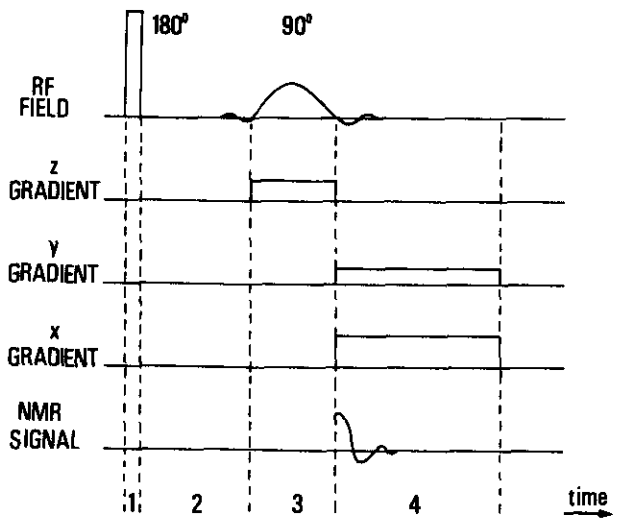


Fig. 2. – Inversion-recovery sequence used to produce T_1 images by means of the projection-reconstruction method.

The 2DFT imaging method proposed in 1975 by Kumar, Welti and Ernst [5] has several technical difficulties thus, although it entirely exploits the physical information that can be taken from the spin system, it has remained a theoretical proposal. With the modification introduced by the Aberdeen group [6] this approach has become the most successful and used one (also called spin-warp). The main change (Fig. 3) consists in varying the amplitude of the field gradient rather than the duration as it occurred in the previous proposal. The x-gradient is normally used to produce the echoes and as reading gradient while the y-gradient is varied in amplitude to change the phase of the nuclear magnetization to get then the information in the second dimension. The great advantage of this approach is the easy technical r. f. pulse sequence and above all the elimination of the "reconstruction" procedure which is cause of blurring over the whole image. In fact the spin-warp method is also not too sensitive to the inhomogeneities of the main static magnetic field.

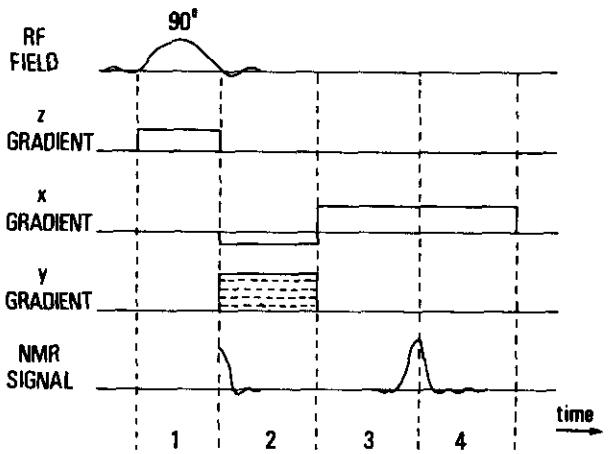


Fig. 3. – Spin-warp sequence. The magnetic field along the y axis (time interval 2) is used to phase encode the spin system.

Both the PR and spin-warp methods can be extended to 3D imaging [7]. 3D imaging seems too slow to be technically useful for clinical diagnosis although it can be quite useful for research in medicine and other fields [8]. Rather than 3D imaging the future of diagnosis seems to be in multi-slice imaging [9]. Multi-slice imaging exploits the "dead" times due to spin-lattice relaxation time, after taking some data from a slice, to get information from other slices, whose spin system have not been perturbed before. With this approach, in 5 minutes or so, images from about 15 slices can be obtain obtained.

It is worthwhile mentioning the "real time" imaging proposed by Mansfied and Pykett [10]. The approach makes use of a selective pulse (Fig. 4) and then of a series of fast inverting y-gradient fields which refocus the magnetization several times. The presence of a weak x gradient allows the simultaneous imaging of a whole plane in a time which, in principle, could be of about 30 ms. Real time imaging was actually achieved [11]

for beating hearts. Unfortunately at the moment the S/N is rather low to get good images. The velocity of the method makes it quite interesting anyway, as improvements could arise from some modification.

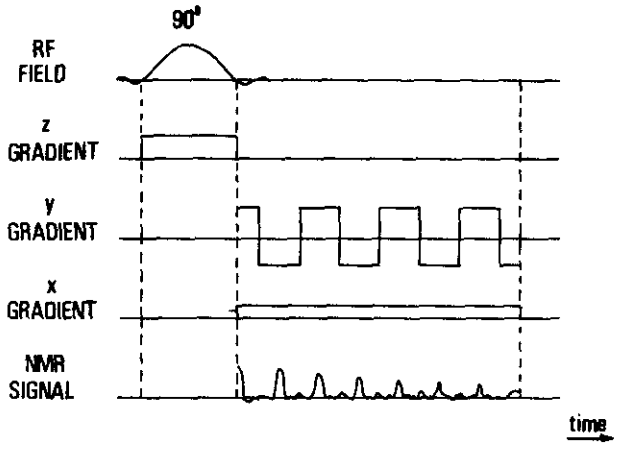


Fig. 4. – Echo planar sequence. It is used for fast imaging.

Instrumentation

Within the framework of NMR imaging methods which are now prevailing, there are some substantial differences for some hardware components. Notwithstanding this differences, the general scheme of the apparatus does not change much (Fig. 5). Let us consider a simplified schematic of an imaging apparatus and let us describe it by starting from the radio frequency pulse generation until the NMR signal production and detection.

By means of the computer the radio frequency generated by a synthesizer is gated and phase controlled to produce the wanted pulse sequence. Tayloring of the r.f. pulses for plane selection is obtained by mixing the r. f. wave with the wanted function which is produced by the computer and converted from digital to analog. The pulse sequence is then amplified by a class A power amplifier, whose maximum power output depends very much on the imaging method used. Usual power required for spin-warp are of the order of 1 KW while projection reconstruction requires powers in the range of 4 to 8 KW. The requirement of the power amplifier to be class A is obviously bound to a need of linear amplification of the taylored pulses.

The NMR probe is made with saddle coils for head scanning. Either one or two orthogonal saddle coils are used. Elliptical coils are instead frequently used for trunk imaging. The NMR probe must be screened from external r. f. disturbances. Thus a Faraday screen is required. Such a screen is normally built large enough as to contain the whole magnetic system.

The magnets used to produce the static field are commonly either resistive or superconductive. Permanent maghets have been considered but found so far not convenient. Resistive magnets are air core and generally made either of four or six helmoltz coils, which are placed in a way as to define a sphere. Typical field strengths range from 0.1 to 0.17 Tesla with power supplies from 20 to 70 KW. Field homogeneities are of the order of 50 ppm over a sphere of 30 cm diameter. The advantage of resistive magnet is related to the lower cost and the fact that their maintainance is limited to their actual use as they can be switched off or on at will. Superconductive magnets on the other hand have

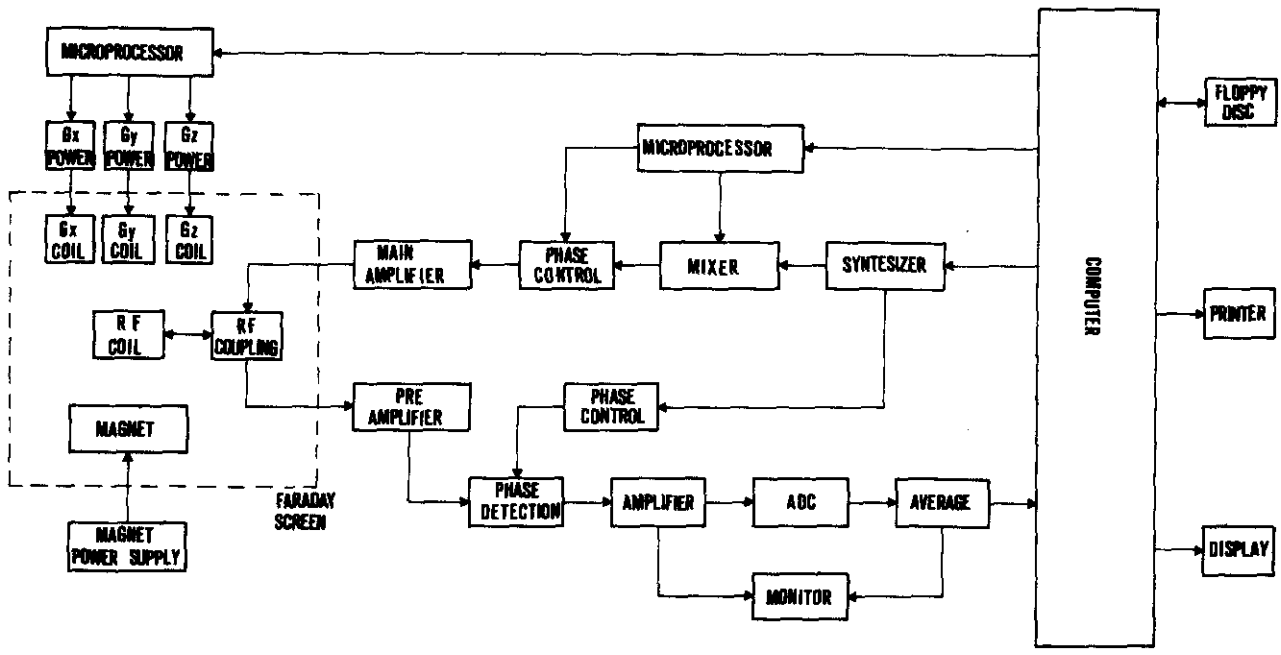


Fig. 5. – Simplified schematic of a NMR imaging apparatus.

higher field stability (although the resistive magnets stability is normally sufficient), can reach higher fields (from 0.3 to 1.5 Tesla) and have larger homogeneous volumes. Negative aspects are cost, more technical assistance required and large amounts of cryogenic liquids (typically 500 liters of liquid helium per month).

The magnetic field gradients power supplies are a rather critical component of the whole apparatus. They must have high power (normally above 1 KW per channel) and must be fast switchable in a bipolar fashion (time constant should be of the order or less than a msec). This latter aspect is not trivial to obtain as rather high inductive loads due to the gradient coils are always present. The NMR signal is produced and detected in a rather traditional way. Tuned pre-amplifiers are advisable. The signal is demodulated and digitized.

The need of a very fast acquisition and averaging makes the digitizer and averaging component a rather crucial one. While signals are digitized they should also be averaged before transfer to the computer. Preliminary hardware operations on the signal can be implemented at this stage, like fast Fourier transform.

The stored signal undergoes computational operations which of course depend on the method used.

The equipment required for *in vivo* spectroscopy like topical NMR is very standard. The only peculiarities arise from a surface r.f. coil and a focusing magnetic field added to the static one [12]. With this combination a chosen volume with the required field homogeneity can be detected. Study of perfused organs is also a standard NMR spectroscopy and thus we will not discuss it here.

REFERENCES

1. LAUTERBUR, P.C. 1973. Image formation by induced local interaction: examples employing nuclear resonance. *Nature.* **242**: 190-191.

2. YOUNG, I.R., BAILES, D.R., BURL, M., COLLINS, A.G., SMITH, D.T., Mc DONNEL, M.J., ORR, J.S., BANKS L.M., BYDDER, G.M., GREENSPAN, R.H., & STEINER, R.E. 1982. Initial clinical evaluation of a whole body NMR Tomograph. *J.Comput. Assist. Tomogr.* **6**: 1-18.

3. MOORE, W.S., HOLLAND, G.N., & KREEL L. 1980. The NMR C.A.T. scanner: a new look at the brain. *J. Comput. Assist. Tomogr.* **4**: 1-7.

4. HINSHAW, W.S. 1976. Image formation by nuclear magnetic resonance: the sensitive point method. *J. Appl. Phys.* **47**: 3709-3721.

5. KUMAR, A., WELTI, D. & ERNST, R.R. 1975. NMR Fourier zeugmatography. *J. Magn. Reson.* **18**: 69-83.

6. EDELSTEIN, W.A., HUTCHINSON, J.M.S., JOHNSON, G. & REDPATH, T. 1978. Spin warp NMR imaging and applications to human whole-body imaging and applications to human whole-body imaging. *Phys. Med. Biol.* **25**: 751-756.

7. KRAMER, O.M., SCHNEIDER, J.S., RUDIN, A.M. & LAUTERBUR, P.C. 1981. True three dimensional NMR Zeugmatographic images of a human brain. *Neuroradiology* **21**: 239-244.

8. DE LUCA, F., DE SIMONE, B.C. & MARAVIGLIA, B. Role of threedimensional imaging in small scale NMR tomography. *Magn. Reson. Imaging.* **1**: N. 4 (in print).

9. CROOKS, L., ARAKAWA, M., HOENNINGER, J., WALTS, J., McREE, R., KAUFMAN, L., DAVIS, P.L., MARGULIS, A.R. & DE GROOT J. 1982. NMR whole-body images operating at 3.5 KGauss. *Radiology* **143**: 169-174.

10. MANSFIELD, P. & PYKETT, I.L. 1978. Biological and medical imaging by NMR. *J. Magn. Reson.* **29**: 355-373.

11. ORDIDGE, R.J., MANSFIELD, P., DOYLE, M. & COUPLAND, R.E. 1982. Real time moving images by NMR. *Radiology* **142**: 244-247.

12. SHAW, D. 1981. In vivo chemistry with NMR. In: *NMR Imaging in Medicine.* L. Kaufman, L. E. Crooks & A. R. Margulis (Eds.). Igaku-Shoin, New York, pp. 147-183.

Ann. Ist. Super. Sanità
Vol. 19, N. 1 (1983) pp. 13-18

EXPRESSION OF RELAXATION PROPERTIES WITHIN THE NMR IMAGE

J.S. ORR, R.A. LERSKI and K. STRAUGHAN

Department of Medical Physics, Royal Postgraduate Medical School, Hammersmith Hospital, London, U.K.

Summary . – *Methods of collecting NMR signal data for imaging can largely be separated from the methods used to construct the image from the data. It is helpful to confine discussion to the imaging of single planes in which every voxel contributes to every FID. The collection of data for imaging from the partially relaxed condition must be made at the same time after excitation for all regions. This time cannot be optimal for all regions. Therefore, some regions will have either poor contrast or poor spatial resolution.*

Since there are several methods of constructing images with differing properties of contrast, resolution, and time, the same NMR signal data can result in quite different images. Some may be suited for visual observation with clear edges, while others may be less good visually but with more accurate or precise values. The possible use of absolute or relative relaxation properties of single regions for specific characterisation of tissues is a complex problem and novel methods of describing such properties will be required, possibly using frequency dependence.

Riassunto. – *I metodi utilizzati per raccogliere i dati NMR al fine di ottenere immagini possono essere separati da quelli usati per costruire dai dati l'immagine stessa. E' utile limitare la discussione alle immagini di piani singoli, in cui ciascun "voxel" contribuisce a ciascun segnale di decadimento libero (FID). I dati ottenuti in condizioni di rilassamento parziale debbono essere raccolti per tutte le regioni allo stesso tempo dopo l'eccitazione. Pertanto alcune regioni presenteranno o un cattivo contrasto o una cattiva risoluzione spaziale.*

Dato che, per costruire le immagini, esistono diversi metodi caratterizzati da diverse proprietà di contrasto, risoluzione e tempo, gli stessi dati NMR possono dare luogo a immagini assai diverse. Alcune possono prestarsi ad una osservazione visiva con contorni netti, mentre altre possono essere visivamente assai meno buone, ma contenere valori più accurati e precisi. La possibilità di utilizzare le proprietà di rilassamento relative o assolute di singole regioni per una specifica caratterizzazione tissutale è un problema complesso e, per descrivere queste proprietà, si richiedono metodi nuovi che possibilmente utilizzano anche la dipendenza dalla frequenza.

Introduction

This paper is a survey of some aspects of the expression of relaxation properties within the NMR image. The subject lies between the construction of specific imaging machines and the biophysics of complex tissues. It is addressed to two separate but closely related topics. One is the way in which NMR signals from a slice of the body are used to produce a complete image of some measure of relaxation properties. The other is the possibility of extracting very full information on relaxation from a few small regions of the body.

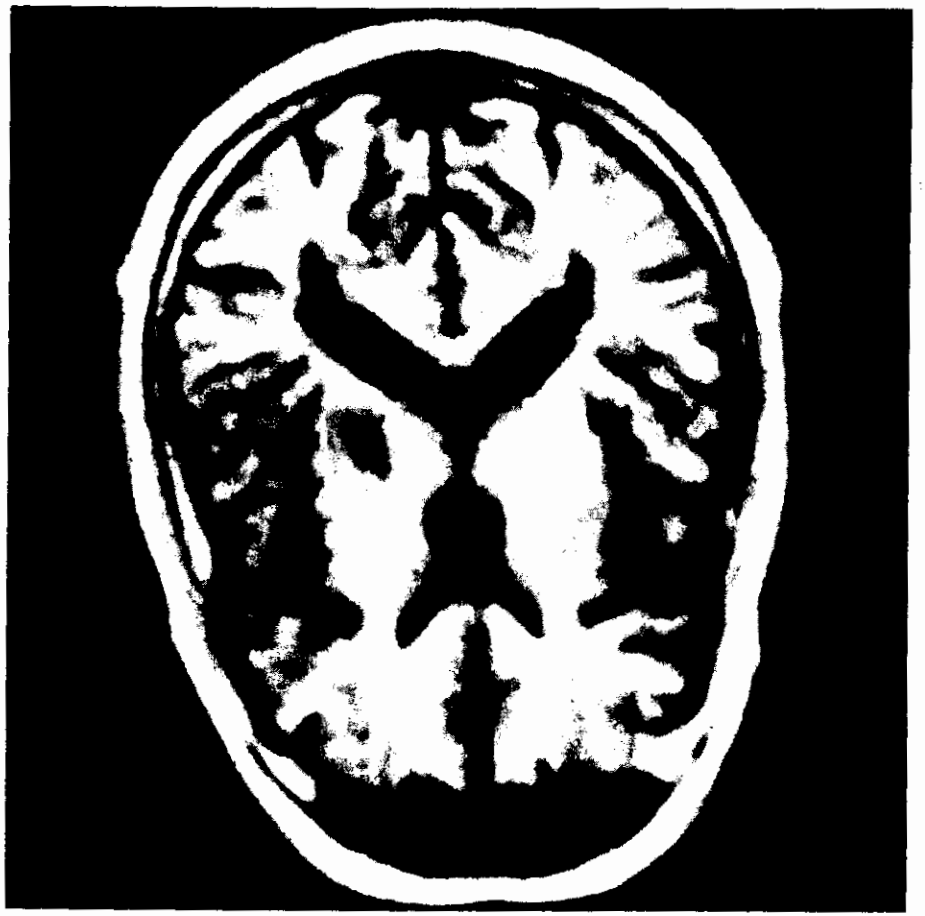
The whole subject is not only relatively new but also very complex. The problems will be hard to solve and in some ways even harder to define. Some assumptions will therefore be suggested to allow simplification and structuring of the subject.

Clinical experience has uniformly found that imaging times are longer than desirable. It is therefore suggested that only the simplest measures of relaxation properties can be presented in clinical images and that these are useful (Fig. 1) [1, 2]. It is suggested further that when much fuller information is sought it should be confined to small selected regions. Finally, for the present purpose it will be assumed that both these functions – imaging and characterisation – can be performed on the same machine.

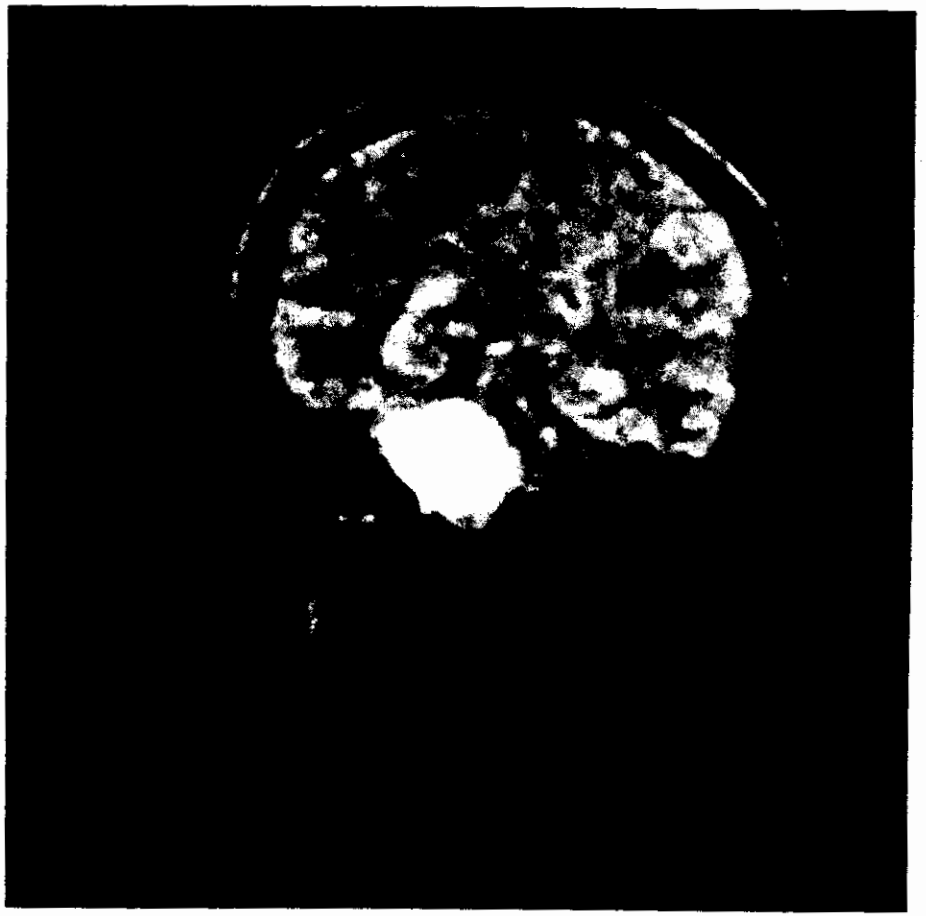
NMR signal data for imaging.

Methods of collecting NMR signal data for imaging can be separated to a considerable extent from the methods used to construct the image from the data.

The constraint of the requirement to produce a highly structured image in a short time can be used to further simplify the present discussion. Since for fundamental physical reasons NMR is a slow and insensitive



(a)



(b)

Fig.1. – Example of clinically useful NMR images in which the brightness in each pixel is largely dependent on a simple measure of one relaxation property : (a) T_1 ; (b) T_2.

technique with poor signal to noise, useful clinical studies today can only be done by accepting a compromise between speed, detail and expense. If a machine is sufficiently well constructed to have a reasonably long T_2^* a single frequency free induction decay (FID) will overdetermine the information collected. Thus all imaging methods worth discussing will use a Fourier approach. Although 3-dimensional volume scanning has theoretical advantages it takes impractically long or makes impracticable demands on computing power. While point and line scanning are simple they are also slow and not very efficient.

Most useful discussion will therefore be confined to imaging of single planes in which every voxel contributes to every FID. It will of course be applicable to several single planes when they are imaged in the one scan. It will further be confined to the use of only 90° and 180° RF pulses and to the use of X,Y and Z gradients to provide spatial information.

Since all signal data is to be derived from Fourier transforms, and since spatial information comes from frequency values or phase values, poor signal to noise will detract both from spatial resolution and from contrast.

A further implication of the poor signal to noise is that many features of the quality of the image produced will closely correlate to the number of FIDs collected. In a sense the number of FIDs corresponds to the X-ray dose in CT where the image information is photon limited. It may therefore be useful to normalise all studies on images by the number of FIDs used.

The expression of spin-lattice relaxation in images illustrates some of the special features of NMR imaging. This relaxation after a 180° pulse has the property, unusual among medical imaging methods, that the signal passes through a zero value between two high signal periods. When the signal obtainable by a 90° pulse is potentially large, that is when the magnetisation vector is aligned either with or against the Z field, the signal to noise will be at its best. When the signal obtainable by a 90° pulse is potentially small, that is when the magnetisation vector in the Z direction is nearly zero, the signal to noise will be poor.

Evidently if the objective were to obtain maximum signal or signal to noise, data would be collected from the high signal period. Similarly, if a relaxation curve were to be collected, a series of values of the falling and rising signal would be obtained by increasing τ between the 180° and the 90° pulses of a series of inversion recovery sequences.

However in imaging the objective is to distinguish regions of tissue that have different relaxation properties. The constraint is that the collection of data from the partially relaxed condition of all regions being studied must be made at the same time τ after excitation. Since the FID signal strengths for most tissues start with closely similar values immediately after a 180° excitation, and end with closely similar values after relaxation is complete, the two periods of high signal correspond to minimum difference between regions. On the other hand the period when the difference in signal between regions is maximum (Fig. 2) is when the signals are near zero and thus have the poorest signal to noise.

As mentioned above, poor signal to noise leads to poor spatial resolution. Thus when conditions are such as to maximize contrast and the distinction between regions of tissue, the signals are relatively low and spatial resolution is lost. When signals are high and spatial resolution is good, contrast is lost. Moreover, as pointed out in a later paper, these conditions vary from one part of an image to another.

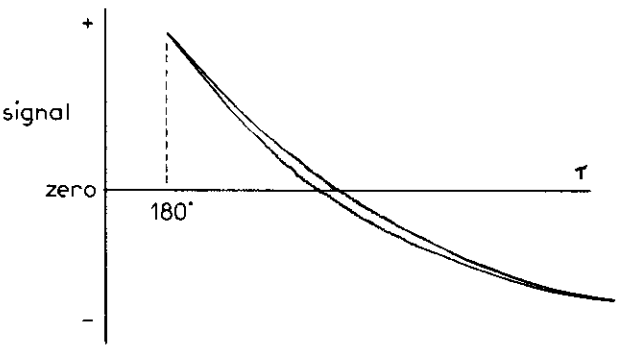


Fig. 2 – Spin-lattice recovery curves from two voxels with slightly different relaxation properties excited by inversion recovery pulse sequences 180°-τ-90°. The separation between the signals is greatest when the signals are small and signal to noise poor.

Image construction

As mentioned above imaging can be carried out by collecting data from points, lines, planes or volumes, although it is suggested that discussion be limited to planes. The imaging methods can also be separated into two groups – reconstruction and mapping (Table 1).

Table 1. – NMR imaging methods

Reconstruction	*Mapping*
Plane integrals	Point
Line integrals	Line
	2 or 3D direct

Where the Z gradient is used to define the selected plane, reconstruction methods generally use simultaneous X and Y gradients, whereas mapping methods use sequential X and Y gradients. The two approaches to imaging a plane have reasonable signal to noise and have roughly equal although different advantages and disadvantages.

The feature of image construction that emphasizes the considerable degree to which it can be separated from the collection of the NMR signal data is illustrated in Fig. 3. In the simplified flow diagram of the system

there are three distinct points from which images can be constructed [3, 4, 5]. The three construction methods are quite different and have different properties and effects with regard to spatial resolution, contrast, signal to noise and construction time. Thus the same NMR signal data can result in quite different images.

In addition to the factors mentioned above, there are several other reasons for distinguishing characterisation from imaging.

The information that the eye and brain need from an image is essentially comparative or relative, so that areas can be distinguished and located, or perhaps more importantly, that edges between areas can be identified. For this purpose absolute values are largely irrelevant. When contrast agents are used it is the differences in concentration and the changes with time that are important.

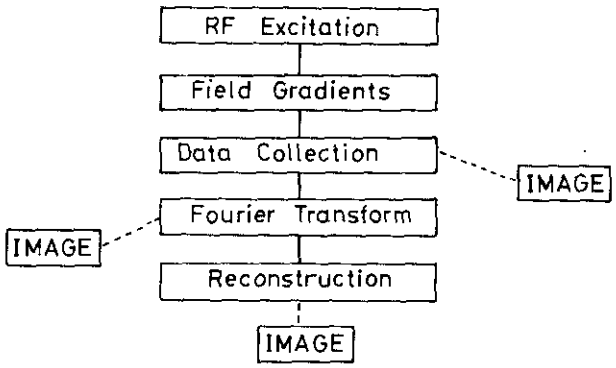


Fig. 3 – Simplified flow diagram of NMR imaging systems demonstrating the three distinct parts of the data flow from which images can be constructed.

It is clear already even from the relatively limited NMR and biophysical studies of real heterogeneous tissues that a wide range of pathological and generalized metabolic factors affect relaxation properties; that these properties can be very complex; and that while general trends can be discerned there is little indication that simple measures of relaxation will be sufficiently specific. Since, as assumed above, imaging will be confined to simple properties, useful and specific characterisation will in general need to be carried out as a separate exercise from imaging.

Characterisation of a single region located and identified within the image

Specific characterisation of tissue by its relaxation properties is in a much more primitive state than imaging. Much of what can be said about it is therefore speculative. However, the interaction between localisation and data content can be compared with the similar problems encountered ^{31}P *in vivo* spectroscopy. It is worth considering the potential value of *in vitro* NMR characterisation of readily obtained body fluids such as blood. Here NMR is in direct competition with biochemistry and cell pathology. If there is real value in such NMR studies then much greater value could be obtained by studies along similar lines of *in vivo* tissues where NMR has the great advantage of non-invasiveness.

The complexities of the NMR biophysics of cells and the heterogeneities of tissues will be discussed at length in later papers. For the present purposes it is sufficient to consider a volume of tissue in which the distribution against frequency of the energy in molecular movements could be represented by, for example, the curve in Fig. 4. While this distribution will have a dependence on temperature, *in vivo* studies do not allow this feature to be used.

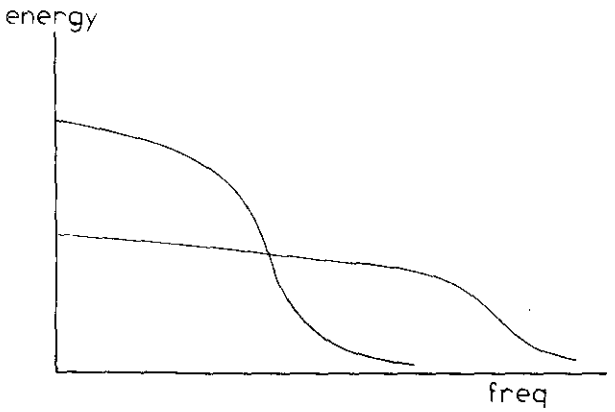


Fig. 4 – Distribution of energy in effective molecular motions against frequency for a two compartment voxel.

Such a volume of tissue can be probed by any one of the variety of standard NMR pulse and gradient sequences. Since the main subject is relaxation it will be assumed here that full spin-lattice and spin-spin relaxation curves can be determined down to almost complete relaxation. It will further be assumed that these relaxation curves can be obtained for a number of frequencies over a range up to the maximum obtainable with the imaging magnet without RF absorption problems.

In later sessions the details of the effects of cell and tissue architecture on relaxation will be discussed. Limited information is also apparently available on the effects of generalized metabolic changes on local cell and tissue hydration. Such effects will be superimposed on the local pathological condition and the relaxation properties will be determined by their combined influence.

The question being addressed is whether the full relaxation curves and their frequency dependencies contain sufficient information to disentangle the local from the generalized properties and then to specify the local tissue in a sufficiently unique manner as to be clinically useful.

There are many possible ways of analyzing relaxation curves. We offer an hypothetical method, partly to stimulate discussion and partly to illustrate the interface with spectroscopy.

One of the methods of analyzing curves that may be multiexponential is to carry out a Fourier transform according to the method of Gardner *et al* (6, 7). This produces a spectrum of the gradients contained in the

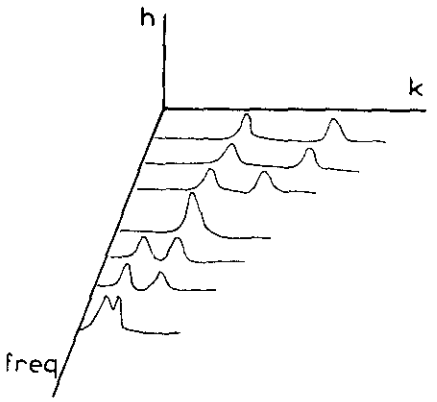


Fig. 5. – Representation of the frequency dependence of the time constants, k, of the bi-exponential spin-lattice recovery curves from the two compartment voxel referred to in Fig. 4.

curve. A series of such spectra from spin-lattice relaxation curves could be presented as shown in Fig. 5. An array of spectra like this could be regarded as the tissue signature or finger print. A similar array could be produced from any combination of spin-lattice and spin-spin relaxation data.

It is possible that data from some region of normal tissue subject to the same generalized metabolic disturbances could be used to help separate the local from the general effects. Nothing is known about the degree of non-linearity of the superposition of these effects.

It must, however, be asked whether the effort involved in producing such relaxation spectra might be better expended in producing chemical NMR line shift spectra. Perhaps both would be required.

Conclusions

The expression of relaxation properties within the NMR image presents many challenging problems but also offers the possibilities of new and clinically useful information both in imaging and in characterisation.

In looking for methods of standardising and calibrating equipment it would be helpful if attention can be focussed on those basic features that have a strong connection to both imaging and characterisation, and from which features specific to these two separate applications can be derived.

Acknowledgements

We are glad to acknowledge the support of the Department of Health and Social Security and the encouragement of Mr. J.L. William and Mr. G.R. Higson. We also acknowledge the collaboration of Dr. G.M. Bydder and colleagues of the Department of Diagnostic Radiology and Dr. I.R. Young and colleagues of Picker International Ltd.

REFERENCES

1. DOYLE F.H., PENNOCK J.M., BANKS L.M., McDONNELL M.J., BYDDER G.M., STEINER R.E., YOUNG I.R., CLARKE G.J., PASMORE I. & GILDERDALE D.G., 1982. Nuclear magnetic resonance imaging of the liver: initial experience. *Amer. J. Roentgenol.* **138**: 193-200.

2. YOUNG I.R., BAILES D.R., BURL M., COLLINS A.G., SMITH D.T., McDONNELL M.J., ORR J.S., BYDDER G.M., GREENSPAN R.H. & STEINER R.E., 1982 Initial clinical evaluation of a whole body nuclear magnetic resonance (NMR) tomograph. *J. Comput. Assist. Tomogr.* **6**(1): 1-18.

3. HINSHAW W.S., 1976. Image formation by nuclear magnetic resonance: the sensitive-point method. *J. Appl. Phys.* **47**: 3709-3721.

4. MANSFIELD P. & MAUDSLEY A.A., 1976. Line scan proton spin imaging in biological structures by NMR. *Phys. Med. Biol.* **21**: 847-852.

5. KUMAR A., WELTI D. & ERNST R., 1975. NMR Fourier zeugmatography. *J. Magn. Reson.* **18**: 69-83.

6. GARDNER D.G., GARDNER J.C., LAUSH G. & MEINKE W.W., 1959. *J. Chem. Phys.* **31**: 978-986.

7. SWINGLER D.N., 1977. A Different Technique for the Fourier Transform Processing of Multicomponent Exponential Functions. *IEEE Trans. Biomed. Eng.* **BME-24**: 408-410.

Ann. Ist. Super. Sanità
Vol. 19, N.1 (1983) pp. 19-24

THE BIOPHYSICS OF THE RELAXATION PROPERTIES OF WATER IN BIOLOGICAL SYSTEMS

R. MATHUR – DE VRE

Institut d'Hygiène et d'Epidémiologie – 14, Rue J. Wytsman, 1050 Brussels, Belgium

Summary. – *The relaxation times of water in biological systems reflect important clinical information by virtue of the fact that relaxation mechanism depend on the intrinsic state of water in cells and tissues. The variations in T_1 and T_2 of tissue water serve as an index for discriminating between the normal and certain pathological conditions, and help to identify the physiological details in soft tissues. A comprehension of the biophysics of relaxation times is essential not only for providing the essential background information for clinical evaluation of NMR applications, but also to provide an insight to practical problems associated with tissue characterization* in vitro.

Tissue characterization is the basic step necessary for upgrading NMR studies from mere empirical correlations of T_1 and T_2 values to obtain precise and meaningful identification of physiological details in tissues.

Riassunto. – *I tempi di rilassamento dell'acqua in sistemi biologici riflettono importanti informazioni cliniche in virtù del fatto che i meccanismi di rilassamento dipendono dallo stato intrinseco dell'acqua in cellule ed in tessuti. Le variazioni in T_1 e T_2 dell'acqua tissutale sono utilizzate come indice per discriminare alcune condizioni patologiche da quelle normali e per aiutare ad identificare alcuni dettagli fisiologici in tessuti molli. La comprensione della biofisica dei tempi di rilassamento è essenziale non soltanto per fornire informazioni fondamentali di interesse primario per la valutazione clinica delle applicazioni NMR, ma anche ai fini di una considerazione dei problemi pratici associati alla caratterizzazione tissutale* in vitro.

Introduction

The widespread applications of NMR in the medical field rely on its ability to discriminate between soft tissues by virtue of the differences in relaxation times T_1 and T_2 of water protons. However, the relaxation mechanism are marked with considerable complexities, and the relaxation data are often found to be inconsistent. In the light of increasing prospects of using T_1 and T_2 for the identification of biological tissues and their dependence on multiple factors, it has become necessary to probe into some biophysical concepts underlying the relaxation behaviour of water in biological systems. A knowledge of the biophysics of relaxation times is essential, not only for providing the background information necessary for clinical evaluation of NMR applications, but also to give insight into some practical problems associated with tissue characterization *in vitro*.

The aim of this article is: (i) to present a discussion of the biophysical aspects of the relaxation behaviour of water in biological systems; (ii) to evaluate the multiparameter character of NMR studies of water and their implications in medical studies; (iii) finally, to point out the underlying basis of some problems involved in tissue characterization that indicate the need for standardizing experimental methods.

I. Biophysical aspects

A full comprehension of the biophysical basis of relaxation times of water in biological systems requires: 1) a clear perspective of the state of water in biological systems; 2) a knowledge of the basic principles of relaxation times and the time scale of relaxation processes; 3) finally, a description of the impact of modified water structures in enhancing the relaxation mechanisms, i.e., underlining the link between 1) and 2).

I.1 Perspective of the state of water in biological systems

All biological macromolecules (proteins, nucleic acids, membranes, etc.) induce a characteristic structure of water (hydration water) in their close vicinity due to macromolecular-water interactions. Some characteristic properties of hydration water are:

i) restricted motion involving translation and rotation of water molecules;

ii) partial orientation of H_2O molecules with respect to macromolecular chains;

iii)multiple motional frequencies and distribution of correlation times (τ_c), where τ_c is the average time between molecular collisions and varies between 10^{-6} to 10^{-9} s in the hydration layer, and has a value of $\sim 10^{-12}$ s for free water;

iv) anisotropic diffusion and proton transfer along the chain;

v) anisotropic rotation, and decoupling of translational and rotational motions of H_2O molecules due to macromolecular-water interactions.

The water molecules in the hydration layer represent a highly complex, organized and heterogeneous network that constitutes an integral part of the macromolecular structure. Furthermore, water plays a crucial role in maintaining the structural and conformational integrity of all biostructures, and water-macromolecular interactions constitute basic phenomena bearing important implications in many essential life processes. Water in biological systems can be implicity described by a twostate model: bound water with multiple motional frequencies and distribution of correlation times in rapid exchange with free or bulk water characterized by a single correlation time. The properties of hydration water delineated above are thus averaged over all the water compartments by fast exchange that influence the relaxation times in a characteristic manner. Frank [1] proposed three types of water in contact with proteins: internal water (inside the macromolecular structure), surface-bound water and the perturbed water shell. Several specific examples illustrating the biophysical aspects of water and the significance of macromolecular hydration based on a variety of physical techniques are treated in a recent publication [2]. The identification of water in the primary sites that produce linking and stabilization of macromolecular structures are elegantly discussed for proteins and DNA by several authors.

A a matter of fact, the behaviour of water in the biological tissues is marked with intrinsic microscopic heterogeneity and overall macroscopic non-homogeneous distribution. The microscopic heterogeneity arises mainly from the nature of modified properties near the macromolecular surfaces and differences in the relative amounts of intracellular water, whereas macroscopic heterogeneity could be attributed to both hydration characteristics as well as variations in the total water content of soft tissues from different organs.

I.2 Relaxation times

Table 1 summarizes the essential features of T_1 and T_2 and outlines the types of molecular motions and their effective motional frequencies. In this article I will not describe the physical significance of relaxation times as they are treated in all text books of NMR spectroscopy, but simply underline the impact of different types of molecular motions and their time scales in enhancing the relaxation mechanism. The experimental methods for determining relaxation times will be treated in a subsequent paper in these proceedings.

Table 1. – *Some characteristics of relaxation times T_1 and T_2.*

T_1	T_2
Spin-lattice relaxation time	Spin-spin relaxation time
Exchange of energy between nuclei and the surrounding lattice	Exchange of energy between spin systems
	Represents average lifetime of a spin state
Sensitive to fast motion (rotational motion)	Sensitive to fast and slow motion (rotational, molecular exchange, diffusion and proton transfer)
Motional frequency MHz	Motional frequency Hz-MHz

I.3 The impact of modified structure of water on relaxation mechanism

The modified and characteristic structure of water molecules near the macromolecular surface summarized in Section I.1 bring into effect very efficient relaxation mechanisms. Packer [3] proposed that the fluctuating fields experienced by hydration water molecules near the macromolecular surface are not totally averaged out due to restricted motion and partial orientation, and their residual effects on relaxation mechanisms constitute a valuable source of information regarding intrinsic molecular states and dynamic structure of water at the macroscopic levels. Non-averaging of local fluctuating fields of the surrounding lattice enhances the T_1 relaxation rates, whereas, non-averaging of the dipolar interactions between nuclei increases the T_2 relaxation rates. Furthermore, T_1 relaxation is sensitive to rapidly fluctuating local fields such as those generated by fast rotational motion corresponding to the resonance (i.e. of the order of MHz); but T_2 senses both the fast and relatively slower fluctuations (c.f. Table 1) due to molecular exchange, proton transfer and diffusion of water molecules. With the results that both T_1 and T_2 of bound water in tissues are reduced relative to free water, but to different extents due to differences in their relaxation mechanisms. Fig 1 illustrates that this difference is more striking at longer τ_c values corresponding to the τ_c range for bound water than for short τ_c values typical of free liquids. Consequently, T_1 and T_2 provide invaluable complementary information about the intrinsic dynamic structure of tissue water. Several authors have stressed upon the need to

employ both T_1 and T_2 times for tissue characterization *in vitro* and for *in vivo* imaging studies [4, 5]. The variations in relaxation times are associated with changes in the structure of macromolecules and the biological state of cells and tissues. As a result, T_1 and T_2 data encode more details about the state of tissues than proton density.

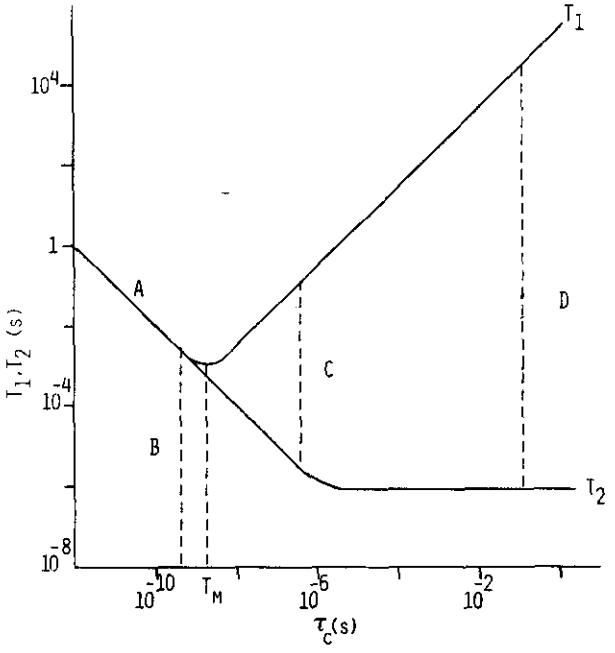


Fig. 1. – Dependence of T_1 and T_2 on correlation times τ_c

A Liquids: free water $T_1 = T_2$
B-C Non-rigid structure, restricted motion (hydration and tissue water); $T_1 > T_2$
D Rigid structure $T_1 \gg T_2$
TM Minimum, $1/\omega_0 \cong \tau_c$
(Based on the scale of Fig. 6.5, Ref. [15])

II. Factors affecting relaxation times

The observed relaxation times of water sensitive to a number of factors that can be classified into three groups: 1) the inherent biological factors; 2) extrinsic physical parameters and sample condition; 3) data treatment.

II.1 The inherent biological factors

The relaxation times of water are influenced by a number of biological processes that alter the "water balance" in cells and tissues. By "water balance" is implied the total water content, microscopic and macroscopic distribution (free/bound) and the nature of macromolecular-water interactions. The values of T_1 and T_2 of pathological tissues, notably of cancerous tissues are generally found to be longer than the corresponding or surrounding normal tissues. This observation attracted much attention in order to develop NMR as a diagnostic tool, and as a research device. Nevertheless, in several instance the distinction is not clear cut, as the relaxation times of normal tissues overlap with those of pathological tissues.

With a view to recognize the underlying processes responsible for discriminating soft tissues and to seek satisfactory explanations for the causes of relative variation in T_1 and T_2, attemps were made to correlate relaxation times with the following characteristics of water in biological systems:

(i) total water conteht;

(ii) the relative distribution of water in different compartments;

(iii) the dynamic structure of intracellular water (e.g. less structuring of water in cancerous cells).

Several authors [6-8] reported that relaxation times T_1 and T_2 are primarily dependent upon the water content of tissue samples. Whereas, Kasturi *et al.* [9] and Kagimoto [10] failed to observe any direct correlation between water proton relaxation times and the water content, Ling and Tucker [11] showed that T_1 is not simply a function of the water content but other factors specific to each tissue type also make important contributions. Their calculations revealed that water content differences between normal tissues and cancerous cells account for less than 10 % of the difference. The observed relaxation rate $(1/T_1)_{obs}$ is the weighted average of relaxation rates characteristic of bound $(1/T_1)_b$ and free $(1/T_1)_f$ water, respectively.

$$(1/T_1)_{obs} = X_b\,(1/T_1)_b + X_f\,(1/T_1)_f$$

Thus, we see that a change in either relative fractions of water in different compartments (X_b, X_f) or in their characteristic relaxation times $(T_1)_b$, $(T_1)_f$ could alter the observed T_1 values. Weisman [12] proposed that longer relaxation times of malignant tumours were not due to longer T_1 and T_2 inside cells but to extracellular water. Shah *et al.* [13] on the other hand, observed T_1 discrimination in the nuclear fractions isolated from the normal and tumour tissues; they concluded that the basis of T_1 differences lies in the intrinsic characteristics of the cellular compartments, in addition to other reasons discussed above.

A number of pathological and physiological conditions perturb the "water balance" of cells and tissues, and their effects are monitored by T_1 and T_2 of tissue water. As a result, a vast number of disorders and abnormal states can be detected by NMR. The changes in the state of water may be affected by natural biological processes such as age, by the impact of stress and strain as well as by the very nature of the species under consideration. The relaxation times of tissue water are likely to be sensitive to radiotherapy. There is some evidence of the effects of γ-irradiation on the hydration characteristics of DNA as studied by NMR [14]. The impact of all these processes needs to be taken into account in order to correlate the variations of T_1 and T_2 with relative differences in the state of pathological and normal tissues.

II.2 Extrinsic physical parameters

The relaxation times depend in a distinct manner on the following extrinsic physical parameters: (a) resonance frequency, (b) temperature, (c) preparation and storage conditions of samples *in vitro*.

(a) Resonance frequency. – Hydration water is characterized by multiple motional frequencies and distribution of correlation times (τ_c). As a result, the relaxation times of tissue water depend on the resonance frequency (ω_0) used for measurements. The effects of the distribution of correlation times on relaxation mechanisms indicate [15] that the most efficient relaxation mechanism occurs at the limiting case of $\omega_0 \cong 1/\tau_c$, resulting in a minimum of T_1 values. The position of the minimum depends on the resonance frequency. Furthermore, the studies of the $1/T_1$ - - frequency dispersion of protein solution demonstrated that the inflection frequency of the dispersion curves is correlated with the rotational time of the protein molecules that determine, in turn, the correlation times of the water molecules in their close vicinity [16, 17].

The foregoing discussion draws attention to carefully selecting the resonance frequency for observing a maximum discrimination of relaxation times.

(b) Temperature dependence. – The observed relaxation times of tissue water are dependent on temperature changes due to temperature sensitivity of the dynamic structures of water in biological systems. The results from the tissues of human liver, bram and liver of mice gave activation energies of 1.5-2.5 kcal/mole for the relaxation processes between $+5^0$ and $+15^0$ C [18]. The effects of temperature changes are all the more significant for measurements performed under the conditions when bound water predominantly contributes to the observed spectra, such as in the case of frozen samples [14, 19]. The T_1-temperature relation between -6^0 and $\sim -40^0$ C for biological samples results in curves with a well-defined minimum located within -12^0 to -18^0 C range (100 MHz) depending on the type of samples.

It is clear that in order to employ T_1 and T_2 as discriminating parameters, the values must be compared at the same frequency and temperature for each sample. The temperature - and frequency - dependent relations of T_1 and T_2 may also serve to identify the nature of relaxation processes. The T_1 at the minimum of T_1 - temperature curves for a given frequency (preferably corresponding to the inflexion frequency of $1/T_1$ vs H_0 curves) for tissue water is likely to be a sensitive discriminating parameters.

(c) Methods of preparation and storage. – In order to obtain reproducible and comparable values of relaxation times of tissue water, the methods of sample preparation and storage must be carefully controlled and standardized. The practical details concerning experimental protocol will be discussed in a subsequent paper.

II.3 Data treatment

The decay curves for tissue water often exhibit multi-exponential behaviour; this renders an adequate analysis of data very complex and difficult for obtaining accurate values of T_1 and T_2. The multi-exponential behaviour arises mainly due to the heterogeneous nature of the state of biological water, and non-averaging of local field fluctuations in the event of restricted motion and partial orientation of water molecules in the intrinsic hydration sites. An understanding of the approach to data analysis is required for specifying the significance of the observed relaxation data. This subject will be treated in detail in a later paper in these Proceedings by J. Reisse (pp. 43-48).

III. Implications of the multiparameter nature of relaxation times

From the preceeding discussion it follows that an important and unique feature of NMR spectroscopy in the perspective of medical applications is the use of three distinct and inter-related variables and the multiparameter dependence of relaxation times. The values of T_1 and T_2 depend in a characteristic manner on a number of biological factors inherent to the particular state of tissues that affect the state of water and on the physical conditions. The multiparameter character of relaxation times gives rise to many practical problems, but it also provides some useful background information. The dependence of T_1 and T_2 values simultaneously on a number of biological factors and experimental variables, requires a strict control of conditions such as: methods of preparation and storage, methods of measurements and analysis of data for meaningful correlation of relative changes in relaxation times with inherent differences in the biological state of tissues.

The state of "water balance" of cells and tissues is readily perturbed by essential biological processes. This gives rise to high sensitivity of NMR relaxation times to detect (*in vitro* and *in vivo)* abnormal states oftissues, lesions and tumours that are associated with changes in the state of water. However, the indiscriminating effects of a number of pathological and physiological processes on "water balance" and relaxation times results in a lack of specifity.

Conclusions

The relaxation times of tissue water are very useful index parameters for the characterization of tissues and identification of their pathological and physiological states. A knowledge of the relaxation behaviour of water in biological systems constitutes an essential base for a full comprehension of the biophysical background of NMR applications in medicine. At the same time, these

tissue characterization for *in vitro* studies, and point out the need to carefully control and standardize the experimental protocol in order to obtain meaningful and consistent correlations between NMR parameters and the biological factors. Some of these are the subject of discussion in subsequent papers in these Proceedings. Finally, a comprehension of the relaxation behaviour of water is likely to provide biophysical basis of many empirical correlations found to be very useful for the clinical applications of NMR. Some of these are: (i) generally, T_1 and T_2 images reveal more information than the proton density images; (ii) certain types of lesions are better seen either in T_1 or in T_2 images, whereas most can be detected when T_1 and T_2 are used in conjunction with each other; (iii) T_2 images often excel in providing information as compared with T_1 images.

REFERENCES

1. FRANKS, F. 1977. Solvation interactions of proteins in solution. *Philos. Trans. R. Soc. London.* B 278: 89-96.

2. FRANKS, F. & MATHIAS, S.F. (Eds.). 1982. *Biophysics of water.* John Wiley & Sons, New York.

3. PACKER, K.J. 1977. The dynamics of water in heterogeneous systems. *Philos. Trans. R. Soc. London* B 278: 59-87.

4. DAVIES, P. L., KAUFMAN, L. E., CROOKS, L. E. & MARGULIS, A. R. 1982. NMR characteristics of normal and abnormal rat tissues. In: *Nuclear Magnetic Resonance Imaging in Medicine.* L. KAUFMAN, L. E. CROOKS & A. R. MARGULIS (Ed.), Igaku-Shoin, New York, pp. 71-100.

5. HAWKES, R. C., HOLLAND, G. N., MOORE, W. S. & WORTHINGTON, B. S. 1981. Nuclear magnetic resonance (NMR) tomography of the normal heart. *J. Comput. Assist. Tomogr.* 5: 605-612.

6. INCH, W.R., McCREDIE, J. A., KNISPEL, R. R., THOMPSON, R. T. & PINTAR, M. M. 1974. Water content and proton spin relaxation time for neoplastic and non-neoplastic tissues from mice and humans. *J. Nat. Cancer Inst.* 52: 353-356.

7. FUNG, B. M., DURHAM, D. L., & WASSIL, D. A. 1975. The state of water as in biological systems as studied by proton and deuterium relaxation. *Biochim. Biophys. Acta* 399: 191-202.

8. EGGLESTON, J. C., SARYAN, L. A., & HOLLIS, D. P. 1975. Nuclear magnetic resonance investigations of human neoplastic and abnormal non-neoplastic tissues. *Cancer Res.* 35: 1326-1332.

9. KASTURI, S. R., RANADE, S.S. & SHAH, S. 1976. Tissue hydration of malignant and uninvolved tissues and its relevance to proton spin-lattice relaxation mechanisms. *Proc. Ind. Acad. Sci.* 84 B: 60-74.

10. KAGIMOTO, T., YAMASAKI, M., MORINO, Y., AKASAKA, K & KISHIMOTO, B. 1977. Nuclear magnetic resonance study of human bone marrows from patients with leukemia, anemia and myeloma. *J. Nat. Cancer Inst.* 59: 335-337.

11. LING, G. N. & TUCKER, M. 1981. NMR relaxation and water contents in normal tissues and cancer cells. In: *NMR in Medicine.* R. Damadian (Ed.) Springer-Verlag, Berlin, pp. 161-174.

12. WEISMAN, I. D., BENNETT, L. H., MAXWELL, L. R., WOODS, M. W., & BURK, D. 1972. Recognition of cancer *in vivo* by nuclear magnetic resonance. *Science* 178: 1288-1290.

13. SHAH, S., RANADE, S. S., KASTURI, S. R., PHADKE, R. S. & ADVANI, S. H. 1982. Distinction between normal and leukemic bone marrow by water protons nuclear magnetic resonance relaxation times. *Magnetic Resonance Imaging* 1: 23-28.

14. MATHUR-DE VRÉ, R., BERTINCHAMPS, A. J., & BERENDSEN, H. J. C. 1976. The effects of γ-irrradiation on the hydration characteristics of DNA and polynucleotides. I. An NMR study of frozen H_2O solutions. *Radiat. Res.* 68: 197-214.

15. FARRAR, T. C. & BECKER, E. D. 1971. *Pulse and Fourier transform NMR.* Academic Press, New York.

16. HALLENGA, K. & KOENIG, S. H. 1976. Protein rotational relaxation as studied by solvent ^1H and ^2H magnetic relaxation. *Biochemistry* 15: 4255-4263.

17. KOENIG, S. H. & SCHILLINGER, W. E. 1969. Nuclear magnetic relaxation dispersion in protein solutions. *J. Biol. Chem.* 244: 3283-3289.

18. MATHUR-DE VRÉ, R., GRIMÉE, R. & ROSA, M. P. 1983. Experimental protocol for tissue discrimination *in-vitro* by NMR. ***Bioscience Reports.*** **3: 599-608.**

19. MATHUR-DE VRÉ, R. 1979. The NMR studies of water in biological systems. *Prog. Biophys. Mol. Biol.* **35:** 103-134.

Ann. Ist. Super. Sanità
Vol. 19, N. 1 (1983) pp. 25-30

HETEROGENEITIES IN SPACE, TIME, AND CLINICAL CONDITION INFLUENCING THE NMR RESPONSE OF TISSUES

K. H. SCHMIDT

Department of Surgery, University of Tübingen, D-7400 Tübingen, Federal Republic of Germany

Summary. – *During the last decade NMR studies on biological tissues have branched off into a number of directions. One approach is NMR imaging which is the spatial resolution of NMR parameters such as spin density or relaxation times, in order to obtain a NMR model of the architecture and organisation of tissues, organs and organisms. This descriptive approach adds valuable NMR information to available descriptive techniques such as X-ray scanning or ultrasound reflection. The potential of this NMR technique for tissue identification or characterization, however, is so far very limited, mainly due to heterogeneities in space, time and clinical conditions of the biological tissues.*

In the present paper attempts are made to define the biological tissue as the substratum of the NMR measurements according to principles of morphology and physiology.

Riassunto. – *Nell'ultimo decennio gli studi NMR di sistemi biologici si sono ramificati in diverse direzioni. Uno degli approcci è l'*imaging *NMR che consiste nella risoluzione spaziale di parametri NMR quali la densità di spin o i tempi di rilassamento, al fine di ottenere un modello NMR dell'architettura e dell'organizzazione di tessuti, organi o organismi. Questo approccio descrittivo aggiunge utili informazioni NMR alle tecniche descrittive disponibili, quali la scansione a raggi X o la riflessione di ultrasuoni. La potenzialità di questa tecnica NMR nell'identificazione e caratterizzazione tissutali sono state tuttavia sinora limitate soprattutto dalle eterogeneità nello spazio, nel tempo e nelle condizioni cliniche dei tessuti biologici.*

In questo lavoro si tenta di definire il tessuto biologico come il substrato delle misure NMR, secondo i principi della morfologia e della fisiologia.

Introduction

Biological tissue is an extremely heterogeneous material for physical measurements such as nuclear magnetic resonance. Not a single element of the periodic system is uniformly distributed on the subcellular, cellular or tissue level. It is therefore logical to relate NMR data measured in biological tissues to the known heterogeneities of this substratum. Under physiological conditions the spatial heterogeneities of cells and tissues change with different functional activities. It is therefore necessary to define the functional state of a tissue or organ before valuable interpretations of NMR data of biological tissues can be made. Presently the potential of NMR techniques for tissue identification and characterization is limited by these heterogeneities in space and time giving a mean NMR response which is difficult to interpret. Under pathological conditions of tissues the range of possible NMR data is further extended and additional overlaps are observed.

The present review describes some of the methods used for separation of NMR signals from different compartments of tissues, as well as for standardisation of functional activity [1–9]. In addition attempts are made to relate the NMR model of pathological tissues to principles of general pathology.

During the last 20 years a number of non invasive techniques such as emission tomography, X-ray computed tomography, ultrasound, digital subtraction angiography, nuclear magnetic resonance tomography, etc. have been successfully applied to biomedical problems. Among these techniques nuclear magnetic resonance and emission tomography have the unique capability of non invasively measuring functional parameters of various organs in relation to metabolic processes. Thus nuclear magnetic resonance can not only provide important contributions to the detection of various pathologies but also allows mechanistic studies on the etiology and pathogenesis of many diseases. In addition nuclear magnetic resonance can contribute to the selection and surveillance of treatment regimens for various disorders. In contrast to most of the other descriptive diagnostic radiology methods nuclear magnetic resonance of tissues has a basic science potential leading to sound hypotheses regarding the cause and mechanism of a disease rather than the morphology. Until now NMR as a tool for tissue characterisation is far from being a push button

method, despite of the fact that the number of manufacturers producing the instrumentation is successively increasing. In this paper an attempt is made to relate NMR parameters to tissue characteristics, particularly in terms of compartmentation, metabolic or functional condition, diseased states of the living tissue, ageing etc. of the living tissue. It is tried to review some of the experimental systems described as useful tools in evaluating the relevance of changes in the NMR response to tissue characterisation.

Usually five parameters are measured in a typical NMR experiment in living tissue: *spin density* (a) of different nuclei which have the appropriate nuclear characteristics, *spin--lattice* relaxation time T_1(b) as a measure of energy relaxation and *spin--spin* relaxation time T_2(c) as a measure of phase relaxation. In addition *flow* (d) information can be obtained by measuring changes in signal intensity owing to movement of nuclei through the resonance region or *chemical shifts* (e) are measured, representing small changes in resonant frequency due to chemical bonds neighbouring the observed nuclei.

It is important to note that the different nuclei analyzed in living tissue by NMR such as hydrogen, carbon, sodium, phosphorus, etc. cannot be measured with equal sensitivity. This is one reason for the fact that NMR studies for identification and characterization of biological tissues are carried out in two fundamentally different modes: imaging and sampling. In both modes the nuclei of interest can be of exogenous or endogenous origin and all five important NMR parameters can be obtained according to the actual detection sensitivity. In the sampling mode NMR parameters can be measured *in vivo* noninvasively using the topical NMR technique (TMR) or invasively using perfused organs, biopsy material, or cultured cells *in vitro*. For the interpretation of the NMR result it is essential to know in which way and to what extent the NMR parameters are influenced by tissue heterogeneities in space and time and how these alterations relate to pathological or clinical conditions.

Tissue heterogeneities in space

Living tissue is organized in functional units whereby the functional demands determine number and spatial distribution of cells and organelles. This structural organisation can be found on the level of whole organs, on the cellular, and subcellular level. Thus, whatever the living tissue or the spatial resolution of the NMR instrumentation may be, one has necessarily to deal with a heterogeneous substratum. On the cellular level the number and size of different organelles such as nucleus, mitochondria, endoplasmic reticulum, lysosomes, and a variety of granules significantly influence the NMR response. The subcellular organelles are separated from the aqueous phase of the cytoplasm by lipid membranes which represent a barrier against free diffusion of cellular constituents. Important physical and chemical parameters such as ionic strength, metabolite concentration, osmolality or oxygen pressure can therefore vary considerably inside and outside a specific organelle.

As a very informative example NMR determinations of the intracellular pH value should be mentioned. In a homogeneous system the pH value can be determined with sufficient accuracy by NMR from the chemical shift of the phosphorus signal. The intracellular pH of a living cell, however, is a composite number due to the very different pH values in different compartments of a single cell such as for example lysosomes (pH 4.5) or cytoplasm (pH 7.4). In this case the interpretation of NMR data is severely hampered by heterogeneities in space on the subcellular level.

Similarly measurements of proton relaxation times of cells result in a mean T_1 or T_2 due to the fact that the different species of protons in a particular cell population cannot be analyzed separately. This difficulty becomes apparent in experiments of whole tissues, where the contribution of protons in the lipid phase and the aqueous phase to the NMR result is not differentiated.

On the cellular and subcellular level it is presently very difficult to relate NMR parameters to structural or functional properties of single compartments. Studies using isolated organelles such as mitochondria or nuclei as a substratum could give additional insight on the contribution of single compartments of a cell to NMR parameters measured. On the level of tissues and whole organs heterogeneities between the cellular compartment and the extracellular space significantly influence the NMR result. Due to differences in the ionic *milieu* in the two compartments the spin density can vary within wide ranges. As an example the concentration ratio of sodium for the extracellular to the intracellular space is about 15:1. Although the sensitivity of sodium measurements is some hundred times less than that for protons, these tissue heterogeneities can be detected by NMR.

In addition to differences in spin density, tissue heterogeneities can be monitored by measuring relaxion times. This is possible by use of paramagnetic ions such as manganese which lower the T_1 of protons and do normally not cross the cellular membrane. Thus the contribution of the two compartments to the T_1 value of a tissue can be separated. In a different approach chemical shift reagents such as lanthanides are used for separation of the extracellular and intracellular NMR signals. This is particularly useful in the NMR studies of whole blood where signals from the plasma can be shifted, whereas signals from the cells are unaffected by the shift reagent. Further research on the development of nontoxic contrast agents should open the way for a highly selective measurement of specific compartments and structures of tissues and organs by NMR. Tissue heterogeneities in space are often caused by differences in the blood supply to different organs or cells. Due to the effect of oxygen on the relaxation times these heterogeneities are reflected by NMR measurements. As an example, it was demonstrated that T_1 increases with increasing oxyhemoglobin contents of tissues. Similarly T_2 changes were measured in association with the oxygen content of the blood. The mechanism of this effects is still

unclear, but it is of great clinical significance in many diseases such as circulatory shock where the oxygen supply to tissues is altered.

In a variety of organs the specific functional organisation of tissues requires macroscopic heterogeneities in space. A characteristic example is the lung where the gas–filled alveolar lumen equilibrates with the tissue. This heterogeneity has been successfully used for the assessment of the distribution and exchange of gases within the lung by NMR of the gas phase. ^{19}F–NMR measurements of the lung after breathing an oxygen/carbon tetrafluoride mixture is one possible method.

In a similar approach the vascular system was filled by intravenous infusion of perfluorocarbons with subsequent measurement of ^{19}F–NMR signals.

In this case the contrast agent has the advantage of simultaneously transporting oxygen molecules to the tissues.

In the later phases of the experiment the perfluorocarbons are converted to volatile fluorinated compounds which are eliminated by the lung. This again allows NMR measurements of the gas phase in the lung.

An additional idea was to use fluorinated lipophilic compounds such as halothane as finger–print markers of different compartments due to differences in the ^{19}F–signal in an aqueous or lipid environment.

Similar heterogeneities are involved in examinations of the human heart, where the myocardium can be distinguished from blood by NMR techniques. In high resolution images mostly gating is used in order to avoid motion. Although ungated heart imaging studies give seemingly good resolutions, the NMR data measured are complex results derived from pericardial lipids, myocardium, and paradoxical T_1 enhancement due to motion. In addition the NMR parameters will vary with hematocrit and oxygenation of the blood.

An important spatial heterogeneity in brain tissue is the blood–brain barrier which controls the access of compounds to the nerve cells. The permeability of this barrier is an important diagnostic parameter which can be measured by NMR after intravenous injection of a paramagnetic contrast agent.

Another heterogeneity in space which can be detected in the brain by NMR is the white and grey matter. The differences in the proton T_1 value between the white and grey matter of the central nervous system appears to be due to the lipids of the myelin. Approximately 80% of the myelin is lipids. An additional characteristic heterogeneity within the brain is the structure of the ventricles filled with cerebrospinal fluid. Using NMR techniques it is possible to evaluate anatomical details of the three dimensional architecture of the ventricles.

Heterogeneities in the kidney such as cortex, medulla, and pelvis can be differentiated by NMR as well. This can add valuable information to available techniques, particularly in diseased states.

An additional potential has the NMR technology in studies of fetal tissues because of the negligible hazards as compared to other methods.

Heterogeneities in time

The dependence of a whole organism on single tissues for carrying out specialized functions implies communication between organs and feedback control. This regulation involves continuous control of metabolic activity, energy metabolism, and performance of specialized functions, such as biosynthesis and biodegradation, growth and involution. In the adaptive response of tissues and organs to changing needs, these control mechanisms play a significant role. In this context we notice that cell proliferation or biosynthetic activity in an organism can vary among different tissues and within the same tissue from time to time. It is well established that some tissues cease to divide after maturation, whereas in others the total cell number is maintained and continuously balanced despite of active proliferation. It can be expected that these heterogeneities in time influence the results of NMR measurements. Experimental studies on relaxation times of water protons of tumor cell lines demonstrated that T_1 changes significantly as a function of the cell cycle. Cells synchronized in the mitosis show T_1 values higher than 1000 ms whereas the T_1 of the same cell line in the phase of DNA synthesis is only about 500 ms. This demonstrates the effects of heterogeneities in time during growth and division of cells on the NMR parameters measured. In a real tissue the cells are normally not synchronized indicating that the relaxation times measured are mean values. Speculations on the mechanism of the cyclic changes of NMR signals are frequent and sometimes contradictory. Conformational changes of macromolecules or differences in water content were proposed to be of etiological significance.

Further heterogeneities in time can be identified during maturation of tissues. Obviously in developing tissues changes in the cellular organisation occur which influence NMR parameters such as T_1 or T_2. It was demonstrated that the relaxation time of immature muscular tissue is of the order of two times longer than relaxation of mature muscle. Similarly the sodium concentration in developing tissues declines with age in very young animals and stabilizes as they reach maturity. These experimental results are interpreted as being a consequence of water–macromolecule interactions. The loss of tissue water generally observed during ageing increases the ratio of bound water to bulk water, thus causing shorter relaxation times. Recurrent changes in NMR signals are observed in different tissues as a function of endocrine responses. As an example centrifuged sediments of human vaginal epithelia sampled at varying times over the menstrual cycle yielded significantly different NMR signals at the midcycle as compared to other times. It was therefrom speculated that the exchange times of intracellular and extracellular water are significantly longer at the midcycle due to the hormonal effects on the cellular membrane. Little information is available at the present time on endocrine effects on NMR parameters of different tissues,

The influence of muscle contraction on line width

and line splitting was studied in uterine smooth muscle samples. Obviously the functional state influences the NMR results measured in tissues. This topic will be further outlined in the chapter on clinical conditions of tissues.

Heterogeneities in time can arise from flow or motion of whole tissues or organs. In this context the motion of organs during inspiration and expiration is an important problem. Deep inspiration can move single organs such as the kidneys over some centimeters causing difficulties in NMR imaging. Techniques requiring more than a few seconds for data acquisition will give images which have the smearing effect of motion and distortion of T_1 values by the motion depending on pulse duration.

In the heart ^{23}Na could act as a natural contrast agent allowing measurements of the ejected blood volume per beat by imaging ^{23}Na. Conventionally blood flow is measured by doppler ultrasound. NMR provides a number of possibilities to noninvasively measure flow: phase dispersion techniques, magneto hydrodynamic effects, time-of-flight of tagged nuclei, or the relation of T_1 to the pulse timing have been used. Valuable flow information of organs can be obtained from changes in T_1 after injection of paramagnetic agents. This also allows studies on the regulation of blood supply under varying conditions such as exercise or in diseased states.

Tissue heterogeneities in time occur during storage of organs for transplantation. This is particularly known for banked blood which undergoes marked changes in the first days of conservation. Similar alterations are known to occur in other organs due to removal from circulation and changes in the environmental conditions. By NMR measurements it is possible to follow the process in stored tissues and organs.

Similarly the immune responses leading to rejection of an implanted tissue can be monitored noninvasively by NMR. Again the tissues heterogeneities influence the NMR signals and allow quantitative estimation of the tissue compatibility after transplantation. In addition it is possible on the basis of NMR measurements to select the appropriate media and conditions for storage of different organs.

Heterogeneities in clinical conditions

NMR not only can provide important contributions to the detection of various pathologies, but also allows mechanistic studies on the etiology and pathogenesis of disorders. It is therefore important to relate changes in measured NMR parameters to tissue heterogeneities, according to the principles of general pathology.

Experimental *in vivo* and *in vitro* models have been developed by which various pathologies can be simulated under standardized conditions. In these experiments it is an important aspect of the NMR approach that the microenvironment of tissues is not distorted by the analytical technique itself. To the most part our current knowledge about tissue characteristics in diseased states is based on invasive techniques such as chemical analysis after freeze clamping of the tissue or upon histomorphology of tissue sections after chemical fixation. Both techniques are sensitive to artifacts and do normally not allow a functional characterization of the tissue *in vivo*.

As an example tissue reactions to circulatory shock should be mentioned, where invasive procedures yield questionable results. In this case NMR analysis allows one to follow some of the mechanisms by which the nervous system, the vascular system, the endocrine system, or the immune system modulate the tissue response.

The heterogeneities in clinical condition can be separated in two major complexes: heterogeneities due to systemic disorders and heterogeneities consequent to localized processes.

Among the systemic modulators of tissue characteristic nutrition plays an important role because of the critical dependence of many organ functions upon an adequate nutritional supply. This is of particular interest under clinical conditions where patients are under parental nutrition. The great number of formulas for calculating the adequate infusion therapy indicates the difficulties in this area of intensive care. NMR could here be of great value in the surveillance of patients. Similar problems arise in diabetics where frequently uncontrolled metabolic aberrations occur. Additional systemic influences on organ functions with relevance to NMR can be summarized as follows: hypoxia, hyperthemia, malnutrition, blood supply, biorhythm, age, allergy and anergy, shock, dialysis, intoxication, assisted respiration, blood transfusion, transplantation, pregnancy, psychological conditions, etc. It is at the present time impossible to predict the areas in which NMR techniques are most appropriate for tissue characterization under clinical conditions.

A similar listing of local conditions affecting the NMR results of tissues can be made: edema, hematoma, cyst, fibrosis, cirrhosis, mineralisation, atrophy, involution, necrosis, necrobiosis, neoplasia, metastasis, ischemia, storage, plaque, exudation, demyelinisation, emphysema, empyema, etc. Some of these local tissue heterogeneities have been studied in patients or in experimental animals.

The validation of NMR methods for diagnostic value will require further detailed animal studies under highly standardized conditions with emphasis to potential artifacts. Furthermore a systematic organization of the research data on animals and man is required in order to understand how image contrast is related to instrument parameters and tissue properties.

REFERENCES

1. BATTOCLETTI, J.H., HALBACH, R.E., SALLES–CUNHA, S.X., & SANCES, A. 1981. The NMR blood flow meter theory and history. *Med. Phys.* 8: 435-443.

2. BUDINGER, T. F. 1981. Nuclear magnetic resonance *in vivo* studies: known thresholds for health effects. *J. Comput. Assist. Tomogr.* 5: 800-811.

3. CHANCE, B., NAKASE, Y. & BOND, M. 1978. Detection of ^{31}Pnuclear magnetic resonance signals in brain by *in vivo* and freeze trapped assays. *Proc. Natl. Acad. Sci. U.S.* 75: 4925-4929.

4. DAMADIAN, R. 1971. Tumor detection by NMR. *Science*, 171: 1151-1153.

5. GADIAN, D.G. & RADDA, G. K. 1981. NMR studies of tissue metabolism. *Ann. Rev. Biochem.* 50: 69-84.

6. JACOBUS, W. E., TAYLOR, G.J., HOLLIS, D.P. & NUNNALLY, R. L. 1977. Phosphorus nuclear magnetic resonance of perfused working hearts. *Nature*, 256: 756-758.

7. KAUFMAN, L., CROOKS, L.E. & MARGULIS, A. R. (Eds.). 1981. *Nuclear Magnetic Resonance Imaging*. Igaku–Shoin. New York.

8. LAUTERBUR, P.C., DIAS, M. H. M. & RUDIN, M. 1978. Augmentation of tissue water proton spin–lattice relaxation ratio by *in vivo* addition of paramagnetic ions. In: *Frontiers of biological energetics. 1.* Academic Press, New York. pp.752-759.

9. YOUNG, I.R., HALL, A.S., PALLIS, C.A., LEGG, N.J., BYDDER, G.M. & STEINER, R.E. 1981. Nuclear magnetic resonance imaging of the brain in multiple sclerosis. *Lancet. II* (8255): 1063-1066.

Ann. Ist. Super. Sanità
Vol. 19, N. 1 (1983) pp. 31-40

Discussion
Moderator: F. Podo

PODO - In the first session of this Meeting we have considered several general problems encountered in the characterization of biological tissues on the basis of their nuclear magnetic relaxation behaviour. The main goal of this workshop is to elaborate a feasible and realistic working plan for a possible joint European cooperation. In order to properly prepare this plan, it is urgent to review the present state of art pertaining to the project, to identify the most significant research problems involved, and the different ways and means of coping with them. Prof. Maraviglia has summarized principles and basic applications of NMR in medicine, from *in vivo* spectroscopy to imaging. We heard about the use of the various approaches and the main physical information derived from them, together with some aspects concerning the instrumentation that might be relevant in trying to cope with the problems, the choices to be made, and the knowledge required to carry out the work. Prof. Orr has given a survey of the principal aspects of the expression of relaxation properties within the NMR image. This is a subject which lies between the problem of instrumentation and the biophysics of complex tissues. Dr. De Vré has presented a scientific review on the relaxation times of water in biological systems, their physical basis and clinical significance. A better understanding of the biophysics of relaxation properties in tissues appears to be essential, not only for providing the scientific background necessary to the clinical evaluation of medical applications of NMR but also for providing an insight into practical problems associated with tissue characterization *in vitro*. Finally Prof. Schmidt has given an excellent survey on heterogeneities in space, time and clinical conditions of biological tissues. These presentations are now open for discussion.

BENE - I noted in the paper presented by Dr. De Vré that T_1 is generally only sensitive to fast motion, while T_2 is sensitive to both fast and slow motions. Do you agree with the fact that when fast and slow motions both occur within a sample, it should be considered that T_1 is clearly sensitive to fast motions, but T_2 is mainly sensitive to slow motions when relaxation processes are controlled by magnetic dipolar interactions?

DE VRE - I agree with your point. In fact T_2 can be used for detecting the effects of proton transfer and molecular diffusion. The diffusion constant of water is determined from T_2 values of the tissue water.

RADDA - Can I make a general point before I make some specific observations, particularly on Prof. Schmidt's beautiful presentation. The general point I'd like to make is that I think there are thirty-five of you here interested in protons and one of us interested in spectroscopy, and the statement that you make about NMR does not apply to spectroscopy in anywhere the same way as they apply to T_1 and T_2 relaxation times. I think that when you make statements that tissue characterization by NMR is difficult, we don't know what clinical conditions could be studied. I'm prepared to be challenged on such statements with respect to phosphorus NMR. It can tell us about certain clinical conditions. I believe we have much better ideas about metabolism and on what we might expect on the basis of animal and other related studies than perhaps one can have in relation to T_1 and T_2 relaxation times. So I think I'd like to have this distinction fairly clear throughout the discussion because otherwise we are going to get very confused whenever we lump NMR as one single measurement. That's the general point I'd like to make. On specific issues, I would like to take an example where that sort of confusion arises. I think Prof. Schmidt discussed the difficulties of looking at compartmentation by NMR. I'd like to say how easy it is to study compartmentation by specific phosphorus NMR rather than difficult. We have obtained new information about pH distribution. We are not confused about what we are measuring from phosphorus NMR in terms of cellular pH. We do know that in a majority of the cases – and it is well-proved – we measure cytoplasmic pH and not therefore indiscriminate pH. We have examples where we can see the differences between mitochondrial pH and cytoplasmic pH in the whole cell. Rather than it being confusing, therefore, I think it in fact clarifies one of the main biological difficulties. We can, with high resolution spectroscopy, look at compartmentation even within the cell, and we can certainly study tissue heterogeneity. Here is one example where T_1 and T_2 measurements might not tell you that, but spectroscopy can. I could go on and pick many others, but perhaps that's enough for the time being.

SCHMIDT - I would answer that if you have additional information on the compartmentation, then NMR can give you the specific results. But if you don't know which compartment you are actually observing, then spectroscopy doesn't give you any idea where the signals are derived from. Only if you have additional parameters localizing your signals into the specific compartments, then I accept that your spectra represent something which contains compartmentation in the model. But intrinsically NMR is not able to distinguish between compartments as such.

RADDA - Well, I think that's true that intrinsically NMR can't, but in combination with biochemical knowledge and biochemical tricks, and not using isolated systems, it can. For example, the use of deoxyglucose to produce deoxyglucose 6–phosphate which we know is entirely cytoplasmic in comparison to what you get from phosphorus would be just one way of doing it, and there are many other tricks that we can use in intact tissues. But when you talk about contrast agents, in a sense

you do biochemical contrasting – you use the biochemistry to distinguish the compartments in conjunction with spectroscopy.

SCHMIDT - This was the message I wanted to get over: that if you characterize the heterogeneities by additional investigation, such as biochemical sampling of compartments, then NMR data can give you valuable information on the micro–environment within a compartment. That's clear.

VILLA - Again with reference to Prof. Schmidt's talk, I found several points very interesting. First of all, I believe that it is very interesting to assert that we really obtain an "average" response from a given compartment when we are looking at mobile spins and not when we consider localized spins such as phosphorus nuclei in intracellular metabolites. I think that a knowledge of this ignorance is fundamental if we want to try to understand what is going on. But there are several things that I didn't understand in that talk, which was so complex and rich, that I need some explanations. You said that you succeeded in detecting compartment effects by doping the system with manganese. However, during your experimental time your water molecules go back and forth across membranes, so that what you essentially obtained was again an average information. You actually perturbed the external environment outside the membrane, so I don't know exactly how you could assess this compartment effect. My second question concerns the ^{19}F NMR experiment. I have made some measurements on fluorinated compounds and I found an extremely complicated spectrum about a thousand p.p.m. wide, with dozens and dozens of different lines. I see instead that you could detect only a couple of ^{19}F NMR signals from your perfluorocarbons. I would like to have some more technical details about this experiment. My last question is only due to my ignorance about biochemistry. Do really our tissues contain 2.6 millimoles per gram of ^{19}F atoms? I didn't think that we had such a high concentration of fluorine naturally occurring in our tissues. Perhaps this value is actually the result of some type of doping the tissues with some fluorocarbon–type compounds?

SCHMIDT - In relation to your first question, how compartments can be separated by addition of manganese ions to tissues or to blood, it is correct that water goes in and out across membranes, but it is not water which is important in this case. The compartmentation is resolved by manganese ions which do not normally penetrate the cellular membrane. This can change under some pathological conditions in which membranes can become leaky. This was the case illustrated in one of the slides shown, in which after myocardial infarction the T_1 relaxation rate increased tremendously because the manganese ions could penetrate into the intracellular compartment and then the intra–cellular water showed the same effect as the extra–cellular one.

VILLA - In my opinion, the possibility of resolving this compartment requires that you could actually separate signals from the inside and the outside respectively. Otherwise in some way, the average relaxation time should be altered.

SCHMIDT - Yes, that's true. You change the average, and the change is significant indeed. In effect the exact meaning of this still remains to be clarified. In the experiments on myocardial tissues we could detect changes in NMR values related to the pathological effects of infarction. This is what I meant with the title "Heterogeneity in clinical condition". The second question was that related to ^{19}F NMR. The experiment shown was not carried out in our laboratory, but was taken from the literature, and did not refer to the use of perfluorocarbons but rather to that of halothane. The separation shown referred to that between halothane in a lipid environment and halothane in an agarose environment. Under these conditions the signals are well separated, so you can distinguish between the two phases.

ERMANS - We can observe with obvious interest the developments of NMR spectroscopy in the field of tissue characterization. The potentiality of this approach was clearly shown by Dr. Schmidt and Dr. Radda. I would like to recall now Fig. 1 shown by Dr. Maraviglia (p.9). He showed that NMR imaging could also possibly give information about tissue characterization – which is rather a guess at the present time. Since spectroscopy is a scientific tool apparatus, and not a clinical one, I think that the real possibility that NMR imaging could give the same information is actually the key problem.

MARAVIGLIA - One of the main points that I was trying to establish is that it's possible with NMR imaging to make images that map pure parameter distribution. Today there are many laboratories where people make a distribution of a complicated function of these parameters. Laboratories can also make a pure parameter distribution, although it takes longer. This is exactly spectroscopy, I think, because one can obtain at each coordinate, the pure NMR parameters which are normally measured with NMR spectroscopy. Now I do not mean at all that this is equivalent – or close to what normally is considered NMR spectroscopy, as it was referred to by Dr. Radda. In fact I am completely convinced by his position that chemical shift spectroscopy gives rather direct information – it's reasonably understandable and it's meaningful for many potential applications. Whereas, as I understand it, T_1 and T_2 and other NMR parameters are possibly easily obtainable, they are however very complicated to explain. Even if one could really obtain T_1 from a single compartment I am sure that nobody would understand how it comes about. I'm completely convinced of this. That's why I think that probably imaging as used today, without special requirements for pure parameter distribution, will be pushed ahead for clinical purposes and spectroscopy will be limited to very specific and clarifying aspects like, say, ^{31}P.

ERMANS - You say that probably NMR spectroscopy will be useful for ^{31}P studies and perhaps therefore imply that imaging will not be useful for phosphorus. Do you think that NMR imaging can be suitable for definite measurements concerning ^{31}P?

MARAVIGLIA - This is something I didn't touch.

Chemical shift maps are something that people are working on, especially in rotating frame imaging. It's something we could talk about but I do not forsee personally the possibility of doing whole body imaging with ^{31}P, if this is what you are wondering. One can do topical NMR on the whole body if there are no unpredicted difficulties which may arise technically. But this is quite different. The spectroscopy I was talking about is just classic NMR parameter distribution in a heterogeneous system. These parameters can be obtained, but then what? They are too complicated to understand – that's my opinion.

LUITEN - The point is that we presently are in what I would call the "honeymoon" of NMR in medicine, and the marriage is between the imaging device and the analytic device and there are here experts of the various aspects. All measurements and discussions on the properties of relaxation behaviour and on high resolution spectra have for their target that of understanding what is going on in the tissue and the cells. The information is actually obtained by biopsy *in vivo*, measurements being made on a certain small part which is representative for the disease to be studied. If you look at the imaging side, imaging is actually the name for making up any spatial distribution. At the moment NMR in medicine is having great success in becoming another imaging modality and that is why people are very enthusiastic about it. The question now, however, remains unanswered – will NMR only be an alternative to X–ray or will NMR be an analytic device? NMR imaging as an analytical tool generally gives you severe limitations in your measurement possibilities. For instance, if you want to measure T_1 thoroughly it's very difficult to do it at more than one measuring point. Measuring multi-dimensional characteristics will become difficult. So it's a very interesting thing to find out in the near future what the greatest strengths of NMR in medicine will be. Will it be local analysis at the expense of spatial distributions, or will it be another way of doing morphology, I mean, looking for instance at the kidney in another representation. I think to some extent we can do both. It should be possible to do spectroscopy over a whole volume by dividing the object in a number of voxels and measuring the spectrum in every voxel. Would it be going too far to suggest that the results obtained in this way are as good as the results you could obtain if you do *in vitro* experiments of a sample? I think that if the importance of NMR goes into the direction of the analytic properties measured at the point, then it will give another impetus to the development of imaging equipment than can be more of a path-finding device – you know where you have to measure – and then change over to a probing device. So when we come to a better conclusion on the final importance of analytic measurements *in situ*, that will give a great influence on the way NMR equipment in medicine will come to a final shape or development.

RADDA - There was a question to which this partially was an answer, or in fact a statement that NMR spectroscopy is not a clinical method. I'd like to take that up, in that, of course, NMR spectroscopy in principle is a much stronger clinical method than imaging. What you are sayng is that it is not necessarily a practical clinical method in the sense that it may be difficult to do the measurement, it may not be practical for big Companies to go and sell that equipment because it might be too expensive, you may need too big teams to run it, or you may need special facilities. That's a very different statement from the fact that clinically there can be little doubt that having analytical information about human biochemistry from any part of the body at a relatively low resolution but still at a resolution where you can look at parts of the organ, *must* be clinically extremely useful. I don't think there is any doubt on that. Whether that is going to be converted into something that every hospital will want to have or only a major group of hospitals will want to have or only three in the world will have is a different question altogether.

ERMANS - Dr. Radda, I realize that the objective of a clinician is to detect the nature of a tissue without taking it out from the body. If you have to take out the piece or a biopsy and put it in the spectrometer, the technique loses large part of its clinical interest.

RADDA - We have been running a clinical NMR apparatus for muscle diseases for the last year and a half and we have seen with no biopsies over 200 patients where we can analyse the biochemical composition of muscle tissue in normal and diseased conditions in a variety of states of exercise, hypoxia, and systemic disorders. You don't need to take a sample – you can just look at a small region of the muscle and do what biopsy would have done. So that technique is already well–proved and it's not far off from a state where you can take a whole human and look at any part of the body with no biopsies. Spectroscopy can be done without biopsies.

TAYLOR - Several speakers have given some throw –away comments about imaging other nuclei apart from hydrogen. If you've done that calculation, it's very depressing to think of imaging anything else but hydrogen. If you take phosphorus, you're talking about at least a decrease in resolution of a thirty in every dimension. I think it is also highly unlikely that you could dope the patient with other substances to a sufficient level that he would survive and you'd still be able to take an image of them. So I think that for the forseeable future we will not be imaging in any useful way in anything but hydrogen.

REISSE - I would like to come back to one remark made by Prof. Maraviglia. I am in complete agreement with him when he says that it is possible to measure different T_1 values and to use these differences to obtain contrasted images but there is a lot of doubts about the possibility of finding explanations for these differences. To be clear, I would like to say that even for pure liquids, in many cases, it remains extremely difficult or even impossible to a simple (or even a complex) interpretation to explain why T_1 (or T_2) has such or such value. In the case of water in cells, the relaxation mechanisms are very complex and not purely intramo-

lecular. Of course, qualitatively, it is possible to explain some observations by using the very simple formula shown previously, but phenomena like cross– correlation, cross–relaxation, etc., which will be discussed, I hope, this week, are very important for water in pathological or normal cells. So personally, as a chemist, I am convinced that it is very difficult and probably hopeless, at this moment at least, to find a general interpretation for T_1 and T_2 values in tissues. For example, let me consider the following problem: why T_1 values are longer in cancerous cells than in normal cells? It is certainly a very interesting problem, but I have really some doubts about the fact that it will be possible to find a simple explanation for such an important fact. So, and this is probably in agreement with what Mr. Luiten said, we must be clear on the following point: it is important to be able to measure T_1 or T_2 differences because it can lead to images, but it is a completely different problem to find interpretations in terms of what I would call biochemical, chemical or physical arguments.

DERBYSHIRE - One of the main problems we have is that NMR spectroscopists, and certainly NMR relaxation people, don't measure anything that is directly of itself of use to anyone at all. They measure things like chemical shifts, spin–spin interactions, T_1, T_2, $T_{1\rho}$, T_{1D} – think of any combination of letters and numbers and they will measure it. Now these are measured, and I speak as an NMR spectroscopist myself, we also measure these parameters because if we've got a suitable theory, we can relate these quantities to something that is of use. These theories work for nice simple systems. They do not work, or at least they creak considerably, when applied to something as complex as a biological system. Relaxation provides an example. Several of the speakers have drawn graphs of T_1s and T_2s, against the temperature or the frequency, but what they've drawn is a graph that's applicable to the theory of simple solutions or simple liquids, in small molecular weight liquids. It's the classic BPP equation. Now this theory does not apply to complex biological systems and that makes it very difficult to predict the effect of temperature and of change in frequency. I'm not talking of the heterogeneity. Even a model biological system that is homogeneous, does not obey the BPP equations. Even if we look at just the bare water component the BPP equation is not appropriate.

MARAVIGLIA - I completely agree with what Prof. Reisse and Dr. Derbyshire have been saying. I think that at this stage something comes out which may be useful for this Workshop. A T_1 distribution, namely a pure parameter image, or a T_2 image, is something which can be interesting for diagnosis, but, I do not believe that they characterize the tissue if I understand what characterizing means. I don't think in fact that T_1 will be understood in terms of its connections with properties of the volume element, which one is actually studying. At this stage I think that it is important to understand what is the purpose of the study of relaxation times in tissue as a piece outside the body and to attempt to correlate this with imaging. Studying correlation among many excised pieces taken from healthy and pathological parts of the body and making averages – is not real characterization. In no case do I see a real analogy with what, say, ^{31}P spectroscopy is giving as information. So in a way we could try to conclude that some aspects of NMR information are not really characterising but are just giving correlation and information for images.

FOSTER - Could I possibly show a couple of slides? This is just a little comment on something that Dr. Derbyshire was talking about – normally we look at simple systems and then extrapolate these to more complex ones. He instanced temperature effects upon T_1. When you look at these simple systems you can sometimes do this. Fig. 1 shows the case of a manganese cloride solution, simply doped water. The T_1 values were measured at different temperatures.

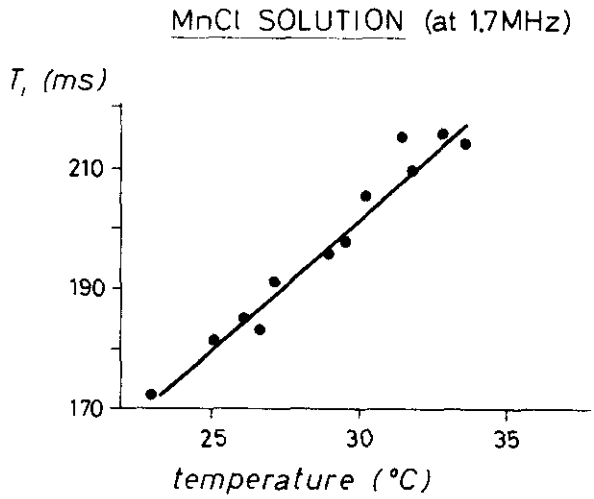


Fig.1. – Temperature dependence of proton spin-lattice relaxation time in a $Mn\ Cl_2$ doped aqueous solution.

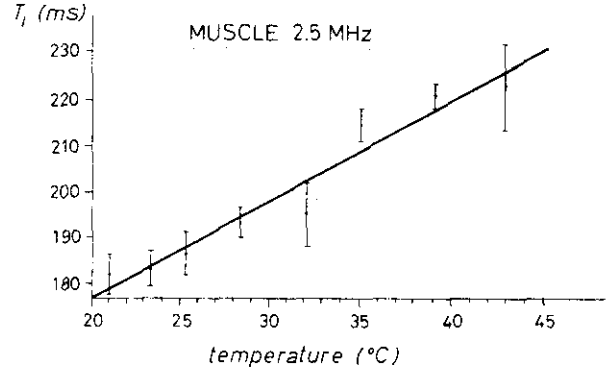


Fig. 2. Temperature dependence of proton spin lattice relaxation time in a dissected muscle.

The T_1 values in Fig. 2 weren't plotted on the same axes but the slope is fairly similar and you can see that here in muscle (also done at a series of different temperatures) we're getting a very similar type of effect. The standard deviation is larger because we are dealing with a more complex system, and some of the pieces have had to wait until after death before measurements, but it's not necessarily untrue to extrapolate from the

simple to the complex system.

REISSE - What you show is T_1 versus temperature. What are the T_2 values?

FOSTER - They were not measured in this experiment.

REISSE - In this case you are probably in such experimental conditions that T_1 and T_2 are the same but, of course, the situation is different for water in muscle cells. You observe the same T_1 temperature dependence, for the solution on the one hand, for the muscle on the other hand, but I think that it is dangerous to say on the basis of this argument alone that the solution is a good model. I am personally convinced that T_2 value in your model solution is different from the value you could observe in the muscle.

FOSTER - It's possibly true, although we didn't measure T_2. I was really making the point that you can extrapolate in some instances and the one we were discussing was one where you could.

REISSE - Yes, but for the solution you are probably in the extreme narrowing conditions, whereas it's of course not so for the muscle.

FOSTER - Yes, that's true.

LHOSTE - Just a very rapid answer to this point. In the presence of manganese ions T_2 will be much shorter than T_1 because manganese has a large scalar interaction. It could hold with gadolinium not with manganese ions. We have to make a much more general comment. I think that water is a very messy molecule and I feel we have the situation we were in in biochemistry about fifteen years ago. I'm not so pessimistic for other nuclei. There are very serious possibilities of interpreting relaxation times even *in vivo* with other nuclei. There's some very fine work already done with ^{13}C for example with other molecules. My third comment is still more general. I think there is a big contradiction at the moment because on the one side you have the spectroscopists or the biochemists or whatever name you want to give to them, who want to make T_1 and T_2 measurements (why not $T_{1\rho}$ which is a very interesting parameter as well?) and look at large dispersion in a large range of frequencies, and on the other hand you have the imaging techniques which are climbing up very high in field strength now (1 Tesla or 1.5 Tesla). My comment is double: I don't see how with these imaging instruments you will be able to make this kind of detailed investigation, which is the only way to understand the relaxation times, specially using low field and low frequency. With the same equipment it cannot be done. Secondly with such high field machines there are probably very severe difficulties for measuring true T_1 and T_2 maps. I agree that for one point it can probably be done, but I can't see how it could be done for the whole image.

ORR - May I take issue with Bruno Maraviglia about the use that we make of the word "characterization". I think that my understanding of our intention was in the sense that we can recognize a character without necessarily understanding how it was created, and I think that we are using the world in the sense of tissue signature or fingerprint or some means of recognizing the tissue with great specificity. I think it is unnecessary to understand how that characterization was created. It may be helpful towards finding a good characterization if we have some understanding of the biophysics. If we find a good characterization that is useful it would be a very great encouragement to biophysicists to try to understand what created that tissue signature. But I think it would be a great mistake to be pessimistic and to expect that because we are faced with a very difficult biophysics problem there may therefore be little value in tissue characterization. I think the opposite is the case. In medicine very many valuable things are done and the physicians have no understanding of the mechanisms. The whole of the treatment of cancer by radiotherapy is based on known facts of the existence of repair and recovery of cells, but there is virtually no understanding of the mechanisms or even the cellular constituents that are primarily involved. That, however, doesn't reduce the clinical value of fractionated treatments. It is important to be clear about what we mean by characterization. Perhaps Franca Podo could repeat to us what she thinks we actually mean by that.

PODO - Before saying something on that, I would like to ask George Radda to complete what he was saying before, because it was just restricted between two other discussions. You haven't presented a report on that, whereas we had three reports fully devoted to relaxation properties of tissues. Could Dr. Radda, please say a few words on the clinical applications of ^{31}P NMR spectroscopy before we try to summarize the most interesting aspects of tissue characterization by NMR? Only on the basis of all these parameters, namely chemical shifts and relaxation times we should try to give a more reasonable definition of our field of interest in relation to tissue characterization and identification by clinical NMR.

RADDA - All I was saying was that I think the majority of the presentations at this meeting are obviously going to be concerned with T_1 and T_2 relaxation times of protons in relation to imaging. I just wanted to make it clear that I think that some of the criticisms or the points of view that you might apply to those measurements may not apply to spectroscopy. So I was simply asking to keep the two separate in the discussions because we get into the sort of confusion that, in fact, we've already seen just now – one statement is immediately applied to the other form of NMR and then it is not valid. Actually in this context I was also going to defend T_1 and T_2 measurements, just the same way as Stewart Orr has done. One of the things that I've learnt since I started to work on clinical NMR is that when a clinician said that he'd diagnosed something, I used to think that he understood it. In fact, I now quite clearly know that diagnosis and understanding are two totally different things. Diagnosis is a way of recognising some symptom in some form or some combination of uncertain facts which is then translated into practice so that on the basis of past experience, the patient can be treated in a given way. That's really what in a majority of cases the clinician says – maybe it sounds cynical but it's true. In many cases that's sufficient for diagnosis, because it has to be quick, on the spot, and some decision has to be

made, whether it's right or wrong. As scientists we are obviously trying to look for much more perfection in our measurements. Now if you are going to look for perfection in T_1 and T_2 measurements, then I think it's a waste of time. But if you are doing to do these measurements on isolated tissues, on whole organs, on animals, on humans, in order to help the use of this very exciting technique of NMR imaging – then I think there is an awful lot of point of doing many T_1 and T_2 measurements on a whole range of conditions. As Stewart Orr said, if at the end of that you have some understanding of the biophysics, then that's an extra bonus but it's by no means a necessary requirement.

PODO - I would completely agree with what Stewart Orr and George Radda have said. These physical parameters are tools for us in several ways. For some people for instance, relaxation times are useful "tools" for enhancing soft tissue contrast in an image. On the other hand the present experimental evidence indicates that modulations of relaxation properties are associated with some pathological states and to their evolution in time, and this can be very useful, although satisfactory theoretical explanations of these phenomena are still lacking. Further biophysical studies would not only help in better clarifying the structural bases of these effects, but could also prevent the mistakes deriving from over simplified interpretations of data collected from complex systems such as biological tissues. Once a rigorous treatment of data and good protocols have been established, so that measurements can be really considered physically sound, then one should try to make use of these parameters in the clinical practice and diagnosis.

MARAVIGLIA - I think I've heard proper answers to the question I had posed about T_1 and T_2 and whether that meant characterization or not. Although I am a physicist, I can completely accept the idea that it's just a label that one knows in a certain way is correlated with a certain state, and this can be useful for diagnosis. This is probably the way one should limit oneself so far for considering NMR characterization. At any rate I'd like also to add to this point that T_1 (and T_2) is very sensitive to the conditions of a system and to impurities. As a living organism is an open system, the statistical variations of T_1s are unbelievably broad. It is therefore very difficult, even with statistics, to establish correlations between T_1s in the same pathological state of different individuals. On the other hand, I believe instead in the possible use of relative variations of relaxation times for tissue characterization. Relative variations within the piece of tissue which contains the pathological part can be obtained by taking small sections, starting from the normal to the pathological part, thus measuring the relative variations of T_1 rather than its absolute value. So if T_1 and T_2 have to be characterizing parameters, one should probably establish relative variations rather than their absolute values.

LUTTEN - I'd like to pick up two points on the previous discussion. On the one hand I could get along very much with Dr. Radda saying that we measure relaxation times, they can give an interesting diagnostic indication and this is a help to come to therapy, whether you actually understand what the origin of the value of T_1s is or not. A problem that arises here is – and this follows from the previous discussion – to what accuracy should we measure them. I mean, if the variations are in the order of a factor of 2, then of course the equipment can be very simple. As soon as one expects on the basis of the biophysical understanding that the relevant information is in the order of the factor of one tenth or a more accurate order of magnitude, then the experimental problems become much more severe.

BENE - I have a general comment to make about the parameters useful in the application of NMR to medical diagnosis. We spoke about chemical shift, T_1 and T_2. Clearly the chemical shift is directly connected to the molecular structure but it is not the same for the relaxation times. Relaxation times are correlated with the molecular system through the dynamical movements. And then these dynamics may be directly obtained by the correlation times and also by the energy associated with each correlation time. We have a step to make between the measurement of relaxation times and the description of molecular dynamics. I think that all the measurements of relaxation times have to be connected with the dynamics at the molecular level and not directly to the status of a biological sample or its pathology.

LHOSTE - Just a last comment on the value of the relaxation times. I feel that they could be used very empirically for imaging. There is another problem which is a technical one. At the present time most imaging machines work in a very large range of frequencies from 6 MHz to 60 MHz. For water it's not really a problem because diffusional correlation times and additional correlation times are probably much shorter than the corresponding frequencies. But for lipids it's much more critical, and if you are speaking about characterization of tissue you have to be very careful because you are not sure you have the same type of contrast with one machine or with the other, once you mix the spin density and T_2.

SERVOZ-GAVIN - As a physicist I am a little confused about the semantics of the characterization of tissues discussed here. In materials science we know very well what characterization means. We can characterize the atomic distribution, we can characterize the crystalline structures, defects and impurities, we can characterize its dynamics and its physical properties. I really don't see how T_1 and T_2 can characterize anything. It seems to me that tissue characterization – but I'm not a biologist – should be either a molecular characterization or a structure characterization, or something of this kind. I would like to hear some accurate definition of characterization of tissue here, because it seems really messy to me. It seems to me that ^{31}P NMR spectroscopy is really a characterization method, as it is an analytical method. When you look at the molecule of ATP or at phosphocreatine, you have then a real characterization, but I really don't see how T_1 and T_2, and therefore NMR imaging up to this point,

may be a real characterization method in the sense we define characterization in physics at least. So is there another medical or biological definition for this – it's an important problem, because it may induce errors. NMR imaging to me is not a characterization method. It may be a diagnostic method, directly or indirectly through correlations with other physiological parameters, it may be an index of water content, but this is not characterization of tissues. It may be an index of water dynamics, but again this is not characterization of tissues. So my question is asked to the biologists, the biochemists and physiologists.

BAKKER - When you look at proton spectra of tissues, all you see is two broad peaks of water and fat, so all you possibly can derive from that is an index for water content and fat content of tissues. Additional information for characterizing tissues possibly can be obtained by multi-exponential analysis of water proton relaxation curves. This might yield an index for the protein content of tissues. By combining relaxation measurements with biochemical manipulation, like Prof. Schmidt and Prof. Radda have said, for instance by labelling the extra-cellular water, you may obtain additional information on the amount of extra - and intercellular water in tissues. Proceeding along this line may lead to a set of parameters which can be used for tissue characterization.

ERMANS - For a medical doctor, when one is speaking about characterization, he has in mind the pathological data, because all our work has, as its final reference, the anatomical-pathological data. For instance, what is the difference between cancer, cysts or nodules of a gland? It is only the pathological information which gives the answer. At the first approach, we would expect that the NMR would give us the same type of answer as the pathology, but obviously it is impossible. Anyway we need a reference and I would say that when we are speaking about characterization, we have mostly in mind the differentiation between different important diseases. Possibly after some time, if the technique of NMR is sensitive enough we will have a new classification of the diseases which will depend on the technique. We had the same process in electrocardiagraphy: there are indeed pathological situations which are characterized more by well defined electrical abnormalities of the heart activity than by clearcut pathological findings. My view is therefore that tissue characterization must be taken in a broad sense including all the pathological, biochemical and clinical aspects of a given disease.

DERBYSHIRE - It does strike me that we are not going to satisfy the purists amongst us as far as NMR imaging is concerned, because, as stated earlier, we aren't really recording the fat or the water. We are recording something to do with the dynamics of the water. Water is an anomalous liquid and we don't even understand the properties of pure bulk water. Here we've got a much more complex situation than that. We've got water in confined spaces, we've got water in contact with membranes, with big macromolecules which are slowly tumbling, we've got proton exchange between water molecules, we've got proton exchange with the substrate proteins and membranes. we've got slow exchange across membranes. We really have a very complex situation. I suspect that a satisfaction for the purists is a long time off and I don't think we can look to it. Could I now move on to ask a specific question to Dr. Schmidt? In your graphs of relaxation enhancement against manganese concentration you showed graphs which started off slowly and then proceeded to increase more rapidly. Presumably that change of slope is trying to tell us something about the partition between intra-cellular and extra-cellular. Well, it's not telling me anything at the moment, but presumably it's telling you. I wonder if you could tell me what it is.

SCHMIDT - I didn't want to point to the fine structure of this graph, how this slope or the deviation from the linear behaviour is to be interpreted in terms of compartmentation. What I wanted to show was only that there are differences in different organs if you add certain concentrations of manganese to the tissues. I wanted to show that there are heterogeneities even in those experiments where you add a contrast agent.

REINHARDT - My background is image analysis and, as I understand, the problem which is being discussed here is a multi-parameter analysis problem. Therefore you have great difficulties in finding an analytical model. One possibility is to define in terms of formal, and not analytical models. You have to apply a multi-dimensional parameter analysis because even for four-dimensional processes, it is really impossible to have an intuitive impression of how the dependencies of the different parameters of your system are correlated. Therefore you have to apply powerful but well-known numerical methods to find out significant correlations and therefore you have to carry out and build up what we call a classified data bank system. Then it seems to be possible to characterize tissues, not in the sense that there is a direct understanding of the measured parameter and the biochemical process, but on the basis of the existence of a strong correlation which can be evaluated with a lot of experience.

PODO - The possibility of having a data bank will be further discussed in this Workshop, together with the present difficulties of having a data bank, arising from the fact that different results are aften obtained by different laboratories today, in the absence of an appropriate standardization of the experimental procedures. Once a standardization of the methods is identified and adapted by various centres, a data bank would be extremely useful.

FOSTER - What I was going to say has been slightly superseded now. I was just going to make the comment that we seem to be approaching two different

things simultaneosly. On the one hand we are heading towards a look at the question "what is T_1 in tissue?", and I agree with Dr. Derbyshire that this is a question that is extremely complex and probably not answerable at the present time at all. Simultaneously we seem to be looking at the problem of tissue characterization, using the term not as a physicist but as a physiologist where, as with Prof. Ermans, to me it means differentiation rather than characterization in its strictest sense. I think that perhaps we ought to try and sort ourselves out a little bit and decide which of these we are going to give priority to or perhaps to try and split them a little further. As far as direct characterization is concerned, we certainly have to bear in mind that, taking the NMR image which obviously I am most familiar with, there is nothing that you can ever do by taking out a single pixel, looking at it and trying to say 'well, this belongs to such and such a tissue or such and such a pathology'. It is impossible. Even taking the simplest case of the proton density image, you must apply a second piece of information, and that is, your own knowledge of the anatomy of the body. Once we get into T_1 and T_2 images, then I agree with Prof. Orr that we need another second feature before we start any form of direct interpretation. So really the plea that I'm making is for a decision on what we are going to be actually looking at and which direction we are heading in.

CHAMBRON - I think that we cannot discuss the biological significance of T_1 and T_2 values in terms of structural function without the help of a tissue model. Generally two models are proposed: a multiphase model with fast exchange, or else an average structure with restricted diffusions or restricted molecular motions. But I would like to know your opinion here about the validity of these tissue models. I'll take as example the muscle. You can interpret variations of T_1 and T_2 in the muscle in terms of the first model with two phases – free water and bound water – in fast exchange. You can see that T_2 is very sensitive to the state of the muscles, in particular to contractions, and to the solid–like structure of the tissue, but T_1 is not. We have here an example in which the model can explain variations of T_2 and T_1 in terms of functions.

STROM - Being a medical biochemist, I would like to express a word of caution toward the uncritical use of techniques which, although they yield results, even reproducible ones, escape comprehension as for the physical or chemical meaning of these same results. I cannot help remembering that some fifty years ago there was, in clinical chemistry, a popular, wide-spread use of what were called the "eucolloidality" tests – i. e. the ability of plasma of giving a precipitate upon mixing with some denaturing agents such as phenol; for years and years a variety of these tests was developed, their positivity being correlated to a number of diseases, and a relevant diagnostic importance being attached to them. Then, in the last ten years, people realized that too many factors were involved, and that each of these tests lent itself to so many interpretations that it could not be relied upon to make a diagnosis.

I think that, in the absence of a physico-chemical basis as a background for the interpretation of the results obtained by a given procedure, or for the comparison between sets of results obtained with different procedures, the only possibility is to draw conclusions, i. e. diagnosis, by either analogy or exclusion. In both cases, the range of possible diagnostic errors is likely to be large – although I can admit that, as Prof. Radda has said, in medicine diagnostics seldom means comprehension, and that in certain cases even such analogical procedures can eventually be of help in reaching the right diagnosis. But I am afraid that a large portion of the effort devoted to develop procedures which are only superficially understood may lead to dead-ends or to results which escape rationalisation, due to the large number of variables involved. In the specific case of NMR imaging, I furthermore believe that, as far as clinicians are concerned, the complexity of the technique and a superficial information of its rationale can really lead to a most dangerous establishment of uncritical correlations between results and clinical interpretations.

ERMANS - I would answer Prof. Strom about the need to know what we are doing, and the need for a complete understandable situation before we can interpret things. At this point of view, I guess that 50 % of our medical practice is still empirical because some of the most important problems in medicine (atherosclerosis, cancer, rheumatism) remain partly unexplained. We cannot therefore hope that we shall be able to explain everything in medicine by T_1 and T_2.

STROM - Excuse me, Prof. Ermans, I did not say that only fully-understood activities should be pursued. I just said that people working in the field of NMR imaging should have some caution, i. e. that they should keep under control every technical detail of the procedure(s) they use, and remember the danger that these technicalities prevail, leading to dead-ends in the comprehension and in the interpretation of the results.

ORR - May I pursue Margaret Foster's question a little? It is quite clear from what a number of people have said that there are very many biological variables influencing the information or the data that do exist. In a sense this meeting is a reponse to a faith that there is valuable data somewhere in there. There is a biological blurring, a defocussing, and the contribution of the biophysicists in answering the question – "What is the mechanism and the understanding of T_1?" – is not to answer that for its own sake but to provide enough to refocus or to sharpen the information that exists by removing at least some of the biological variables. At this stage not much more should be asked of the biophysicists or of the

question about understanding the mechanism of T_1 than to remove some of the variables, so that studies *in vitro* will be sharper and crisper and comparable from one laboratory to another. There may also be the benefit that there will be a feed–back to imaging to suggest different or new methods of using sequences that could give a better image. Therefore although I would certainly say that the question we are not asking at this meeting is to understand T_1 in its full mechanistic way, it is necessary to get some biophysics contribution in order to sharpen up our view of the information that is there.

PODO - I think that we have reached the end of our work today. Tomorrow we've got quite a heavy day and we can go on with detailed discussions on various, more detailed aspects of tissue characterization by NMR.

Ann. Ist. Super. Sanità
Vol. 19, N. 1 (1983) pp. 41-42

TECHNICAL ASPECTS OF RELAXATION MEASUREMENTS AND THEIR RELATIONSHIP WITH TISSUE PROPERTIES

C. CASIERI, F. DE LUCA, B.C. DE SIMONE and B. MARAVIGLIA

Dipartimento di Fisica, Università di Roma "La Sapienza" – 00185, Roma, Italy

Summary. – *Some of the most relevant technical problems for relaxation measurement of protons in tissues* in vitro *and* in vivo *are proposed and shortly discussed. The possibility of pure relaxation parameters mapping is also reported.*

Riassunto. – *Sono presentati e brevemente discussi alcuni fra i più importanti problemi tecnici per le misure* in vitro *e* in vivo *del rilassamento dei protoni nei tessuti. Inoltre è discussa la possibilità di realizzare mappe dei soli tempi di rilassamento.*

Introduction

The complexity of a living organism structure both on the microscopic and macroscopic scale makes the measurements of NMR relaxation in tissues difficult to interpret and not straight forward to perform. In this brief report we try to propose for further analysis and discussion most of the problems which arise in relaxation measurement.

It is convenient to separate the discussion of measurements into A) *in vitro* and B) *in vivo*.

A) The pulse sequences used to measure T_1 (Fig. 1) and T_2 *In vitro* are standard. The procedure used is not unimportant anyway because of the following reasons:
a) non exponentiality is common in spin-lattice relaxation measurement in tissues. Agreement among laboratories for measurement protocols could thus follow two lines. One is the determination of T_1 from the initial slope of the magnetization recovery and a magnetization value at a definite instant. The other could be the complete determination of the recovery which would imply tentative fits with multiexponential functions. Non exponentiality of T_2 is obvious as the system is heterogeneous. Criteria to cut off the short T_2 components (protein, etc.) should be decided; this aspect is also related to technical features like the after pulse dead time and thus the Larmor frequency of the measurement. Exponentiality, after the fast decays have occurred, could be assumed;
b) inhomogeneity of the static field B_0 and of the r.f. field B_1, should be known for each experiment. For T_2 measurements the Carr-Purcell - Meiboom-Gill (CPMG) sequence is advisable for its insensitivity to B_0 inhomogeneity. The relevance of B_1 inhomogeneity for each experiment should be reported giving, for example, the residual amplitude of the FID after a $\pi/2$ pulse and a $(3\pi)/4$ pulse, or other criteria;
c) repetition rate of the sequence. This measuring procedure is obviously critical in influencing the relaxation behaviours. This aspect should be then clearly analysed to fix the protocols;
d) offset of frequency and pulse lengths must be clearly included in the analysis of the measuring procedures. The spectrum of the pulse sequence and the physically acting frequency components can this way be stated.

B) All the problems involved in the *in vitro* relaxation measurements are also relevant for the corresponding measurements *in vivo*. Some other important aspects arise though in the latter case. If we limit the discussion to T_1 and T_2 mapping, namely pure spin-lattice and spin-spin relaxation imaging, the complications arise from the conflict between the enormous interest to get an image in the possible shortest time and the requirement of displaying pure parameters maps. In fact all imaging methods produce maps of functions of most of the NMR parameters together, except possibly a subtractive method used in spin warp [1]. Many of these methods would be able to produce pure relaxation parameters maps by simply slowing and properly choosing the pulse sequence. As a limiting case simply consider the example of the Projection-Reconstruction approach [2]. One projection at a certain angle gives the degree of recovery of the nuclear magnetization at each frequency. So if, for example, one applies the pulse sequence shown in Fig. 2 with $\tau' \ll \tau$, the echo obtained for each delay time τ (at constant τ') gives the degree of spin-lattice relaxation at each frequency and thus at each coordinate. By repeating the measurement at different τ's one can plot the recovery of the longitudinal magnetization at each coordinate. This way one gets a pure T_1 projection, which of course

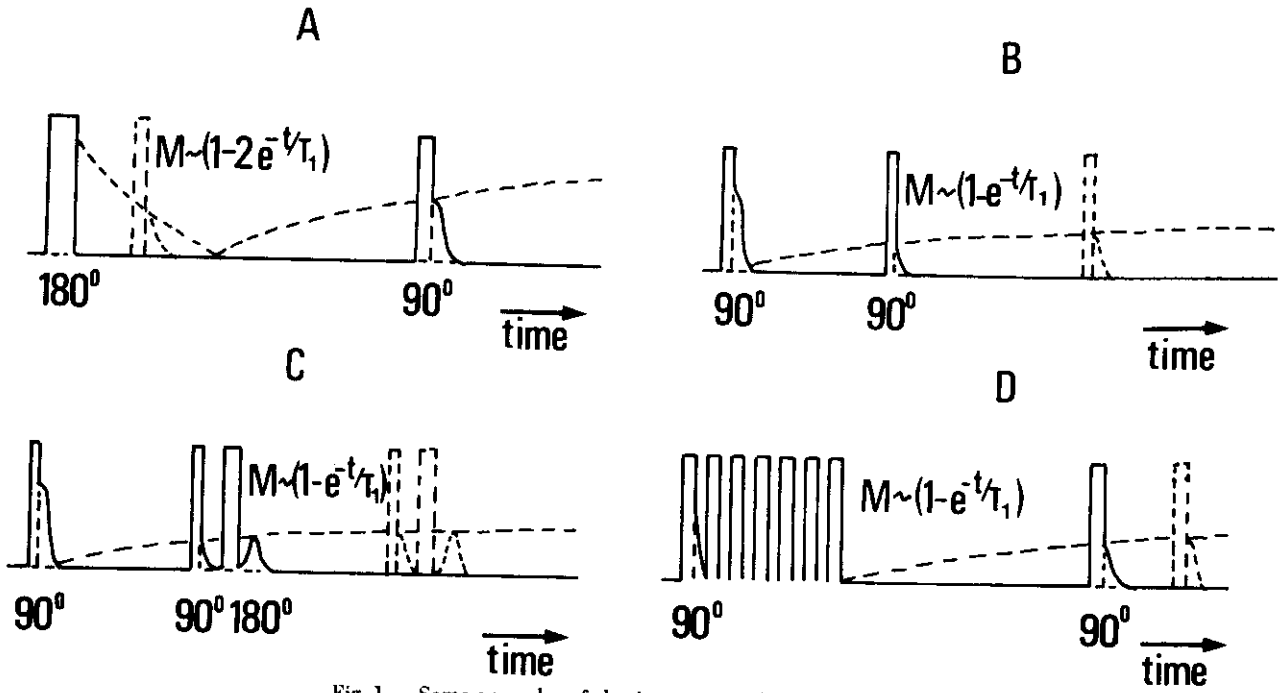


Fig. 1. – Some examples of classic sequences for T_1 measurements

would require an acquisition time that is much longer than that needed for an usual projection. By repeating this operation at different angles, the necessary projections can be taken and back projected to form the pure T_1 image. Similar procedures can be used for pure T_2 images.

In a way this is a limiting case. Analogous procedure can be applied to a spin-warp sequence, as shown in

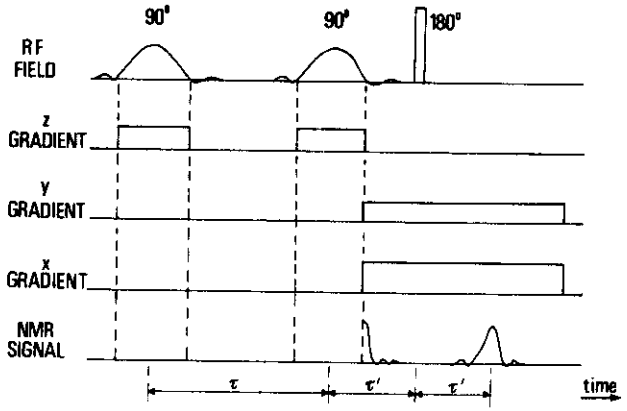


Fig. 2. – Projection-reconstruction sequence with which a pure T_1 image can be obtained. The delay time τ' is much shorter than τ. Echoes must be recorded for different values of τ; ideally only two values should be sufficient, in practice their number depends on signal-to-noise ratio and on the wanted accuracy for the T_1 values.

Figure 3. In fact particular conditions to image pure parameters can possibly be found with less waste of time [3] but still with procedures longer than those used normally for NMR imaging.

In conclusion we can say that pure relaxation imaging can be already used for research and to correlate the *in vivo* with the *in vitro* results. Until pure T_1 and pure T_2 imaging will not be as fast as the most efficient imaging methods, it is predictable anyway that diagnostic imaging will provide essentially insufficient information to extract the local relaxation behaviour of living tissues.

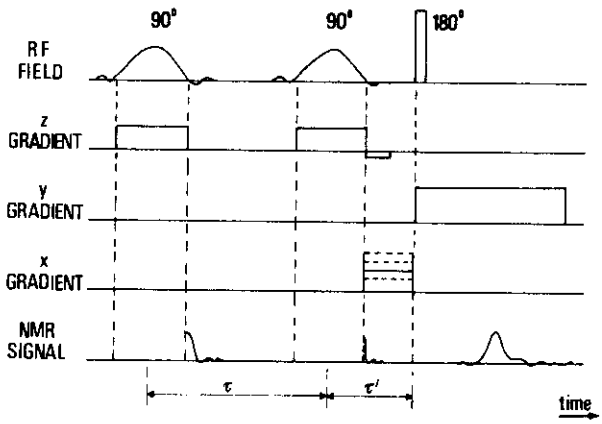


Fig. 3. – Spin-warp sequence with which pure relaxation parameters imaging is possible.

REFERENCES

1. HUTCHISON, J.M.S., EDELSTEIN, W.A. & JOHNSON, G. 1980. A whole-body NMR imaging machine. *J.Phys. E.* 13: 947-955.

2. LAUTERBUR, P.C. 1973. Image formation by induced local interaction: examples employing nuclear magnetic resonance. *Nature.* 242: 190-191.

3. See for example: LUITEN, A.L. TAYLOR, D.G. & ERICSSON, A. in these Proceedings.

Ann. Ist. Super. Sanità
Vol. 19, N. 1 (1983) pp. 43-46

EFFECTS OF RELAXATION TIME CONSTANTS IN NMR IMAGING

A.L. LUITEN

Medical Systems Division, N.V. Philips Gloeilampenfabrieken, Eindhoven, The Netherlands

Summary. — *The contrast between the tissues in an NMR image is strongly determined by the differences in T_1 and T_2. This contrast varies strongly with different imaging techniques and also with the setting of the various time parameters in each technique. The comparision of two or more images with different T_1 or T_2 contrast enables the calculation of the T_1 and T_2 values of the tissues in all pixels of the NMR image.*

In the calculation of T_1 from the intensities of a saturation recovery image and a simultaneously recorded inversion recovery image (or a partial recovery image) a mono-exponential recovery function is assumed and the best accuracy is obtained for the T_1 values in the region of the applied recovery time. The T_2 values can be determined from two or more images reconstructed from successive echo signals using a Carr-Purcell pulse technique in the same measurements to determine the T_1 values. The possibility of using more than 2 echoes within the same recording time improves the obtainable accuracies or the calculation of a multi-exponential decay function.

Riassunto. — *Il contrasto tra i tessuti in una immagine NMR dipende fortemente dalle differenze in T_1 e T_2. Questo contrasto d'altra parte varia fortemente con le diverse tecniche di immagine usate, nonché con i valori dei parametri utilizzati in ciascuna tecnica. Il confronto di due o più immagini con diverso contrasto in T_1 o T_2, consente di calcolare i valori di T_1 e T_2 nei tessuti in tutti i "pixels" dell'immagine.*

Nel calcolo del T_1 dalle intensità di una immagine ottenuta in "saturation recovery" e di una immagine registrata in "inversion recovery", si ipotizza che il ritorno all'equilibrio della magnetizzazione sia mono-esponenziale e l'accuratezza maggiore si ottiene per valori di T_1 dell'ordine del tempo di recupero applicato. I valori di T_2 possono essere determinati da due o più immagini ricostruite da segnali di eco successivi, usando una sequenza di impulsi tipo Carr-Purcell nelle stesse misure eseguite per determinare i valori di T_1.

La possibilità di usare più di due segnali di eco nello stesso tempo di registrazione migliora l'accuratezza ottenibile o il calcolo di una funzione di decadimento multi-esponenziale.

Introduction

In NMR imaging an important part of the contrasts between organs, tissues, lesions, fluids, etc. is related with differences in relaxation time constants.

In general the intensity of the resonance signal depends on:

i) the proton density;

ii) the degree of magnetisation, determined by the ratio between the recovery time and the relaxation time T_1;

iii) the degree of signal decay, determined by the time interval between excitation and echo signal detection relative to the relaxation time T_2.

In presently used imaging techniques 90° and 180° pulses are applied. The 90° pulses cause the rotation of magnetisation into the transverse direction necessary to produce resonance signal emission. The 180° pulse is either the magnetisation inversion pulse preceding the 90° pulse, or an echo creating pulse following the 90° pulse. More sequential echo signals can be obtained by repetition of the 180° pulse after the echo signal.

The well-known imaging techniques to be discussed here, are

a) Spin-echo imaging;
   The 90° pulse is followed by a single 180° pulse after an interval t_1 creating an echo signal at $2t_1$.
   The sequence is repeated at a time t_2 after the 180° pulse. The repetition time is thus $t_1 + t_2$ after the 180° pulse. The repetition time is thus $t_1 + t_2$.
b) Inversion Recovery Imaging;
   The 90° pulse is now preceded by an 180° pulse with a time interval t_3, and followed by another 180° pulse with a time interval t_1. The total cycle is $t_3 + t_1 + t_2$.

Calculation of the relative magnetisation and signal intensity

The following calculation method is described in more detail by Hinshaw [1].

The magnetisation vector M can be described in the rotating frame.

$$\underline{M} = M_x \underline{i} + M_y \underline{j} + M_z \underline{k}$$

If the B_1 field is applied in the $\underline{i}$ direction, then the rotation of $\underline{M}$ can be effected by application of the operators

$$P(90°) = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 0 & 1 \\ 0 & -1 & 0 \end{pmatrix} \text{ and } P(180°) = \begin{pmatrix} 1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & -1 \end{pmatrix}$$

The T_1 and T_2 relaxation during an interval t_i can be described by the simplified Bloch equation at the resonance frequency in a homogeneous B_0 field.

$$\frac{d\underline{M}}{dt} = -\frac{M_x}{T_2}\underline{i} - \frac{M_y}{T_2}\underline{j} - \frac{(M_z - M_0)}{T_1}\underline{k}$$

Introduction of the operator $R(t_i) = \begin{pmatrix} \epsilon_i & 0 & 0 \\ 0 & \epsilon_i & 0 \\ 0 & 0 & E_i \end{pmatrix}$

in which $E_i = \exp(-t_i/T_1)$ and $\epsilon_i = \exp(-t_i/T_2)$, leads to the formula

$$\underline{M}(t_i) = R(t_i)\,\underline{M}(0) + M_0\,[1 - E_i]\,\underline{k} \qquad (1)$$

Application to various techniques

Spin-echo imaging (M_0 = equilibrium, M_z 1)

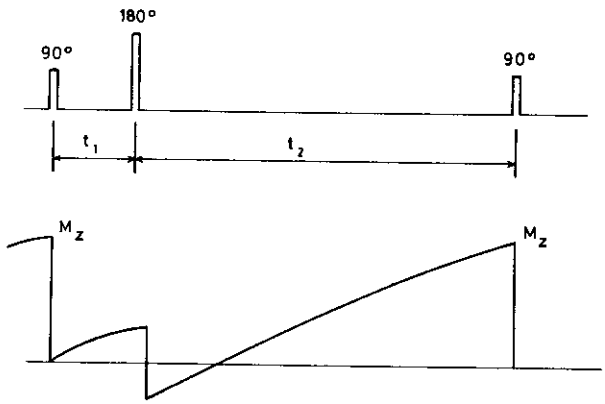


Fig. 1

The calculated M_z just before the 90° pulse is

$$M_z = \frac{1 - 2E_2 + E_1 E_2}{1 + E_1 E_2 \epsilon_1 \epsilon_2} \qquad (2)$$

in practice $\epsilon_i \approx 0$, thus (Figure 1)

$$M_z = 1 - E_2(2 - E_1) \qquad (3)$$

(if $t_2 \gg T_1$, then $M_z \to 1$)
The signal amplitude

$$S = C\ 1 - E_2(2-E_1)\ \exp(-2t_1/T_2)$$
$$\text{or}\quad S = C\ 1 - E_2(2-E_1)\ \epsilon_1^2 \qquad (4)$$

C is the constant of proportionality.
In case of two echo signals (Figure 2)

$$M_z = \frac{1 - 2E_2 + 2E_1{}^2E_2 - E_1{}^3E_2}{1 + E_1{}^3E_2\,\epsilon_1{}^3\epsilon_2} \qquad (5)$$

or if $\epsilon_2 \approx 0$

$$M_z = 1 - E_2(2 - E_1{}^2 + E_1{}^3) \qquad (6)$$

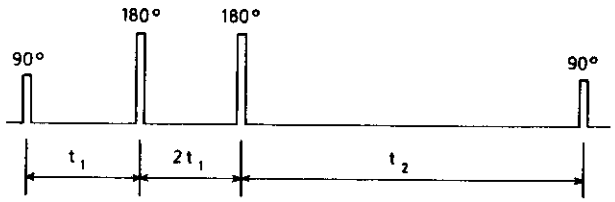


Fig. 2

The first echo signals are $S_1 = CM_z\,\epsilon_1^2$ (7)

and $S_2 = CM_z\,\epsilon_1^4$ (8)

The calculation of T_2 is simply $T_2 = -\dfrac{2t_1}{\ln \dfrac{S_1}{S_2}}$

By using more than 2 echo signals a higher accuracy is obtainable.

Inversion recovery imaging

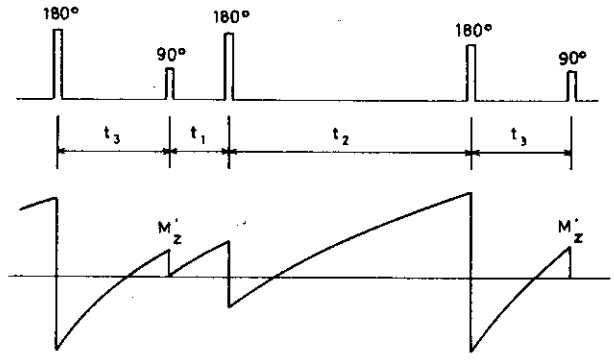


Fig. 3

The value of M_z' at the time of the 90° pulse determines the amplitude of the resonance signal S'.

For reasons of mathematical uniformity, the interval of time length t_3 preceding the 90° pulse is treated as the last interval in the pulse cycle, which begins with t_1.

Application of the appropriate rotation and relaxation operators P and R on M_z, yields the formula (Figure 3):

$$M_z' = \frac{1-2E_3 + 3E_2 E_3 - E_1 E_2 E_3}{1 + E_1 E_2 E_3 \epsilon_1 \epsilon_2 \epsilon_3} \qquad (9)$$

where $\epsilon_2 \approx 0$, and $\epsilon_3 \approx 0$ this formula can be reduced to

$$M_z' = 1 - 2E_3\,(1 - E_2 + 1/2E_1 E_2) \qquad (10)$$

The signal amplitude of the first echo is then

$$S' = C \cdot \left\{1 - 2E_3\,(1 - E_2 + 1/2E_1 E_2)\epsilon_1^{\,2}\right\} \qquad (11)$$

In those situations where $t_2 \gg T_1$, $E_2 \approx 0$, the formulae can be further simplified to

$$M_z' = 1 - 2E_3 \qquad (12)$$

$$S' = C(1 - 2E_3)\epsilon_1^{\,2} \qquad (13)$$

Using the formulae (6). (7) and (11) yields the following equation from which T_1 can be calculated

$$\frac{S'}{S} = \frac{1-2E_3(1-E_2 + 1/2E_2E_3)}{1-E_2(2-E_1^{\,2} + E_3^{\,3})} \qquad (14)$$

$E_2 = \exp(-t_2/T_1)$, $E_3 = \exp(-t_3/T_1)$

Optimum value of t_3 for minimum error in T_1

The noise fluctuation (N) in the reconstructed image causes a random error in the values for S' and S_1 in each pixel. This leads to a random error in the calculated T_1:

$$\Delta T_1 = \left\{\left(\frac{\delta T_1}{\delta S'}\right)^2 + \left(\frac{\delta T_1}{\delta S}\right)^2\right\}^{\frac{1}{2}} N$$

For the determination of the optimum t_3 the equation (14) can be simplified by assuming the $E_2 \ll 1$, which leads to

$$\frac{S'}{S} = 1 - 2E_3 = 1 - 2\exp(-t_3/T_1)$$

$$\text{or } \exp\left(\frac{-t_3}{T_1}\right) = \frac{S - S'}{2S}$$

The relative error in T_1 then becomes:

$$\frac{\Delta T_1}{T_1} = \frac{(2 - 4E_3 + 4E^2)^{\frac{1}{2}}}{2E_3\, t_3/T_1} \cdot \frac{N}{S} = \xi \left(\frac{S}{N}\right)^{-1}$$

The error in T_1 is a factor ξ times the reciprocal value of the signal-to-noise-ratio in the spin echo image.

The value of ξ depends on t_3 but the optimum value is not critical as can be seen in the following Table 1:

t_3/T_1	ξ	rel. increase of error
0.5	1.70	21%
0.7	1.42	1%
1.0	1.40	0
1.5	1.70	21%

The noise in the T_1-map is some 50% larger than in the spin echo image, but the accuracy of T_1 is practically constant in the range of 2/3 t_3 through $2t_3$.

REFERENCES

1) HINSHAW, W.S. 1976. Image formation by nuclear magnetic resonance: the sensitive-point method. *J. Appl. Phys.* 47: 3709-3721.

Ann. Ist. Super. Sanità
Vol. 19, N. 1 (1983) pp. 47-50

TECHNIQUE DEPENDENCE IN NMR IMAGING MEASUREMENTS

D.G. TAYLOR (*a*) and ERICSSON (*b*)

(a) Department of Physics, University of Surrey, Guilford, Surrey, U.K.
(b) Arrhenius Laboratory, University of Stockholm, Sweden

Summary. — *A theoretical investigation is reported of the response of a nuclear spin system to the repeated application of the pulse sequences, 90-τ-180 and 180-τ-90, at a rate $>1/5T_1$. The magnetisation at any time during the excitation sequence is calculated in terms of the relaxation parameters, T_1 and T_2. It is shown that providing the sequence repetition time is much greater than the longest T_2 in an NMR image cross-section, then a steady state is established for all pixels and an accurate determination of T_1 and T_2 is possible.*

Riassunto. — *Viene qui riportato uno studio teorico della risposta di un sistema di spin nucleari all'applicazione di sequenze di impulsi ripetute; 90-τ-180 e 180-τ-90, con tempi di attesa tra le sequenze più brevi di $5T_1$. L'andamento nel tempo della magnetizzazione durante la sequenza di eccitazione è calcolata in dipendenza dei parametri di rilassamento T_1 e T_2. Viene dimostrato che, purché il tempo di ripetizione della sequenza sia molto più grande del T_2 più lungo nella immagine NMR di una data sezione, si stabilisce uno stato di equilibrio per la magnetizzazione di tutti i "pixels" ed è possibile determinare i valori di T_1 e di T_2 in modo accurato.*

Introduction

It is conventional in nuclear magnetic resonance to allow greater than five times the longest spin-lattice relaxation time, T_1, to lapse before further measurements are made in order to permit the nuclear spin system to return to thermal equilibrium. In NMR imaging, where one is dealing with live subjects, this imposes a severe restraint on the minimum data collection time. In practice a compromise is made with radiofrequency excitation repeated at times less than $5T_1$. However, when a system is pulsed repeatedly, in this case a minimum of 180 times, at a rate of $>1/5T_1$ a steady state condition may be rapidly established. The magnetisation measured in a given pixel is then, in general, a complex function of the parameters, proton density, T_1 and T_2.

For the determination of T_1 and T_2 it is in fact essential that a steady state be set up in the imaging experiment i.e. in the presence of transient magnetic field gradients, and for off resonant nuclei. A theoretical investigation is reported in which the bulk nuclear magnetisation at any time during the excitation sequence is calculated by means of matrix equations for each of the commonly used excitation methods (180-τ-90, 90-τ-180, 90-τ-90). The dependence of the magnetisation amplitude on T_1 and T_2 is determined for any position on the image plane. Further, the consequences for the accurate determination of single and multi-exponential relaxation parameters for the range of tissues within a single cross-section are discussed.

Behaviour of spins subjected to imaging pulse sequences

The response of a nuclear spin system to a regular sequence of identical pulses has been treated by Ernst and Anderson [1] based on the Bloch equations. This work was extended by Freeman and Hill [2] and by Hinshaw [3] to describe the phenomenon of "steady state free precession". The aim of the calculations described below is to further extend the treatment to the rapid repetition of the 180-τ-90 and 90-τ-180 pulse sequences.

For a sample with gyromagnetic ratio γ, the Bloch equations of motion for the magnetisation, M, can be written

$$\frac{d\underline{M}}{dt} = \gamma \underline{M} \times \underline{B}_r - (M_x \underline{i} + M_y \underline{j})/T_2 - (M_z - M_0)\underline{k}/T_1 \quad (1)$$

with

$$\underline{B}_r = (B_0 - \omega/\gamma)\underline{k} + B_1 \underline{i}$$

$\underline{i}$, $\underline{j}$ and $\underline{k}$ are the unit vectors of a frame rotating with frequency ω about the common z axis of the laboratory frame. B_0 is the static field applied in the z direction and B_1 is the r.f. field which is in the x direction of the rotating frame. Equation (1) is solved for two situations. During the r.f. pulse, B_1 is much larger than $B_0 - \omega/\gamma$

and relaxation effects are negligible. A pulse of length t_p rotates $\underline{M}$ through an angle $\beta = \gamma B_1 t_p$ about the x-axis. Using the rotation operator R, the effect of the rotation on the magnetisation can be written

$$\underline{M}(t_p) = R(t_p)\,\underline{M}(0) \qquad (2)$$

where

$$R(t_p) = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos\beta & \sin\beta \\ 0 & -\sin\beta & \cos\beta \end{bmatrix}$$

Between pulses, the solution of equation (1) is

$$\underline{M}(t) = D(t)\underline{M}(0) + M_0[1 - \exp(-t/T_1)]\,\underline{k} \qquad (3)$$

where the relaxation operator D(t) is given by

$$D(t) = \begin{bmatrix} \exp(-t/T_2)\cos\delta\omega t & -\exp(-t/T_2)\sin\delta\omega t & 0 \\ \exp(-t/T_2)\sin\delta\omega t & \exp(-t/T_2)\cos\delta\omega t & 0 \\ 0 & 0 & \exp(-t/T_1) \end{bmatrix}$$

with $\delta\omega = \omega - \omega_0$ and $\omega_0 = \gamma B_0$.

The effect on the magnetisation of any pulse sequence may be obtained by successively combining equations (2) and (3).

a) 90-τ-90

The magnetisation at time t = 0 immediately after a pulse in the sequence is

$$\underline{M}^+(0) = R(\beta)D(\tau)\underline{M}^+(-\tau) + M_0(1 - e_1)R(\beta)\,\underline{k} \qquad (4)$$

where $\underline{M}^+(-\tau)$ is the magnetisation immediately after the previous pulse, and $e_1 = \exp(-\tau/T_1)$. For a steady state solution $\underline{M}^+(0) = \underline{M}^+(-\tau)$. Substituting in equation (4)

$$\underline{M}^+(0) = (R^{-1}(\beta) - D(\tau))^{-1}\,M_0\,(1 - e_1)\,\underline{k} \qquad (5)$$

The solution to this equation for $M^+_{xy}(0)$ is

$$\begin{aligned} M^+_x(0) &= M_0 Q\, e_2 \sin\beta \sin\delta\omega\tau \\ M^+_y(0) &= -M_0 Q \sin\beta (1 - e_2\cos\delta\omega\tau) \end{aligned} \qquad (6)$$

and $$Q = \frac{(1 - e_1)}{(1 - e_1\cos\beta)(1 - e_2\cos\delta\omega\tau) - e_2(e_1 - \cos\beta)(e_2 - \cos\delta\omega\tau)}$$

where

$$e_2 = \exp(-\tau/T_2) \text{ and } \beta = \pi/2$$

b) 180-τ-90-T-

The magnetisation at time $t = \tau$ immediately after a 90 pulse is

$$\underline{M}^+(\tau) = R(\pi/2)D(\tau)R(\pi)D(T)\underline{M}^+(-T) + R(\pi/2)D(\tau)R(\pi)M_0(1 - E_1)\,\underline{k} + R(\pi/2)M_0(1 - e_1)\,\underline{k} \qquad (7)$$

where $E_1 = \exp(-T/T_1)$, and again $e_1 = \exp(-\tau/T_1)$. For the steady state $M^+(\tau) = M^+(-T)$. Substituting and solving

$$\begin{aligned} M^+_x(\tau) &= M_0\, u\epsilon_2 \sin\delta\omega(T - \tau)[1 - 2e_1 + \epsilon_1] \\ M^+_y(\tau) &= M_0\, u(\epsilon_2\cos\delta\omega(T - \tau) - 1)[1 - 2e_1 + \epsilon_1] \end{aligned} \qquad (8)$$

where $u = [1 - \epsilon_1\epsilon_2^2 + \epsilon_2\cos\delta\omega(T - \tau)[\epsilon_1 - 1]]^{-1}$

and $\epsilon_1 = e_1 E_1$, $\epsilon_2 = e_2 E_2$, $E_2 = \exp(-T/T_2)$.

c) 90-τ-180-τ-T-

The magnetisation at time $t = 2\tau$ i.e. at the spin echo centre is

$$M(2\tau) = D(\tau)R(\pi)D(\tau)R(\pi/2)D(T)\underline{M}(-T) + M_0(1 - E_1)D(\tau)R(\pi)D(\tau)R(\pi/2)\,\underline{k} + M_0(1 - e_1)D(\tau)R(\pi)\,\underline{k} + M_0(1 - e_1)\,\underline{k}$$

For steady state $\underline{M}(2\tau) = \underline{M}(-T)$. Substituting and solving for $M_{xy}(2\tau)$

$$\begin{aligned} M_x(2\tau) &= -M_0 V\epsilon_2 e_2^2 \sin\delta\omega T[1 - 2E_1 e_1 + \epsilon_1] \\ M_y(2\tau) &= M_0 V e_2^2(1 - \epsilon_2\cos\delta\omega T)[1 - 2E_1 e_1 + \epsilon_1] \end{aligned} \qquad (9)$$

where $V = [1 - \epsilon_2\cos\delta\omega T - \epsilon_1\epsilon_2(\epsilon_2 - \cos\delta\omega T)]^{-1}$

and now $\epsilon_1 = E_1 e_1^2$ and $\epsilon_2 = E_2 e_2^2$.

Discussion

The three irradiation sequences analysed above, are routinely used to measure the parameters proton density, T_1 and T_2. Examination of equations (6), (8) and (9) yields the conditions for which this is possible. It is immediately obvious, for example, for the inversion recovery sequence (180-τ-90) which is used to measure T_1, that in the steady state there is a very complex dependence on both T_1 and T_2. However, these equations may be simplified considerably if T_2 is much shorter than the repetition time of the sequence and give results in agreement with Young *et al.* [4].

For the repeated 90 pulse (90-τ -90), $e_2 = \exp(-\tau/T_2) \sim 0$ if $\tau \gg T_2$ and, as expected,

$$\begin{aligned} M^+_x(0) &= 0 \\ M^+_y(0) &= -M_0(1 - \exp(-\tau/T_1)) \end{aligned}$$

For the 180-τ -90 sequence, $\epsilon_2 = \exp(-(\tau + T)/T_2) \sim 0$ if $(2\tau + T) \gg T_2$, and

$$M_x^+(\tau) = 0$$

$$M_y^+(\tau) = M_0[1 - 2\exp(-\tau/T_1) + \exp(-(\tau + T)/T_1)].$$

Finally, for the $90 - \tau - 180$ sequence,
$\epsilon_2 = \exp(-(2\tau + T)/T_2) \sim 0$ if $(2\tau + T) \gg T_2$, and

$$M_x(2\tau) = 0$$

$$M_y(2\tau) = M_0 \exp(-2\tau/T_2)[1 - 2\exp(-(\tau + T)/T_1) + \exp(-(2\tau + T)/T_1)].$$

All the above equations are independent of the parameter $\delta\omega$. In an imaging experiment, magnet field gradients are applied whose effect is to vary $\delta\omega$ as a function of position. Thus, providing the T_2 condition is satisfied, the equations above hold for any position on the image plane. It is interesting to consider the response when the repetition time is of the order of T_2. The magnetisation, equations (6), (8) and (9), is now frequency dependent. There are two possible regimes. Firstly, the sequence is repeated many times for a given image "view" e.g. projection angle. Again a steady state condition may be established, as is the case for steady state free precision technique of Hinshaw [3].

However, if there are only a limited number of repetitions per gradient position, then a steady state will not be established due to the variation of $\delta\omega$. Because of the wide variation in T_1 and T_2 values in biological tissue this latter situation could happen inadvertenly for some spins in an image cross-section. Care must be taken when choosing the τ values and repetition times. This point is not trivial as it is not yet clear how to select these times for optimum tissue discrimination signal-to-noise per unit time. Should one have short times and many signal averages or long times and few if any averages? What is the optimum τ value if there is multi-exponential relaxation behaviour? This is the subject of further work.

REFERENCES

1. ERNST, R.R. & ANDERSON, W.A. 1965. Application of Fourier transform spectroscopy to magnetic resonance. *Rev. Sci. Instrum.* 37: 93-102.

2. FREEMAN, R. & HILL, H.D.W. 1971. Phase and intensity anomalies in Fourier transform NMR. *J. Magn. Reson.* 4: 366-383.

3. HINSHAW, W.S. 1976. Image formation by nuclear magnetic resonance: the sensitive point method. *J. Appl. Phys.* 47: 3709-3721.

4. YOUNG, I.R., BAILES, D.R., BURL, M., COLLINS, A.G., SMITH, D.J., McDONNELL, M.J., ORR, J.S., BANKS, L.M., BYDDER, G.M., GREENSPAN, R.H. & STEINER, R.E. 1982. Initial clinical evaluation of a whole body Nuclear Magnetic Resonance (NMR) tomograph. *J. Comput. Assist. Tomogr.* 6: 1-18.