

Magnetic Resonance
in
Medicine and Biology

M. A. FOSTER

Magnetic Resonance in Medicine and Biology

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PREFACE

It has been remarked by many philosophers throughout the long period of written history that at least one of the major aims in the pursuit of knowledge is the complete understanding of mankind, physically, mentally and socially. It is certainly possible to see the physical aspect of this aim being furthered, either consciously or unconsciously, by those associated with scientific and technological development since the renaissance of intellectual thought in Europe. Almost every discovery of a new physical principle has been followed rapidly by its application, in some way, to the human organism. In many cases, certainly until quite late in the last century, this seems to have been done more in the pious hope of curing some of the ills of mankind than to further the deeper understanding of the workings of the human organism. Perhaps the limited understanding of the basic principles involved made such an approach inevitable, and we see both magnetism and electricity abused in this way in the early days of their study. During the last hundred years, however, there has been a more reasoned, sequential approach, involving first some understanding of the physical principles of the new phenomenon followed by a development of the applications of these principles initially to simple systems and then more complex ones. Ultimately, in some cases, has come the application of the new phenomenon to the study of the human system.

Magnetic resonance is a prime example of this development of understanding of a phenomena and its subsequent application to systems of increasing complexity. The earliest papers on the subject, produced independently by Bloch and Purcell in 1946 are mainly concerned with the existence of magnetic resonance and relaxation phenomena. Within a very short time several laboratories were investigating the possibilities of using both ESR and NMR to further the understanding of simple chemical systems. The complexity of the system under study as well as the technology available to make the study gradually increased (though very valuable ESR and NMR work is still being done, applying modern advanced techniques to simple chemical systems). Eventually the time and technology became right for the first applications, during the 1950s, of ESR and NMR to the detailed investigation of cellular systems. Soon normal and pathological excised tissues were being studied by both techniques and eventually NMR techniques were developed which have, in the past few years, enabled a new generation of instrumentation experts to design and build instruments for the observation of NMR characteristics of atomic nuclei deep within the bodies of living animals, including man.

As would be expected, a major aim of many of the tissue and whole-body studies undertaken using magnetic resonance techniques is the furtherance of medical knowledge. The intent of this book is to summarise the variety of ways in which ESR and NMR can be, and have been, applied to the study of normal and of pathological living systems. Once the basic phenomenon of magnetic resonance was discovered, its many possibilities became obvious. So we see that ESR techniques have been developed either to assist conventional ESR spectroscopy, for example low temperature methods, the use of tissue cells and freeze drying of biological material, all of which help to overcome the problems created by tissue water, or to extend the range of problems to which the technique can be applied as, for example, in the development of spin labelling and spin trapping methods. The variety of applications of NMR techniques to biological problems is even greater. Conventional NMR spectroscopic methods have been used to study a variety of naturally-occurring or artificially-introduced atomic nuclei in living systems and special methods like the shaping of the magnetic field (topical magnetic resonance)

have enabled such studies to be extended to localised regions within living beings. The study of NMR relaxation behaviour, especially when applied to the protons of tissue water molecules, has been of major importance in understanding some aspects of living systems. This field has been greatly developed in a variety of directions, including NMR imaging techniques, methods for the study of flow in blood vessels and even suggestions for the development of a form of NMR microscopy.

Some developments of magnetic resonance techniques will not be covered in this volume. In particular, these include the various double resonance techniques, ELDOR (electron-electron double resonance), ENDOR (electron-nuclear double resonance) and INDOR (nuclear-nuclear double resonance). As yet, these are mainly tools for the chemist and biochemist, but the history of developments in the application of magnetic resonance techniques to biological problems strongly suggest that it may not be long before the double resonance technique will have earned a place in any discussion of the biological applications of magnetic resonance.

I would like to conclude this preface by thanking the many people who have helped in the preparation and production of this book. Firstly, the three colleagues who have provided me with chapters on specialist topics which I did not feel competent to tackle in depth. Nick Dodd of the Paterson Laboratories, Manchester, is an ESR spectroscopist who has worked for many years in the general field of biological applications of ESR. Recently he has turned most of his attention to spin label studies, a field into which I have not yet ventured. I am grateful to him both for providing such an excellent review of this field and also because, in doing so, he has stimulated me to extend my own studies a little further in that direction. Frank Smith is a colleague and friend at Aberdeen Royal Infirmary. As Consultant in Nuclear Medicine here he was able to make an early assessment of the clinical possibilities of the NMR imaging system being developed in our Department. His keen interest in, and clinical use of, the NMR imager from its first moment of coming "on line" has been one of the most powerful driving forces for the NMR group. His involvement in NMR imaging from its earliest days makes him by far the best qualified person available to write a chapter on the clinical applications of this technique. The author of the third specialist chapter deserves a double vote of thanks from me. As the focal point in the team who have designed and built the Aberdeen NMR imaging system he has won a world-wide reputation for himself. In this capacity I am grateful to him for writing a chapter summarising the variety of NMR imaging techniques as well as that in use at Aberdeen. But I must also thank him for the vast amount of help and encouragement that he has given to me at every stage of the preparation of this volume, both as a knowledgeable colleague and as an understanding husband. The latter was probably at times the more difficult role for him to take.

The labour of typing, from a first interpretation of my, at times terrible, hand-writing through various stages to the production of copy for the publisher was undertaken by Wendy Harris. Despite my various problems, delays and re-writes, she has uncomplainingly got ahead with the job and has been a constant source of encouragement to me. I would also like to thank Raymond Hutcheon and Cameron Forbes for all their help in the preparation of photographic material for this book.

Finally, I would like to offer more general thanks to Professor J.R. Mallard, Head of the Department of Bio-Medical Physics and Bio-Engineering at Aberdeen University. It was Professor Mallard who first, many years ago, suggested to me the possibilities of applying ESR to the study of human disease and he has offered constant advice and encouragement since that time. I would also like to thank the Cancer Research Campaign and the Medical Research Council for providing funds for me over the years.

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CHAPTER 1

MAGNETIC RESONANCE — THE PHENOMENON

Since its initial application by Bloch and Purcell, the phenomenon of magnetic resonance has been used widely by chemists and biochemists in the study of both inorganic and isolated organic compounds. Over the last twenty years, with the increasing interest in, and knowledge of, the fundamental chemistry and physics of biological processes, magnetic resonance spectroscopy has also been applied in the study of organised biological systems. Its use has yielded considerable amounts of information about the life processes both in simple organisms and in isolated pieces of more complex living systems. In very recent years magnetic resonance phenomena have even been used to obtain information about metabolic processes and structural details in mammals *in vivo*.

Magnetic resonance is used in two forms of spectroscopy — electron spin resonance (ESR, sometimes called electron paramagnetic resonance or EPR) and nuclear magnetic resonance (NMR). The essential difference between ESR and NMR, as their names imply, is that in ESR the magnetic resonance is achieved by the electron whilst in NMR it is achieved by the nucleus of the energy-absorbing system.

Magnetic resonance spectroscopy is essentially similar to other forms of absorption spectroscopy in that the specimen is bombarded with electromagnetic radiation with the intention of inducing an absorption of this incident radiation during the process of raising a low energy ground state to a high energy

excited state somewhere within a molecule. It is different from the shorter wavelength spectroscopies (infra-red to X-ray) in that the interaction is with the magnetic rather than the electric component of the electromagnetic radiation and the input energy is not used to probe the bonding energies of the atoms of the molecule, but to provide the much smaller amount of energy needed to induce a change in spin (or magnetic moment) orientation of an electron or nucleus. In all forms of spectroscopy transition from the ground to the excited state can only occur if the quantum energy of the incident radiation is the same as the energy gap between the ground and excited states (usually referred to as ΔE). The quantum energy ($h\nu$) is related to the frequency (ν) by Planck's constant (h) so that we have:

$$h\nu = \Delta E$$

as a common denominator in absorption spectroscopy. Since ΔE is relatively small in both forms of magnetic resonance spectroscopy, ν is correspondingly small, and so in these studies we are dealing with the microwave and radiowave parts of the electromagnetic spectrum.

The use of longer wavelength radiations can provide an additional bonus for biological and medical studies in that microwaves and radiowaves have a much deeper penetration into biological (i.e. "wet")

material than do most of the wide band of frequencies between infra-red and ultra-violet. To the biologist this means that not only can considerable amounts of chemical information be obtained from small samples of isolated cell components, body fluids or pieces of tissue, but there is, for both ESR and NMR, the possibility of studying the internal chemistry of much larger tissue samples, whole organs or even whole bodies whilst they perform their normal life functions. This has led, in recent years, to a considerable amount of effort being put into investigating the possibility of medical whole body imaging by NMR. This will be discussed in detail in a later chapter.

Electrons, protons and certain nuclei possess the property of spin and all charged particles with spin possess a magnetic moment. If the particle is placed in an external magnetic field, therefore, it will act like a bar magnet and align itself with that external field. Normally the bar magnet will align itself so that its north pole faces the south pole of the external magnet, a low energy state. By careful positioning, however, it is possible to place the bar magnet so that its north pole faces the north pole of the external magnet. In this state there is no lateral force so it cannot move out of this alignment. However, if given the smallest perturbation it will flip round to the north-to-south orientation and it would be possible to recover energy from the system during this flip. North-to-north is, therefore, a high energy state similar to the excited state in ESR and NMR.

In most forms of absorption spectroscopy the sample is directly receptive to the absorption of the incident energy, but in magnetic resonance spectroscopy an artificial state has to be produced before a split in energy levels can be observed, and into which energy can be absorbed. In a sample which is not exposed to an external magnetic field the magnetic moments of the electrons or nuclei are randomly oriented (Fig. 1.1). It is only when the experimenter applies the external field that they orient themselves into specific directions and hence into different populations with different energy levels. The small difference in energy levels between the different orientations of the magnetic moments means that there is, among the large number of electrons or nuclei in any sample, a chance that some will go into the low and others into the high energy orientation when the field is applied. As would be expected, the minority will enter the high energy

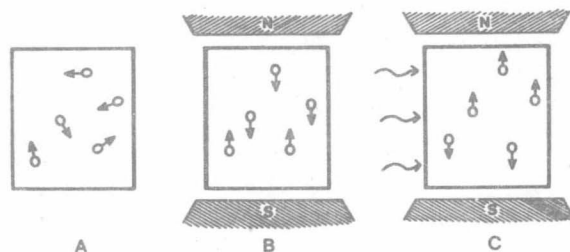


Fig. 1.1. Spin orientation. (a) Classical picture of random spin orientation in absence of magnetic field, (B) Quantum picture of spin alignments in an external magnetic field and (C) Spin orientations inverted after application of EM radiation at precession (Larmor) frequency.

state, but the proportions in the two states are affected by factors such as the temperature of the sample and the energy difference between the states (ΔE but, as was seen earlier, this can be expressed as $h\nu$). The proportion can be calculated from the Boltzman expression:

$$(N_{\text{down}}/N_{\text{up}}) = e^{h\nu/kT} \quad (1)$$

where N_{down} is the low energy and N_{up} is the high energy orientation, k is Boltzman's constant and T the temperature in K.

To find the effective fraction of spins in the two states (and hence to interpret an ESR or NMR absorption spectrum quantitatively) the hyperbolic tangent (\tanh) is used, where $\tanh x$ is defined as:

$$e^{2x} - 1 / e^{2x} + 1 \quad (2)$$

The effective fraction of spins, i.e. the difference in size of the populations (N_{eff}) is:

$$N_{\text{eff}} = (N_{\text{down}} - N_{\text{up}}) / (N_{\text{down}} + N_{\text{up}})$$

and so, by re-arrangement and substituting from (1):

$$N_{\text{eff}} = (e^{h\nu/kT} - 1) / (e^{h\nu/kT} + 1) \quad (3)$$

Now let us say that $2x = h\nu/kT$ and insert this into (3):

$$N_{\text{eff}} = (e^{2x} - 1) / (e^{2x} + 1)$$

Which, by comparison with (2) shows that:

$$N_{\text{eff}} = \tanh x = \tanh h\nu/2kT$$

In fact, on calculating this out, the tanh makes so little difference that for most purposes it can be said that:

$$N_{\text{eff}} = h\nu/2kT$$

To see what kind of differences we can expect between the numbers of spins in the two energy states let us insert numbers into this formula, assuming that we are doing an ESR study at room temperature and at one of the common frequencies, the X-band of radar (9.3 GHz). Hence:

$$h \text{ is } 6.62 \times 10^{-34}$$

$$\nu \text{ is } 9.3 \times 10^9 \text{ Hz}$$

$$k \text{ is } 1.38 \times 10^{-23}$$

$$T \text{ is } 300 \text{ K}$$

so the effective fraction is 7.435×10^{-4} . It can be seen that the difference between the sizes of the two populations is very small, although it can be increased by reducing the temperature. ΔE in NMR is much smaller than in ESR, so the effective fraction in NMR is extremely small. For this reason the use of very high magnetic fields and special techniques such as Fourier transform have been developed to overcome the relative insensitivity caused by the small N_{eff} .

To induce a change in orientation of the magnetic moment of the spin system after application of the external magnetic field it is necessary to either donate (low to high energy transition) or to remove (high to low energy transition) a quantum of energy, ΔE , from the spinning electron or nucleus. However, the value of ΔE and hence the frequency of this radiated energy is dependent upon both the value of the magnetic moment of the spin particle and upon the strength of the applied magnetic field (H). This latter is associated with the fact that it is a spinning system. It was shown by Larmor that a friction-free spinning magnet placed in a magnetic field precesses about the direction of the magnetic field in the same way that a spinning top precesses in the earth's gravitational field. He also demonstrated that the frequency of precession (referred to as the Larmor frequency) is directly proportional to the magnetic field strength and that the proportionality constant

is the ratio of the magnetic moment of the spinning magnet to its angular momentum. This proportionality constant (usually signed γ) is commonly called the gyromagnetic ratio, although certain workers term it the magnetogyric ratio, arguing that it is the ratio between magnetic moment and angular momentum, not the reverse. The argument for "gyromagnetic ratio" comes from the function of the spin system in magnetic resonance experiments. Here we see that γ can be regarded as the ratio of the precession frequency of the spin to the magnetic field. The value of γ derived from these two viewpoints is, however, the same.

From Larmor's work we obtain the basic equation of magnetic resonance:

$$\omega = \gamma H$$

where ω is 2π times the precession frequency ($2\pi\nu$) and H is the strength of the applied magnetic field. However, if working in SI units this equation would be $\omega = \gamma B$, where B is the field strength in Tesla. B will be used where appropriate in this text but the symbol H , measured in Gauss, is more familiar to ESR users. Another form of this equation is more frequently met in ESR (although both forms apply equally to both types of spectroscopy). This involves the use of the Bohr (for electrons) or the nuclear (for NMR studies) magneton. The magneton is associated with the magnetic moment of the spinning particle and can be derived from:

$$\beta = eh/4\pi m$$

where β is the Bohr magneton, e the charge of the spinning particle and m the mass of the electron. For the nuclear magneton β_n this would be:

$$\beta_n = eh/4\pi M$$

β_n is much smaller than β since the proton mass (M) is 1836 times as great as that of the electron. These formulae refer to the calculation in SI units, hence leaving out the speed of light term necessary when using older unit systems. It can be seen that the magnetic moment of the electron is about 2000 times that of the proton. The magneton relates to the basic equation above because:

$$\gamma = g\beta/\hbar$$

where g is a proportionality constant usually referred to as the g -value and \hbar is Planck's constant divided by 2π . Hence:

$$\omega/H = g\beta/\hbar \text{ or } \omega\hbar = g\beta H$$

since ω is $2\pi\nu$, then:

$$h\nu = g\beta H$$

which is the standard equation used in ESR. The g -value is a very useful term in ESR, specifying the position of the spectral absorption from a particular spin system and being characteristic of that system. The ratio of spin magnetic moment to spin angular momentum is twice as large as the ratio of orbital magnetic moment to orbital angular momentum, which means that $g = 2$ for the electron. However, a small relativistic correction has to be inserted producing the free electron g -value of 2.0023. That for the free proton is 5.5855. Several factors, to be determined later, affect the g -value and considerable amounts of information about the immediate molecular or atomic environment of the spin can be obtained by a study of g -value shifts.

To obtain a transition from one energy level to another, i.e. from one spin orientation to another, we have to deliver our energy as EM radiation whose photon energy is equivalent to the energy difference between the two states ($h\nu = \Delta E$). As we have seen, $h\nu = g\beta H$, hence the frequency of the radiation needed to induce transition is the same as the Larmor frequency. Equally if a spin re-orientates from the high to the low spin state it will emit a photon at that same frequency. Since, as we have seen, there is a slight preponderance of spins in the lower energy state the net result of applying a pulse of EM radiation at the Larmor frequency should be the absorption of an amount of incident radiation related to the effective difference between spin-up and spin-down. This will be true if a sufficiently short pulse of radiation is given, and in such cases the size of the absorption spectrum will be related to the size of the effective spin population. Hence, knowing the temperature of the sample, the total size of the spin population should, in theory, be calculable. In fact, this is rarely the case because the spins are neither completely coupled to their environment, i.e. they cannot lose the extra energy totally and immediately, nor are they completely unable to lose the energy

obtained from the EM radiation. In fact, an excited spin population undergoes a process of relaxing during which the additional energy may be dissipated as thermal vibration over a time period whose duration is related to the amount of coupling between the spin and the surrounding molecular or atomic matrix. The processes of energy loss or randomization of spin orientation are referred to as relaxation. A great deal of information, particularly in NMR, can be obtained from a study of relaxation times and mechanisms.

Relaxation and Saturation

If sufficient EM power is applied to the system a stage will be reached at which there is no net absorption of power. The system will have reached a state of effective thermal equilibrium so that spin-up is equal to spin-down and the amount of energy absorbed by low to high energy transition is the same as that released by high to low transition. This is a phenomena known as power saturation. On cessation of EM irradiation of the sample, the spin population relaxes back to the ground state by a combination of two processes: loss of energy as heat by dissipation into the immediate environment, a process known as spin-lattice relaxation, and a loss of spin orientation with respect to the direction of the applied magnetic field (H) known as spin-spin relaxation.

In examining relaxation processes it is often easier to look at the net magnetization of a population of spins rather than to visualise them individually. While a single nucleus should be treated according to quantum mechanical laws a population of spins in the aggregate can be regarded as obeying the laws of classical dynamics. Thus the angle of spin orientation with respect to H can be referred to, rather than the quantum state of the single spin. The direction and field strength of the net magnetization of the spin population is normally referred to as its magnetization vector, this being the vector sum of the magnetic field of each individual spin. When the external magnetic field is applied to the spin population, the magnetization vector rotates around the main field orientation at the Larmor frequency, (Fig. 1.2). If we now regard our system in three dimensions with z being the axis of the main field and x and y the orthogonal axes, then we find that in the absence of applied EM radiation there is no net magnetic moment in the x and y directions ($M_x = M_y = 0$). The

difference between spin-up and spin-down, i.e. the size of the effective spin population, gives a net magnetic moment in the z direction (M_z). This is the equilibrium state. If we now apply our EM radiation at the Larmor frequency we start the spin population precessing, hence angling out from the z -axis. As this proceeds M_z becomes less and M_{xy} becomes greater until M_z is zero and M_{xy} is at a maximum, being equivalent to the original M_z when the precession angle reaches 90° and the spins are precessing on the xy -plane. The amount of power, or duration of EM pulse needed to bring this about is referred to as a 90° or $\pi/2$ pulse. If a larger pulse is applied the magnetization vector can be inverted. A pulse of double the 90° size (either duration or amplitude) will send the spin population into a complete reversal from the equilibrium state – a 180° or π pulse. In this state M_{xy} is again zero and M_z has the same value as in the equilibrium state, with a reversed sign.

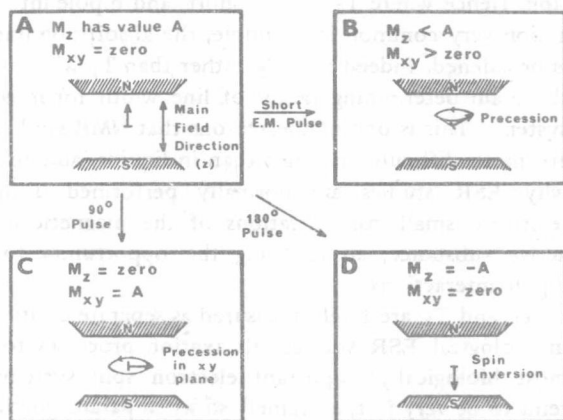


Fig. 1.2. Effect of EM pulses of different size on direction of net magnetization of spin population (shown by arrow between magnet poles).

The relaxation processes allow the excited spin population to return to the equilibrium state. If a 180° pulse has been applied to the system, the spins can relax back directly along the z -axis. Hence M_z (if the sign is ignored) will first decrease, then pass through zero and then increase again until the original

value is achieved, (Fig. 1.3.). This longitudinal relaxation process, which gives no increase in M_{xy} involves a direct loss of energy by the spin system. The energy is dissipated as heat through the surrounding molecular structure, hence longitudinal relaxation can be equated with spin-lattice relaxation. The decay is an exponential process, the time constant of which is referred to as T_1 , and the rate constant as R_1 . The time taken for M_z to decrease to the zero point is known as T_{null} .

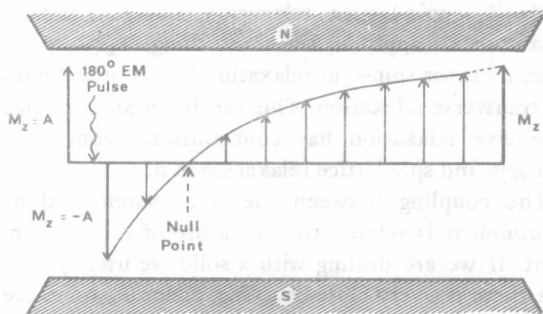


Fig. 1.3. Longitudinal relaxation after the spin system has received an inverting EM pulse. The arrows represent the magnetization vector at various times after spin inversion. The line shows the exponential decay curve whose time constant is T_1 . T_{null} is the point at which the magnetisation vector is zero.

Relaxation after applying a 90° EM pulse is slightly more complex because it is compounded of two elements. One part of this transverse relaxation will be associated with spin-lattice relaxation, but a second part involves the loss of the M_{xy} component directly. This latter occurs through randomization of the spin orientation with respect to the external magnetic field. When this field is first applied to the spin system, the spins become oriented along its main axis. However, it is possible for an excited spin to pass its additional energy on to another spin which has the same Larmor frequency. This second spin may not have the same orientation as the first, hence the vector of magnetization will be changed after the spin-spin interaction. No energy is lost from the

system during this process. If, however, the vector of magnetization of the excited spin population was originally inclined to the main axis of the applied magnetic field it would have been able to induce an EMF in a detector coil. The signal from such a system is a sine-wave, the initial height of which is related to M_{xy} . As the spin orientation is randomized by spin-spin interaction and T_1 relaxation processes also reduce the energy level of the system, so the amount of the induced EMF decreases. This signal is known as the Free Induction Signal (FIS), the time constant of its decay to zero (an exponential process) is known as T_2 , and the rate of decay as R_2 . This decay of the FIS is often referred to as Free Induction Decay or FID. It should be emphasised that, although the terms T_1 , spin-lattice relaxation and longitudinal relaxation all apply to the same thing, T_2 is sometimes used for spin-spin relaxation but at other times for transverse relaxation. This can be deceptive since transverse relaxation has contributions from both spin-spin and spin-lattice relaxation in it.

The coupling between the spin system and its environment is related to the nature of the environment. If we are dealing with a solid we usually find that there is a very close coupling, hence T_1 times are relatively short. If, however, we are dealing with a liquid sample the coupling is usually less strong so T_1 is longer. The T_1 value can affect the size of the spectral line, especially in cases where it is either very short or very long. In the former case it causes a broadening of the spectral line, known as relaxation broadening, which in extreme cases may cause the line to be broadened out until it is lost completely. The underlying cause of relaxation broadening is the uncertainty of the energy level of the excited state if the time spent in this state is extremely short — an application of Heisenberg's Uncertainty Principle. ΔE has a range of possible values, the size of this range being inversely related to the life-time of the excited state (τ). When τ is extremely short ΔE has a wide range and hence the resonance condition can be fulfilled over a wider part of the spectrum. The number of spins involved is just the same so the result is a broader, lower spectral line which may be more difficult to resolve. One can find typical examples of relaxation broadening in ESR studies of paramagnetic metal ions of the transition-metal series, e.g. copper or iron complexes. At room temperature the coupling is so tight that the spectrum is highly broadened. However, if the temperature is reduced

T_1 is increased and hence relaxation broadening is less important. For this reason most ESR studies of metal ion complexes are made at liquid nitrogen or liquid helium temperatures.

If the relaxation time of the system is too long the spins are excited more rapidly than they can relax back to the ground state. This will also occur if a very high power of incident radiation is used even with a system which has a moderately fast T_1 . In such a case, as we noted previously, the system becomes power saturated and there is loss of signal size. Power saturation is often met in low temperature ESR studies in which transition metal ions are not involved. If the electron is fairly loosely coupled to its environment, at low temperature it easily becomes saturated. Working at higher temperatures or with a reduced incident power level can reduce the effects of power saturation.

T_2 also affects the spectral line, in that the ability of the system to distribute its spin orientations among surrounding spins can affect the line width of the absorption line. This re-distribution of spin is known as a dipole interaction and, as we have seen, results in loss of total orientation of the spin population. Hence where T_2 is very short, and dipole interaction very common in a sample, the absorption line is broadened. Indeed it is T_2 rather than T_1 which is the main determining factor of line width for most systems. This is one of the reasons that NMR studies are more difficult on solids than in liquids, and also why ESR studies are normally performed using relatively small concentrations of the magnetically active substance, so reducing the opportunity for dipole interactions.

T_1 and T_2 are rarely measured as separate entities in biological ESR studies. Relaxation processes for most biologically important electron spin systems tend to be very fast, extremely so when paramagnetic metal ions are involved. However, in recent years there has been a great deal of interest in the measurement of NMR T_1 and T_2 times in biological materials. In NMR the rates of the relaxation processes are much slower, T_1 and T_2 on most machines being measured in milliseconds, or even up to seconds for dilute solutions or pure water. A considerable body of work has been produced by researchers looking at NMR T_1 and T_2 values of water protons in tissues, organs or whole living bodies. As will be discussed in a later chapter, the tissue water exists in a semi-structured state. Because of this the available time for