Paramagnetic Contrast Agents in Nuclear Magnetic Resonance Medical Imaging

M. Helena Mendonça-Dias,* Elena Gaggelli,† and Paul C. Lauterbur

Relaxation time differences are the sources of most of the contrast observed in proton NMR images, not only among normal organs and tissues but between lesions and the adjacent tissue. Although these differences are often large, there are low-contrast situations in which it would be desirable to increase the visibility of an organ or region. The study of time-dependent phenomena would also be aided by the ability to change selected relaxation times deliberately. One way to achieve these goals is to administer substances that change proton relaxation times in tissues without causing significant toxic effects or other physiologic changes. Paramagnetic ions and molecules, those with unpaired electrons, may be useful for this purpose because the very large magnetic effects associated with such electrons can drastically decrease water proton relaxation times at concentrations of the order of 100 to 1000 μM, which may be reached in certain organs after doses of 10 to 100 μM/kg. The general characteristics of such paramagnetic substances are described, and specific animal experiments with manganous ion and its complexes, and with stable nitroxide free radicals and molecular oxygen, are reviewed. The paramagnetic contrast agents already studied are effective, and many more are potentially possible, but the most important questions to be answered are whether acute and chronic toxicity are low enough to permit research and diagnosis on humans.

SINCE THE DISCOVERY of nuclear magnetic resonance (NMR) in liquids and solids,[1,2] paramagnetic species have been used to alter the relaxation times T_1 (spin-lattice) and T_2 (spin-spin) of nuclei. The first such experiment was carried out by Bloch and coworkers,[3] who used $Fe(NO_3)_3$ in aqueous solution to decrease the relaxation times of water protons. The addition of paramagnetic ions to pure water results in an increase of the relaxation rates, R_1 and R_2 (where $R_{1,2} = 1/T_{1,2}$), which is directly proportional to the concentration of the paramagnetic species. If macromolecules are also present, as in biologic tissues, there will be an additional change in the water proton relaxation rates. This effect depends on the interactions of water molecules with the macromolecules in ways that are still not well understood. It is often approximately true that the changes in the relaxation rates produced by several substances in a solution or heterogeneous system are simply additive. For example, the relaxation rate change caused by a mixture of paramagnetic ions in solution will be the sum of the rate changes expected for each individual species. If there is an interaction between two species, such as binding of a paramagnetic ion to a macromolecule, an enhancement of the relaxation effect of the ion may be observed.

The first paramagnetic relaxation enhancement (PRE) experiment was described in 1961 and 1962 by Shulman and coworkers,[4,5] who studied the coordination of paramagnetic ions to DNA. In 1962 Cohn and Leigh[6] studied the binding of Mn^{2+} to creatine kinase and enolase, and this study was followed by studies of the binding of the same metal ion to other proteins.[7-10]

In 1971 Reuben[11,12] proposed the use of lanthanide ions as PRE probes for the study of bovine serum albumin; the same class of metal ions was used by Dwek and coworkers,[13] in order to study the three-dimensional conformation of lysozyme in aqueous solution. In this case, paramagnetic ions were used as broadening probes, because their effect on the relaxation rates can be directly correlated with distances from the coordination site (or sites) to the individual protons in a molecule. In 1972 Dwek et al[14] published a detailed review of the PRE technique, which was followed in 1973 by a book on the same subject.[15]

*Instituto Superior Tecnico, Universidade Tecnica de Lisboa

†Istituto di Chimica Generale dell' Università di Siena, Siena, Italy

From the Departments of Chemistry and Radiology, State University of New York at Stony Brook, Stony Brook, NY.

Supported by Grant No. CA15300, awarded by the National Cancer Institute, DHHS, and by Grant No. HL19851, awarded by the National Heart, Lung and Blood Institute, DHHS.

Address reprint requests to M. Helena Mendonca-Dias, Ph.D., D.Sc., Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794.

0001-2998/83/1304-0008$02.00/0

PARAMAGNETIC METAL IONS AS CONTRAST AGENTS IN NMR IMAGING

Theoretical Basis

The study of nuclear relaxation effects caused by paramagnetic probes and free radicals (spin labels) can provide structural and kinetic information by altering selectively the natural differences in proton relaxation times, just as density contrast media are used in x-ray imaging, or radioactive tracers are injected to obtain emission images.

When dealing with the effects produced by unpaired electrons on the nuclear relaxation parameters, we have to take into account the dipolar magnetic field experienced by the nucleus, as well as the strength of the local magnetic fields that result from hyperfine interactions. Since the electronic magnetic moment is relatively large as compared with the nuclear magnetic moment, such local fields may be as high as 10^4 gauss (G). Moreover, the electronic spin is usually characterized by very short relaxation times (typically 10^{-8}-10^{-7} sec for spin labels, and even shorter for some metal ions) that cause rapid fluctuations of the local fields, inducing transitions between the nuclear spin states. Tumbling motions of the whole molecule or internal motions also modulate the dipolar local magnetic fields, whereas the field arising from hyperfine interactions does not change with molecular motions.

In solutions, spin exchange and diffusion processes may carry information from the paramagnetic center to the bulk solution. For example, in the case of two environments, free and bound, the relaxation rates in each one are significantly modified by chemical exchange processes, and the observed rates are given by[15-17]

$$T_{1\,ob.}^{-1} = T_{1f}^{-1} + p_m q\,(T_{1m} + \tau_m)^{-1} \qquad (1)$$

$$T_{2ob.}^{-1} = T_{2f}^{-1} + p_m q \tau_m^{-1}[T_{2m}^{-1}(T_{2m}^{-1} + \tau_m^{-1}) + \Delta\omega_m{}^2]\,[(T_{2m}^{-1} + \tau_m^{-1})^2 + \Delta\omega_m^2], \qquad (2)$$

where $1/T_{1f}$ and $1/T_{2f}$ are the nuclear relaxation rates in the free environment, $1/T_{1m}$ and $1/T_{2m}$ are the nuclear relaxation rates in the bound environment, $1/\tau_m$ is the rate of chemical exchange, $\Delta\omega_m$ is the difference in chemical shifts, and p_m and q are, respectively, the mole fraction and the number of ligand nuclei bound to the paramagnetic center. An experimental paramagnetic contribution can be defined, which is directly related to the relaxation parameters in the bound environment:

$$T_{1p}^{-1} = T_{1ob.}^{-1} - T_{1f}^{-1} = p_m q\, T_{1m}^{-1} \qquad (3)$$

$$T_{2p}^{-1} = T_{2ob.}^{-1} - T_{2f}^{-1} = p_m q\, T_{2m}^{-1}. \qquad (4)$$

Equations 3 and 4 explain why a very low concentration of paramagnetic centers is sufficient to obtain significant paramagnetic contributions to the relaxation rate. For example, for pure water containing Gd^{3+} ions, $T_{1f} = 265$ sec, $T_{1m} = 10^{-5}$ sec, and a concentration of Gd^{3+} of the order of 0.10 mM is sufficient to shorten the T_1 by more than one order of magnitude.

The full equations for $1/T_{1p}$ and $1/T_{2p}$ are complicated and contain several parameters, and usually it is very difficult to determine the various contributions from the experimental parameters.[18-20]

For such reasons we only outline schematic expressions that can be useful when dealing with paramagnetic relaxation phenomena. The effectiveness of certain paramagnetic species acting as relaxation centers depends upon the following variables:

1. the effective magnetic moment
2. the scalar coupling constant
3. the actual correlation time (eg, ions with very short electron spin relaxation times, such as Co^{2+}, are very poor relaxation probes)
4. the temperature (which influences the chemical exchange region)
5. the value of the external magnetic field

It is obvious that the probes that are most effective relaxation agents in vitro are expected to be good contrast agents in vivo, at low concentrations, if they are selectively concentrated in particular organs or tissues, or in normal or pathologic regions in the same organ or tissue.

General Considerations About the Use of Paramagnetic Metal Ions as Contrast Agents for NMR Medical Imaging

It has been recognized[21] that pathologic conditions such as edema and infarction will manifest themselves, as far as the NMR phenomenon is

concerned, by either different water concentration or changes in the relaxation times (both T_1 and T_2) between involved and uninvolved regions. Such differences are in many situations small, sometimes of the order of 10% of the R_1 or R_2 values, which may not be enough to provide good contrast for NMR imaging.[22]

Because it is known that metal ions and their complexes often concentrate selectively in abnormal tissues, and that paramagnetic ions increase the relaxation rates of water protons at low concentrations, several series of experiments were carried out in which rats and dogs were injected with dilute solutions of manganous chloride in physiologic saline solution and the relaxation times and manganese concentrations were measured in vitro in tissue samples. Table 1 shows the average effect of 0.1 mmol/kg of body weight of Mn^{2+} injections on the water proton spin-lattice relaxation rates (at 4 MHz) in dog tissues, sampled 30 min after injection.

The possibility that naturally occurring paramagnetic ions might contribute significantly to the observed relaxation rates in vivo has been raised but never proven.[23] The potential for diagnostic applications of NMR relaxation times has been enhanced by the development of zeugmatographic imaging techniques, which make possible the measurement of relaxation effects in intact macroscopic living organisms.[24-27] The pronounced relaxation effects of very small concentrations of paramagnetic ions on water relaxation in biologic systems[5] have led to the suggestion that the deliberate introduction of such ions into living systems might give rise to interesting and useful changes in relaxation times.[28,29]

Table 1. Average Effects of 0.1 mmol/kg of Mn^{2+} Injections on 4 MHz Water Proton Spin-lattice Rates in Dog Tissues Sampled 30 min After Injection

Tissue	R_1 (s^{-1}) Control	R_1 (s^{-1}) Mn^{2+} Injected
Myocardium (normal)	3.2	16.3
Myocardium (infarcted)	2.9	6.0
Lung	2.7	8.0
Liver	5.8	36.2
Spleen	3.4	7.2
Abdominal muscle	3.7	5.1
Large intestine	3.1	12.7
Small intestine		14.4
Kidney (medulla)	2.0	8.5
Kidney (cortex)	3.8	16.4

As a prelude to the use of contrast agents in NMR imaging, we have studied the effects of injections of manganous salt solutions ($MnCl_2$, Mn-Citrate, Mn-EDTA) on the water proton spin-lattice (T_1) relaxation times in organs of rats and dogs. Manganous ion was chosen for the initial studies because it is both a very effective relaxation reagent[5,14,15] and only moderately toxic. Also, the long-term toxicity has been widely studied and the distribution of injected manganous ion in mammalian tissues and its elimination have been investigated.[30-32] It was also shown that there is a significant enhancement factor for the relaxation of water protons by manganous ion in rat plasma, that manganous ion concentrates in vitro in samples of myocardium, and that the distribution of stable manganese in organs of rats and dogs at dosages in the range of 0.01 to 1.0 mmol/kg of body weight resembles that of much smaller dosages of radioactive manganese isotopes,[29-32] as shown in Figure 1.

Finally, the analysis of Mn^{2+} extracted from the tissues can be easily and precisely done by atomic absorption spectroscopy,[33] allowing correlations between R_1 values and Mn^{2+} concentrations to be established, as shown in Fig. 2. It is interesting to note that although the final distri-

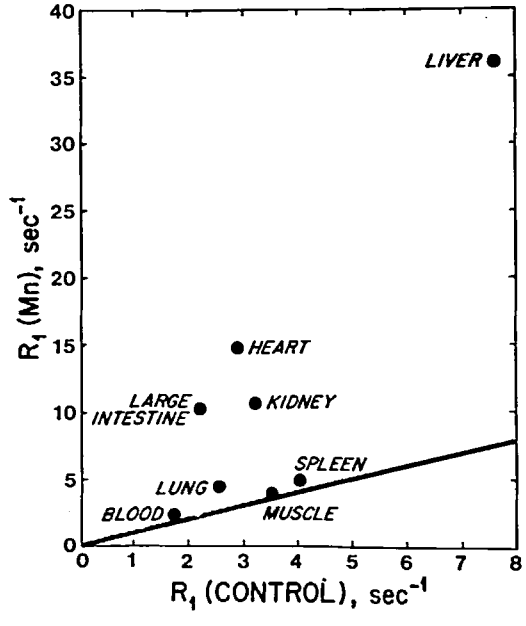

Fig. 1. Comparison of the relaxation rates in rat tissues 1 hr after injection of 0.1 mmol/kg of body weight of $MnCl_2$ with controls.

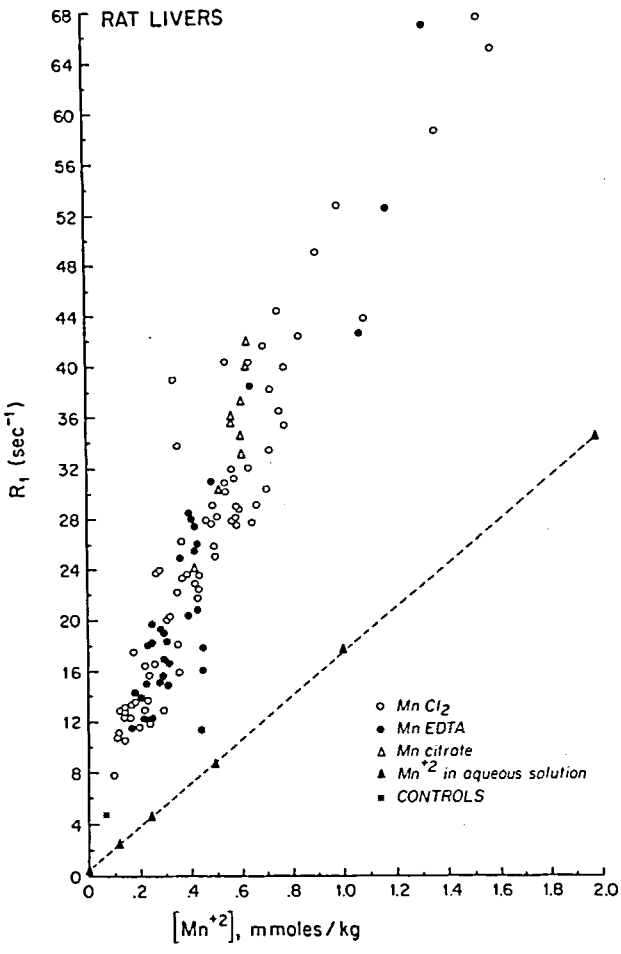

Fig. 2. The relationship between the relaxation rate (T_1) of the water proton NMR signal in rat liver, at 4 MHz, and the total manganese concentration. The dashed line shows the same relationship for manganous ion in pure water.

bution may depend on whether manganese is injected as a free ion or in the form of a complex (such as Mn-citrate or Mn-EDTA), the paramagnetic relaxation enhancement observed for the different organs studied appears to be the same.

In addition to Mn^{2+}, other paramagnetic metal ions such as Gd^{3+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, V^{2+}, and Cr^{3+} could be used. In Table 2 approximate values for the 4-MHz water proton relaxation times of 1.0-m*M* solutions of these paramagnetic ions are given as interpolated from data presented by Morgan and Nolle[34] for the dependence of T_1 and T_2 on the strength of the magnetic field.

Table 2 suggests that Gd^{3+} should be the best in vivo relaxation agent, not only because it is the most effective T_1 relaxation agent in vitro but also because T_2 is equal to T_1, minimizing the spectral broadening proportional to T_2^{-1}, which would reduce image resolution.

Cr^{3+}, Fe^{3+}, Cu^{2+}, and Ni^{2+} are also potentially useful as contrast agents, but the acute toxicities of copper and nickel and even of iron are higher than that of manganese. Also, iron is present in the body at very high levels, making it difficult to relate relaxation effects to the distribution of injected iron. Further efforts need to be directed to the search for the most suitable contrast agents, with low enough toxicity to make possible their eventual use in medical diagnosis.

Table 2. Water Proton Relaxation Times in 1 m*M* Aqueous Solutions of Paramagnetic Ions at 4 MHz[34]

	T_1 (s)	T_2 (s)
Ni^{++}	1.3	1.3
Cu^{++}	0.5	0.5
V^{++}	0.2	0.07
Cr^{+++}	0.1	0.1
Fe^{+++}	0.1	0.05
Mn^{++}	0.06	0.02
Gd^{+++}	0.04	0.04

In addition to metal ions, other paramagnetic substances such as nitroxide stable-free radicals and molecular oxygen may be used, as will be discussed later.

Use of Manganese for the Study of Myocardial Infarction

In 1976 Frank and coworkers[22] showed that 4 hr after ligation of the canine anterior descending coronary artery, a region of increased water proton spin-lattice (T_1) relaxation times and of increased water content developed, distal to the ligation, with small effects on the T_1 values (increases of the order of 10 to 20%) between the involved and uninvolved regions of the myocardium. They also found greater differences between animals than between normal and infarcted myocardium in each animal. Therefore, it seemed desirable to investigate the possibility of differentially altering such effects by the administration of paramagnetic reagents. For the reasons already mentioned, our choice has been manganous ion.[35] A series of five dogs were initially studied, four with manganese injections and one control, with qualitatively consistent results.

The dosage of manganese injected 1 hr after ligation of the descending coronary artery was 0.1 mmol/kg of body weight, and the hearts were excised 30 min after the injection, divided into a number of tissue blocks, and the T_1 values measured at 4 MHz, using a 180°-τ-90° pulse sequence. Least squares semiexponential fits were used to obtain single relaxation times. The water contents were measured, and the Mn^{2+} concentrations in the tissues were analyzed after extraction of the metal ion with dilute nitric acid.[33]

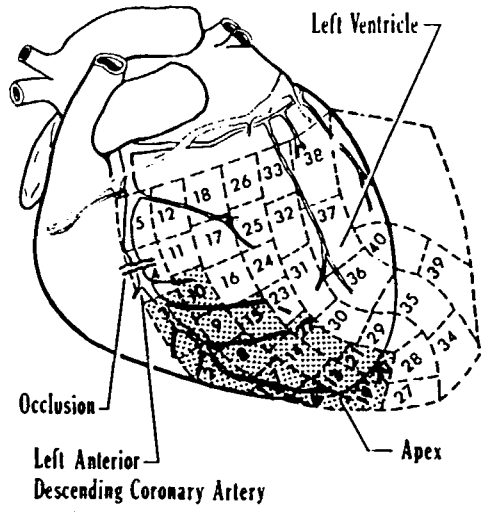

Fig. 3. Diagram of an infarcted dog heart (dog 004 in Table 3), showing the region of infarct (in which R_1 is less than 6 sec^{-1}) stippled.

In Fig. 3 we present the results obtained with one animal; the ischemic region corresponds to the shaded area. A summary of the results obtained with the five animals is given in Table 3 (dog 005 is the control). Figure 4 shows the similar results obtained by Chauncey and coworkers,[36] using Mn-54.

In a related experiment, the entire heart was imaged immediately after being excised. After a 60-min ischemic period a normal saline solution containing manganous ion at a dosage of 0.1 mmol/kg of body weight was injected through a cannula placed in the left lower pulmonary vein. Thirty minutes later the animal was heparinized

Table 3. Paramagnetic Relaxation Effects in Canine Myocardium After Ligation of a Coronary Artery

Dog No.	Mn^{2+} mmol/kg Body Weight	Tissue Classification*	Mean T (msec)	Mean R_1 (sec^{-1})	Mean ΔR_1	$(\Delta R_1)_N/(\Delta R_1)_i$
0005	0.0	Normal	315	3.18	(0)	—
		Ischemic	349	2.86	(0)	—
0004	0.1	Normal	130	7.69	4.51	5.7
		Ischemic	274	3.65	0.79	
0003	0.1	Normal	55	18.5	15.3	4.1
		Ischemic	152	6.58	3.72	
0002	0.01	Normal	272	3.67	0.49	8.2
		Ischemic	343	2.92	0.06	
0001	0.1	Normal	44	22.8	19.6	4.0
		Ischemic	128	7.81	4.95	

*The animal was sacrificed 30 min after injection of the solution and 90 min after ligation.

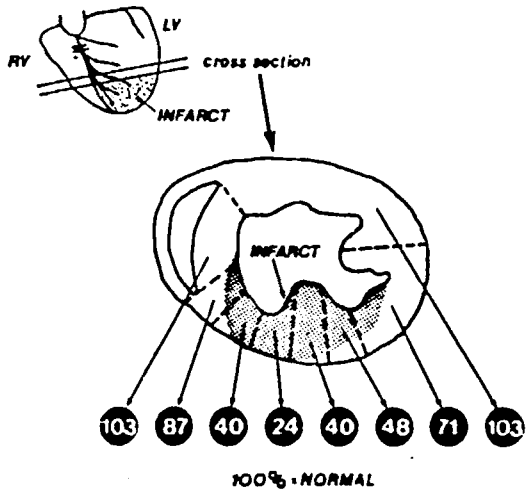

Fig. 4. Cross section of dog heart showing regional concentrations of Mn-54 in normal and infarcted myocardium, 4 hr after administration of Mn-54. The shaded area indicates the region of infarct. Concentration of Mn-54 in normal myocardium is normalized to 100%. Concentration of Mn-54 in center of infarct is only 24% of normal.[36]

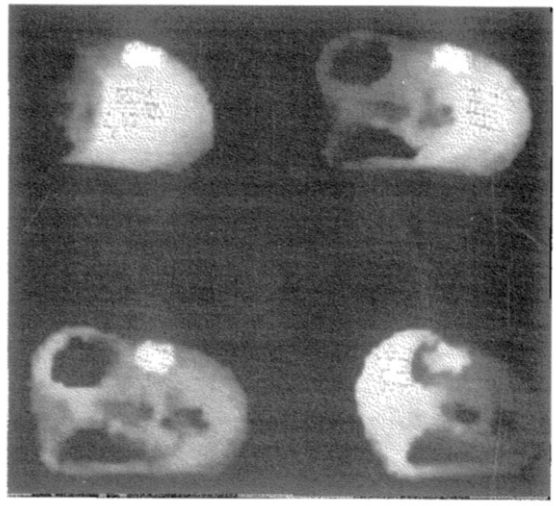

Fig. 5. Four frames from a movie displaying a rotating transparent myocardial surface with an opaque infarct.[37]

and all the vessels to and from the heart ligated in order to excise the heart while both of the ventricles and atria were filled with blood. The image was obtained at 4 MHz, using the saturation-recovery method with 90° pulses. The number of independent projections was 961, in a linear magnetic field gradient corresponding to a resonance frequency variation of 200 Hz/cm. The image was reconstructed by a two-stage filtered back-projection algorithm onto a 65 × 65 × 65 array. In Figure 5 the image is shown, using a special display technique in which an operator-interactive program[37] was employed to identify a region of relatively low signal intensity containing a lower concentration of manganese. Four frames from a movie are shown, with the surface of the myocardium displayed as if it were transparent and the presumed ischemic region of the left ventricle shown as opaque.

This experiment was not actually analogous to the in vivo situation. From in vivo studies it is known that although manganese is cleared from the blood very quickly, becoming concentrated in organs such as the heart, liver, and kidney, the distribution is dynamic and homeostatically controlled. After death such active mechanisms are suppressed, and there will be a tendency for the metal ion to diffuse from the tissues to the blood, until a static equilibrium is reached. This change in distribution of manganese can completely alter the appearance of the image.

Similar experiments were carried out by Goldman and coworkers,[38] Pohost and coworkers,[39] and Brady and coworkers.[40] In such experiments the manganese dosage was 0.05 mmol/kg of body weight, and the contrast observed in the NMR images (performed on the excised hearts

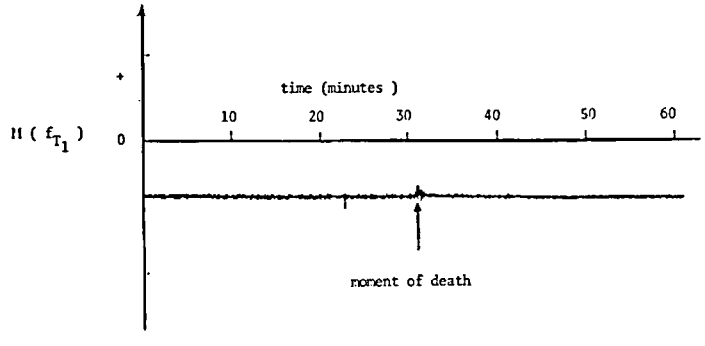

Fig. 6. Behavior of the steady-state magnetization after the 90° pulse, in a repeated (180°-50 msec-90°-1000 msec) sequence in the exposed liver of a rat.

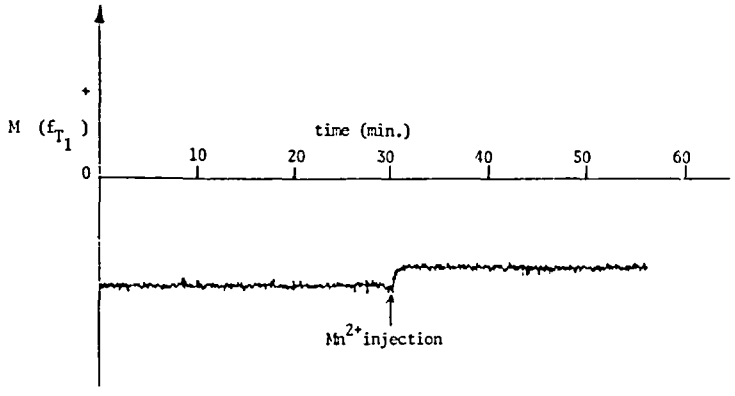

Fig. 7. Behavior of the steady-state magnetization after the 90° pulse in a repeated (180°-50 msec-90°-1000 msec) sequence in the exposed liver of a rat following an i.v. injection of 0.05 mmol/kg of body weight of $MnCl_2$.

empty of blood) was very good, with excellent concordance between the size of the infarct as determined by NMR and by conventional methods. From related experiments, Alfidi and coworkers[41] have concluded that the use of contrast agents such as Mn^{2+} "has proved fruitless when used in concentrations which might be useful to humans." In their work, however, the concentration of Mn^{2+} used was 9×10^{-5} mmol/kg of body weight, much smaller even than the concentration of naturally occurring manganese in animal hearts, and even too low to cause significant changes in simple aqueous solution (eg, pure water has a T_1 of 2500 msec at 4 MHz, and at the same frequency a 0.004 mM solution of Mn^{2+} has a T_1 of 2170 msec).

Gore and coworkers[42] have also used manganese to discriminate between soft tissues of rabbit in vivo and have shown subtraction images of the liver and posterior parts of the lungs, 10 and 40 min, respectively, after injection of 0.018 mmol/kg of body weight of Mn^{2+}, showing very clear discrimination between the gallbladder and the surrounding liver.

One important result we observed in our studies is that the route of excretion of Mn^{2+} may be altered if MnEDTA rather than $MnCl_2$ is injected. The chelated form of manganous ion is excreted through the kidneys rather than through the liver, giving a high concentration of manganese in the urine.

Similar observations have been made by Cotzias et al.[30]

IN VIVO REAL-TIME-STUDIES WITH LOCAL COILS

One important question concerning studies of Mn^{2+} as a paramagnetic relaxation agent in

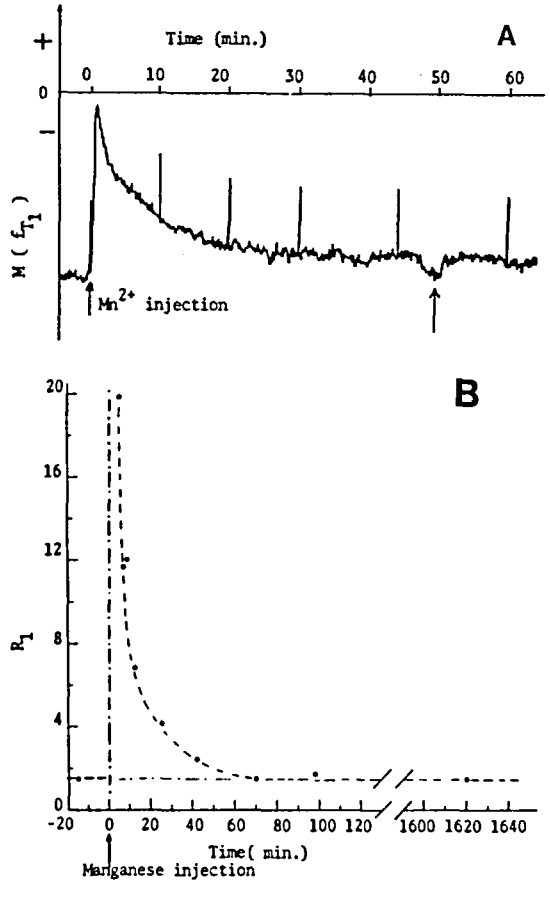

Fig. 8. (A) Behavior of the steady-state magnetization after the 90° pulse in a repeated (180°-50 msec to 90°-1000 msec) sequence in dog blood following an i.v. injection of 0.05 mmol/kg of body weight of $MnCl_2$. The baseline fluctuation marked by the arrow at 50 min was caused by a slightly magnetic hypodermic needle. (B) Behavior of the relaxation rate R_1 in dog blood following an i.v. injection of 0.05 mmol/kg of body weight of $MnCl_2$, monitored in a classical way, taking blood samples and measuring the T_1 in vitro in test tubes.

tissues is whether the effects are the same in living tissues and in excised specimens. Studies done in vivo and postmortem on rat exposed livers, using as receiver a local surface coil, and a 180°-τ-90° pulse sequence from a Helmholtz transmitter coil, monitoring the magnetization with a value of τ of the order of 50 msec, demonstrated that the T_1 does not change with death or for several hours afterward (similar observations had already been made by monitoring the in vitro T_1 of rat hearts over periods of 24 hr). Figure 6 shows the magnetization for 30 min

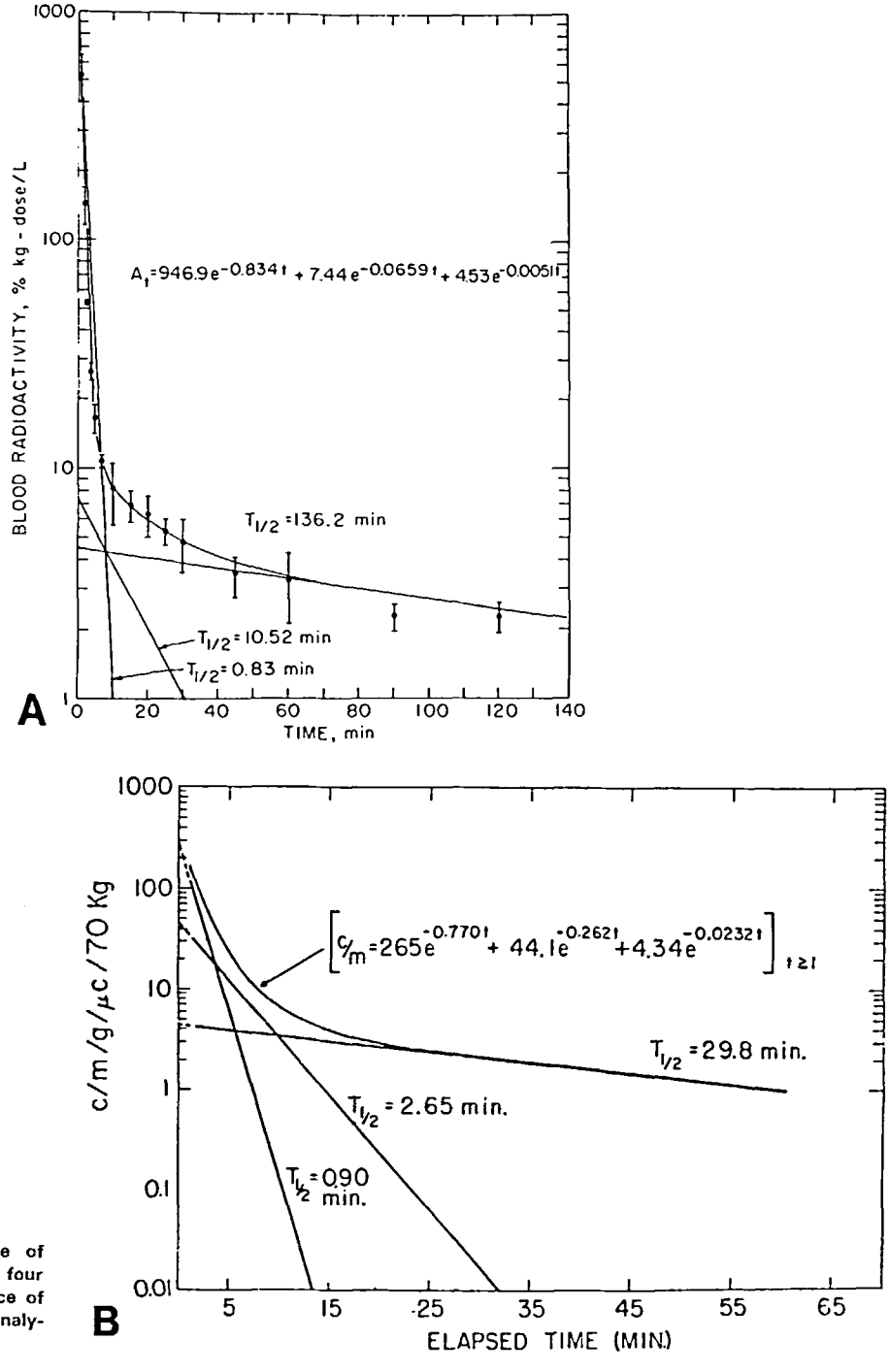

Fig. 9. (A) Clearance of Mn-54 from the blood in four dogs.[43] (B) Blood clearance of Mn-56 in humans and its analysis into components.[30]

before and after death. Using the same technique, the T_1 relaxation in the liver of a living rat before and after an i.v. injection of 0.05 mmol/kg of $MnCl_2$ was monitored, as shown in Figure 7.

The rate of uptake and washout of Mn^{2+} from the blood of dogs, following an injection of 0.05 mmol/kg of body weight, using a similar technique to monitor the magnetization and a solenoidal local coil as receiver, is presented in Figure 8A. In Figure 8B are shown previous results obtained by taking blood samples and measuring the T_1 in vitro in test tubes.

Our results on Mn^{2+} clearance from the blood parallel those of Atkins and coworkers[43] who used Mn-54 (Fig. 9A), Bertinchamps and coworkers[32] who used Mn-54, and Cotzias and coworkers[30] who used Mn-56 (Fig. 9B). In the earlier work, it was found that the disappearance of radioactive manganese from the blood was extremely rapid and could be analyzed in terms of three exponential components.

Atkins[43] concluded from his studies that the rapid clearance of Mn-54 from the blood and the high myocardial concentration of manganese are compatible with it being a good agent for myocardial imaging. His data show also a good correlation between the distribution of microspheres and manganese concentration (Fig. 10) in normal and infarcted myocardium, which support the idea that the different concentrations of manganese in normal and damaged tissue may be due, in great part, to different perfusion.

Experiments on manganese partition and clearance carried out with very small concentrations of radioactive Mn isotopes may give rather different results from those carried out with the higher concentrations of stable manganese used as a relaxation agent, since both turnover rates

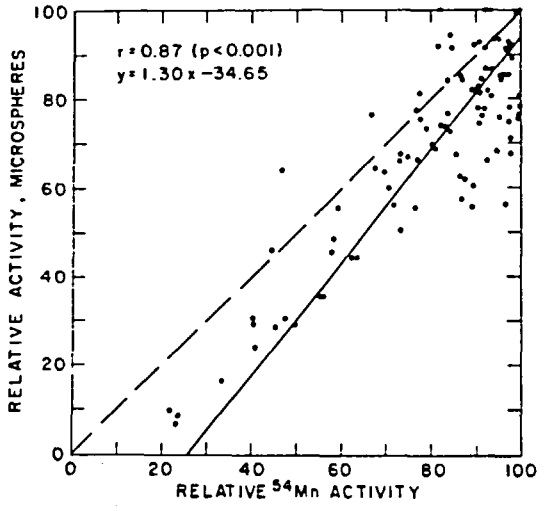

Fig. 10. Relationship of ^{85}Sr-labeled microsphere concentration and ^{54}Mn concentration in samples of myocardium of a dog in whom the left anterior descending coronary artery had been tied.[43]

and distributions have been found to be functions of the total manganese concentration, as shown in Figure 11.[30]

One important point concerning the studies presented up to now is related to the fact that the NMR relaxation processes in tissues must be understood, in order to interpret fully the results obtained by NMR medical imaging. In fact, in dealing with biologic samples, one can expect more than a single relaxation rate, due mainly to tissue heterogeneities. The study of multiple components in tissues, either in vitro or in vivo, is a difficult problem that requires careful computer analysis; the data can best be analyzed by the use of a special computer program[44] for decomposition of multiple exponential data, including statistical analysis of the significance

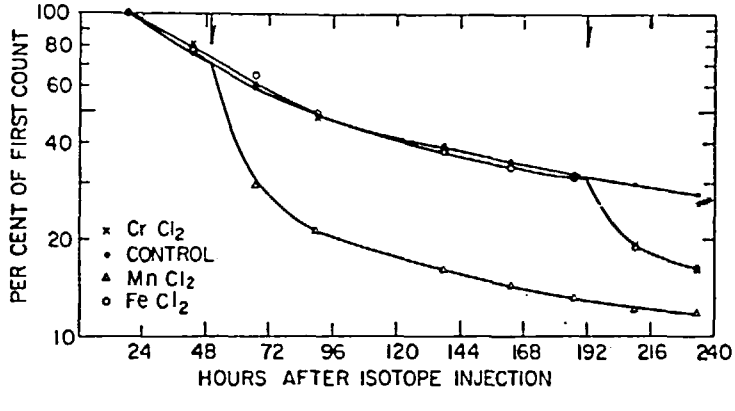

Fig. 11. Effect of injected Cr^{2+}, Mn^{2+}, and Fe^{2+} (first arrow) on the turnover of Mn-54 in mice. The second arrow shows the time of injection of stable $MnCl_2$ into animals previously injected with $FeCl_2$ and $CrCl_2$.[30]

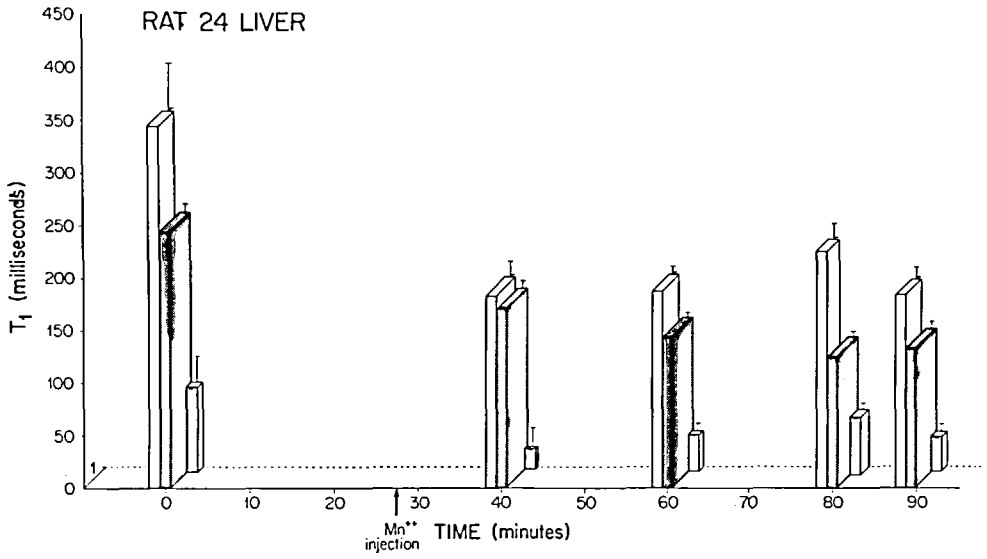

Fig. 12. A comparison of a single-component and two-component analysis of spin-lattice relaxation measured at 4 MHz in the exposed liver of a living rat, using a surface receiver coil. The Mn^{2+} was injected as a 60-m*M* solution of $MnCl_2$ in normal saline, to give a dose of 0.1 mmol/kg of body weight.

of the rates and amplitudes.[45] Figure 12 shows the results of applying such an analysis to in vivo T_1 data measured on rat liver, using a local surface coil and a 180°-τ-90° pulse sequence before and after a $MnCl_2$ injection of 0.05 mmol/kg of body weight.

MANGANESE TOXICITY

The long-term toxic effect, known as chronic manganism, is well known, producing several neurologic and psychotic disorders, which in the late stages resemble Parkinson's disease.[46-55]

The acute toxicity is almost unknown. In Table 4 the few available data are summarized.

In several years of work in this laboratory with rats and dogs the observed acute effects have been primarily cardiac. Dosages of 0.1 mmol/kg of body weight in rats are almost always lethal, but injection of EDTA can block the effect and restore the normal ECG pattern.

For dogs, dosages of $MnCl_2$ at 0.05 mmol/kg of body weight have no serious effect on the heart, and MnEDTA has little or no effect at the same dosage. This suggests that the acute cardiac toxicity of manganese depends upon the concentration of free manganous ion in the blood. Careful studies still need to be done before manganese can be considered for contrast enhancement in human NMR medical imaging.

LANTHANIDE TOXICITY

Studies done by Mela and Chance with mitochondria have shown that lanthanide ions at concentrations of 0.07 to 0.1 mg/g of protein inhibit the accumulation of Ca^{2+} in rat liver mitochondria, as measured by murexide,[58-62] or as shown by the inhibition of other mitochondrial reactions induced by the energy-dependent accumulation of Ca^{2+}, such as pH changes in the membrane and the redox changes of the respiratory chain components.[58-61] On the basis of such studies with Ho^{3+}, La^{3+}, and Pr^{3+}, it seems likely

Table 4. Acute Toxicity of $MnCl_2 \cdot 4H_2O$

Animal	Route	Dose	Dosage Values		Time of Death	Ref.
			mg/kg	mmole/kg		
Mouse	s.c.	LD	180–250	0.91–1.26	—	55
Guinea pig	s.c.	LD	180	0.91	12 Hours	55
Rabbit	s.c.	LD	180	0.91	12 Hours	55
Rabbit	i.v.	MLD	64.8	0.33	—	55
Dog	i.v.	LD_{50}	201.6	1.0	—	56

that serious acute toxicity may be expected from Gd^{3+}

NITROXIDE STABLE FREE RADICALS AS CONTRAST AGENTS

Nitroxide stable-free radicals are synthetic organic molecules with an unpaired electron, and have been used for the last two decades as spin labels for in vitro biologic studies.[63] They have been proposed as contrast agents for NMR medical imaging by Brasch and coworkers,[64] who have suggested the general abbreviation NSFR.

Such compounds have been shown, in recent work,[65,66] to be particularly useful as contrast agents for demonstrating abnormalities of the blood brain barrier (BBB) and for urographic studies. One particular compound of the family, given the designation TES, was used because it is water soluble and rapidly excreted into the urine after intravenous injection. Also according to the same authors, the ability of TES to be covalently bonded to a variety of molecules, drugs, and particles may permit the synthesis of tissue-specific contrast agents. Some of these compounds have high chemical stability in aqueous solution over a broad range of pH (1.7 to 10.0) at temperatures of as high as 100°C.

TES was used as a contrast agent for NMR imaging of the brains of animals having experimentally induced bacterial cerebritis or radiation damage, because TES seems to cross the blood-brain barrier (BBB) only at sites of damage.[65] The authors report an increase of 45% in contrast, in studies of bacterial brain abscesses, for images made 20 min after an intravenous injection of TES, but note that the degree of contrast decreases 60 min after the administration of the contrast agent.

The T_1 values decrease at the center and rim of the abcess, but the T_2 values seem to remain practically unaffected. From postmortem analysis it is apparent that there are variations in the concentration of the contrast agent, which is lower in the necrotic center of the abscess than in the rim, and much smaller 1 cm distal to the rim.

In the case of experimentally induced radiation damage, the authors report a T_1 contrast enhancement of about 19%, but again no changes in the T_2 values. According to the same authors, the potentiality of TES for brain studies is great because of the lack of other contrast agents that are able to identify subtle breakdown in the BBB.[67]

TES was also proposed[66] as a urographic contrast agent and tested in animals with experimentally induced unilateral renal ischemia, renal vascular congestion, and hydronephrosis; TES is rapidly excreted in the urine with a clearance equivalent to glomerular filtration rate, at relatively low dosages (0.04 to 0.9 g/kg of body weight or 0.14 to 3.2 mmol/kg of body weight). The TES-enhanced spin-echo renal images demonstrate the presence of diseases or abnormalities in the kidney, which could not be seen in the nonenhanced images. The relaxation efficiency of this NSFR was stated to be comparable to that of Cu^{2+} or Fe^{3+} in similar concentrations. In the urographic studies, TES did not appear in the cerebral spinal fluid, suggesting again that it does not cross a normal BBB.

The toxicity and metabolic fates of such compounds are poorly known, and no final comparison with paramagnetic transition metal ions is possible at the present moment.

MOLECULAR OXYGEN AS A PARAMAGNETIC CONTRAST AGENT

The results presented with paramagnetic ions, and even with nitroxide stable free radicals, although promising, still pose problems for use in humans, because of the limited information on toxic effects.

It would be preferable to use a paramagnetic substance that is nontoxic and whose metabolism is well known. Many suggestions have been made for the use of molecular oxygen. It is present in all tissues, and at an arterial tension of 100 mm Hg there are ~ 3 ml of O_2 dissolved in each liter of blood,[42] corresponding to a concentration of 6×10^6 molecules of O_2/ml. It has been experimentally proven[68] that in water the relaxation rate change is proportional to the concentration of dissolved O_2. According to Gore and coworkers,[42] in tissues and at normal oxygen tensions, paramagnetic oxygen will contribute only 1 to 2% to the total relaxation rates; this contribution can possibly be increased if oxygen-enriched air is breathed for several minutes. In experiments with rabbits,[42] images of the thoraxic region, obtained before and after 15 min the animal began breathing pure oxygen, have been

obtained. In the presence of oxygen there was an apparent increase in contrast between heart muscle and blood, which disappeared when normal air was breathed again. In a similar experiment with a human volunteer, breathing 100% oxygen during scanning, Alfidi and coworkers reported an increased intensity (corresponding to a decrease in the T_1 values) of the intraventricular septum and of the blood in the left ventricle.[41]

ACKNOWLEDGMENTS

We wish to thank Ms. Ruth Heidelberger for invaluable assistance.

REFERENCES

1. Bloch F: Nuclear induction. Phys Rev 70:460, 1946

2. Purcell EU, Torrey HC, Pound RV: Resonance absorption by nuclear magnetic moments in a solid. Phys Rev 69:37, 1946

3. Bloch F, Hansen WW, and Packard M: The nuclear induction experiment. Physiol Rev 70:474, 1946

4. Eisinger J, Shulman RG, Blumberg WE: Relaxation enhancement by paramagnetic ion binding in deoxyribonucleic acid solutions. Nature 192:963, 1961

5. Eisinger J, Shulman RG, Szymanski BM: Transition metal binding in DNA solutions. J Chem Phys 36:1721, 1962

6. Cohn M, Leigh JS: Magnetic resonance investigations of ternary complexes of enzyme-metal-substrate. Nature 193:1037, 1962

7. Reuben J, Cohn M: Magnetic resonance studies of manganese (II) binding sites of pyruvate kinase-temperature effects and frequency dependency of proton relaxation rates in water. J Biol Chem 245:662, 1970

8. Navon G: Proton relaxation times and hydration numbers of manganese ions bound to enzymes. Chem Phys Lett 7:390, 1970

9. Fabry MH, Koenig SH, Schillinger WE: Nuclear magnetic relaxation dispersion in protein solutions. IV—Proton relaxation at the active site of carbonic anhydrase. J Biol Chem 245:4256, 1970

10. Danchin A, Gueron M: Proton magnetic relaxation study of manganese transfer-RNA complex. J Chem Phys 53:3599, 1979

11. Reuben J: Gadolinium (III) as a paramagnetic probe for proton relaxation studies of biological molecules. Binding to bovine serum albumin. Biochemistry 10:2834, 1971

12. Reuben J: Electron spin relaxation in aqueous solutions of gadolinium (III). Aquo, cacodylate, and bovine serum albumin complexes. J Phys Chem 75:3164, 1971

13. Dwek RA, Richards RE, Morallee KG, et al: The lanthanide cations as probes in biological systems. Proton relaxation studies for model systems and lysozyme. Eur J Biochem 21:204, 1971

14. Dwek RA: Proton relaxation enhancement probes. Applications and limitations to systems containing macromolecules. Adv Mol Relax Proc 4:1, 1972

15. Dwek RA: Nuclear Magnetic Resonance in Biochemistry; Applications to Enzyme Systems. Oxford, Clarendon Press, 1973

16. Farrar TC, Becker ED: Pulse and Fourier Transform NMR: Introduction to Theory and Methods. New York, Academic Press, 1971

17. Swiff TJ, Connick RE: NMR relaxation mechanisms of O^{17} in aqueous solutions of paramagnetic cations and the lifetime of water molecules in the first coordination sphere. J Chem Phys 37:307, 1962

18. Burton DR, Forsen S, Karlstrom G, et al: Proton relaxation enhancement (PRE) in biochemistry: A critical survey. In Progress in NMR Spectroscopy, vol. 13. New York, Pergamon Press, 1979, p 1

19. Niccolai N, Tiezzi E, Valensin G: Manganese (II) as a paramagnetic relaxation probe in the study of biomechanisms and biomolecules. Chem Rev 82:384, 1982

20. Burton R, Forsen S, Karlstrom, G, et al: The determination of molecular motion parameters from proton relaxation-enhancements in a number of Gd (III) antibody-fragment complexes: A comparative study. Eur J Biochem 75:445, 1977

21. Lauterbur PC, Frank JA, Jacobson MJ: Water proton spin-lattice relaxation times in normal and edematous dogs lungs. Special Issue, Digest of the Fourth International Conference in Medical Physics. 33:9, 1976 (abstract)

22. Frank JA, House WV, Lauterbur PC, et al: Measurements of proton nuclear magnetic resonance longitudinal relaxation times and water content in infarcted canine myocardium and induced pulmonary injury. Clin Res 24:217A, 1976

23. Block RE, Maxwell GP, Branam DL: Nuclear spin relaxation in solutions prepared from normal and tumor hepatic tissues; brief communication. J Natl Cancer Inst 54:1731, 1977

24. Lauterbur PC: Image formation by induced local interactions: Examples employing nuclear magnetic resonance. Nature 242:190, 1973

25. Mansfield P, Maudsley AA: Medical imaging by NMR. Br J Radiol 50:188, 1977

26. Damadian R, Goldsmith M, Minkoff L: NMR in Cancer: XVI Fonar image of the live human body. Physiol Chem Phys 9:97, 1977

27. Hinshaw WS, Bottomley OA, Holland GM: Radiographic thin-section image of the human wrist by nuclear magnetic resonance. Nature 270:722, 1977

28. Hollis DP, Bulkley BH, Nunnally RL, et al: Effect of manganese ion in the phosphorus NMR spectra of perfused rabbit heart: A possible new membrane probe. Clin Res 26:240A, 1979

29. Lauterbur PC. Jacobson MJ, Rudin AM, et al: Augmentation of the water proton spin-lattice relaxation in tissues by *in vivo* injection of manganous ion. Abstracts of the 19th ENC, 1978, p B19

30. Cotzias GC, Borg DC, Bertinchamps AJ: Clinical experiences with manganese, in Seven MJ, Jonhson LA (eds): Metal Binding in Medicine. Philadelphia, Lippincott, 1960, p 50

31. Davies IJT; The Clinical Significance of the Essential Biological Metals. London, Heinerman, 1972, chap IV

32. Bertinchamps AJ, Miller ST, Cotzias GC: Interdependence of the routes excreting manganese. Am J Physiol 211:217, 1966

33. Hinners TA: Atomic absorbion analysis of liver without ashing. Z Anal Chem 277:377, 1975

34. Morgan LO, Nolle AW: Proton spin relaxation in aqueous solutions of paramagnetic ions. J Chem Phys 31:365, 1959

35. Lauterbur PC, Mendonca-Dias MH, Rudin AM: Augmentation of tissue water proton spin-lattice relaxation rates by the *in vivo* addition of paramagnetic ions, in Dutton PO, Leigh JS, Scarpa A (eds): Frontiers of Biological Energetics. New York, Academic Press, 1978

36. Chauncey DP Jr, Hagen PL: Tissue distribution studies with radioactive manganese: A potential for myocardial imaging. J Nucl Med 18:933, 1977

37. Herman GT, Udupa JK, Kramer DM, et al: Three-dimensional display of nuclear magnetic resonance images. Opt Eng 23:923, 1982

38. Goldman MR, Hinshaw WS, Pohost GM: Quantification of experimental infarction using NMR imaging and paramagnetic ion contrast enhancement in excised canine hearts. Circulation 66:1012, 1982

39. Pohost GM, Goldman MR, Pykett IL, et al: Gated NMR imaging in canine myocardial infarction. Circulation-Part II 66: Communication 155, 1015; October 1982

40. Brady TJ, Goldman MR, Hinshaw WS, et al: Nuclear magnetic resonance imaging for high resolution delineation of myocardial infarction using paramagnetic enhancement of proton signal. Radiology 144:343, 1982

41. Alfidi RJ, Haaga JR, Elyousef SJ, et al: Preliminary experimental results in humans and animals with superconducting whole-body NMR scanner. Radiology 143:175, 1982

42. Gore JC, Doyle FH, Dennock JM: Relaxation rate enhancement observed *in vivo* by NMR imaging, in Partain CL, James AE, Rollo FD, et al (eds): Nuclear Magnetic Resonance (NMR) Imaging. Philadelphia, WB Saunders Co, 1983

43. Atkins HL, Som P, ScM DVM, et al: Myocardial positron tomography with manganese-52m. Radiology 133:769, 1979

44. Provencher SW: A Fourier method for the analysis of exponential decay curves. Biophys J 16:27, 1976

45. Mun SK, Mendonça-Dias MH, Lauterbur PC, et al: Multicomponent water proton T_1 analysis in organs and tissues from animals injected with manganese. Abstracts of the First Annual Meeting of the SMRM, August 16-18, Boston, Mass, 1982, p 111

46. Cotzias GC: Manganese in health and disease. Physiol Rev 35:503, 1958

47. Sollmon TA: Manual of Pharmacology. Philadelphia, WB Saunders Co, 1949, p 936

48. Van Oettingen WF: Manganese: Its distribution, pharmacology and health hazards. Physiol Rev 15:175, 1935

49. DiPaolo JA: The potentiation of lymphosarcomas in the mouse by manganese chloride. Fed Proc 23:393, 1974

50. Resenstock HA, Simons DG, Meyer JS: Chronic manganism: Neurologic and laboratory studies during treatement with Levodopa. JAMA 6:1354, 1971

51. Chandra SVM, Srivastava SP: Experimental production of early brain lesions in rats by parenteral administration of manganese chloride. Acta Pharmacol Toxicol 28:177, 1970

52. Pentschew A, Ebner FD, Kovatch RM: Experimental manganese encephalopathy in monkeys. J Neuropathol Exp Neurol 23:488, 1963

53. Meiri U, Rahamimoff R: Neuromuscular transmition: Inhibition by manganese ions. Science 176:308, 1972

54. Venugopal B: Metal Toxicity in Mammals. New York, Plenum Press, 1978, chap 7

55. Schubert J: Heavy metals; toxicity and environmental pollution, in Dahar SK (ed): Metal Ions in Biological Systems. New York, Plenum Press, 1973, p 239

56. Langecker A: Heffter's Handbook Experimentellen Pharmacology 3.2:1346, 1923

57. Carvinka F: Pharmacologie et toxicologie du manganèse. C R Soc Biol 102:262, 1929

58. Mela L, Chance B: Spectrophotometric measurements of the kinetics of Ca^{2+} and Mn^{2+} accumulation in mitochondria. Biochemistry 7:4059, 1968

59. Mela L: La^{3+} as a specific inhibitor of divalent cation-induced mitochondrial electron transfer. Fed Proc 27:828, 1968

60. Mela L: Interactions of La^{3+} and local anesthetic drugs with mitochondrial Ca^{2+} and Mn^{2+} uptake. Arch Biochem Biophys 122:286, 1968

61. Chance B, Mela L: Hydrogen ion concentration changes in mitochondrial membranes. J Biol Chem 241:4588, 1966

62. Mela L, Chance B: Calcium carrier and the "high affinity calcium binding site" in mitochondria. Biochem Biophys Res Commun 35:556, 1969

63. Stone TJ, Buckman T, Nardio PL, et al: Spin-labeled biomolecules. Proc Natl Acad Sci 54:1010, 1965

64. Brasch RC, Nitecki DE, London D, et al: Evaluation of nitroxide stable free radicals for contrast enhancement in NMR imaging. Abstracts of the First Annual Meeting of the SMRM, August 16-18, Boston, Mass 1982, p 25

65. Brasch RC, Nitecki DE, Brant-Zawadzki M, et al: Brain NMR imaging enhanced by a paramagnetic nitroxide contrast agent. Preliminary report. AJNR (in press)

66. Brasch RC, London DA, Wesby GE, et al: Nuclear magnetic resonance urography enhanced by a paramagnetic nitroxide contrast agent. Preliminary report. Radiology (in press)

67. Bydder GM, Steiner RF, Young IR, et al: Clinical NMR imaging of the brain: 140 cases. AJR 139:215, 1982

68. Chiarotti G, Cristiani G, Guilotto L: Proton relaxation in pure liquids and in liquids containing paramagnetic gases in solution. Nuovo Cimento 1:863, 1955