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

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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.
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0001-2988/83/1304-0008$02.00/0

Seminars in Nuclear Medicine, Vol. XIII, No. 4 (October) 1983

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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

\[
\frac{1}{T_{1\text{ob}}} = \frac{1}{T_{1f}} + \frac{1}{T_{1m}} + \frac{1}{T_{1m}} \cdot \tau_{m}^{-1} \cdot p_{n} q (T_{1m} + \tau_{m}) \cdot^{-1} \quad (1)
\]

\[
\frac{1}{T_{2\text{ob}}} = \frac{1}{T_{2f}} + \frac{1}{T_{2m}} \cdot \tau_{m}^{-1} \cdot \left[ \frac{1}{T_{2m}} \cdot (T_{2m}^{-1} + \tau_{m}^{-1}) + \Delta \omega_m^2 \right] \cdot^{-1} \cdot \tau_{m}^{-1} \cdot \left[ (T_{2m}^{-1} + \Delta \omega_m^2) \right] \cdot (2)
\]

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 \(p_{n}\) 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:

\[
T_{1p}^{-1} = \frac{1}{T_{1\text{ob}}} - \frac{1}{T_{1f}} - \frac{1}{T_{1m}} \cdot \rho_n q \cdot T_{1m}^{-1} \quad (3)
\]

\[
T_{2p}^{-1} = \frac{1}{T_{2\text{ob}}} - \frac{1}{T_{2f}} - \frac{1}{T_{2m}} \cdot \rho_n q \cdot T_{2m}^{-1}. \quad (4)
\]

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.

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. The potential for diagnostic applications of NMR relaxation times has been enhanced by the development of zeugmagraphic imaging techniques, which make possible the measurement of relaxation effects in intact macroscopic living organisms. The pronounced relaxation effects of very small concentrations of paramagnetic ions on water relaxation in biologic systems 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.

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. Manganese ion was chosen for the initial studies because it is both a very effective relaxation reagent 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. 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, 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, 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-

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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

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Mn$^{2+}$ Injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardium (normal)</td>
<td>3.2</td>
<td>16.3</td>
</tr>
<tr>
<td>Myocardium (infarcted)</td>
<td>2.9</td>
<td>6.0</td>
</tr>
<tr>
<td>Lung</td>
<td>2.7</td>
<td>8.0</td>
</tr>
<tr>
<td>Liver</td>
<td>5.8</td>
<td>36.2</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.4</td>
<td>7.2</td>
</tr>
<tr>
<td>Abdominal muscle</td>
<td>3.7</td>
<td>5.1</td>
</tr>
<tr>
<td>Large intestine</td>
<td>3.1</td>
<td>12.7</td>
</tr>
<tr>
<td>Small intestine</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>Kidney (medulla)</td>
<td>2.0</td>
<td>8.5</td>
</tr>
<tr>
<td>Kidney (cortex)</td>
<td>3.8</td>
<td>16.4</td>
</tr>
</tbody>
</table>

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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.
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 manganese ion in pure water.

Distribution 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-mM solutions of these paramagnetic ions are given as interpolated from data presented by Morgan and Nolle\(^3\) 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>
<thead>
<tr>
<th>Ion</th>
<th>( T_1 ) (s)</th>
<th>( T_2 ) (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni(^{2+})</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Cu(^{2+})</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>V(^{2+})</td>
<td>0.2</td>
<td>0.07</td>
</tr>
<tr>
<td>Cr(^{3+})</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Fe(^{3+})</td>
<td>0.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Mn(^{2+})</td>
<td>0.06</td>
<td>0.02</td>
</tr>
<tr>
<td>Gd(^{3+})</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>
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\(^2\) 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.\(^3\) 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°-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.\(^23\)

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,\(^3\) 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>
<thead>
<tr>
<th>Dog No.</th>
<th>Mn$$^{2+}$ mmol/kg Body Weight</th>
<th>Tissue Classification*</th>
<th>Mean $T_1$ (msec)</th>
<th>Mean $R_1$ (sec$^{-1}$)</th>
<th>Mean $\Delta R_1$</th>
<th>$\left(\Delta R_1 \right)/\left(\Delta R_1\right)_{\text{ normal}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0005</td>
<td>0.0</td>
<td>Normal</td>
<td>315</td>
<td>3.18</td>
<td>(0)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ischemic</td>
<td>349</td>
<td>2.66</td>
<td>(0)</td>
<td>—</td>
</tr>
<tr>
<td>0004</td>
<td>0.1</td>
<td>Normal</td>
<td>130</td>
<td>7.69</td>
<td>4.51</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ischemic</td>
<td>274</td>
<td>3.65</td>
<td>0.79</td>
<td>—</td>
</tr>
<tr>
<td>0003</td>
<td>0.1</td>
<td>Normal</td>
<td>55</td>
<td>18.5</td>
<td>15.3</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ischemic</td>
<td>152</td>
<td>6.58</td>
<td>3.72</td>
<td>—</td>
</tr>
<tr>
<td>0002</td>
<td>0.01</td>
<td>Normal</td>
<td>272</td>
<td>3.67</td>
<td>0.49</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ischemic</td>
<td>343</td>
<td>2.92</td>
<td>0.06</td>
<td>—</td>
</tr>
<tr>
<td>0001</td>
<td>0.1</td>
<td>Normal</td>
<td>44</td>
<td>22.8</td>
<td>19.6</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ischemic</td>
<td>128</td>
<td>7.81</td>
<td>4.95</td>
<td>—</td>
</tr>
</tbody>
</table>

*The animal was sacrificed 30 min after injection of the solution and 90 min after ligation.
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 x 65 x 65 array. In Figure 5 the image is shown, using a special display technique in which an operator-interactive program 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, Pohost and coworkers, and Brady and coworkers. 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...
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 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 $9 \times 10^{-5}$ 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 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.

**IN VIVO REAL-TIME-STUDIES WITH LOCAL COILS**

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

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**Fig. 9.** (A) Clearance of Mn-54 from the blood in four dogs.\(^6\) (B) Blood clearance of Mn-56 in humans and its analysis into components.\(^\text{36}\)**
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 and distributions have been found to be functions of the total manganese concentration, as shown in Figure 11.$^{20}$

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.

![Graph showing relationship between 54Mn concentration and Sr-labeled microsphere concentration](image-url)
of the rates and amplitudes. 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°-70° 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.

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, 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. On the basis of such studies with Ho$^{3+}$, La$^{3+}$, and Pr$^{3+}$, it seems likely

Table 4. Acute Toxicity of MnCl$_2$·4H$_2$O

<table>
<thead>
<tr>
<th>Animal</th>
<th>Route</th>
<th>Dose</th>
<th>Dosage Values</th>
<th>Time of Death</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>s.c.</td>
<td>LD</td>
<td>180-250 mg/kg</td>
<td>—</td>
<td>55</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>s.c.</td>
<td>LD</td>
<td>180 mg/kg</td>
<td>12 Hours</td>
<td>55</td>
</tr>
<tr>
<td>Rabbit</td>
<td>s.c.</td>
<td>LD</td>
<td>180 mg/kg</td>
<td>12 Hours</td>
<td>55</td>
</tr>
<tr>
<td>Rabbit</td>
<td>i.v.</td>
<td>MLD</td>
<td>64.8 mg/kg</td>
<td>—</td>
<td>55</td>
</tr>
<tr>
<td>Dog</td>
<td>i.v.</td>
<td>LD$_{50}$</td>
<td>201.6 mg/kg</td>
<td>—</td>
<td>56</td>
</tr>
</tbody>
</table>
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. They have been proposed as contrast agents for NMR medical imaging by Brasch and coworkers, who have suggested the general abbreviation NSFR.

Such compounds have been shown, in recent work, 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. 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 abscess, 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.

TES was also proposed 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, corresponding to a concentration of $6 \times 10^4$ molecules of O$_2$/ml. It has been experimentally proven that in water the relaxation rate change is proportional to the concentration of dissolved O$_2$. According to Gore and coworkers, 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, images of the thoracic 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.

ACKNOWLEDGMENTS

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

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