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Transverse Relaxation of Solvent Protons Induced by Magnetized Spheres: Application to Ferritin, Erythrocytes, and Magnetite

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Since $1/T_2$ of protons of tissue water is generally much greater than $1/T_1$ at typical imaging fields, small single-ion contrast agents—such as Gd(DTPA), which make comparable incremental contributions and therefore smaller fractional contributions to $1/T_2$ compared to $1/T_1$—are not as desirable for contrast-enhancement as agents that could enhance $1/T_2$ preferentially. In principle, such specialized agents will only be effective at higher fields because the field dependence (dispersion) of $1/T_1$ is such that it approaches zero at high fields whereas $1/T_2$ approaches a constant value. The residual $1/T_2$ is called the "secular" contribution and arises from fluctuations in time—as sensed by the protons of diffusing solvent or tissue water molecules—of the component of the magnetic field parallel to the static applied field. For solutions or suspensions of sufficiently large paramagnetic or ferromagnetic particles (≥250 Å diameter), the paramagnetic contributions to the relaxation rates satisfy $1/T_2 \gg 1/T_1$ at typical imaging fields. We examine the theory of secular relaxation in some detail, particularly as it applies to systems relevant to magnetic resonance imaging, and then analyze the data for solutions, suspensions, or tissue containing ferritin, erythrocytes, agar-bound magnetite particles, and liver with low-density composite polymer-coated magnetite. In most cases we can explain the relaxation data, often quantitatively, in terms of the theory of relaxation of protons (water molecules) diffusing in the outer sphere environments of magnetized particles. The dipolar field produced by these particles has a strong spatial dependence, and its apparent fluctuations in time as seen by the diffusing protons produce spin transitions that contribute to both $1/T_1$ and $/T_2$ comparably at low fields; for the larger particles, because of dispersion, the secular term dominates at fields of interest. On the basis of the agreement of theory with data for solutions of small paramagnetic complexes, large magnetite particles, and liver containing low-density polymer-coated magnetite agglomerates, it is argued that the theory is sufficiently reliable so that, e.g., for ferritin—for which $1/T_2$ is unexpectedly large—the source of its large relaxivity must reside in nonideal chemistry of the ferritin core. For blood, it appears that diffusion through intracellular gradients determines $1/T_2$.

INTRODUCTION

At typical imaging fields, the transverse relaxation rate $1/T_2$ of protons of soft tissue is much greater than their longitudinal rate $1/T_1$. Moreover, these rates are different functions of the strength of the magnetic field and, at very low imaging fields, appear to become equal (cf. (*1–3*)). The dependence of these rates on magnetic field is known as nuclear magnetic relaxation dispersion (NMRD). The trend toward equality of the two relaxation rates at low fields relates to the fact that tissue water is highly mobile and, as a result, the relaxation properties of tissue protons—including tissue containing

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paramagnetic agents—are much like those of water solutions with analogous macromolecular and ionic contents (*1, 4–6*); for such liquids, $1/T_1 = 1/T_2$ in the limit of zero field (cf. (*7*)). Dispersion of the relaxation rates with increasing field occurs when a representative tissue proton diffusing in the local internal magnetic field experiences field fluctuations that become comparable and then slow compared to the proton Larmor precession frequency. In diamagnetic tissue, the fluctuating component of the local field results mainly from the magnetic moment of the other proton of the water molecule, whereas in tissue containing paramagnetic agents the field is contributed to by these agents as well. The times that characterize fluctuations of the components of the local field are called correlation times and can range over many orders of magnitude, depending on the particular relaxation mechanism. The phenomenological basis for this view of tissue water being much like solvent water, as well as the essentials of relaxation theory in this context (the concept of motional narrowing), has been recently reviewed (*1, 4, 7*).

A major distinction between $1/T_1$ and $1/T_2$, which follows directly from very general aspects of the theory of motional narrowing, is that $1/T_1$ always decreases (disperses) toward zero for sufficiently high external fields whereas $1/T_2$ disperses to a nonzero, field-independent value, usually to about 20% of its low field limit, the precise value depending on the relaxation mechanism. This residual transverse relaxation rate at high fields is sometimes termed the "secular" contribution (*8*), whereas all other contributions to both $1/T_1$ and $1/T_2$ are "nonsecular." The secular, nondispersive, term results from fluctuations of the component of the internal field parallel to the external static field B_0; these cause phase shifts in the Larmor precession of the magnetization of the proton ensemble which, when they have a random component, contribute to $1/T_2$. The nonsecular terms result from fluctuations of components of the local field within the transverse plane; these alter the longitudinal magnetization and therefore contribute to $1/T_1$ as well.

The half-way point of any dispersive component of the NMRD profile occurs at a field for which the product of the corresponding correlation time and Larmor angular frequency is of order unity. In body fluids (e.g., blood and CSF, in contrast to soft tissue), the relevant correlation times are short (10^{-10} to 10^{-11} s) and, as a result, typical imaging fields correspond to relatively low fields and, accordingly, $1/T_1$ and $1/T_2$ are about equal. Similarly, the correlation times that characterize the interaction of the protons of body fluids with small paramagnetic chelates ($\leqslant$25 Å diameter) are also short, causing their paramagnetic contributions to $1/T_1$ and $1/T_2$ to be comparable as well, on both a relative and an absolute scale. For this reason, such agents, typified by Gd(DTPA), can be of great utility in a clinical setting for examining the peripheral circulation. However, in soft tissue (where correlation times are often much longer), because of dispersion, the values of $1/T_2$ are about 10-fold greater than $1/T_1$ in the imaging range, and images are generally $1/T_2$-weighted to speed data acquisition. It follows that agents such as Gd(DTPA) are not too useful in this environment; paramagnetic agents that enhance $1/T_2$ of soft tissue preferentially at the fields of interest would accordingly be of greater clinical utility. The problem is to find a paramagnetic agent for which the relevant correlation time is appropriately long. Conventional thinking would suggest that these agents be rather large: perhaps clusters of paramagnetic ions acting collectively rather than individually and independently. For example,

from the darkening of liver and spleen in $1/T_2$-weighted images of thalassemic patients with iron-overload disease (*9, 10*), one would infer that the rather large iron-storage proteins ferritin and hemosiderin induce a substantial secular relaxation in tissue water protons. Reasoning from there, as well as from high-resolution data on deoxygenated blood (*11, 12*), dominance of the secular term might also be expected from pools of blood, e.g., from trauma-induced hematomas.

In a sample immersed in an ideally uniform field, all the protons of the sample precess at the same Larmor frequency and the transverse components of the precessing magnetization of each spin add constructively to produce the proton signal. However, if the field is not uniform, the phases of the proton precession in different parts of a single sample can lose synchronization with each other and contribute destructively to the signal amplitude because of a spatially dependent Larmor precession frequency (which can arise from nonuniformities of the static field); the result can be an apparent increase in the rate of decay of the transverse magnetization, i.e., in $1/T_2$. That the extent of this increase depends on the method of measurement is well known; the original spin-echo method of Hahn (*13*), and its subsequent generalizations by Carr and Purcell (*14*) and Meiboom and Gill (15) are all ways of recovering, in part, a loss in net transverse magnetization (arising from field inhomogeneities) that otherwise appears as an increase in $1/T_2$. The associated nonrecoverable part arises from diffusion of the protons in the inhomogeneous field, a process that destroys any underlying coherence in the relative precessional motion of all the protons of a sample. As will be seen, such diffusion in the neighborhood of large paramagnetic particles can lead to a dominant secular term. Clearly, however, care must be exercised in defining $1/T_2$ in these circumstances, since its observed value can depend on the method of measurement.

The theory of transverse relaxation in the presence of nonuniformities of the static field, either external or internal, has not been systematically examined, particularly for the secular terms. Perhaps more precisely put, a realistic model from which reliable predictions can be extracted in the general case has not yet been given. In the present work, we consider the many aspects of transverse relaxation of solvent protons in the presence of impenetrable magnetized spheres of uniform size ("particles"), as a function of particle concentration, with emphasis on those aspects of the problem that cause the secular term to dominate at imaging fields. Larger spheres, containing many paramagnetic ions, will become important; their magnetization can arise from the paramagnetism of their ions (*12*) or the paramagnetism of the solvent in which they are embedded (*11*). We obtain quantitative results that are directly applicable to ferritin and hemosiderin (*16*) and to protons diffusing inside and outside of deoxygenated erythrocytes (*11, 12*). The magnetization of the particles can also arise from the permanent magnetization of small ferromagnetic and ferrimagnetic particles such as magnetite (*17, 18*). For such systems, precise theoretical results are computationally difficult to obtain, though it is not difficult to describe the essence of the phenomena and estimate the magnitude of the relaxation effects.

What is special about paramagnetic spheres is that their magnetization is oriented along the external field; it does not fluctuate in direction as a result of either paramagnetic relaxation processes—as does that of single-ion complexes such as Gd(DPTA)—or rotation of the particles. With the elimination of these rapidly fluc-

tuating contributions to the local field, the correlation time may lengthen sufficiently to make possible a dominant secular term at imaging fields. We first review the reasonably well-developed theory of relaxation of solvent protons diffusing in the outer sphere environment of single, noninteracting, paramagnetic ionic complexes (*19–23*), called "outer sphere relaxation," and then compare these theoretical results, rewritten in a form convenient for the purpose, with available data for systems of relevance to contrast enhancement in MRI. The interesting and germane effects will be seen to depend on the geometrical scale of the particles as well as on the strength of the external static field; it then becomes important to be able to estimate the particle diameter at which the transition from one type of dependence of the relaxation rates on particle concentration to another occurs.

THEORETICAL BACKGROUND

Microscopic Outer Sphere Theory

The problem of outer sphere relaxation of solvent protons in a dilute solution of small isotropic paramagnetic complexes, for which the contributions to the relaxation rates are assumed to be independent and additive, has been addressed by many authors (*19–22*); their results have been summarized recently in a form relevant to the present concerns (*23*). For a concentration $[C]$ of particles in an external field B_0, with spin S and gyromagnetic ratio γ_s (γ_I is the proton ratio), diffusion constant D relative to the solvent protons, for which the protons can approach no closer than R, and for which the only contribution to the fluctuations in the local field arises from diffusion, the paramagnetic contributions to the longitudinal and transverse relaxation rates are

$$\frac{1}{T_1} = \left(\frac{32\pi}{405}\right)\gamma_I^2\gamma_S^2\hbar^2 S(S+1)\frac{N_A}{1000}\left(\frac{[C]}{RD}\right)[7\,j(\omega_S\tau_R) + 3\,j(\omega_I\tau_R)] \qquad [1a]$$

and

$$\frac{1}{T_2} = \left(\frac{32\pi}{405}\right)\gamma_I^2\gamma_S^2\hbar^2 S(S+1)\frac{N_A}{1000}\left(\frac{[C]}{RD}\right)[6.5\,j(\omega_S\tau_R) + 1.5\,j(\omega_I\tau_R) + 2\,j(0)], \qquad [1b]$$

where

$$\tau_R = R^2/D \qquad [1c]$$

is the time required to diffuse a distance of the order of R;

$$\omega_S = \gamma_S B_0, \qquad \omega_I = \gamma_I B_0 \qquad [1d]$$

are the paramagnetic ion and proton Larmor angular frequencies, respectively; and the spectral density functions $j(\omega)$ are given by

$$j(\omega) = \mathrm{Re}\left\{\frac{1 + (1/4)(i\omega\tau_R)^{1/2}}{1 + (i\omega\tau_R)^{1/2} + (4/9)(i\omega\tau_R) + (1/9)(i\omega\tau_R)^{3/2}}\right\}, \qquad [1e]$$

where the secular term

$$j(0) = 1. \qquad [1f]$$

Re means "the real part of."

In this system, even though the paramagnetic moments themselves are aligned along B_0, the orientation of their dipolar fields assumes all directions in space, de-

pending on the spatial position relative to that of the dipole. Accordingly, the diffusing protons see comparable (but not identical) fluctuations of all components of the local field, with the result that the magnitudes of the secular and nonsecular contributions are always comparable. It follows that the only way in which one can have $1/T_2 \gg 1/T_1$ is for the $j(\omega)$ terms to have dispersed substantially. The variation with field of $1/T_1$ and $1/T_2$ as expressed by the spectral density terms in Eqs. [1a] and [1b] is shown in Fig. 1 for 100-Å-radius spheres at 35°C, just large enough so that the secular terms dominate relaxation at higher imaging fields.

Implicit in the derivation of $j(0)$ is the assumption that the radiofrequency (rf) field B_1 is negligibly small; more specifically, $\gamma_1 B_1 \tau_R \ll 0$. When this condition fails, the secular term itself decreases with increasing B_1. The result is a more general transverse relaxation known as "longitudinal relaxation in the rotating frame," or $1/T_{1\rho}$ (cf. (*2, 3, 24*)). For typical values of B_1, about 10^{-4} T, dispersion of the secular term will become significant for $R \gtrsim 0.5$ μm. Moreover, such effects can be emphasized by increasing the strength of the rf field, and images based on the dispersion of $1/T_{1\rho}$ have been reported (*2*).

The coefficient of the dispersive terms, Eqs. [1a] and [1b], can be rewritten in a form that is particularly convenient for the present purposes. The magnetic moment μ of each particle is given by

$$\mu^2 = \gamma_S^2 \hbar^2 S(S+1), \qquad [2a]$$

and $\delta\omega$, the equatorial magnetic field at the surfaces of the particles (in units of the proton Larmor frequency) is

$$\delta\omega = \gamma_1 \mu / R^3. \qquad [2b]$$

Therefore, at zero field,

$$\frac{1}{T_2(0)} = \frac{1}{T_1(0)} = \frac{16}{27} f(\delta\omega)^2 \tau_R \qquad [2c]$$

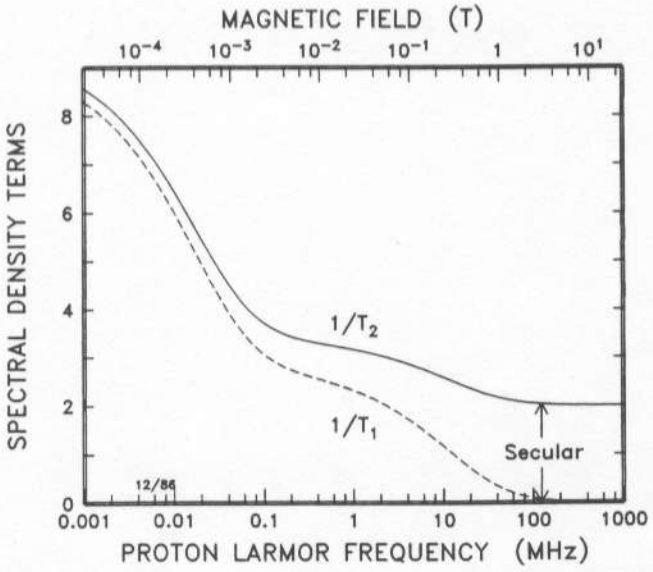

FIG. 1. The dispersive spectral density terms of Eqs. [1a] and [1b], showing the field dependence, at 35°C, of outer sphere relaxation of solvent protons due to diffusion of solvent in the local dipolar magnetic field of magnetized solute spheres. The size of the spheres (100 Å radius) was chosen to make the secular (non-dispersive) component of $1/T_2$ dominate at higher imaging fields.

and the secular term becomes

$$\frac{1}{T_{2\text{sec}}} = \frac{16}{135} f(\delta\omega)^2 \tau_R, \qquad [2d]$$

where f is the volume fraction from which solvent is excluded. A major condition for the validity of the theory is that

$$(\delta\omega)\tau_R \ll 1, \qquad [2e]$$

a condition that (for the present purposes) only breaks down for large, solid, ferromagnetic particles, $\geq$250 Å diameter. The spatial distribution of magnetic dipolar moments within radius R must be spherically symmetric, but may be otherwise arbitrary, for Eqs. [1a] and [1b] to hold. For large particles, composed of many paramagnetic ions, it becomes convenient to relate μ to M_0, the magnetization (magnetic moment per unit volume) of the particle, by

$$\mu = 4\pi M_0 R^3/3; \qquad [2f]$$

$\delta\omega$ is then seen to be proportional to M_0 and independent of R.

Equations [2c] and [2d], together with Eq. [1e], show how all contributions to the outer sphere relaxation rates vary with particle size for a fixed volume fraction of uniformly magnetized particles, i.e., for fixed f and $\delta\omega$. The correlation time τ_R is the only variable that depends on the size of the particles; from Eq. [1c], $1/T_1$ and $1/T_2$ increase as the square of the radius of the particles. Put another way, for outer sphere relaxation, when Eq. [2e] holds, *the same amount of magnetized material is much more effective when distributed as fewer large particles than as a greater number of smaller ones.*

The several derivations of these equations (*19–22*) all use the traditional quantum-mechanical approach (*25*) in which rate equations are set up for the "flip-rates" of the solvent protons in terms of transition probabilities, which in turn are computed using time-dependent perturbation theory in a standard way. The interaction responsible for relaxation is the magnetic dipolar interaction of the proton spins with the fields produced by the paramagnetic ions, an interaction that fluctuates in time because of the relative diffusive motion of solvent molecules and particles. In the earlier work (*19, 20*), no boundary conditions are imposed on the diffusing water molecules; the volume occupied by the particles is only excluded in the final integration that gives the relaxation rate. In the later work (*21, 22*), the impenetrability of the particles is included explicitly in the boundary conditions on the diffusion equation as well as in the final integration. In both cases, however, the diffusing waters can get infinitely far from a paramagnetic particle. (As noted earlier (*25*), the predictions of the two approaches are indistinguishable except that the first are uniformly lower by a factor 9/10). The correlation time that characterizes the interaction is τ_R, Eq. [1c]. An important, but implicit, assumption of the calculation is that the time-averaged field experienced by a single proton as it diffuses through the long-range, spatially varying dipolar fields of the paramagnetic centers can be replaced by a spatial average, at any given time, over all the solvent protons. This assumption is rarely violated for small particles. As an example, even for particles as large as, say, ferritin (130 Å diameter) at the density needed to store the normal iron content of liver (about 0.1 mg/ml), the intersphere spacing $2A$ can readily be estimated to be about 1200 Å, and $\tau_A = A^2/D$,

the time required for solvent to diffuse this distance, is about 1 μs. By comparison, typical relaxation times are a few tenths of a second (*16*) and the assumption is valid. However, for large particles (e.g., erythrocytes) at high densities, the assumption may lose its validity.

Two-site Exchange and Macroscopic Theory

Equation [2c] is essentially identical in form to the results for a well-defined two-site model obtained by Swift and Connick (*26*) and independently by Luz and Meiboom (*27*). These authors considered exchange of water protons between solvent and a class of binding sites (in low concentration) that have a characteristic chemical shift, i.e., a site-specific local field. For a difference of chemical shift $\Delta\omega$ and rapid exchange between sites and solvent, their computed contribution to $1/T_2$, which is secular because $\Delta\omega$ is an effective field parallel to B_0, is

$$1/T_{2\text{sec}} = f(\Delta\omega)^2\tau_M, \qquad [3a]$$

where τ_M is the mean lifetime of a proton in the binding sites and f is the fraction of protons bound.

There is a fundamental distinction between the model of Swift and Connick and that of Luz and Meiboom, a distinction that will become germane when considering relaxation in blood, below. For Luz and Meiboom, $\Delta\omega$ represents a contact interaction that is taken as the sole relaxation-producing interaction at the binding site. By contrast, the Swift-Connick result, Eq. [3a], is a limiting case of a model in which protons have a characteristic transverse relaxation rate T_{2M} when bound as well as a chemical shift relative to solvent. One criterion for Eq. [3a] to hold is that exchange be rapid; i.e., $\tau_M \ll T_{2M}$. A second is that $(\Delta\omega)^2\tau_M T_{2M} \gg 1$ with $(\Delta\omega)\tau_M \ll 1$ to guarantee rapid exchange. Both these criteria can obviously be satisfied when T_{2M} is long. However, if the criterion for rapid exchange fails (as it can for water in the interior of erythrocytes, as seen below), then the Swift-Connick model gives the usual result for exchange-limited relaxation

$$1/T_{2\text{sec}} = f/\tau_M. \qquad [3b]$$

Note that at the critical condition, $(\Delta\omega)\tau_M = 1$, Eqs. [3a] and [3b] give the same relaxation rate. However, when exchange is rapid, $1/T_{2\text{sec}}$ *increases* as τ_M increases whereas the reverse is true for slow exchange.

In the Swift-Connick model (*26*), the water molecules experience two environments and the boundary between these environments is sharp. By contrast, in outer sphere computation leading to Eq. [2c], water molecules may be considered either near the magnetized spheres, where there is a chemical shift of order $\delta\omega$, or far away where there is none. In the latter case, the distinction between the two environments is not well defined because of the long range of the dipolar field. However, as long as $A \gg R$, the contributions of each particle should be independent. More quantitatively, it is seen from Eqs. [1a] and [1b] that for $A = 10R$, the volume beyond a sphere of radius A contributes 0.1 of the total relaxation rate. Thus, these equations should be valid to better than 10% for $A \geq 10R$, corresponding to $f \leq 10^{-3}$. For larger values of f, the rates will be less than the linear prediction since the range of $\delta\omega$ decreases when the fields of neighboring particles overlap.

It should be noted that the Swift-Connick (*26*) and Luz–Meiboom (*27*) approaches are classical; rather than solve the appropriate quantum-mechanical problem, they formulated the problem in terms of the Bloch equations (*28*), a set of equations that describes the motion of the magnetization of the proton ensemble. This magnetization is a macroscopic, potentially spatially varying quantity that is an average of the behavior of many protons, each of which must be treated quantum mechanically if handled individually. However, one may either solve the quantum-mechanical problem and average the results over all protons, as was done for the outer sphere calculation leading to Eqs. [1a], and [b], or else derive a classical equation (analogous to Newton's Laws) that describes the motion of the macroscopic parameters (in the present case the magnetization of the proton ensemble) directly. That is what the Bloch equations do, even in the presence of spatially varying static fields, once a diffusion term has been incorporated (*29*).

Equations [2c] and [3a] are identical to the expression of motional narrowing of high-resolution NMR linewidths, usually derived using the Bloch equations, as was discussed by Packer (*30*) in a related connection. For lines from two environments with weighting f, chemical-shift difference $\Delta\omega$, and lifetime in the minority site τ_M, Eq. [3a] is the proper expression for the width (a measure of $1/T_2$) of the motionally narrowed line; the separation $\Delta\omega$ is narrowed by the factor $(\Delta\omega)\tau_M$, which must be $\ll 1$ for merging of the lines and subsequent narrowing to occur. Indeed, the expressions for a motionally narrowed secular transverse relaxation are very general and arise from the fact that boundary conditions are imposed on the diffusing protons, either in space or in time, depending on the experimental conditions. Before applying the theory of specific situations, this point will be pursued further.

Temporal and Spatial Boundaries

Hahn, in his first observation of spin echoes (*13*), realized that the time course of the free-induction decay after a 90° pulse was determined in the main by the non-uniformities of the static magnetic field (given the magnet technology of that time), but that much of the lost transverse magnetization could be "refocused" by application of a second pulse (also 90° in his early work) a time τ_H later to produce an echo of the decay of the initial transverse magnetization. He, and subsequently Torrey (*29*), solved the equations that describe these events (the Bloch equations with a diffusion term), assuming a magnetic field with a constant gradient, and showed that the amplitude of the echo is multiplied by a factor that was exponential in $-(C_3\tau_H^3 + C_1\tau_H)$. The coefficient C_3 of this cubic term depended on the diffusion constant D of the water molecules, the gyromagnetic ratio γ_I of the protons, and G, the component of the gradient parallel to B_0:

$$C_3 = 2(\gamma_I G)^2 D/3. \qquad [4a]$$

This was for the case of unrestricted diffusion, i.e., no boundary conditions other than the initial 90° pulse; for progressively longer times, a given molecule can diffuse infinitely far from its position at the time of the initial pulse and accordingly accumulate an unlimited precessional phase shift. The $-t^3$ behavior follows as a consequence. The case of restricted diffusion is quite different.

Equation [4a] can be cast into a form similar to that for outer sphere relaxation and motional narrowing. Thus, for an imaging field with a constant gradient, it is possible to compute an apparent $1/T_2$ from the echo associated with a given τ_H, which is $T_E/2$ in MRI parlance. The apparent $1/T_2$ will be a function of τ_H; the longer τ_H, the greater this $1/T_2$. In a time τ_H, a water molecule will diffuse a distance with a mean component L_H along the direction of B_0 given by

$$L_H^2 = 2D\tau_H. \quad [4b]$$

Defining

$$\Delta\omega_H = \gamma_I G L_H, \quad [4c]$$

where $\Delta\omega_H$ is the range of field (in frequency units) experienced by the protons during τ_H, one obtains (by direct substitution) the expression for the apparent τ_H-dependent $1/T_{2H}$, as determined from the amplitude of the Hahn echo at time $2\tau_H$:

$$1/T_{2H} = (\Delta\omega_H)^2\tau_H/6. \quad [4d]$$

This is the same functional form as Eqs. [2d] and [3a], except that τ_H, the analog of τ_M and τ_D—parameters related to the microscopic properties of the samples—is determined by the parameters of the experiment.

The spin-echo experiment requires, of course, that $\tau_H \ll T_2$, i.e., that the water molecules diffuse many times L_H and average their local environments in an observation time nT_2. From Eq. [4d], this leads to

$$(\Delta\omega_H)\tau_H \ll 1, \quad [4e]$$

analogous to Eq. [2e].

Restricted diffusion results when boundary conditions are imposed on the sample that limit the excursion in local field that the protons experience. Such boundary conditions may be imposed in either space or time. The second pulse in a Hahn 90–180° experiment is a temporal boundary condition, reversing the phase of each precessing sign. Extending Hahn's ideas, Carr and Purcell (*14*) showed how the apparent transverse relaxation rate can be decreased by more restrictive temporal boundary conditions. They applied a sequence of n 180° pulses, the first a time $\tau_{CP} = \tau_H/n$ after the 90° pulse, and the remainder separated by $2\tau_{CP}$. The result for the apparent relaxation rate, with the echo amplitude measured at the same τ_H as in Eq. [4d], is

$$1/T_{2CP} = (\Delta\omega_{CP})^2\tau_{CP}/6 = (1/T_{2H})/n^2, \quad [4f]$$

where

$$(\Delta\omega_{CP})^2 = (\Delta\omega_H)^2/n. \quad [4g]$$

The more closely spaced (temporal) boundaries both narrow the range of fields experienced by the protons and increase the rate at which this field distribution is averaged, hence the quadratic dependence of the decrease in the relaxation rate on n.

Several authors have suggested other experiments in which the boundary conditions are applied in time rather than in space, experiments quite familiar but not often considered this way explicitly. One example, above, is the Carr–Purcell sequence (*14*), in which periodic reversal of the gradient (as it appears to the proton spins) restricts the range of field that any proton can experience. Another is due to Stejskal and Tanner (*31*), who applied a strong gradient for a short time τ_{ST} between the 90–180°

and 180–90° intervals in the spin-echo sequence, thereby directly limiting the time that the protons experience a significant gradient. The description of their results can also be put in a form similar to Eq. [2d],

$$1/T_{2\text{sec}} = F(\Delta\omega_{\text{ST}})^2\tau_{\text{ST}}, \qquad [5]$$

where τ_{ST} is the rate of decay of the transverse magnetization, which is exponential in $-t$; $\Delta\omega_{\text{ST}}$ is the range of field encountered during time τ_{ST}, and F is the fraction of time between pulses that the gradient is on. Once again, the shorter τ_{ST} becomes (and thus more restricting the boundary conditions), the less the influence of the heterogeneous environment on $1/T_2$, quite consistent with the language of motional narrowing.

Robertson (*32*) addressed the problem of proton relaxation in a sample constrained between parallel planes, separated by a distance d, that are normal to the direction of B_0 and its constant gradient G. The planes impose spatial boundary conditions that require the flux of magnetization normal to the planes to be zero. He solved the Bloch equations, with a diffusion term, subject to the boundary conditions, and found that the secular contribution to the long-time delay of the transverse magentization was exponential in $-t$, with a rate that depends on an average of the fields experienced. Interestingly, the very short-time behavior involved an exponential in $-t^3$, with a coefficient precisely that found by Hahn (*13*) for diffusion in an unbounded medium with a constant gradient. The Hahn-like dependence persists until the protons have sufficient time to sense that their spatial excursions are bounded before they lose precessional phase in the gradient. For a distant scale even as large as microns (cell-sized), the transition from $-t^3$ to $-t$ behavior occurs well below a millisecond, and the cubic term will usually be unimportant. For longer times, the secular relaxation rate contribution can be put in the form

$$1/T_{2\text{sec}} = (\Delta\omega_R)^2\tau_d/120 \qquad [6]$$

where again $\Delta\omega_R = \gamma_1 dG$ is the range of field experienced by the diffusing protons and $\tau_d = d^2/D$ is the approximate time required to diffuse between the planar boundaries. In this problem, there is no equivalent of f since the constant gradient pervades the entire volume. Nonetheless, Eq. [6] has the same form as Eqs. [2c] and [3a]. The critical problem for this and more complex geometries is to compute the magnitude of the coefficient on the right-hand side equivalent to f, which here is very small (1/120).

Motional narrowing in the geometry of Robertson (*32*) causes $1/T_2$ to *increase* as the fourth power of the separation of the planes; i.e., as d *increases* and the occurrence of boundaries is diluted, both the range of field experienced by the water protons and the time required to average this field increase, with a resultant *increase* in the secular relaxation rate.

We have previously considered (*33*) an assemblage of identical magnetic dipoles, spherical and impenetrable, distributed periodically in space (which implies that their diffusive motion can be neglected relative to that of solvent). This, too, is a model of restricted diffusion since periodic boundary conditions are equivalent to physically delimiting a region in space: the diffusive flow of magnetization normal to the boundaries must be zero ($\nabla_n \mathbf{M} = 0$). This problem can be transformed to a form analogous

to Robertson's, though somewhat more complex. For both dia- and paramagnetic particles in a uniform external field, the periodicity of the local field arises directly from the spatial periodicity of the particles since the magnetic moment of each particle is proportional to the magnitude of the applied field and aligned with it. This periodicity also holds for ferromagnetic particles, since their field-independent magnetization at reasonable fields must necessarily be correlated with the field direction. The system of spherical particles is fully characterized by the magnetic moment μ of the particles, their radius R, and their separation $2A$. The volume fraction f occupied by the particles is readily found to be $f = \pi\lambda^3/6$, where $\lambda = R/A$.

The problem is to calculate the magnitude of the transverse magnetization (the spin-echo amplitude) at time $t = 2\tau$ following a 90° pulse applied at $t = 0$ and a 180° pulse applied at $t = \tau$. These are the same experimental conditions considered by Robertson. Our calculations proceed much as his do, except that the gradient is not constant; it arises from the moments of the particles, which generate fields that decrease as $1/r^3$, where r is the distance from the center of a particle. This three-dimensional geometry is different from Robertson's, which is one-dimensional, but can be reduced to his by means of a simplification that should not seriously affect the results—mainly because of the similarity of the boundary conditions. In the present case, a water molecule also moves between two boundaries: the surfaces of the impenetrable spheres and the midpoints of two neighboring spheres and the condition zero flux of the magnetization holds at both. Our simplification is to neglect the curvature of the magnetized spheres, which is akin to neglecting the angular dependence of the dipolar field. This makes the problem one-dimensional and equivalent to that of Robertson; it clearly characterizes the limit of high particle density. However, in contrast to the true one-dimensional problem of Robertson, our problem is characterized by two time scales: the time to diffuse out of range of a particle ($\tau_R = R^2/D$, which is a measure of the range of the nonuniform field), and the time to diffuse between particles ($\tau_A = A^2/D$). The ratio of these two times is related to the volume fraction f, which does not appear in Robertson's formulation.

The details of the computation follow those of Robertson quite closely, and the short- and long-time results are similar as well. One difference is that only the mean squared fluctuations of the component of the local field parallel to B_0 contribute to the secular term of $1/T_2$, which introduces a factor 0.8 into the results (a factor not included previously (*33*)). The other components of the fluctuations produce the dispersive, nonsecular terms. The result for the exponential secular relaxation becomes

$$1/T_{2\text{sec}} = C_2(f)(\delta\omega)^2\tau_A = (C_2/(6f/\pi)^{2/3})(\delta\omega)^2\tau_R = C_2(4\pi\gamma_1 M_0/3)^2A^2/D, \qquad [7]$$

where $M_0(=3\mu/4\pi R^3)$ and the other quantities have been defined earlier. For uniformly magnetized particles that differ only in size, M_0 is independent of size and therefore particularly useful in computations. For paramagnetic particles, M_0 can be computed knowing B_0 and the paramagnetic susceptibility of the particles (which can be obtained using Curie's law). Equation [7] should afford a good qualitative description of the relaxation behavior at high particle densities, $f \gtrsim 0.01$, a limit in which $1/T_2$ should decrease with increasing f. However, because of the neglect of the curvature of the particles, Eq. [7] will overestimate $1/T_2$ except at the highest densities. By contrast, the quantum mechanical outer sphere theory, with the assumption of independent

particles and essentially unrestricted diffusion, represents the limit of low particle density. The overall behavior is seen quite clearly in Fig. 2, in which the predictions of Eqs. [2d] and [7] are plotted.

In a system for which the averaging time is defined by spatial boundary conditions, it is possible to shorten this time and reduce the secular relaxation rate by application of a Carr–Purcell pulse sequence in a time that is short compared to the spatially determined correlation time. Thus, in the Swift-Connick case (*26*) with rapid exchange, Eq. [3a] applies, and a sequence with spacings that are short compared to τ_M will reduce $1/T_{2sec}$. If not, Eq. [3b] applies, relaxation is exchange limited, and a Carr–Purcell sequence will have no effect. A Carr–Purcell sequence can also influence a measurement of outer sphere relaxation, as characterized by τ_R, Eq. [1c], though the experiment is only realistic for cell-sized particles. This point, though demonstrated by Luz and Meiboom (*27*) both theoretically and experimentally, appears to be not generally recognized. Rather, the view is often expressed that a Carr–Purcell sequence can only convert a dependence of the decay of the transverse magnetization that is exponential in $-t^3$ (arising from unrestricted diffusion in a constant gradient) to one that is exponential in $-t$; this, though true, is a special case of the more general utility of Carr–Purcell and related pulse sequences.

APPLICATIONS

Small Paramagnetic Centers

It is known that Eq. [1a] affords an excellent quantitative description of the $1/T_1$ NMRD profile of aqueous solutions of Mn^{2+} ions chelated by ligands that exclude all inner-coordinated water (*33*); moreover, the $1/T_2$ data extant agree with the prediction

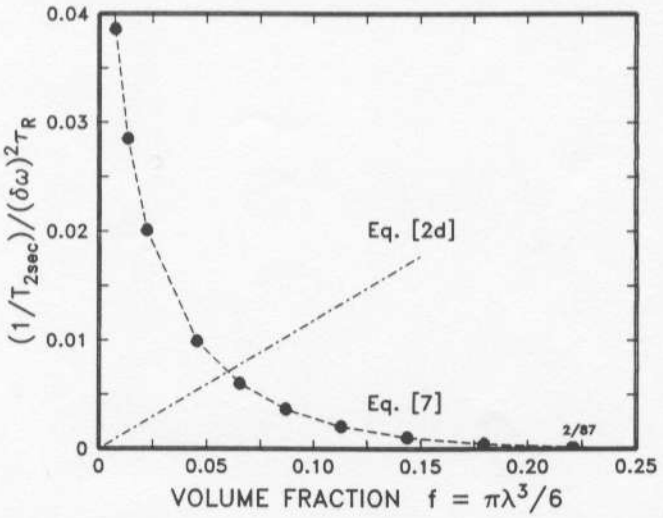

FIG. 2. The secular contribution to transverse outer sphere relaxivity of solvent protons as a function of the volume fraction occupied by solute spherical particles. The dot–dashed curve expresses Eq. [2d], valid when the contributions of each particle are independent and additive. The dashed curve connects points computed using an extension (*33*) of the model of Robertson (*32*), given by Eq. [7], in which overlap of the magnetic fields of neighboring particles is incorporated in an approximate way; it overestimates the relaxation rate at low values of f, but becomes a progressively better approximation at high particle densities.

of Eq. [1b] that $1/T_2$ and $1/T_1$ should be about equal over most of the accessible field range for such small complexes. Similarly, the $1/T_1$ NMRD profiles of aqueous solutions of nitroxide free radicals are also readily accounted for by Eq. [1a], though the anisotropic geometry of these centers precludes the same level of quantitation as for chelated Mn^{2+} ions (*23*). The situation if more complex, however, for ferritin, the only paramagnetic complex that is much larger than the small, single-ion, chelate complexes but is still small on a biological scale, and for which relaxation data are available.

Ferritin

Ferritin is an iron-storage protein that can hold about 3000 ferric ions in a paramagnetic oxyhydroxide core that is surrounded by a spherical shell of protein 130 Å in diameter and 30 Å thick (*35*). The demetallized protein is synthesized *in vivo,* predominantly in the liver and spleen, in response to the presence of excess iron, a condition associated with, for example, idiopathic hemochromatosis and transfusional therapy of thalassemic patients. Such individuals may have a concentration of ferric iron in the liver more than 10-fold above the normal concentration (about 0.1 mg/ml or 2 m*M*). MRI of such patients shows a pronounced darkening of these organs (*9, 10*), perhaps not intuitively surprising but not explicable from the usual theory of relaxation and the assumption of ideal structure for the ferritin core (*16*).

Figure 3 shows both the longitudinal and the transverse NMRD profiles, computed using Eqs. [1a] and [1b], for a solution of paramagnetic ferritin containing 1000 ferric ions per protein molecule. This loading, about one-third the maximum, was chosen for ease of comparison with earlier data (*16*). The results are expressed in units of relaxivity (relaxation rates per m*M* of ferritin). The value of μ^2, Eq. [2a], is a function

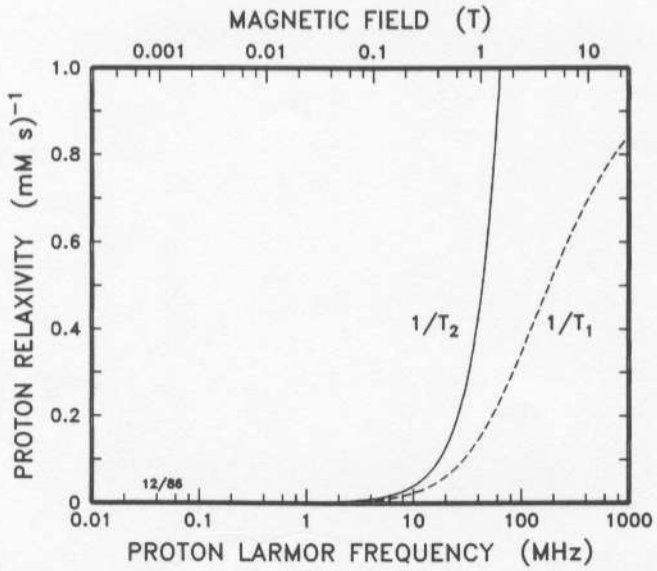

FIG. 3. The NMRD profiles expected for solutions of ferritin molecules (1000 Fe^{3+} per core), at 35°C, with about 30% of their maximum core loading of iron. The core was assumed to be an ideal paramagnetic, and its magnetic moment was computed using Curie's law. The increase in relaxivity at high fields, in contrast to the behavior in Fig. 1, arises from the increase in magnetization of the core with increasing external field.

of magnetic field for paramagnetic particles and was calculated using Curie's law; the density of paramagnetic ions in the 70-Å-diameter ferritin cores was obtained by taking 3000 molecules as the number that fills them. The observed paramagnetic part of the transverse relaxivity at 20 MHz is 150 (mM s)$^{-1}$ ($1/T_2 \sim 3$ s^{-1} for a 20 μM sample), more than *three orders of magnitude greater* than the computed value shown in Fig. 3. The paramagnetic component of the longitudinal relaxivity due to the core ions is unobservably small at all fields, $\ll 5$ (mM s)$^{-1}$, in agreement with Fig. 3. However, the predicted field dependence of $1/T_2$ does agree with that reported (*36*) for a limited range of field (8–60 MHz).

There appears to be no way to account for the relaxivity of ferritin solutions unless, as noted earlier (*16, 33*), it is assumed that the magnetization of the cores is far greater than the paramagnetic value computed assuming ideal chemistry of the solid-state polycrystalline ferric oxyhydroxide core. Such a condition could arise from incomplete oxidation of a few percent of the ferric core ions, resulting in inclusions of the ferromagnetic mixed-valence oxide magnetite. This is not unreasonable since the ferritin core can be loaded only by presenting it with ferrous ions from solution, which are then catalytically oxidized by the protein to ferric as they become incorporated in the core. Figure 4 shows the $1/T_1$ and $1/T_2$ NMRD profiles computed, using Eqs. [1a], assuming 76 ferric ions per core aligned ferromagnetically; this gives a relatively large moment that is independent of field. (The paramagnetic response to a field of 1 T would be an alignment of only 5 out of 1000 spins. Only at higher fields, above about 100 MHz, will the induced paramagnetic moment play a role and contribute a quadratic dependence of $1/T_2$ on magnetic field.) The number of aligned spins was chosen to produce agreement with the $1/T_2$ data at 20 MHz (*16*). However, the computed $1/T_2$

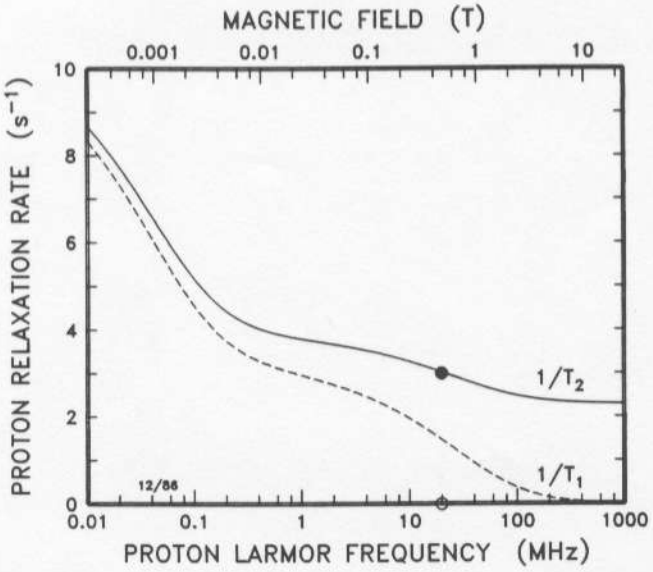

FIG. 4. The NMRD profiles of 20 μM ferritin, calculated at 35°C, assuming that the chemistry of the core is nonideal. It is postulated that the oxidation of the iron ions is incomplete so that a single region of mixed ferrous–ferric oxides (e.g., magnetite) exists in the core, with their spins permanently aligned. In particular, the curves were computed for the case of 76 aligned ferric ions (Fe^{3+}), chosen to have the computed $1/T_2$ agree with the single data point (filled circle) at 20 MHz (*16*). The computation agrees neither with the reported field dependence of $1/T_2$ (*36*), nor with the single $1/T_1$ result (open circle) (*16*).

NMRD profile does not exhibit the reported field dependence (*36*), and that computed for $1/T_1$ does not agree with the observed magnitude of $1/T_1$, which is essentially zero at all fields (*16*). A possible explanation within the present conceptual framework is that small regions of the core are ferromagnetic, with the directions of their magnetization (which, because of magnetic anisotropy energy, would be aligned along one of several equivalent crystallographic axes) random at zero field. As B_0 is increased, the entire core would become magnetized, thus behaving much as a paramagnetic with a very large susceptibility. Moreover, a high field is required to magnetize magnetite since it has a large crystalline anisotropy energy. This could account for the magnitude of $1/T_2$ and its dependence on field, but may overestimate $1/T_1$. In summary, unless one assumes that nonideal chemistry plays a role in the transverse relaxation of ferritin solutions, there is no ready explanation for the solution data (*16*) and the MRI observations (*9, 10*). There appear to be no published data inconsistent with the conjecture presented here.

Blood (Erythrocyte Suspensions)

Upon deoxygenation, the spin state of the heme-iron moiety of hemoglobin changes from $S = 0$ to $S = 2$ (diamagnetic to paramagnetic). Thulborn *et al.* (*12*) reported changes in $1/T_2$ of the protons of whole blood that reflect this magnetic transition and which disappear when the cells are lysed. The increment in the secular component of $1/T_2$ upon deoxygenation is 44 s^{-1} at 182 MHz, with an approximately quadratic dependence of this rate on field. Thulborn *et al.* used a Carr–Purcell–Meiboom–Gill sequence (*14, 15*), from which a correlation time of 0.6 ms was derived from the dependence of $1/T_2$ on pulse spacing (*27*). Not only did they note that this correlation time was of the order of the τ_R expected for particles the size of erythrocytes (and which, because of high concentration of cells, is about the same as τ_A), but they also noted that the value of $\Delta\omega$ needed to explain their data, using the equivalent of Eq. [3a] above, could be accounted for by the known value of the magnetic susceptibility of hemoglobin ($\chi = 2 \times 10^{-7}$, which they checked independently). Moreover, they pointed out that the measured correlation time is an order of magnitude shorter than the residence lifetime of a water molecule in an erythrocyte and concluded that "the dependence of blood T_2 on oxygenation results from the diffusion of water protons through local field gradients arising from the increased volume susceptibility of deoxygenated erythrocyte cytoplasm." However, they did not indicate whether this diffusion was predominantly intra- or extracellular. The question at hand now is the extent to which these data for blood (and comparable systems) can be explained, with reasonable quantitation, by the outer sphere theory discussed here applied to a model for blood, or whether intracellular relaxation is also important.

Erythrocytes are neither spherical nor impenetrable; the fact that the protons of all the water of blood (one-third intracellular and two-thirds extracellular; correction for the volume occupied by the hemoglobin gives the hematocrit value of 0.45) relax identically demonstrates that mixing is rapid on a T_2 time scale. However, exchange is sufficiently slow (the intracellular water lifetime is about 8 ms for human erythrocytes (*37, 38*)) so that only about 1 encounter in 10 allows a water molecule to cross the erythrocyte membrane; therefore the boundary conditions for outer sphere diffusion

should be little affected and the theoretical results essentially unchanged by the limited permeability of the membranes. Accordingly, we first consider as a model a water suspension of impenetrable "spherical erythrocytes" with the same volume and volume fraction as those in blood: a radius of 2.8 μm and a hematocrit (volume fraction) of 0.45.

The major distinctions between model erythrocytes suspensions and the ferritin solutions considered above—similar in that they both involve paramagnetic spheres—are first that the density of Fe^{3+} ions in erythrocytes is ~6 mM compared to ~8 M in the ferritin cores; thus the magnetization of the erythrocytes and therefore the equatorial field at the surface is 1/400 that of ferritin. Second, because the diameter of erythrocytes is about 600 times that of ferritin, the correlation time is much longer. For fields up to ~50 MHz, Eq. [2e], the condition for motional narrowing holds very well for erythrocyte suspensions (moreover, a modest violation of this condition is known to have but a small effect on the predictions).

Figure 5 shows the changes of $1/T_2$ induced by deoxygenation of rat blood, at 37°C, reported by Thulborn *et al.* (*12*). We first use Eq. [7], obtained for outer sphere relaxation using the Bloch equations, to compare theory with the data of Thulborn *et al.* Though the computational results are not shown for values of f as great as the hematocrit, even choosing a value for f of 0.22 (about half that of the model, which might be expected to compensate, in part, for the nonspherical shape of erythrocytes) gives a prediction that fails by two orders of magnitude to account for the data. Thus, the ordinate, Fig. 2, that goes with the point $f = 0.22$ is 2×10^{-4}, obtained as follows. Assuming a cell content of 20 mM Fe^{2+} ions in the high spin $S = 2$ state, one can calculate the susceptibility (or use the measured value (*12*)) and conclude that $\Delta\omega = 960\ s^{-1}$ at 182 MHz. Taking the measured correlation time of 0.6 ms gives $1/T_{2sec} \sim 0.1\ s^{-1}$, in contrast to the measured value of 44 s^{-1}, Fig. 5. Thus, it is quite clear that outer sphere relaxation, on the spherical model, is inadequate to account for the data.

The next consideration is whether outer sphere relaxation calculated for a more realistic cell shape can account for the data; the following argument shows that it only makes things worse. Consider the erythrocytes to be flat disks. As such, they would orient, in a time of about 20 s, with their planes parallel to the field, though not necessarily parallel to each other. Nonetheless, a reasonable approximation to this would be a one-dimensional array of alternating slabs of blood plasma and hemoglobin, the latter about 2 μm thick, with the spacing adjusted to match the hematocrit. To first order, the boundary conditions on H_0, the magnetic field intensity, require that there be no discontinuity at the interfaces; therefore H_0 will be uniform throughout space. As a result, there will be no extracellular field gradients and therefore no outer sphere contribution to the relaxation. There will be a discontinuity in B across the boundaries of magnitude $4\pi\chi H_0$, the largest possible for any geometry, corresponding to $\Delta\omega = 2.5$ ppm or $2900\ s^{-1}$ at 182 MHz. The contribution to $1/T_{2sec}$ will be the lesser of the results of Eqs. [3a] and [3b]. For $\tau_M = 8$ ms, the value for human erythrocytes, Eq. [3b] dominates above 50 MHz, giving $1/T_{2sec} = 40\ s^{-1}$ as an upper bound on the relaxation rate. This is less than half the maximum rate observed, Fig. 5, and if this is to be ascribed to intracellular relaxation—which appears to be necessary—then the lifetime of water in rat red cells must be less than that in human cells by at

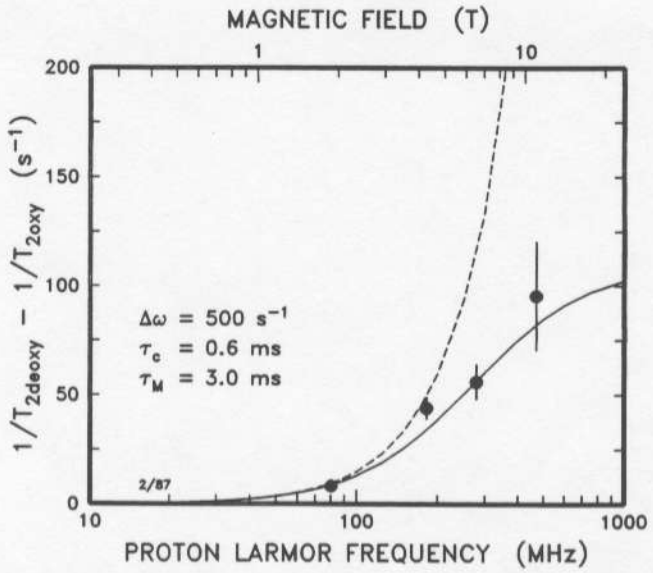

FIG. 5. The filled circles show the contribution to $1/T_2$ of water protons of rat blood, at 37°C, induced by deoxygenation (*12*) and the vertical bars show the estimated uncertainty. In the present work, it is argued that this relaxation contribution arises from diffusion of blood water in the *intracellular* gradients of the local magnetic field that arise upn deoxygenation, which causes hemoglobin to become paramagnetic. The solid curve results from the application of the theory of secular relaxation in nonuniform fields to the interior of erythrocytes, assuming an intracellular lifetime of 3 ms (see text). The corresponding correlation time of 0.6 ms was taken from Thulborn *et al.* (*12*). The internal gradients are related to the toroidal shape of the blood cells (*11*) and a value of 500 s^{-1} was chosen as the renge of intracellular field variation. The dashed curve is the prediction assuming no limitations on the relaxation rate because of slow exchange.

least this factor. The solid curve, Fig. 5, assumes this, as well as the idea that the observed correlation time is associated with *intracellular* diffusion in the intracellular field gradients that arise from the toroidal shape of erythrocytes.

A realistic model for erythrocytes is a torus with a filled center, the latter with about 20% of the volume (*39*). Once aligned with the external field, different regions within the cells will have different fields because of differing demagnetization factors (*11*), which depend on geometry. Taking an average range for $\Delta\omega$ within the cells of 500 s^{-1} for an external field of 182 MHz and the measured correlation time of 0.6 ms, one can calculate the value of $1/T_{2Msec}$, the intracellular secular relaxation rate, for any field. Postulating a water lifetime τ_M in rat erythrocyte of 3 ms gives the solid curve in Fig. 5, using the expression (*26*)

$$1/T_{2sec} = f/(T_{2Msec} + \tau_M) \qquad [8]$$

for exchange between two environments. The dashed curve results if the limits set by exchange are ignored. (The value 500 s^{-1}, compared with the maximum of 2900 s^{-1} above, can readily be argued from the toroidal model and the proper expression for averaging the internal gradients, Eq. [22] of Ref. (*27*).)

Thus, we see that for deoxygenated, paramagnetic blood, the observed field-dependent values of $1/T_2$ can be encompassed by the theory of outer sphere relaxation as embodied in Eqs. [1a] and [1b], by applying it to diffusion in the intracellular field gradients of the erythrocytes, a novel result. Diffusion of water in the external field gradients adds little to $1/T_{2sec}$. Implicit in this interpretation is that $1/T_{2sec}$ of blood

(with intact erythrocytes) should be quite sensitive to temperature and to the shape of the erythrocytes, which can be altered drastically by altering the tonicity of the plasma, predictions which can be readily checked experimentally.

Ferromagnetic Particles

Solid magnetite. Lauterbur *et al.* (*40*) have shown microscopic MR images of ferromagnetic (magnetite) particles, ~ 5 μm diameter, suspended in agar gels. The images mimic the shape of the *d*-like dipolar distribution of magnetic field intensity produced by the particles and are relatively dark throughout a volume over 10 times the particle diameter. The very fact that the images display the shape of the local field indicates that the criterion for motional narrowing fails: in a time of order T_2, which is no longer than a few tens of milliseconds, the water protons do not diffuse very far compared to the scale of the particles. (The time for water to diffuse 25 μm is about 0.5 s, even assuming that the diffusion of water is unimpeded by the agar; it would be longer otherwise.) Thus, unless a Carr–Purcell or similar series of pulses is applied, each proton must be assumed relatively stationary in space with an apparent $1/T_2$ given by the Hahn expression, Eq. [4d], for the magnitude of the gradient at the position of that proton. The field at the surface of magnetized magnetite is about 0.13 T (*41*) and, for a particle with $R = 2.5$ μm, the gradient at the surface is 1.5×10^7 G cm^{-1}, but almost 10^6-fold smaller 75 μm away. With a pulse separation of, say, 5 ms, one can readily estimate the rate of decay of the transverse relaxation 75 μm away to be about 40 s^{-1}, corresponding to an apparent T_2 of 25 ms; this would produce a relatively dark region in an image. Because of the radial dependence of the gradient, this relaxation time increases as the eighth power of the distance of the protons from the particle. As a result, the particle should not alter image contrast much beyond 75 μm, a conclusion supported by their data.

Thus, for large, solid, ferromagnetic particles, the situation is almost macroscopic. Diffusion does not carry water very far compared to the range of the local fields experienced by a proton during a time of the order of a typical T_E pulse separation; therefore the usual Hahn computation (Eq. [4d]) can be used to find $1/T_{2sec}$ at each position in space around a particle. As noted by Lauterbur *et al.* (*40*), for particles the size of cells, the gradients are sufficient to produce a dark area over a volume ~ 1000 times that of the particle. For MRI microscopy, in which pixel sizes are not yet as small as cell dimensions (about 10-fold larger, linearly), this magnification is extremely useful. However, for clinical imaging, with much larger pixels, the contrast enhancement will be diluted by the size of the pixel volume relative to the volume of the region with significant gradients.

As the size of the particles is reduced, two things occur that influence the behavior of ferromagnetic agents: the surface field remains unchanged but the surface gradient increases, and the time τ_R required to diffuse out of range of a particle decreases. For $R \leqslant 100$ Å $= 10^{-2}$ μm, $\tau_R \leqslant 0.02$ μs, which is short enough for Eq. [2e] to hold and Eqs. [1a] and [1b] to be appropriate for computing both the secular and the nonsecular contributions that determine $1/T_2$ and $1/T_1$. In this limit, the procedure is precisely that used here in the analysis of ferritin, and thus should be straightforward. Mendonça-Dias *et al.* (*42*) have presented data for particles somewhat larger than this limit, 0.05

μ diameter magnetite. They find $1/T_2 = 10\ s^{-1}$ for 10 μm magnetite, corresponding to $\sim 4 \times 10^{-11}$ M particles. One can readily compute $\tau_R = 2.5 \times 10^{-7}$ s, at 25°C, $\delta\omega = 3.4 \times 10^7\ s^{-1}$, $f = 1.6 \times 10^{-6}$, and, from Eq. [2d], $1/T_2 = 55\ s^{-1}$, a rate not too far from the observed value.

For particles of solid magnetite of intermediate dimensions, the situation becomes more complex than for small particles in several ways. Though $\delta\omega$, the strength of the interaction, remains fixed, τ_R, the correlation time, becomes larger. As noted above, $1/T_{2sec}$ increases for fixed f until τ_R becomes so large that the condition for motional narrowing no longer holds. In essence, $1/T_2$ in the vicinity of the particles becomes so great that the particles relax before they "escape"; the situation becomes essentially one of slow exchange, as expressed by Eq. [3b]. At this point, the dependence of $1/T_{2sec}$ on particle size reverses sign. This occurs for the larger ferromagnetic particles as well; however, in this case, the volume around the particles is so large that one can compute a meaningful spatially dependent $1/T_2$. This is not so for the ferromagnetic particles of intermediate size for which the size scale is much too small compared to the smallest reasonable pixel size. Because of slow exchange, the observed relaxation rates will be less than predicted by Eqs. [1a] and [1b], but otherwise difficult to compute.

"Low-density magnetite." Recently, a novel composite material has been investigated for its potential utility as a contrast agent for the reticuloendothelial (RE) system (*43, 44*). These are proprietary materials (Advanced Magnetics, Inc., Cambridge, MA) that appear to be made of small (100–200 Å)-diameter magnetite ($FeO \cdot Fe_2O_3$) particles held together by a hydrophilic organic binder to form large, roughly spherical, entities about 0.5–1.0 μm in diameter. The magnetic material in the larger particles is highly diluted, to about 1/250 the density of magnetite, so that $\delta\omega$, the equatorial field at the surface, is also reduced by this same factor, to $\sim 5 \times 10^{-4}$ T at room temperature when these particles are magnetically saturated. There are about 200–2000 magnetite particles in each composite particle, presumably randomly oriented. We will refer to this composite material as "low-density magnetite," or LDM.

As in the conjectured model for the core of ferritin, LDM will be unmagnetized at zero field, even though each of their small magnetite core particles will always be fully magnetized (because of their ferromagnetism), since their orientations will be random and the net magnetization of each LDM particle will sum to zero. However, by application of B_0 sufficiently large to overcome the crystalline anisotropy energy of magnetite (*41*), LDM particles will become magnetized to the full saturation magnetization of magnetite at that temperature (corrected of course by the large dilution factor for these agents). Such behavior is sometimes called "superparamagnetism," since the field dependence of the magnetization of these materials has the form of a typical paramagnet, but the resulting magnetization is typically 200-fold greater than that of their paramagnetic analogs.

When introduced intravenously, LDM is scavenged by the RE system and, in liver, appears only in the Kupffer cells and not in the hepatocytes (*43*). Kupffer cells, about one-fifth the diameter of hepatocytes, make up but a few percent of the liver volume. The liver, magnetically, then becomes analogous to deoxygenated blood, with superparamagnetic Kupffer cells being the analog of erythrocytes, and the water of the hepatocytes, known to be very mobile (*7*), being the analog of plasma water. A major difference, however, is that f is much less for Kupffer cells in liver than for erythrocytes

in blood (cf. Fig. 2) and that the secular relaxation is all due to extracellular interactions (see below). Saini *et al.* (*43*) have reported a significant increase in $1/T_2$ of excised rat liver for rather large intravenous doses of LDM (333 μmol Fe/kg, at which dose the liver relaxation rate, and presumably the Kupffer cells, are saturated) and significant changes in MRI contrast for dosages 40-fold lower. Unfortunately, they do not report the concentration of LDM (Fe) that settled in the liver other than to demonstrate its location in the RE system. The question is whether the present theory can explain the observed relaxation rates.

LDM in liver. We will assume that 25% of the total bolus of 8 μmol/kg of LDM reached the liver RE system, a dosage that was found to "influence the signal intensity from the entire liver," which means that $1/T_{2sec}$ is of the order of $1/T_2$ of undoped liver, or $\sim 30\ s^{-1}$. Taking liver to be about 5% of the mass of a 500-g rat and the Kupffer cells to be 3% of the liver gives 1 g, or about 1 ml of tissue containing 1 μmol—or 1 mM—of Fe ions. The Kupffer cells then each contain four LDM particles, on average. Since each paramagnetic ion has an average moment of 2.8 Bohr magnetons at room temperature (*41*), one can readily calculate (using Eqs. [2b] and [2f]) that the equatorial field is small. Thus, $\delta\omega = 1.7 \times 10^{-3}\ s^{-1}$; $\tau_R = 3.3 \times 10^{-4}$ s, assuming 1 μm radius for the Kupffer cells; and $(\delta\omega)\tau_R = 0.5$, indicating that the limit of motional narrowing should be a good approximation. Substituting values for the terms in Eq. [2d] gives $1/T_{2sec} = 30\ s^{-1}$ for the outer sphere contribution to relaxation, in remarkable agreement with the data.

Exchange of water from the Kupffer cells is demonstrably too slow to contribute to $1/T_1$ and $1/T_2$. This can be estimated from the results of Holtz and Klaveness (*45*), who introduced starch granules labeled with Gd(DTPA) into Kupffer cells and saw no change in $1/T_1$ until the tissue was mechanically homogenized and the paramagnetic agent made accessible to all the liver water. Without going through the numerical details, it is readily shown that if exchange is too slow for the intracellular Gd(DTPA) to influence $1/T_1$ at the concentrations used, it is certainly too slow for LDM to contribute to $1/T_2$ by exchange of water between Kupffer cells and the rest of the liver.

LDM in blood. Saina *et al.* (*43*) also show that the effect of LDM on $1/T_2$ of whole blood is about 10-fold less than that for liver. Again, though quantitation is lacking, one can readily account for this difference; indeed it is just what one would expect for comparable concentrations of LDM in blood and liver, i.e., for the same value of f. In blood, the LDM is extracellular. In liver, the LDM is contained in the Kupffer cells, with a radius of about three times that of the LDM particles, which from Eq. [2b] gives a factor of 10 in τ_R and therefore in $1/T_{2sec}$.

DISCUSSION

The secular contribution to $1/T_2$ is produced by fluctuations in the component of the magnetic field that is parallel to the external static field. When these fluctuations arise from the diffusive motion of the protons through gradients in the external field B_0, the intrinsic fluctuation rate is relatively slow, and the nonsecular contributions to relaxation produced by fluctuations transverse to the direction of B_0 disperse away well below any imaging field. In this limit, the correlation time often becomes so long that B_1 cannot be conveniently made sufficiently small to avoid dispersive $1/T_{1\rho}$ effects. In addition, application of a Carr–Purcell–Meiboom–Gill sequence, with its phase-

shifted B_1 parallel to the transverse magnetization, can also introduce unsuspected $1/T_{1\rho}$-like effects (*46*).

It is often convenient to handle the situation of external gradients classically, using the Bloch equations. The same results can be obtained quantum mechanically by Fourier analysis of the fluctuations and computation of the appropriate transition rates of the protons. This latter procedure is usually reserved for the limit in which the scale of the spatial variation of the local field is of macromolecular dimensions or less. In this limit, one generally derives expressions for both the secular and the non-secular components of the relaxation rates, and the close connection of the respective contributions is readily apparent (Eqs. [1a] and [1b]). In the present work, we reviewed the theory of relaxation by diffusion of protons in the outer sphere environment of small uniformly magnetized spheres and demonstrated its relation to the case of larger particles, paralleled by the corresponding relation between the quantum-mechanical and classical approaches.

There appears to be no problem in describing (or at least rationalizing) relaxation by paramagnetic cells (e.g., deoxygenated erythrocyte suspensions and LDM-loaded Kupffer cells) and cell-sized ferromagnetic particles. Moreover, we would agree with the earlier conclusion of Packer (*30*) that diamagnetic cells (since the magnitude of their susceptibility is 100- to 1000-fold less than their paramagnetic counterparts) would make no observable contribution to $1/T_2$. For magnetized particles of intermediate size ($\sim$200 Å diameter; virus size), the situation is somewhat different. When ferromagnetic, the condition for motional narrowing breaks down and computation becomes difficult. For ferritin (and hemosiderin), which ideally is paramagnetic, there is a major discrepancy between theory and observation for $1/T_2$ at imaging fields. However, the demonstrated validity of the theory of outer sphere relaxation for much smaller paramagnetic complexes makes us conclude that the problem is in the material and not in the theory. We conjecture that there are regions of the ferritin core that are ferromagnetic; the problem has been relegated to biochemistry. It remains an important one, however, since ferritin deposits are often invoked to explain problematic dark spots in MRI of the brain (*47, 48*).

An important point that is apparent from the present work is that the nature of the spatial distribution of a fixed quantity of magnetic material can influence $1/T_2$ markedly; for improved relaxation enhancement, a smaller number of larger particles is preferable. As seen from Eq. [2d], both $1/T_1$ and $1/T_2$ increase as the square of the radius of the particles as long as the criterion for rapid exchange is not violated. Experimentally, this effect was used to explain the greater efficacy of LDM in liver compared to blood (*43*). Similar considerations will also apply to liposomes and synthetic vesicles loaded with paramagnetic cores, as have been investigated recently (*49*).

The application of the present work to relaxation in and near hematomas as they evolve clinically (*50*), an important application not considered here, should not be too difficult. The effects should be more akin to those of paramagnetic cells than ferromagnetic particles, since the density of Fe^{2+} and Fe^{3+} ions is in the range of millimolar rather than molar, as it is in erythrocytes and the cores of ferritin. The major difficulty in this case—and, we suspect, in many other cases—will most likely be in modeling the condition rather than in predicting the results of the model, judging from the ideas and conclusions presented here.

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