Review of Two Models for Water Proton Relaxation in Tissue

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I. INTRODUCTION

Pulsed NMR spectroscopy has been used extensively to study the motion of water in tissue by proton and deuteron resonance methods. While some studies (1-4) of this type had been done in the 1960's, activity in this area received a great stimulus from the report by Damadian (5) in 1971 that the proton spin-lattice relaxation time in the laboratory frame (T_1) was substantially longer for rat tumors than for apparently healthy tissue. In the many studies which followed, generally one of two types of measurements was made. In most studies, relaxation times were measured—mainly T_1 and the spin-spin relaxation time T_2. The results (6) were markedly different than for comparable measurements on pure water. T_1 generally was in the 300 900-ms range and T_2 was of the order of 50 ms, while for pure water at 20°C, T_1 and T_2 are equal and have values of 2.5 to 3.3 s (7), depending on purity. In other studies, the self-diffusion constant (D) of the water molecules was measured via proton resonance. For tissue, the value of D was found to be about half that observed for pure water (8).

At the simplest level, the fact that the diffusion constant for water in tissue was lower than that of pure water by only about a factor of two was usually interpreted to indicate that the mobility of most of the water in tissue was comparable to that of pure water (8). On the other hand, the relaxation times (both T_1 and T_2 are shorter in tissue than in pure water, and T_2 is about an order of magnitude shorter than T_1) were more like those in solids than in liquids. Interpretations (1,9) of these results have tended to center around models in which water may be in one of several phases or situations. Two examples of models used to interpret such relaxation time and diffusion constant results are reviewed below.

II. TWO-PHASE MODELS

Within a heterogeneous environment such as tissue, many situations would appear possible for water molecules, ranging from the free liquid to water of hydration bound to sites on macromolecules. The simplest model based on this realization is a two-phase model in which the water molecules of one phase have more rotational and/or translational freedom than those of the other.

Referring to the water phase with a longer relaxation time as "free" and to that with a shorter relaxation time as "bound," the differential equations for

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the time dependence of the observed magnetization are

$$\frac{dM_f}{dt} = \frac{-M_f}{T_f} - \frac{M_f}{\tau_f} + \frac{M_b}{\tau_b} \quad (1)$$

$$\frac{dM_b}{dt} = \frac{-M_b}{T_b} - \frac{M_b}{\tau_b} + \frac{M_f}{\tau_f}$$

where τ_f and τ_b are, respectively, the mean times of a molecule in the free and bound phases. That is, for T_f, $T_b = T_{1f}$, T_{1b}, respectively,

$$M_f = M_{zf(t)} - M_{zf(0)} \quad (2)$$
$$M_b = M_{zb(t)} = M_{zb(0)}$$

while for T_f, $T_b = T_{2f}$, T_{2b} respectively,

$$M_f = M_{xf(t)} \quad (3)$$
$$M_b = M_{xb(t)}$$

in the rotating frame.

Several extreme cases are noteworthy:

1. Very slow exchange: τ_f, $\tau_b >> T_f$, T_b

$$M_{(t)} = M_o\{f_b e^{-t/T_b} + f_f e^{-t/T_f}\} \quad (4)$$

where f_b and f_f are the equilibrium fractions of the populations in the two phases. This case has not appeared to be applicable to tissues or biopolymer water systems for T_1 because the plots are very nearly linear. However there is one notable exception (11). By comparing proton T_1 in the liver of live and dead mice, it was found that the decay of the magnetization from the liver of the live mouse was not exponential. This result was interpreted assuming that the two water phases were intercellular and intracellular: in the live state, the exchange time for water molecules is long and the relaxations of the two phases are distinguishable, while after the animal has been dead for at least a few minutes, exchange between inter- and intracellular water is faster and the data may be represented by a single relaxation time (11).

2. Fast exchange: τ_f, $\tau_b >> T_f$, T_b

A single relaxation rate is observed

$$1/T_{obs} = (f_b/T_b) + (f_f/T_f) \quad (5)$$

3. Minor fraction: $f_b << f_f$ $T_b < T_f$

$$1/T_{obs} = [f_b/T_b + \tau_b] + f_f/T_f \quad (6)$$

If $\tau_b << T_b$ as well, this case is identical to that of fast exchange.

In applying the fast-exchange model to the measurements in tissues, it has been assumed that the correlation time for motion of the free water, τ_{cf}, is short, *i.e.*, $\omega_L^2\tau_{cf}^2 << 1$, so

$$1/T_{1f} = 5K_f\tau_{cf} \quad (7)$$
$$1/T_{2f} = 5K_f\tau_{cf}$$

ω_L is the Larmor frequency and K_f is a constant representing the strength of the local magnetic field fluctuations from the motion responsible for the relaxation. Molecular motion for the bound water is assumed to be slow, *i.e.*, $\omega_L^2\tau_{cb}^2 << 1$, so

$$1/T_{2b} = 3K_b\tau_{cb}/2 \quad (8)$$

$$1/T_{1b} = K_b\left[\frac{\tau_{cb}}{1 + \omega_L 2\tau_{cb}2} + \frac{4\tau_{cb}}{1 + 4\omega_L 2\tau_{cb}2}\right]$$

Unknown parameters are K_b, K_f, τ_{cb}, τ_{cf}, τ_f, τ_b, and f_f. Both estimates of K_f and K_b and measurements for two frequencies are needed to characterize all parameters. Depending on estimates of certain parameters, it was concluded (12) that T_{1f} is equal to T_1 of free water, but other studies (13) indicated that T_{1f} may be significantly less than the value for free water.

For tissue, the two-phase model is a useful starting point but an over-simplification. It was suggested (9) that the bound fraction is very small, so the relaxation rates for the minor fraction approximation would be more appropriate, but neither model is general enough to account for all observed results. However, the minor fraction model was successfully applied (14) to the relaxation of water in dilute solutions of globular proteins where the correlation time for the bound water relaxation process is associated with random reorientation of the solute molecule.

III. EXPERIMENTAL STUDIES

Studies of spin relaxation of water in tissue may be divided into two types: 1. those done on related systems or on tissue at various temperatures, and 2. those using NMR parameters other than proton T_1 and T_2.

Studies of simpler or model systems. Examples range from protein-water systems (15) to single-cell studies, for example, on egg (16) or a single nerve (17). Such systems have the immediate advantage of

eliminating possible confusion between inter- and intracellular water.

Temperature dependence of spin relaxation time. At least two aspects of the theory have been tested with such studies. One is the expectation that for correlation times between the fast and slow exchange limits for a two-phase situation, there should be a temperature region in which the slope of T_2 vs temperature is reversed. This was found in measurements on a giant barnacle muscle cell (17) but not for other systems. Another aspect is the phenomenon commonly referred to as "non-freezeable water" (18,19). Below 0°C, where the proton signal of ice gives a T_2 of about 5 μs, a fraction of the water molecules in tissue and protein-water systems appear to retain higher mobility and a relatively long T_2 (about 100 μs). The temperature dependence of this water has been studied to try to determine an expression for the correlation time which could be extrapolated to represent the bound water in the 0-50°C range.

Comparison of relaxation times for different types of tissue. These include comparisons of measurements on the livers of live and dead mice (11), measurements on different organs (20), measurements on healthy vs tumorous tissue (21-23), and measurements on mature and growing tissues (20). Many of the mature-vs-growing and healthy-vs-tumorous tissue differences in T_1 were found to be related to water content: tissues with longer T_1 values contain more water (21-24). These studies were recently reviewed in this journal (25).

More precise measurements of magnetization decay. When measured carefully over a wider range of magnetization values, non-exponential decay has been observed in T_1 measurements on live mouse liver (11) and in $T_{1\varrho}$ (25) and T_2 (13) measurements on many samples. Line width measurements yield comparable results (2). At least three components were identified in the T_2 decay of the proton signal in rat muscle tissue (13). These studies show the need for interpretation in terms of a multi-component model.

Frequency dependence (dispersion) of relaxation rates. Such studies have been done for T_1 over a wide frequency range (26) and for $T_{1\varrho}$ over a wide (two orders of magnitude) H_1 range (27). Some of these dispersion curves could be fitted fairly well with a single correlation time but a distribution of correlation times gave a closer fit.

Relaxation times of other isotopes, notably deuterons and ^{17}O. For free water, the relaxation rates of different isotopes should be in simple ratios. Such studies (27) should therefore be useful in determining the fraction of water which is free in a system and the correlation time for its motion.

The self-diffusion constant D of water. In measurements made on tissue (6), the observed values of D were generally not much different from those for free water. These results were interpreted to indicate that the mobility of most water in tissue is similar to that of pure water. Measurements of the D of deuterated water in rat muscle and brain tissue were interpreted with the assumption of a D comparable to that of free water and the presence of many closely spaced barriers to diffusion (28).

IV. DIFFUSION-BASED MODEL

If one places relatively greater confidence in the reliability and interpretation of the diffusion constant measurements than in relaxation time measurements, a different starting point many be chosen for interpreting relaxation time measurements (29,30). The model assumes that water molecules diffuse through the system and that all significant relaxation occurs at sites (volumes and/or surface regions) where deviations of the magnetization from equilibrium are quickly reduced to zero (magnetization sinks).

The observed magnetization decay then depends on geometry (locations of magnetization sinks) and on the water molecule diffusion. The magnetic moment per unit volume $\varrho(r,t)$ *is determined by the equation*

$$\nabla \cdot D \cdot \nabla \varrho - \gamma \varrho = \frac{\partial \varrho}{\partial t} \qquad (9)$$

where D is the tensor of diffusion coefficients and γ is the volume magnetization sink strength density. The solutions $\varrho(r,t)$ are a sum of normal decay modes

$$\varrho(r,t) = \sum_{n=0}^{\infty} A_n F_n(r) \exp\{-t/T_n\} \qquad (10)$$

where the A_n are constant coefficients and the F_n are spatially dependent orthogonal eigenfunctions which must satisfy the boundary conditions

$$-\nabla \cdot (D \cdot \nabla F_n) + \gamma F_n = F_n / T_n \qquad (11)$$

$$(n \cdot D \cdot \nabla F_n + \sigma F_n)_{surfaces} = 0$$

where σ is a surface magnetization sink strength density. The resulting time constants are ordered: $T_o > T_1 > T_2 - - - > 0$ and may represent spin-lattice or spin-spin decay. This equation is solved for three simple geometries (planar with boundaries at $z = 0, a$; cylindrical of radius a; and spherical of radius a) with the approximations that $\gamma = 0$, D is a scalar, and the active surface is at z or $r = a$ (29,30). The diffusion rate may be characterized by three regions:

Fast diffusion—$Sa/D << 1$

Decay is via a single exponential, i.e., only the lowest decay mode is evident.

Intermediate diffusion—$1 < Sa/D < 10$

The first few modes above the lowest contribute a few percent to the intensity.

Slow diffusion—$Sa/D >> 1$

The lowest mode contributes 60-80%, and several higher modes each contribute a few percent to the intensity.

In these equations, $S = (1/A) \int \sigma (r)\, da$, is the average of σ over the active surfaces. For fast diffusion, the mode T_o depends on $1/S$ but not on D, while in the slow diffusion limit, T_o is determined by D and is independent of S. To exhibit the higher decay modes, conditions must meet or very nearly meet those of the slow diffusion region. For two decay modes to be observable within the typical NMR range of 10 μs to 0.2 s, "a" must be in the range of 1 to 40 x 10^{-6} m for the geometries solved, if D is assumed to be 2.5 x 10^{-9} m^2/s. This range for "a" is of the order of the diameter of a muscle cell.

The treatment of an annular cylindrical region was applied to T_2 data for rat muscle which had been analyzed assuming four time constants (13). The decay components were identified as:

Proton Source	T_i	*Relative Amplitude*
protein molecules	20 μs	0.2
bound water	0.4 ms	0.08
intracellular non-bound water	45 ms	0.82
extracellular water	200 ms	0.1

The "bound water" and "intracellular non-bound water" portions were viewed as two components within the two-phase model, possibly with intermediate exchange.

Within the diffusion model, the longest-lived component was again assumed to be due to extracellular water, and the remaining magnetization was analyzed in terms of normal decay modes

$$\frac{M_t - A_{extra} \exp(-t/T_{extra})}{M_o} = (1 - A_{extra}) \sum_{n=o}^{\infty} A_n \exp(-t/T_n) \qquad (12)$$

The first three modes and their contributions to the total signal are

n	T_n, ms	Fraction of Signal, %
0	45	88.4
1	11	0.8
2	4.7	6.8

The calculation assumed that the ratio of the outer radius "b" to the inner radius "a" was $R > 2.37$, with σ infinite at "a" and finite at "b". For D equal to half the value in free water (i.e. 1.25 x 10^{-9} m^2/s), the cell diameter "$2b$" is 62 x 10^{-6} m.

In comparing the two models, it should be noted that both span the regions of exponential decay and fast spin transport (exchange or diffusion) and non-exponential decay and slow exchange or diffusion. It should also be noted that in the intermediate exchange region of the two-phase model, the two decay times are eigenvalues which do not correspond directly to the decay times of the two original populations of the model (10,13).

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