MODELING OF PROTON SPIN RELAXATION IN MUSCLE TISSUE USING NUCLEAR MAGNETIC RESONANCE SPIN GROUPING AND EXCHANGE ANALYSIS

W. T. SOBOL, I. G. CAMERON, W. R. INCH AND M. M. PINTAR
Department of Physics, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1

ABSTRACT NMR spin relaxation experiments performed on healthy mouse muscle tissue at 40 MHz and 293 K are reported. The spin-lattice relaxation experiments were performed using different combinations of selective and nonselective radio frequency pulses. Relaxation experiments in the rotating frame at H_1 = 10, 5 and 1 G are also reported. The experimental results were analyzed using the spin-grouping method, which yields the sizes of the resolved magnetization components as well as their T_2's and T_1's (or $T_{1\rho}$'s) for the nonexponential relaxation functions. These results were analyzed further for the exchange between different spin groups. It has been found that to explain all of these experimental data it was necessary to use a four-compartment model of the muscle tissue that consists of a lipid spin group, a "solid-like" spin group (mainly proteins), a "bulk water" spin group and a "bound water" spin group. The chemical exchange rate between "bulk" and "bound" water was found to be 29 ± 9s^{-1} at room temperature. The exchange rate between the bound water and the solid moderator was estimated to be ~500 s^{-1}.

INTRODUCTION

When NMR was first used to study tissues it was felt that at high magnetic fields there should always be strong mixing of different proton spin groups by fast exchange of magnetization. Thus the overall spin-lattice relaxation was expected to be characterized by one relaxation time and no real effort was made to determine how good this approximation was. Indeed, the experimental recovery function, unless studied very carefully, does appear to be a single exponential recovery. The early experimental results at high fields showed good agreement with the predictions of this picture, however, when the first experiments in the rotating frame were performed, it became quite clear that the results of these experiments could not be explained using the strong mixing assumption (1–3). For lack of a better approach, though, the results were still analyzed using this approach.

If it were true that mixing by exchange is indeed fast in all situations then the probing of its value would not be very important in NMR since its only effect would be to average the properties of the individual spin groups. If, on the other hand, the mixing is not strong, an analysis of the effect of mixing on the recovery of the proton spin magnetization in tissues is quite necessary since it may have a drastic effect on the NMR parameters. In 1978 this research was steered in the proper direction by a very timely experiment in which a direct observation of the rate of mixing of distinct proton spin groups was made (4). At about the same time a new method of observation was proposed for improving the resolution of the recovery function (5). This technique, now called spin grouping, enables the determination of the component magnetization recoveries from the measured composite recovery function. Having a new method for the study of the relaxation recovery as well as new experiments to disentangle the exchange rate with which the major component magnetizations mix in tissues, it was then possible to obtain from the observed relaxation parameters in tissues the inherent (or "true") relaxation rates and the actual sizes of the spin groups involved. With these inherent parameters a realistic two-mode dynamics has been employed to model the three relaxation times and their dispersivity. The results obtained for mouse muscle tissue are reported in this article.

METHOD OF EXCHANGE ANALYSIS

The formulas used in the exchange analysis of spin grouping results are obtained with the method first described by D. E. Woessner in 1961 (7) although several other methods are also available (4, 6, 8–13).

Assuming that the sample consists of two coupled spin reservoirs "a" and "b," with respective equilibrium magnetizations M_{oa} and M_{ob}, the normalized relaxation recovery functions are introduced for a 180°-τ-90° inversion recov-

W. T. Sobol is on leave from the Silesian University, Katowice, Poland.

W. R. Inch's present address is Experimental Oncology Group, The Ontario Cancer Treatment and Research Foundation, London Clinic, London, Ontario, Canada N6A 4G5.

ery spin-lattice relaxation experiment. They are given by

$$m_a(\tau) = -\frac{M_{za}(\tau) - M_{oa}}{2M_{oa}}, \quad (1)$$

$$m_b(\tau) = -\frac{M_{zb}(\tau) - M_{ob}}{2M_{ob}}. \quad (2)$$

The initial conditions for both spin reservoirs, $m_a(0)$ and $m_b(0)$ are created by the (first) preparation pulse (180°), which is of duration $\Delta = \pi/\gamma H_1$. In this expression γ is the gyromagnetic ratio for protons and H_1 is the amplitude of the r.f. field. If the pulse is very short ($\Delta \simeq 1\mu s$), but H_1 is large, it will be described as a "hard" pulse, while if it is a long pulse ($\Delta \simeq 200$ μs, or more), with a correspondingly smaller value of H_1, it will be referred to as a "soft" pulse. Sometimes these pulses are also described as "nonselective" and "selective" excitation pulses, respectively. We are primarily interested in the case in which one of the spin groups exhibits a solid-like behavior (the free induction decay, FID, is Gaussian in shape and the spin-spin relaxation time, T_2, is of the order of 10 μs), while the other spin group is liquid-like (the FID is exponential with T_2 of the order of 10 ms or longer). In this case a "hard" preparation pulse inverts the magnetization components of both groups while a "soft" pulse, with $\Delta > M_2^{-1/2}$, where M_2 is the second moment of the solid line, inverts the liquid magnetization (if $\Delta < T_2$ of the liquid) without affecting the solid magnetization. Thus, if we identify the solid-like spin group with the "a" reservoir and the liquid-like group with the "b" reservoir, we find that for a "hard" preparation pulse the initial conditions are

$$m_a(0) = 1 \text{ and } m_b(0) = 1, \quad (3)$$

whereas for a "soft" preparation pulse we have:

$$m_a(0) = 0 \text{ and } m_b(0) = 1. \quad (4)$$

In the 180°-τ-90° spin-lattice relaxation experiment there are three combinations of "hard" and "soft" pulses which are of interest. In high power NMR the h-h sequence, which has a "hard" preparation as well as a "hard" monitoring pulse, is used most frequently whereas in high resolution NMR and in NMR imaging the s-s sequence, where both pulses are "soft," is more common. The s-h sequence, which consists of a "soft" preparation and a "hard" monitoring pulse, was first introduced by Edzes and Samulski (4) for studying the rate of mixing of distinct proton spin groups.

In the case of a h-h experiment the observed response of a sample can be written as

$$F(t,\tau) = \{[M_{oa} - M_{za}(\tau)] \exp(-M_{2a}t^2/2)\}/2(M_{oa} + M_{ob}) + \{[M_{ob} - M_{zb}(\tau)] \exp(-t/T_{2b})/2(M_{oa} + M_{ob}) \quad (5)$$

where the measured equilibrium magnetization M_o is equal to $M_o = M_{oa} + M_{ob}$. The calculation leads to the result

$$F(t,\tau) = [p_a C_a^+ \exp(-M_{2a}t^2/2) + p_b C_b^+ \exp(-t/T_{2b})] \exp(-\lambda^+\tau) + [p_a C_a^- \exp(-M_{2a}t^2/2) + p_b C_b^- \exp(-t/T_{2b})] \exp(-\lambda^-\tau). \quad (6)$$

In this formula p_a and p_b denote the relative sizes of both spin groups while λ^+ and λ^- are the apparent relaxation rates. The observed (apparent) magnetization fractions $p_a C_a^{\pm}$ and $p_b C_b^{\pm}$ of the relaxation recovery function $F(t, \tau)$ are determined by the initial conditions (3) and the inherent relaxation parameters of the studied system.

The explicit formulas for coefficients $C_{a,b}^+$, $C_{a,b}^-$ and rates λ^+, λ^- were published earlier. (4, 7, 9).

When a s-h experiment is performed the experimental function is still given by Eq. 6 with $M_o = M_{oa} + M_{ob}$ but the initial conditions in this case are given by Eq. 4. This causes the relaxation function $F(t, \tau)$ to change considerably and, as a result, the observed magnetization fractions in the s-h experiment are substantially different from those obtained when the h-h sequence is used. In the s-s experiment a "soft" 90° pulse is used initially to determine the equilibrium value of the magnetization $M_o = M_{ob}$. Consequently,

$$F(t,\tau) = \frac{[M_{ob} - M_{zb}(\tau)] \exp(-t/T_{2b})}{2M_{ob}} = F(t,\tau) = (C_b^+ e^{-\lambda^+\tau} + C_b^- e^{-\lambda^-\tau}) \exp(-t/T_{2b}). \quad (7)$$

In the case of a $T_{1\rho}$ experiment, where the pulse sequence consists of a 90° pulse followed by a spin-locking pulse of duration τ, the preparation pulse (90°) can be either "hard" or "soft." The use of a "soft" preparation pulse, however, does not offer any advantage and will not be considered here. For a $T_{1\rho}$ experiment with a "hard" preparation pulse the measured response of the sample can be written as

$$F(t,\tau) = \frac{M_{x'a}(\tau) \exp(-M_{2a}t^2/2) + M_{x'b}(\tau) \exp(-t/T_{2b})}{M_{x'a}(0) + M_{x'b}(0)} \quad (8)$$

The theoretical result has the form of Eq. 6 with values of all the variables describing the $T_{1\rho}$ experiment.

In this case, we define $m_a(\tau)$ and $m_b(\tau)$ by

$$m_a(\tau) = \frac{M_{x'a}(\tau)}{M_{x'a}(0)}, \; m_a(0) = 1, \quad (9a)$$

$$m_b(\tau) = \frac{M_{x'b}(\tau)}{M_{x'a}(0)}, \; m_b(0) = 1, \quad (9b)$$

instead of by Eqs. 1 and 2.

The presented results describe the relaxation behavior of any exchange rate in a two-compartment spin system. All of the properties of the relaxation recovery functions can be described, provided that we know the size of one spin reservoir (e.g. p_a), one exchange rate (e.g. k_a), and the inherent relaxation rates R_a and R_b. In practice, however, the situation is reversed since normally the available

information is on the recovery function. Thus, we actually want to recover from the experimental (or apparent) relaxation data the inherent (true) relaxation rates R_a and R_b, the true spin reservoir sizes p_a and p_b and the exchange rates k_a and k_b. Usually spin grouping analysis can be used to provide clues about the size of p_a (from the magnetization fractions $p_aC_a^{\pm}$, $p_bC_b^{\pm}$) as well as information about the exchange rate k_a (from the value of the apparent fast relaxation rate λ^+, measured in a s-h or s-s relaxation experiment). One can then use this initial information, parametrize the values of p_a and k_a and solve the set of rate equations for R_a and R_b. This procedure yields

$$R_a^{1,2} = \frac{1}{2}[-2k_a + (\lambda^+ + \lambda^-) \pm \sqrt{(\lambda^+ - \lambda^-)^2 - 4k_ak_b}] \quad (10)$$

$$R_b^{1,2} = \frac{1}{2}[-2k_b + (\lambda^+ + \lambda^-) \pm \sqrt{(\lambda^+ - \lambda^-)^2 - 4k_ak_b}]. \quad (11)$$

Only one set of these solutions $R_{a,b}^1$ or $R_{a,b}^2$ is physically meaningful. The choice has to be made using the calculated values of $C_{a,b}^{\pm}$.

The magnetization fractions $p_aC_a^{\pm}$ and $p_bC_b^{\pm}$ can be calculated in terms of p_a and k_a. This procedure can be repeated iteratively until reasonable values of R_a and R_b are determined and an acceptable fit for the magnetization fractions in all experiments (h-h, s-h, s-s and $T_{1\rho}$) is obtained. In addition, this correlation-iteration procedure yields values for p_a, p_b, k_a, and k_b.

The main weakness of the model presented above when used in the study of tissues is obviously the assumption that the sample can be considered as a system with only two spin reservoirs. The detailed analysis of $T_{1\rho}$ experiments indicates that at least three compartments have to be considered: the "solid" compartment (protons on rigid macromolecules), the "bound" water compartment (protons on the water molecules bonded within the macromolecular hydration shells) and the "free" or bulk water compartment. However, it is not practical to generalize the procedure outlined above to describe a three compartment spin system analytically since the increased number of free parameters would make the description ambiguous and the procedure laborious. Fortunately, from $T_{1\rho}$ experiments in tissues there is experimental evidence that the "solid" and "bound water" spin reservoirs are approximately equal in size and that the exchange rate between them (k_s) is much faster than the exchange rate between the "bound" and "free" water (k_a). In this case the relaxation recovery function $F(t, \tau)$ is

$$F(t, \tau) = \{[M_{os} - M_{zs}(\tau)] \exp(-M_{2s}t^2/2) + [M_{or} - M_{zr}(\tau)] \exp(-t/T_{2r}) + [M_{ob} - M_{2b}(\tau)] \cdot \exp(-t/T_{2b})\}/2(M_{os} + M_{or} + M_{ob}), \quad (12)$$

where the subscripts s, r, and b refer to the "solid-like" component, the "bound" water component and the "free" water component, respectively.

To account for the very fast exchange process consider the phenomena on a time scale which is long compared to k_s^{-1}, but short in comparison with k_a^{-1}. Since the exchange between the "solid" reservoir and the "bound" water reservoir is assumed to be fast we can use the results from the two-site case to write the following:

$$m_s(\tau) = [m_s(0) - m_r(0)]\, p_s' e^{-2k_s\tau} + \{m_s(0) - p_s'[m_s(0) - m_r(0)]\}e^{-R_s\tau}, \quad (13a)$$

$$m_r(\tau) = [m_r(0) - m_s(0)]\, p_s' e^{-2k_s\tau} + \{m_r(0) - p_s'[m_r(0) - m_s(0)]\}e^{-R_s\tau}, \quad (13b)$$

where we consider $p_s' = p_r' = 0.5$, and

$$R_a = p_s'(R_s + R_r), \quad (14)$$

where R_s and R_r denote the inherent relaxation rates for "solid"-like protons and "bound" water protons, respectively. Since it is quite possible that the rate $2k_s$ (which is very fast) may not be seen in the experimental recovery function, we have neglected the first term in Eqs. 13a and 13b and have incorporated them into the two-site exchange equations for the description of the slower chemical exchange processes between "bound" water protons and "bulk" water protons. The result for the experimental recovery function (12) becomes

$$NF(t, \tau) = \{p_s[n \exp(-M_{2s}t^2/2) + \exp(-t/T_{2r})]C_a^+ + p_bC_b^+ \exp(-t/T_{2b})\}e^{-\lambda^+\tau} + \{p_s[n \exp(-M_{2s}t^2) + \exp(-t/T_{2r})]C_a^- + p_bC_b^- \exp(-t/T_{2b})\}e^{-\lambda^-\tau}, \quad (15)$$

where

$m_a(0) = 1$, $m_b(0) = 1$, $n = 1$, and $N = 2p_s + p_b = 1$ for the h-h sequence;

$m_a(0) = 0.5$, $m_b(0) = 1$, $n = 1$, and $N = 2p_s + p_b$ for the s-h sequence;

and

$m_a(0) = 0.5$, $m_b(0) = 1$, $n = 0$, and $N = p_s + p_b$ for the s-s sequence.

This methodology will now be applied to analyze the proton spin relaxation in mice muscle. On the basis of the results of this analysis molecular dynamics of water will be modeled.

EXPERIMENTS AND MATERIAL

All experiments were performed in vitro on samples prepared from young mice of the C3He/J (10 animals) or Balb/c (six animals) strains. Hind leg striated muscles were blotted free of excess blood and then placed in 8 mm OD glass tubes. A typical sample volume was $\simeq$0.1 cm^3. All samples were flame sealed to prevent water losses and reduce catabolic changes of the tissue and stored at ~1°C. The measurements were performed on the tissues between 3 and 32 h after the tissue was obtained. No change in the relaxation times was observed over this time period.

NMR relaxation measurements were performed at 20°C using a Bruker SXP 65 spectrometer operating at 40 MHz. The spin-lattice relaxation data were obtained using a 180°-τ-90° pulse sequence. The value of the H_1 field created by both preparation (180°) pulse and monitoring (90°) pulse was independently hardware controlled by using a $T_{1\rho}$ circuit board and by adjusting the power amplifier gain. Each pulse could be sent as a short, high power "hard" pulse (Δ for 180° $\simeq$ 3 μs, for 90° $\simeq$ 1.5 μs) or as a longer, low power "soft" pulse (Δ for 180° $\simeq$ 200 μs, for 90° $\simeq$ 100 μs). These pulses were combined to give three 180°-τ-90° sequences. The first of these was when both pulses were "hard" (h-h sequence) while the second one consisted of a "soft" preparation pulse followed by a "hard" monitoring pulse (s-h sequence). The third sequence which we found useful had "soft" pulses for both preparation and monitoring (s-s sequence). Experiments in the rotating frame were performed using a standard spin locking sequence consisting of a 90° pulse followed immediately by a spin-locking pulse of duration τ. The preparation (90°) pulse in this sequence was always "hard." In each experiment up to 33 free induction decay (FID) signals (for different τ's) were recorded and digitized using a Biomation 805 sample and hold unit. These signals were then stored for further analysis using a Hewlett-Packard 9845A computer.

The experimental data were analyzed with the spin-grouping technique (5). With this technique the composite decay functions were resolved into component decays and the fraction of the total magnetization which is associated with each component (having different T_1 or T_2 values) was determined. Since the T_2 data were recovered from the shape of the FID signals, the longest values obtained were limited by the inhomogeneity of the static magnetic field.

RESULTS

The relaxation function for muscle tissue, which was measured at 40 MHz, was resolved into components using the spin grouping method (5). The high field results for the h-h, s-h and s-s excitations are shown in Table I and Figs 1 *a* to 1 *f*. A few characteristic recovery functions are presented in Figs 2 *a* to 2 *d*. The characterization of the h-h recovery function at high fields was rather ambiguous although the results of the analysis, in which the recovery function was considered to be a sum of two exponential components, agree quite well with the analysis of the recovery function in the rotating frame. When the s-h excitation is used we observe a fast relaxing component, which we associate with exchange. From this component the exchange rate and the amount of magnetization involved in the exchange can be determined. The FID's associated with the medium and long T_1 values (see Fig. 1 *d*), are similar to the ones obtained with the h-h excitation. The exchange component, however, grows from a rather small value to $\simeq$6% of the total magnetization. This indicates that some of the inverted liquid exchanges magnetization quickly with the solid magnetization which initially was unaffected by the selective "soft" inversion pulse.

The effect of the selective inversion can also be seen in the recovery function. In Fig 2 *b* the fulfilment of the selective inversion condition is indicated by a jump in the magnetization at zero time (seen as a difference between the star at 0.1 of the magnetization and the third component intercept). The value of this jump should be equal to the size of the solid magnetization. The negative solid magnetization FID can be seen clearly at short time windows on the FID corresponding to the exchange compo-

TABLE I
NORMAL MOUSE MUSCLE TISSUE: PROTON NMR RELAXATION PARAMETERS

Experimental mode	T_2	Measured apparent values			Modeled apparent values				Inherent (true) values (derived by modeling)	
		T_1	M_o		T_1	M_0	p_bC_b	p_aC_a		
			Total	No lipid					T_1	M_o
	ms	*ms*	%	%	*ms*	%			*ms*	%
h-h	4 ± 1*	850 ± 50	73.8 ± 3	87.6 ± 3	860 ± 1700	89.7 ± 30	79.4 ± 30	10.3 ± 2	1045 ± 1450	79§
	0.025 ± 0.003		10.4 ± 2	12.4 ± 2		10.3 ± 2		10.3 ± 2	511 ± 1350‡	10.5‖
	1 ± 0.5	420 ± 40	15.8 ± 2							
	—	—	—	—	26 ± 3.8	−0.2 ± 2	−0.4 ± 4	0.2 ± 2	511 ± 1350‡	10.5¶
						0.2 ± 2		0.2 ± 2		
s-h	4 ± 1*	860 ± 30	67 ± 3	82.2 ± 3	860 ± 1700	80.4 ± 30	71.1 ± 30	9.3 ± 2		
	0.025 ± 0.003		6.5 ± 2	8.0 ± 2		9.3 ± 2		9.3 ± 2		
	1 ± 0.5	480 ± 30	18.5 ± 2							
	1 ± 0.5	50 ± 10	6 ± 2	7.4 ± 2	26 ± 3.8	3.8 ± 4	7.8 ± 2	−4 ± 2		
			−6 ± 2	−7.4 ± 2		−4.0 ± 2		−4 ± 2		
		0	8 ± 2	9.8 ± 2		10.5				
s-s	4 ± 1*	860 ± 30	72.1 ± 3	89.0 ± 3	860 ± 3.8	90.2 ± 20	90.2 ± 20			
	1 ± 0.5	480 ± 15	19.0 ± 2							
	1 ± 0.5	26 ± 2	8.9 ± 2	11.0 ± 2	26 ± 3.8	9.8 ± 3	9.8 ± 3			

*Apparent T_2 determined by the inhomogeneity of the magnetic field.
‡Common relaxation rates of the solid and bound water protons.
§"Free", or bulk, water protons.
‖"Bound" water protons.
¶"Solid" protons.

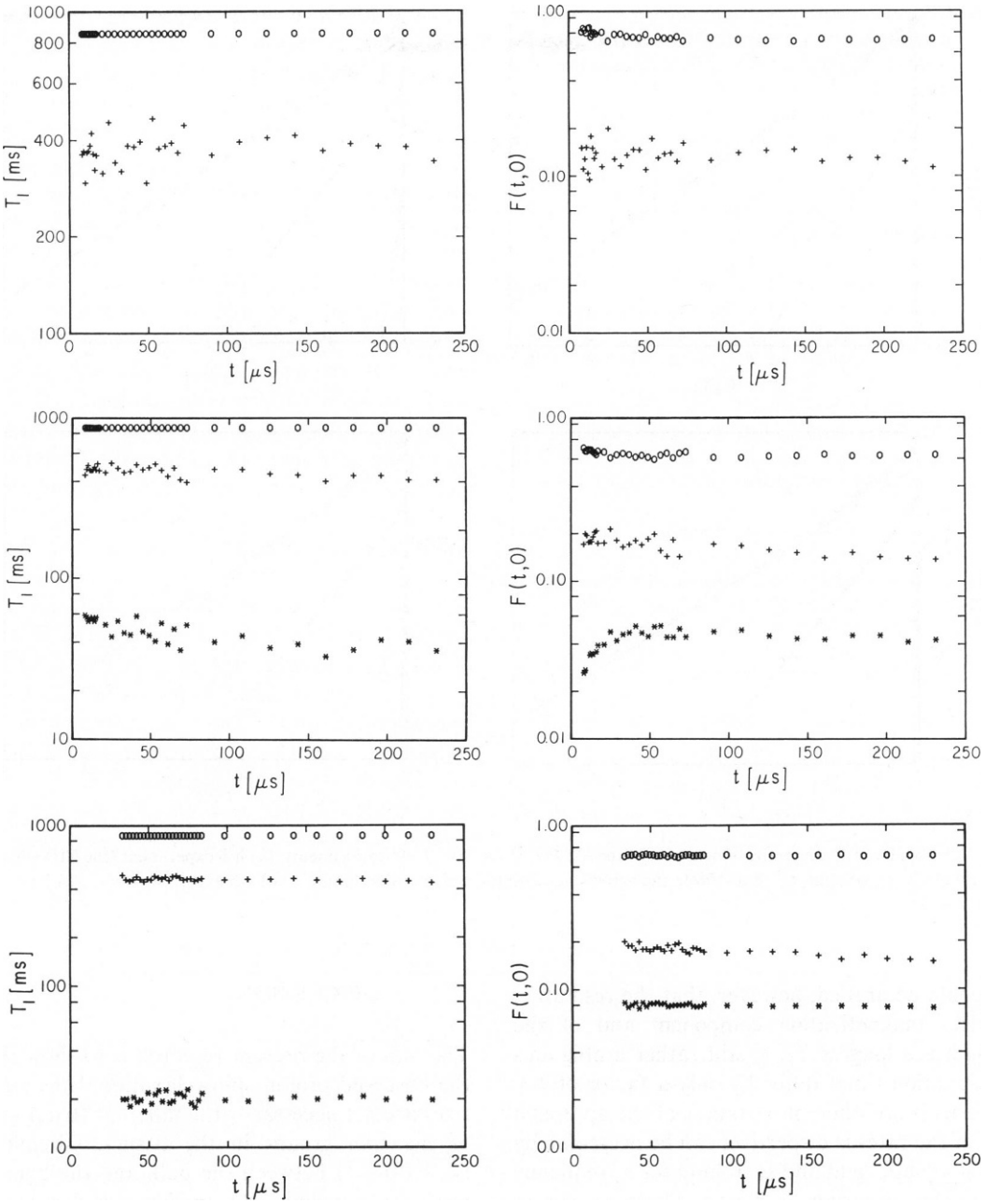

FIGURE 1 The results of the spin-grouping analysis of T_1 experiments in a mouse muscle tissue: relaxation time window dependences for the h-h (*a*), s-h (*c*), s-s (*e*) experiments and the reconstructed component FID shapes for the h-h (*b*), s-h (*d*) and s-s (*f*) experiments, respectively.

nent with the shortest relaxation time, see Fig 1 *d*. At time windows further along on the FID, however, (see Fig. 2), the exchange component is no longer overlapped by the solid magnetization component. Furthermore, the magnetization associated with the short relaxation time at these windows has increased as a result of the magnetization transfer, which by this point is complete.

The s-s excitation also shows the effect of exchange which, in this case, is not obscured even at the first windows on the FID because the monitoring pulse in the s-s sequence does not monitor the solid magnetization. Consequently the total magnetization in the s-s experiment is different in magnitude from the total magnetization in a s-h or h-h experiment. The difference in total magnetizations is comparable to the size of the solid proton spin-group signal ($\simeq$10%).

The recovery function following the rotating frame spin locking pulse sequence agrees well with the high field spin grouping analysis. Fortunately, in the rotating frame the observed relaxation rates differ more than the corresponding rates in the lab frame. This makes the spin grouping analysis in the rotating frame more reliable (see Fig.

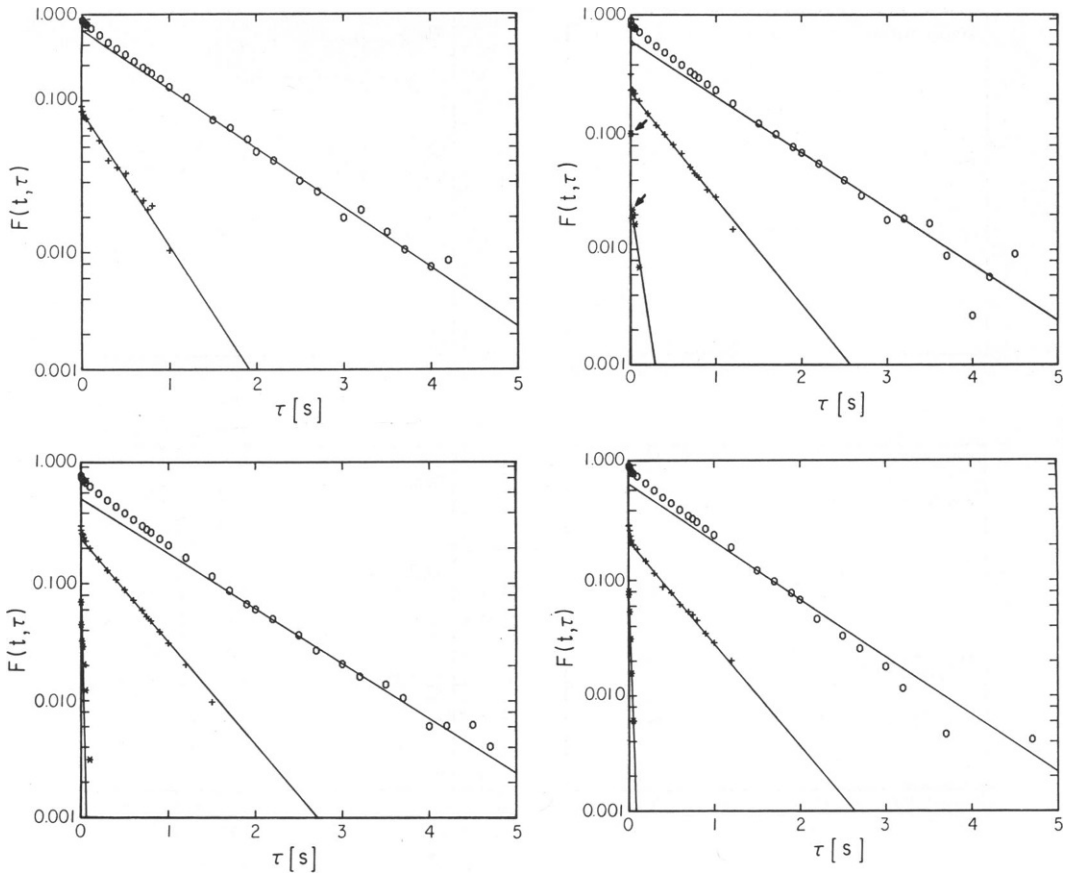

FIGURE 2 Examples of the magnetization recovery function $F(t, \tau)$ for 180°-τ-90° experiments: (*a*) h-h experiment (the FID window was set at $t = 8$ μs); (*b*) s-h experiment, $t = 8$ μs (Note the arrows indicating the size of the "jump"); (*c*) s-h experiment, $t = 196.2$ μs; and (*d*) s-s experiment, $t = 80$ μs.

3 *a*, *b*, *c*). It could be argued, however, that the resolution of the majority magnetization component and of the component with the longest $T_{1\rho}$ is still rather ambiguous since their relaxation times differ by only a factor of $\simeq 3$, see Fig. 3 *a*. This is an inherent weakness of the approach which, if one of the rates is dispersive, can be overcome by changing the magnetic field and searching for a frequency domain where the resolution is better. These resolution difficulties are compensated for by the correlation of the results from all of our observations. In addition, to the best of our understanding, neither the system nor the approach has shown a critical dependence on the above resolution.

Fig. 3 *c* represents the most important experimental result. There it is shown that all of the solid and some of the liquid (see also Table II), relax with the same $T_{1\rho}$ while the rest of the liquid relaxes with a substantially different time constant (Fig. 3 *a*, *b*, *c*). This tells us the rather important fact that a small liquid component is in thermal contact with the solid and the rest of the liquid is relaxed by a different mechanism. Therefore, on the time scale of the longest $T_{1\rho}$, the proton magnetization of a mouse muscle is effectively a four group system.

DISCUSSION

The aim of the present research is to show that in tissues the observed proton spin relaxation rates and spin group sizes are not necessarily the inherent (true) rates and sizes of the spin groups in the tissues. Chemical exchange ($k_a \simeq 30$ s^{-1}) between the bulk and the bound water and proton spin exchange (carried by spin flips in the solid local fields) between the bound water and the solid moderator ($k_s \simeq 10^3$s^{-1}) have a substantial influence on the measured rates and the spin group sizes (all of which are therefore only apparent).

To investigate the effects of these exchange processes it was necessary to resolve the composite recovery functions into components to obtain the experimental (apparent) rates and spin group sizes. However, such an analysis can be ambiguous at high fields if the standard h-h pulse sequence is the only sequence used. Therefore three sequences (h-h, s-h and s-s) were used in the present study. The value of λ^- can be determined using any one of these three but to obtain the value for λ^+ either the selective s-h or s-s excitation must be used. The results obtained from

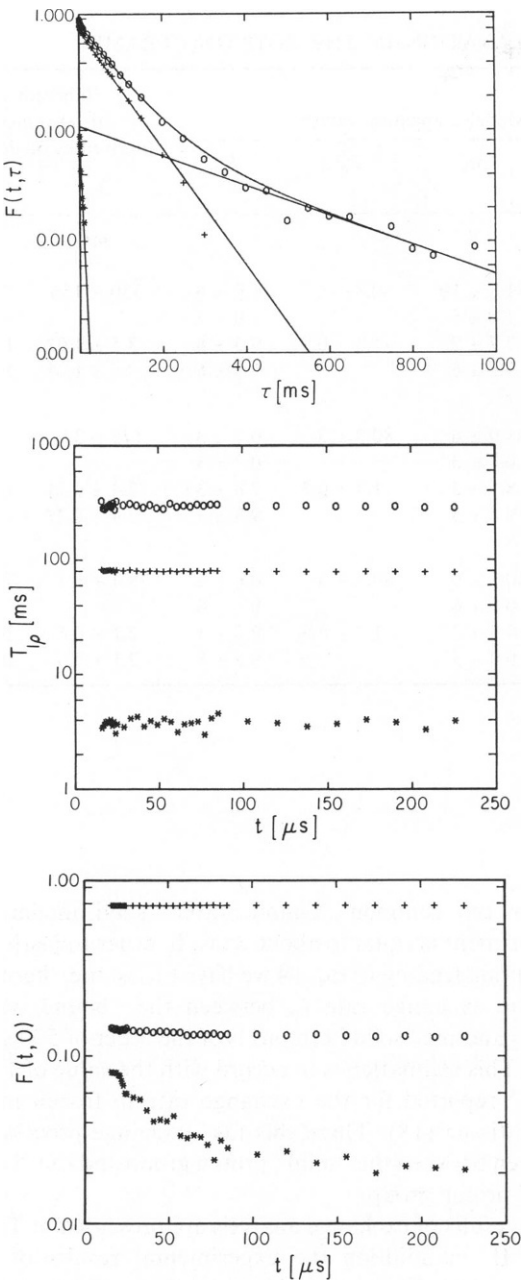

FIGURE 3 An example of the spin-grouping analysis of a $T_{1\rho}$ experiment of $H_1 = 10$ G: (*a*) the decay function $F(t, \tau)$, (the FID window was set at $t = 16$ μs); (*b*) the window dependence of the relaxation times; and (*c*) the reconstructed FID shapes for the corresponding components.

these measurements are further supported by the results of spin grouping analysis in the rotating frame. In particular, the rather ambiguous determination of the spin group at high fields with $T_1 \simeq 450$ ms and $M_o \simeq 17\%$ is corroborated by the spin group in the rotating frame with $T_{1\rho} \approx 300$ ms and $M_o = 17\%$ of the total magnetization.

As was mentioned earlier in the text, the spin grouping analysis of high and low field NMR data for mouse muscle tissue strongly indicates the presence of four spin groups. To investigate this problem with the exchange analysis we had to make several assumptions.

First, we identified tentatively the spin group with $M_o \approx 17\%$, $T_1 \approx 450$ ms and $T_{1\rho} \approx 300$ ms at $H_1 = 10$ G as the spin group of the lipids in muscle tissue. The size of this spin group is somewhat larger on average than the reported amount of lipid in mouse muscle tissue which is ≃10% (14). However, the lipid percentage was independently checked with a relaxation time experiment at 200 MHz and 295 K using the h-h 180°-τ-90° sequence and the FT technique. Two lines were observed. The smaller one ($M_o = 11 \pm 3\%$) was shifted upfield by 3.9 ppm from the larger one. The measured spin-lattice relaxation times were 1030 ± 20 ms for the bigger peak and 505 ± 20 ms for the smaller one. This observation supports very strongly the tentative interpretation above (15–17). It is proposed also that in the muscle tissue the lipid proton spin group is essentially isolated from the water protons and from the protons on large molecules. This is supported by the values of the observed lipid T_1 and $T_{1\rho}$ as well as by their ratio $T_1/T_{1\rho} = 1.9$. For this reason the lipid spin group was excluded from the exchange analysis. Only three spin compartments, a spin group associated with the bulk water, a spin group associated with the bound water, and a spin group associated with the solid-like macromolecular protons (the moderator) were analyzed for the effect of exchange. The data used in this analysis are the measured apparent values, where the apparent magnetization fractions were renormalized to 100% after the exclusion of the lipid spin group (for this reason the label "No lipid" appears above the second magnetization columns in Tables I and II).

To derive the inherent (or true) relaxation times from these experimental data the three-compartment exchange model, which was introduced in section II was used. On the basis of experimental clues (in particular the magnetization of liquid and solid protons in the $T_{1\rho}$ components with shortest relaxation time, Table II) it was assumed that the sizes of the "bound water" spin group and the "solid" spin group are equal. This left the values of only two parameters unknown: the "true" size of the solid proton magnetization fraction, p_s, and the chemical exchange rate, k_a, from the "bound water" to the "bulk water." These parameters were varied iteratively until the best agreement between the modeled values of the apparent quantities (relaxation times and magnetization fractions) and the results of the spin-grouping analysis was reached. Using this procedure the following characterization of the three proton compartments was arrived at:

—the solid proton spin group is 10.5% of the total spin mass and has a T_2 of 25 μs;

—the bound water spin group is 10.5% and has a T_2 of ~ 1 ms;

—the above two proton spin groups are strongly coupled

TABLE II
NORMAL MOUSE MUSCLE TISSUE: PROTON NMR RELAXATION IN THE ROTATING FRAME

H_1	T_2	Measured apparent values			Modeled apparent values				Inherent (true) values (derived by modeling)	
		$T_{1\rho}$	M_0		$T_{1\rho}$	M_0	P_bC_b	P_aC_a		
			Total	No lipid§					$T_{1\rho}$	M_0
G	*ms*	*ms*	%	%	*ms*	%			*ms*	*ms*
10	1 ± 0.5	300 ± 50	17.9 ± 2							
	4 ± 1*	100 ± 4	72.6 ± 3	88.5 ± 3	100 ± 20	81.8 ± 10	80.8 ± 4.7	1.0 ± 6	330 ± 156	79‖
						1.0 ± 6		1.0 ± 6		
	1 ± 0.5	3.2 ± 0.8	4.8 ± 2	5.8 ± 2	3.2 ± 0.8	7.7 ± 7	−1.8 ± 0.9	9.5 ± 6	3.5 ± 1.0†	10.5¶
	0.025 ± 0.003		4.7 ± 2	5.7 ± 2		9.5 ± 6		9.5 ± 6	3.5 ± 1.0†	10.5**
5	1 ± 0.5	270 ± 10	16.3 ± 2							
	4 ± 1*	77 ± 2	75.3 ± 3	89.9 ± 3	77 ± 10	81.0 ± 6	80.3 ± 3	0.7 ± 3	173 ± 37	79‖
						0.7 ± 3		0.7 ± 3		
	1 ± 0.5	2.2 ± 0.2	3.9 ± 2	4.7 ± 2	2.2 ± 0.2	8.5 ± 3	−1.3 ± 0.3	9.8 ± 3	2.4 ± 0.2‡	10.5¶
	0.025 ± 0.003		4.5 ± 2	5.4 ± 2		9.8 ± 3		9.8 ± 3	2.4 ± 0.2‡	10.5**
1	1 ± 0.5	190 ± 2	9.2 ± 2							
	4 ± 1*	50 ± 1	84.5 ± 3	89.8 ± 3	50 ± 5	80.8 ± 9	80.2 ± 3	0.6 ± 6	78.4 ± 8.5	79
						0.6 ± 6		0.6 ± 6		
	1 ± 0.5	2 ± 0.5	3.6 ± 2	3.8 ± 2	2 ± 0.5	8.6 ± 3	−1.2 ± 0.5	9.8 ± 3	2.1 ± 0.6	10.5
	0.025 ± 0.003		2.6 ± 2	6.4 ± 2		9.8 ± 3		9.8 ± 3	2.1 ± 0.6	10.5

*Apparent T_2 determined by the inhomogeneity of the magnetic field.
‡Common relaxation rates of the solid and bound water protons.
§M_0 corrected for the local field effect.
‖"Free" or bulk water protons.
¶"Bound" water protons.
**"Solid" protons.

by spin flips into a magnetization reservoir "a" of the size 21%;

—the bulk water protons form a magnetization reservoir "b" of the size 79% having an effective T_2^* of ~ 4 ms.

The chemical exchange rate, which provides the optimal fit for all the experimental results, is found to be $k_a = 29 \pm 9\ s^{-1}$.

An example of the modeling procedure for the $T_{1\rho}$ experiment at $H_1 = 10$ G is presented in Fig. 4. In Fig. 4 *a* the inherent relaxation rates R_a and R_b are plotted vs. the exchange rate k_a with fixed values of the apparent rates λ^+ and λ^- (taken from the spin-grouping analysis of the experimental data). In Fig. 4 *b* the magnetization components $p_aC_a^{\pm}$, $p_bC_b^{\pm}$ are plotted. The vertical line in both figures represents the optimal value of the exchange rate k_a. To evaluate the exchange regime for the relaxation processes in muscle the inherent relaxation rates obtained in the previous step were used to plot the apparent rates λ^+ and λ^- vs. the exchange rate k_a (Fig. 4 *c*). The corresponding magnetization components are shown in Fig. 4 *d*. The true k_a of 29 s^{-1}, its value indicated by the vertical line in all figures, shows that in the $T_{1\rho}$ experiments the rate of chemical exchange is only moderate compared with the inherent $T_{1\rho}^{-1}$ values. However, the same exchange rate is much faster than T_1^{-1}. The inherent values of the relaxation times and magnetization fractions, derived from the modeling, are listed in Tables I and II. The inherent value of a $T_{1\rho}$ for the common "bound water—solid moderator" compartment is equal to about 2 ms. In order to justify the assumption leading to Eq. 14 we have to assume, therefore, that the exchange rate k_s between the "bound water" protons and the "solid" protons is of the order of 500 s^{-1} or faster. This estimation is in accord with the value of 160 ± 250 s^{-1} reported for the exchange rate in frozen mouse muscle tissue (18). There this fast exchange process was only seen between the "solid" proton group and the "bound water" proton group.

The results of exchange analysis are presented in Tables I and II, in addition the experimental results of spin grouping are also quoted. The experimental errors were estimated during the spin-grouping analysis. The errors include the statistical fluctuations due to the noise on top of the measured signal as well as the scatter introduced by the analysis itself (error propagation). The errors for modeled values were estimated using the standard deviations calculated with experimental errors for the apparent values. All modeled values agree very reasonably with the experimental data (apparent values). However, the smaller inherent relaxation rate has always the largest error. This is the imminent feature of the error propagation in the exchange analysis. This error propagation is determined by the analytical properties of the formulas used and cannot be improved upon at a given experimental error.

It should be noted that although the magnetization associated with the "solid moderator" proton spin group

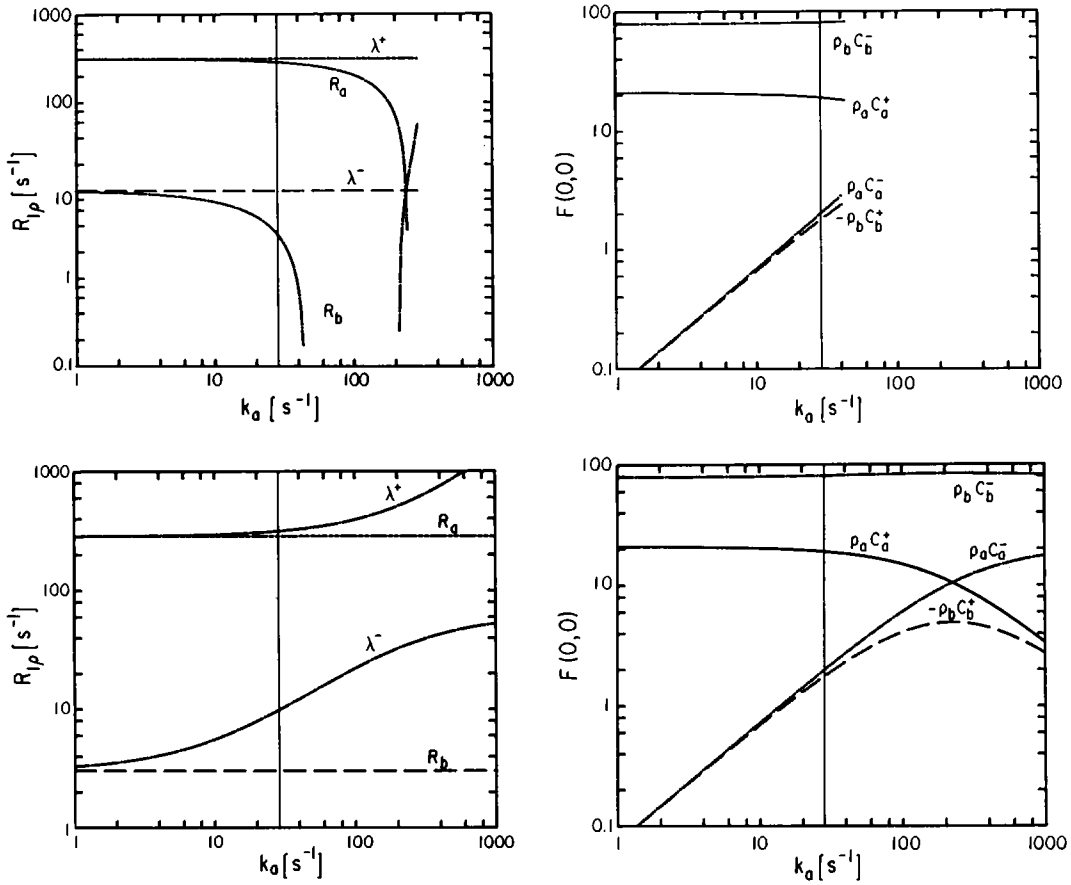

FIGURE 4 The results of the spin-exchange modeling for a $T_{1\rho}$ experiment at H_1 = 10 G. The following values are plotted vs. the exchange rate k_a: (*a*) the inherent relaxation rates R_a, R_b for fixed apparent rates λ^+, λ^-; and (*b*) the corresponding apparent magnetization components. (*c*) the apparent relaxation rates λ^+, λ^- for fixed inherent rates R_a, R_b; and (*d*) the corresponding apparent magnetization components. The vertical line indicates the value of the exchange rate k_a = 29 s^{-1}, which satisfies optimally the exchange analysis at high and low fields (Tables I, II).

corresponds to 8.8% of the total magnetization (when the lipid magnetization is included) it actually corresponds to a much larger percentage of the mass of muscle tissue. This is because the spin density of water is ~1.7 times larger than that of proteins.

The major reservoir of the spin magnetization in the sample is formed by the "bulk" water proton group. Its inherent relaxation time is 1,045 ± 1,450 ms at high fields and it is dispersive at low fields (330 ± 156 ms at H_1 = 10 G, 78.4 ± 8.5 ms at 1 G). Since the error of the above results of the exchange analysis is unusually high, a unique description of the dynamics of bulk water molecules cannot be proposed. For bulk water with fast and isotropic molecular reorientation and diffusion it is expected that $T_1 = T_{1\rho} = T_2$ as all molecular processes are much faster than the Larmor frequency ($\tau_c\omega_o \ll 1$). However, the $T_{1\rho}$ data show some H_1 field dependence and the relaxation times in the rotating frame are significantly shorter than T_1. The observed $T_{1\rho}$ dispersion of the bulk water shows clearly that part of this proton group is substantially moderated in spite of the fact that it is not within the reach of the large local fields that surround the solid moderators.

The "solid"-"bound water" group is characterized by substantially shorter T_1 and $T_{1\rho}$ values with considerable dispersion. This is in accord with the rather short T_2 (~1 ms) of the "bound water" spin group. It should be recalled that in tissue the bulk water T_2 is ~50 ms (10). All of the observed relaxation processes indicate severe moderation of bound water dynamics by the solid moderator (2, 3, 19–22). In particular one may expect the occurrence of strong anisotropic modes due to the "trapping" of water molecules on the surface of the moderator.

An exact discussion of the molecular dynamics of bound water molecules is unfortunately not possible, as the only reliable relation for the inherent relaxation times of bound water and solid moderator molecules is given by Eq. 14. This reads for T_1 at 40 MHz (in units of ms)

$$\frac{1}{511} = \frac{1}{2}\frac{1}{T_{1r}} + \frac{1}{2}\frac{1}{T_{1s}}. \tag{16}$$

Assuming that T_{1s} is much longer than T_{1r}, the lower limit for T_{1r} is estimated to be 256 ms. If the solid moderator also contributes to the observed T_1 the T_{1r} would become longer. However, it is almost certain that $T_{1r} < T_{1s}$.

For $T_{1\rho s}$ at 10 G and 40 MHz we get from Eq. 14 (in units of ms)

$$\frac{1}{3.5} = \frac{1}{2}\frac{1}{T_{1\rho r}} + \frac{1}{2}\frac{1}{T_{1\rho s}}. \quad (17)$$

The lower limit for $T_{1\rho r}$ is 1.8 ms and a similar discussion applies.

Since T_2 for the solid protons is 25 ± 3 μs, while T_2 for the bound water protons is ~1 ms, the assumptions that $T_{1r} < T_{1s}$ and $T_{1\rho r} < T_{1\rho s}$ are well justified. It follows that the dynamics of the "bound" water proton spin group plays a key role in the relaxation behavior of the muscle tissue at high (λ^-) and low (λ_ρ^-) fields (3, 20–29).

CONCLUSIONS

The presented study consists of four steps. In the experimental part of the application of various combinations of selective and nonselective pulses for T_1 as well as $T_{1\rho}$ determination provide a broad and rather accurate experimental basis for further analysis. In particular, T_1 experiments using h-h, s-h and s-s pulse sequences give explicit information about the exchange, which is otherwise hidden. The spin grouping method of analysis enables the extraction of experimental parameters with reliable correlations between the various sets of data. The information obtained from this procedure about the magnetization fractions and their relaxation rates makes it possible to further analyze these results for the effect of exchange. Using this approach it was found that the exchange rate between the bulk and the bound water protons is 29 ± 9 s^{-1}. This rather significant exchange rate has not been determined as accurately for a tissue before. In the last step the discussion of the molecular dynamics of protons in different spin compartments is presented. This discussion is quite limited as a result of the complexity of the system studied. However, it was still possible to arrive at a few conclusions.

In mouse muscle tissue the "bulk water" spin group forms a major reservoir. The proton motion in this group is considerably slower than in pure water, yet fast enough to average out most of the interactions between water molecules. For this reason the bulk water spin group, although the largest in the tissue, represents only a minor proton spin relaxation sink. On the other hand, the so called "bound water" spin group, which is much smaller than the "bulk water" group, plays a major role in determining the NMR relaxation in tissues. The motion of water molecules in this spin group is most likely anisotropic with a distribution of correlation times due to the great variety of environments on the macromolecular surfaces which are covered by the water molecules. As it has been proposed earlier the "tumbling" of water molecules or slow diffusion within the hydration shell in conjunction with the unknown distribution of bound water environments governs both the spin-spin relaxation as well as the spin-lattice relaxation time in the rotating frame while the spin-lattice relaxation time in the laboratory frame is caused by fast reorientation around the hydrogen bonds formed between the water molecules and the macromolecular host (2, 3). The "solid spin" group, which is approximately equal in size to the "bound water" spin group, does not appear to make a significant contribution to the relaxation processes since the protons in this group undergo much slower motion. The solid however, physically supports the "bound water" and moderates its dynamics. This in turn brings about bound water dispersive properties at high and low fields.

In conclusion the presented four step correlation approach provides the inherent relaxation parameters. With these parameters more realistic modelling of molecular processes governing NMR relaxation phenomena in tissues is possible. A systematic study of other mouse tissues is underway in Waterloo.

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