will gradually decrease with time as given by

$$R_t = 2\ KD \sum_{n=1}^{\infty} (-1)^{n+1} \exp\left(\frac{-n^2 \pi^2 Dt}{l^2}\right)$$

The fractionation in the sample permeating at time t is given by

$$F_t = \frac{D_{20}}{D_{22}} \left[\frac{1 + 2\sum_{n=1}^{\infty} (-1)^n \exp\left(\frac{-n^2 \pi^2 D_{20} t}{l^2}\right)}{1 + 2\sum_{n=1}^{\infty} (-1)^n \exp\left(\frac{-n^2 \pi^2 D_{22} t}{l^2}\right)} \right]$$

An expression for the fractionation for the decay can be similarly written. If it is assumed that the diffusion coefficients for the two isotopes are in the ratio of the inverses of the square roots of their respective masses, for which there is evidence both from our experiment and the experiment of Frank, Swets, and Lee (*2*), the fractionation factors are calculated using $D_{20} = 1.05\ D_{22}$.

From the foregoing equations, one can obtain F_t as a function of R_t/KD (the ratio of the permeation rate at time t to the permeation rate at steady state). This function depends only on the ratio D_{20}/D_{22}, and is shown by the solid curve in Fig. 1. This relation, however, is applicable only to instantaneous values of F_t and R_t/KD, while the experimental data are obtained over an interval of R_{t1}/KD to R_{t2}/KD. Therefore, in order to compare the experimental data with theory, an average value of F was calculated by integrating Eq. 1 for proper intervals of R_t/KD. These values of F differ significantly from the instantaneous ones only in the early stages when the permeation rate is changing very rapidly. They are shown by the broken curve. The experimental data are shown by small circles. The fit of the experimental data to the theoretical curve is considered very good at both temperatures; the small apparent departures are not beyond the systematic and random errors in the measurement of F and R/KD.

Fractionations may occur in nature by similar processes. These might be partly responsible for some of the isotopic anomalies observed, for example, in the meteorites.

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Nuclear Magnetic Resonance Studies of Living Muscle

Abstract. *Measurements of nuclear magnetic relaxation times for protons of water in living skeletal frog muscle show the transverse relaxation time,* T_2, *increases when a muscle contracts isometrically. This result and other experimental data suggest that a fraction of the intracellular water molecules have restricted rotational freedom and that this fraction decreases when contraction occurs.*

Nuclear magnetic resonance studies of protons in living muscle point to a correlation or binding or immobilization of some of the water molecules relative to the structure of the muscle cell. Part of the bound water appears to be freed reversibly during isometric contraction and irreversibly in death.

The tissues used were the skeletal muscles of the frog *Rana pipiens*. Most of the experiments were performed on the gastrocnemius muscle, but a few were performed on the semitendinosus and sartorius muscles. Within the limits of error the results were the same. A copper-wire radio-frequency coil was wound on a pyrex tube (inside diameter, 8 mm), and a muscle was suspended within the tube. Tension was continuously recorded from a resistance strain gauge attached to one end of the muscle. Electrical stimulation was delivered through attached platinum electrodes. The complete assembly was centered in the gap between the pole faces of a Varian V-4012A electromagnet equipped with a Varian VK-3519 homogeneity control unit capable of maintaining fields varying less than 5 mgauss over the sample volume.

The preliminary studies were made with slow sweep techniques at a Larmor frequency of 24 Mc/sec. The steady-state response of the protons was obtained by phase-sensitive detection of the first sidebands caused by 1 kc/sec modulation of the main magnetic field. A single, well-defined proton line several milligauss in width was observed, but no broad, superimposed line was detected. From the strength and definition of the line we conclude that this signal came from the protons of water which constitutes approximately 80 percent of the total weight of a muscle. By comparison with proton standards of known line widths and chemical splittings, the width of the line was judged to originate from relaxation processes and not from inhomogeneities of the field alone. When the muscle was stimulated to undergo tetanic isometric contraction, the proton signal was distinctly narrowed by approximately 20 percent. Repeated sweeps confirmed the effect as 20 ± 5 percent.

An increase in line width of the proton signals from water in which various substances have been dissolved or suspended is not unprecedented. Notable examples of organic solutes for which the effect has been observed are DNA (*1*) and agar (*2*). Proton-line broadening has also been observed in samples of human tissues and cells (*3*). Several possible causes are (*4, 5*) paramagnetic impurities, chemical exchange, anisotropy of the local static magnetic fields, and adsorption processes.

To ascertain the cause in our experiment and to eliminate interfering static-field inhomogeneities, the transverse relaxation time, T_2, was measured directly by the Hahn spin echo technique (*6*) which makes use of pairs of pulses of 90° and 180°. The decay in amplitude of repeated spin echoes followed a simple exponential curve and showed thereby the relative unimportance of diffusion effects. The relaxation time, T_2, was also measured by the Carr-Purcell modification (*7*) of the spin echo method (with results that agreed with those obtained by the Hahn method), which has the advantage that a single measurement requires less time—approximately 0.1 second compared to about 1 minute for the Hahn method.

The longitudinal relaxation time, T_1, was also measured with a pulse technique (*7*). The measurements of T_1 and T_2 were made at two field strengths corresponding to Larmor frequencies of 24.0 and 4.30 Mc/sec. The longitudinal relaxation time, T_1, was found to be 400 msec at the higher field strength and 250 msec at the lower field strength. Within experimental error T_1 did not change with changes in the state of a

muscle. At both field strengths the transverse relaxation time, T_2, was found to be 40 msec for fresh, relaxed muscle and increased with tetanic, isometric contraction. Under conditions of exhaustion T_2 increased to greater than 60 msec. We estimate the precision of any one measurement of T_1 to be ± 10 percent and of T_2 to be ± 7.5 percent.

In view of our results, a model of the proton relaxation processes concerned must explain the following facts: (i) The longitudinal relaxation time, T_1, is dependent on the main magnetic field strength but is insensitive to changes in the state of a muscle. (ii) The transverse relaxation time, T_2, is essentially independent of the field strength, but increases with contraction and exhaustion of a muscle. (iii) Finally, T_1 is greater than T_2 at both field strengths used.

These facts cannot be explained satisfactorily by the simplest situation, where one correlation time describes the random thermal motion of the intracellular water molecules and where a single interaction strength exists for the proton pairs of the water molecules. For this homogeneous model (*4*, *8*), the mathematical analysis of the data for T_1 gives a value for the correlation time, t_1, smaller than that required by the field insensitivity and magnitude of T_2. The value of t_1 thus obtained corresponds furthermore to the minimum of the longitudinal relaxation rate as a function of correlation time for a Larmor frequency of 24 Mc/sec. This would explain the insensitivity of T_1 to changes in the state of a muscle at the higher field strength but not the insensitivity at the lower field strength.

Three possible models might account for the results. One relies on the coupling of the water protons to electronic moments. For this model it is assumed that the relaxation times of the electronic moment are the effective correlation times for the proton relaxation processes (*4*). It is difficult, however, to explain the insensitivity of T_1 and the sensitivity of T_2 to changes in the state of a muscle. Several assumptions that have no independent justification must be made concerning the nature of the electronic intermediate and its relaxation processes. We know of no suitable paramagnetic intermediate for coupled relaxation.

For another possible model it is assumed that there is a distribution of correlation times for the intracellular water, characterized by a mean correlation time and a mean width (*9*). Our information does not allow a calculation of these parameters nor a determination of the adequacy of such a model.

However, the data are compatible with a two-phase model (*5*) that places a fraction, f, of the intracellular water in a phase for which the random thermal rotational motion of water molecules is greatly restricted. This phase, which we will call solid-like, is described by a correlation time, t_2, much greater than the Larmor period. The remaining fraction $(1 - f)$ is a liquid phase characterized by a correlation time, t_1, much less than the Larmor period. To explain the experimentally observed single resonance line and the occurrence of simple relaxation times it is further assumed that exchange of water molecules between the two phases occurs at a faster rate than the relaxation rates for any given phase. On this basis, the equations for the relaxation rates are:

$$\frac{1}{T_1} = 5(1-f)K_1 t_1 + fK_2 t_2 \left[\frac{1}{1+(\omega t_2)^2} + \frac{4}{1+4(\omega t_2)^2}\right];$$

$$\frac{1}{T_2} = 5(1-f)K_1 t_1 + \frac{3}{2} fK_2 t_2,$$

where $\hbar K_1^{1/2}$ and $\hbar K_2^{1/2}$ are the energies of interaction of proton moments within the water molecules in the liquid phase and the solid-like phase, respectively, and ω is the Larmor frequency in radians per second ($\hbar$ is Planck's constant divided by 2π).

With the substitution of the experimental data the equations can first be solved for t_2. The result is $t_2 = 1.6 \times 10^{-7}$ sec. Although the data are insufficient to allow K_1, K_2, t_1, and f to be determined separately, fK_2 and $5(1 - f)K_1 t_1$ can be determined. Thus, fK_2 was found to be 9.42×10^7 sec^{-2} and $5(1 - f)K_1 t_1$ was found to be 2.39 sec^{-1}.

If $K_2^{1/2}$ is assumed to be $2.5 \times 10^{+5}$ sec^{-1}, corresponding to the interaction strength estimated from the known transverse relaxation time for ice, f turns out to be 1.5×10^{-3}. On the other hand, if it is assumed that the transverse relaxation rate for the solid-like phase has reached its asymptotic value, that is, $t_2 \geqq 1/K_2^{1/2}$, then the contribution of the solid-like phase to total transverse rate is given by $fK_2^{1/2}$, and $K_2^{1/2}$ is determined by the condition $t_2 \approx 1/K_2^{1/2}$. On this alternate basis, the solution of the relaxation rate equations gives $t_2 = 2 \times 10^{-7}$ sec, and thence $K_2^{1/2} \approx 5.3 \times 10^6$ sec^{-1}. The corresponding fraction f is 4.5×10^{-6}.

Our data interpreted by the two-phase model therefore permits for f a range of values from 4.5×10^{-6} to 1.5×10^{-3}, and a range of interaction strengths which varies from 1 to 20 times that of ice, though the smaller value is most likely. Since the solid-like phase contributes 90 percent of the total transverse relaxation rate, and since the liquid phase tends to dominate the longitudinal relaxation rate, the model further explains the sensitivity of T_2, as well as the insensitivity of T_1, to changes in the state of a muscle.

The two-phase model has been successfully applied to other experiments on simpler systems, such as water adsorbed on silica gel (*5*, *10*) and carbon suspensions (*11*). The model has also been suggested for water in contact with glass beads (*12*). The concept of bound water has often been helpful in explaining the properties of protein solutions. We therefore strongly incline to the model of a two-phase system of free and bound water as the most likely explanation of our results.

It has only to be assumed that the change in tension is associated with a liberation of about 20 percent of the fraction f of bound water. This would result in a change in T_2 of the magnitude and sign observed, but would not affect T_1 within experimental error. Earlier microwave studies on cardiac muscle indicate that aproximately the same amount of bound water is released during contraction (*13*).

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