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During the periodcovered by this contract [AT(11-1)-1626] the following studies were attempted on muscle, bone and model systems.

Measurements of the longitudinal relaxation time T_1, the transverse relaxation time T_2 and the self-diffusion coefficient D for water molecules in colloidal suspensions of silica particles (Ludox) showed a temperature dependence essentially like that found for pure water by Simpson and Carr (1). The more interesting result, however, was the small increase in the self-diffusion coefficient D compared to the pure water value at a given temperature and a smaller activation energy.

Measurements of T_1 and T_2 for protons in aqueous solutions of the muscle protein myosin have been made as a function of concentration, pH, ionic strength and purity. These solutions were prepared by D. S. Love, Assistant Professor of Anatomy, Case Western Reserve University. The results showed that T_2 is sensitive to the degree of calcium-activated ATP-ase activity exhibited by myosin, particularly in low ionic strength preparations at pH 9.5. On the other hand, T_1 is more sensitive to variations in ambient temperature than to the presence of calcium ions and ATP in solutions. Measurements made on the several fractions obtained from chromatographically purified preparations indicated that T_2 depends on the presence or absence of oligonucleotides bound to myosin molecules. Again, T_1 was more sensitive to temperature variations. We interpreted the behavior of T_1 to mean that the gross viscosity

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of the low ionic strength preparations is not affected by chemical changes during activation. Our dilemma is occasioned by the fact that for myosin preparations T_2 decreased with increasing ATP-ase activity while in living muscle T_2 increases with contraction. We speculate that low ionic strength myosin solutions are models for activation but not the contraction process.

Studies made on bone using both pulse and wideline NMR techniques produced evidence that water exists in at least two distinct phases which, in contradistinction to muscle, do not exchange water molecules rapidly if at all. By progressively dehydrating bone the relative amplitudes of two superimposed proton resonance lines changed; the broad line became more prominent as dehydration progressed. However, this broad line disappeared when the samples were decalcified. In addition, samples cut transverse to the axis of the bone showed a dependence of linewidth on the orientation of the bone relative to the magnetic field direction. This effect also was present in decalcified samples. We conclude that one water pahse is associated with the mineral component of bone, the other phase with the organic fibrillar matrix. Studies of the dependence of linewidth and free induction decay times on applied pressure have not yielded a coherent set of data.

Proton relaxation time measurements were made on muscle to determine the effects on T_1 and T_2 due to fiber orientation in the magnetic field, the state of hydration, concentration of heavy water, active and passive tension, and temperature.

The results of the angular dependence experiments on frog sartorius muscle were negative; no effect of fiber orientation with respect to the direction of the magnetic field could be detected even for muscles which had been dried to 20% of their original weight.

On the other hand, the effect of dehydration on T_1 and T_2 was found to be compatible with the two water-phase model. The graphs of T_1 and T_2 versus normalized weight m/m_o were linear with the exception of the initial region at $m/m_o = 1$ (m_o is the dissected weight; m is the weight at any point during dehydration.) From these data an upper limit on the initial amount of "bound" water m_x was found to be $m_x \leq 0.01\ m_o$. We had originally calculated from our two phase model that $m_x \simeq 0.0015\ m_o$.

The results of the deuteration experiments were ambiguous at best and no conclusions have been made.

The temperature dependence studies showed that T_2 is relatively independent of temperature. However, T_1 increases with increasing temperature. This result is at odds with the assumption that the dominant relaxation mechanism is the fluctuations in the magnetic dipole interaction between protons in a water molecule. The suggested relaxation mechanism is one for which the dominant interaction is a scalar coupling between a proton and either an electron or another nuclear magnetic moment.

This work has not been published because we felt that there are too many unconnected facts which need to be supplemented and many questions yet to be answered. However, a part of the study on bone did result in an Honors Thesis for an undergraduate student and presently a graduate is preparing her doctoral dissertation based on studies of the effect of tension on the proton relaxation times for frog sartorius muscle. In all, one undergraduate student, two graduate students, three postdoctoral fellows and one visiting professor have received training or gained further experience in the application of NMR techniques applied to living systems during the tenure of this contract.

Reference (1) J. H. Simpson and H. Y. Carr, Phys. Rev. 111, 1201 (1958).