

# PULSED NUCLEAR MAGNETIC RESONANCE STUDY OF $^{17}\text{O}$ , $^2\text{D}$ , AND $^1\text{H}$ OF WATER IN FROG STRIATED MUSCLE

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**ABSTRACT** Whole gastrocnemius muscles were incubated in Ringer's solution enriched with  $\text{H}_2^{17}\text{O}$ ; the paired contralateral gastrocnemius muscles were incubated in a similar solution enriched with deuterons, as well. Subsequently, the longitudinal relaxation times ( $T_1$ ) were measured for  $^{17}\text{O}$ ,  $^2\text{D}$ , and  $^1\text{H}$ , both at 8.1 MHz and at 4.3 MHz. The results indicate that: (a) the absolute values of  $T_1$  characterizing the three nuclides are different in muscle and pure water, (b) the longitudinal relaxation rates of all three have an identical frequency dependence over the range studied, (c) the ratio  $(T_1)_{^2\text{D}}/(T_1)_{^{17}\text{O}}$  is the same in muscle water and pure water, while the ratio  $(T_1)_{^1\text{H}}/(T_1)_{^{17}\text{O}}$  is 2.1 times greater in pure water than it is in muscle water, and (d) 30–49% substitution of  $^2\text{D}$  for  $^1\text{H}$  has very little effect on the spin-lattice relaxation of tissue water protons. These data suggest that muscle water is in rapid exchange between a small fraction of immobilized molecules and a large fraction of free water. The results render unlikely the possibility that hypothetical ordering of muscle water significantly contributes to its longitudinal relaxation.

## INTRODUCTION

The state and composition of the intracellular fluids are of great physiologic interest and potential pathophysiologic relevance. Following the initial work of Bratton et al. (1), a number of investigators have attempted to determine the quantitative significance of possible ordering or structuring of the intracellular water, by means of nuclear magnetic resonance (NMR) techniques.

Defined on the basis of NMR parameters, several studies have demonstrated a heterogeneity of the intracellular water. Measurement of the proton transverse relaxation time ( $T_2$ ) of muscle suggests that 8–20% of the total water is immobilized within the tissue (presumably as shells of hydration about macromolecular sites), 10–15% of the muscle water is extracellular, and the remaining 65–82% constitutes the bulk of the intracellular fluid; the  $T_2$  of each of the populations of water protons is considerably shorter than that of bulk water (2, 3).

Study of the continuous wave NMR spectrum of  $^{17}\text{O}$  from  $\text{H}_2^{17}\text{O}$  in muscle water reveals only 75% of the total  $^{17}\text{O}$ , similarly suggesting a heterogeneity of tissue water (4).

Two properties of muscle water are particularly striking. First,  $T_1$  and  $T_2$  of the water protons are not equal. Second, the  $T_1$  of water protons in muscle and other tissues is dependent upon the Larmor frequency ( $\omega$ ) (5-7). Within the formalism of nuclear magnetic resonance, these phenomena must reflect a rate of molecular tumbling which is slower than or comparable to  $\omega$  (i.e. reflect long correlation times). This effect could arise from: (a) some ordering factor or anisotropy of the intracellular fluid (4), or (b) rapid exchange between small immobilized fractions with much larger fractions of water molecules (8, 6).

In order to examine these possibilities in greater detail, the longitudinal behavior of three nuclides ( $^{17}\text{O}$ ,  $^2\text{D}$ , and  $^1\text{H}$ ) of water was studied at two different frequencies within the same samples of frog striated muscle. Given the different axis of interaction for each nuclide within the molecular frame, ordering would be expected to affect each of the three relaxation behaviors differently.

## METHODS

As previously described (4), gastrocnemius muscles were excised intact from doubly-pithed frogs, *Rana esculenta*, and bathed initially in a standard Ringer's solution (9) ( $\text{NaCl}$ , 115.5 mM;  $\text{KCl}$ , 2.5 mM;  $\text{CaCl}_2$ , 1.8 mM;  $\text{Na}_2\text{HPO}_4$ , 2.5 mM;  $\text{NaH}_2\text{PO}_4$ , 0.5 mM; and *d*-tubocurarine chloride, 9 mg/liter). In four experiments, one muscle from each pair was then transferred to a similar solution containing 10-15%  $\text{H}_2^{17}\text{O}$  and 30-49% deuterium; the paired muscle from the same frog was transferred to a similar solution enriched with an identical concentration of  $\text{H}_2^{17}\text{O}$ , but not with deuterium. In one experiment, both muscles of the pair were incubated in the same Ringer's solution containing 10-15%  $\text{H}_2^{17}\text{O}$  alone. After incubations for periods of some 2-3 h, during which time oxygen was bubbled into the solution, the isotope compositions of the water within the muscle and within the bathing media were identical (4). At that time, the muscles were removed, blotted dry on filter paper, and gently packed into thin-walled test tubes, of 10 mm OD.

All measurements were performed with a Bruker Pulsed NMR B-KR Spectrometer (Bruker Physik AG, Karlsruhe, Germany) at a frequency ( $\omega$ ) of 8.1 MHz, or at a frequency approximately half that of 4.3 MHz; the strength ( $H_0$ ) of the steady magnetic field provided by a 12-inch model V 4012 A-HR electromagnet system of a Varian DP-60 NMR spectrometer (Varian Associates, Palo Alto, Calif.) was adjusted appropriately.

As previously described in detail (8),  $T_1$  was measured by standard techniques. The *z*-component of the magnetization was studied following paired pulses of  $180^\circ$  and  $90^\circ$ . The free-induction decay following the  $90^\circ$  pulse was time-averaged in order to enhance the signal: noise ratio. The duration of the  $90^\circ$  pulses for  $^{17}\text{O}$  and  $^2\text{D}$  was 10-20  $\mu\text{s}$ , and for protons was 2-3  $\mu\text{s}$ . The recovery time, even at the lower frequency, was not longer than 60-70  $\mu\text{s}$ , and correspondingly shorter at the higher frequency used.

## RESULTS

Figs. 1-3 present the recovery with time of the *z*-component of the magnetization for  $^{17}\text{O}$ ,  $^2\text{D}$ , and  $^1\text{H}$ , respectively, of muscle water following a pulse of  $180^\circ$ . The abscissa is the time interval separating the initial  $180^\circ$  and subsequent  $90^\circ$  pulse.

The data of Figs. 1 and 3 were obtained from the same muscle. The data of Fig. 2 were obtained from the contralateral gastrocnemius muscle of the same frog. In each

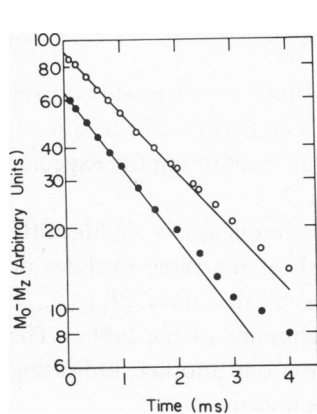


FIGURE 1

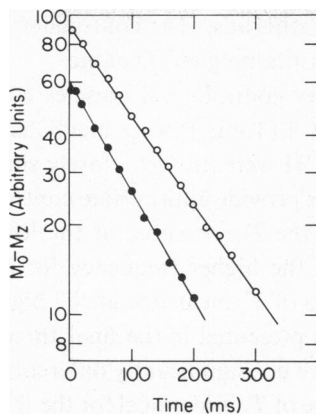


FIGURE 2

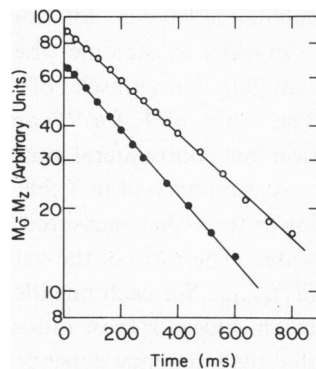


FIGURE 3

FIGURE 1 Spin-lattice relaxation of  $^{17}\text{O}$  from  $\text{H}_2^{17}\text{O}$  in frog gastrocnemius muscle as a function of time and Larmor frequency. The ordinate is the  $\log_{10}$  of the difference between  $M_0$  (the full magnetization), in arbitrary units, following paired pulses of  $180^\circ$  and  $90^\circ$ . The data points presented as open and closed circles were obtained at Larmor frequencies of 8.1 and 4.3 MHz, respectively. The solid lines are empirical best fits of the initial data points. The longitudinal relaxation of  $^{17}\text{O}$  in fresh striated frog muscle is clearly nonexponential. The data of Figs. 1 and 3 were obtained from the same muscle sample; the data of Fig. 2 were obtained from the contralateral muscle from the same frog.

FIGURE 2 Spin-lattice relaxation of  $^2\text{D}$  from water in frog gastrocnemius muscle as a function of time and Larmor frequency. Legend to Fig. 1 applies to Fig. 2, as well. The longitudinal relaxation of the  $^2\text{D}$  is simply exponential.

FIGURE 3 Spin-lattice relaxation of  $^1\text{H}$  from water in frog gastrocnemius muscle as a function of time and Larmor frequency. Legend to Fig. 1 applies to Fig. 3, as well. As noted for  $^2\text{D}$ , the longitudinal relaxation of water protons in muscle is simply exponential.

figure, the data points obtained at 8.1 MHz and at 4.3 MHz are presented as open and closed circles, respectively.

As previously described, the longitudinal relaxation of  $^{17}\text{O}$  from  $\text{H}_2^{17}\text{O}$  in fresh muscle is distinctly nonexponential (Fig. 1), but becomes increasingly exponential with progressive deterioration of the sample over periods of many hours. This phenomenon is apparent at both frequencies studied. The  $T_1$  calculated from the initial slope represents a weighted average of the two or more populations of  $^{17}\text{O}$  contributing to the observed signal (8). It is this value of  $T_1$  for each plot, which is used in all computations of the present study.

On the other hand, the longitudinal relaxation of the water deuterons (Fig. 2) and water protons (Fig. 3), may be fitted by single values of  $T_1$ . This suggests that exchange among the populations of water is sufficiently rapid in comparison with the  $T_1$  of water deuterons to result in a single average rate of longitudinal relaxation both for D and for H.

Because of the possibility that deuterated water might alter or damage the muscle samples, it was considered desirable to incubate only one muscle of each pair in

Ringer's solution containing deuterons. The contralateral muscle in each case was incubated in Ringer's solution containing  $\text{H}_2^{17}\text{O}$  alone.

In order to examine whether contralateral muscles constitute an adequate control sampling, both muscles of exp. I (Table I) were incubated in deuterium-free solutions. The values of  $T_1$  for  $^{17}\text{O}$  and  $^1\text{H}$  were, in fact, closely similar, confirming the expectation that contralateral muscles provide appropriate controls.

As summarized in Table I, the  $T_1$  measured at the lower frequency is significantly lower than that measured at the higher frequency for each of the three nuclides of water. The ratio of the values of  $T_1$  measured at the high and low values,  $(T_1)_{8.1 \text{ MHz}} / (T_1)_{4.3 \text{ MHz}}$ , for each nuclide is presented in the final three columns of the table. The mean values for these ratios are not significantly different from one another, indicating that the frequency-dependence of  $T_1$  is identical for the three nuclei.

It will be noted that the longitudinal relaxation times for  $^{17}\text{O}$ ,  $^2\text{D}$ , and  $^1\text{H}$  of muscle water are different from those of bulk water. Interpolating the data presented in the most careful studies of the temperature dependence of the  $T_1$  of water nuclides, the  $T_1$  of  $^{17}\text{O}$ ,  $^2\text{D}$ , and  $^1\text{H}$  in water at  $23^\circ$  may be estimated to be 6.9 ms (10), 427 ms (11), and 3.4 s (12), respectively. In order to compare these values directly, account must be taken of the relative viscosities of the experimental solutions. The longitudinal

TABLE I  
LONGITUDINAL RELAXATION TIMES ( $T_1$ ) FOR  $^{17}\text{O}$ ,  $^2\text{D}$ , and  $^1\text{H}$  OF  
MUSCLE WATER AS A FUNCTION OF FREQUENCY

Exp.		$T_1$						$(T_1)_{8.1} / (T_1)_{4.3}$		
		$^{17}\text{O}$		$^2\text{D}$		$^1\text{H}$		$^{17}\text{O}$	$^2\text{D}$	$^1\text{H}$
		4.3 MHz	8.1 MHz	4.3 MHz	8.1 MHz	4.3 MHz	8.1 MHz			
		<i>ms</i>		<i>ms</i>		<i>ms</i>				
I	A	1.35	1.74	—	—	306	377	1.29	—	1.23
	B	1.38	1.71	—	—	296	358	1.24	—	1.21
II	A	1.35	1.20	—	—	312	406	1.29	—	1.30
	B	1.26	1.74	100	129	316	414	1.38	1.29	1.31
III	A	1.75	2.07	—	—	368	464	1.18	—	1.26
	B	1.52	1.86	126	152	377	464	1.22	1.21	1.23
IV	A	1.57	1.99	—	—	367	458	1.27	—	1.25
	B	1.54	1.84	122	151	—	—	1.20	1.24	—
V	A	1.43	1.99	—	—	341	442	1.38	—	1.30
	B	1.46	1.81	116	139	338	428	1.24	1.20	1.27
Mean		1.46	1.86	116	143	335	423	1.27	1.24	1.26
±SEM		±0.050	±0.041	±5.7	±5.5	±10.1	±12.8	±0.022	±0.020	±0.013

The muscle samples A of exps. I–V, and muscle sample B of exp. I were incubated in a Ringer's solution enriched with  $\text{H}_2^{17}\text{O}$ . The muscle samples B were obtained from the contralateral muscle of each pair: samples B from exps. II–V were incubated in the same Ringer's solution enriched both with  $\text{H}_2^{17}\text{O}$  and deuterons. Some 30% of the water protons of samples II B, III B, and IV B were exchanged with  $^2\text{D}$ ; 49% of the water protons of V B were replaced with  $^2\text{D}$ . The final three columns present the ratio of the value obtained for  $T_1$  at 8.1 MHz,  $(T_1)_{8.1}$ , to that obtained at 4.3 MHz,  $(T_1)_{4.3}$ , for each of the three nuclides studied.

relaxation time of deuterons was measured in a solution containing more than 99.5%  $^2\text{D}_2\text{O}$ ; therefore, the value given above must be multiplied by 1.24, the relative viscosity of  $^2\text{D}_2\text{O}$  to that of  $\text{H}_2\text{O}$  at  $23^\circ\text{C}$  (13). The correction factor for the result obtained with  $^{17}\text{O}$  is negligible, since lower concentrations of  $^{18}\text{O}$  and  $^{17}\text{O}$  were in the experimental solutions and since the relative viscosity to  $\text{H}_2^{16}\text{O}$  of pure  $\text{H}_2^{18}\text{O}$  is only 1.054 (14).

From these estimates, the ratios of the longitudinal relaxation times in pure water are:  $(T_1)_{2\text{D}}/(T_1)_{17\text{O}} = 77$ , and  $(T_1)_{1\text{H}}/(T_1)_{17\text{O}} = 493$ .

From the data presented in Table I, the means  $\pm$ SEM for the experimental values of  $(T_1)_{2\text{D}}/(T_1)_{17\text{O}}$  are  $79 \pm 1.9$ ,  $80 \pm 0.9$ , and  $79 \pm 1.0$  at 8.1 MHz, at 4.3 MHz, and for the combined data, respectively. The corresponding means  $\pm$ SEM for  $(T_1)_{1\text{H}}/(T_1)_{17\text{O}}$  are  $229 \pm 4.1$ ,  $231 \pm 4.6$ , and  $230 \pm 3.0$  at 8.1 MHz, 4.3 MHz, and for the combined data, respectively.

It will be noted from Table I that the presence of deuterons did not seem to affect significantly the  $T_1$  characterizing the water protons. In order to examine this point more carefully, exp. V was performed with Ringer's solution enriched with 49%, rather than 30%, deuterons. Even at this high concentration, deuterium did not measurably alter the longitudinal relaxation of the  $^1\text{H}$ .

## DISCUSSION

The most striking data of the current study are (Table I): (a) the longitudinal relaxation rates of  $^{17}\text{O}$ ,  $^2\text{D}$ , and  $^1\text{H}$  of muscle water have an identical frequency dependence over the range examined, (b) the absolute values of  $T_1$  characterizing these three nuclides are different in muscle and pure water, (c) the ratio  $(T_1)_{2\text{D}}/(T_1)_{17\text{O}}$  in muscle water is closely similar to that in pure water, while the ratio  $(T_1)_{1\text{H}}/(T_1)_{17\text{O}}$  is 2.1 times greater in pure than in muscle water, and (d) 30–49% substitution of deuterons for  $^1\text{H}$  has very little effect on the spin-lattice relaxation of water protons in the tissue. Even if the values obtained for  $T_1$  of  $^1\text{H}$  in the deuterated samples is appropriately multiplied by approximately 1.1 to account for the enhanced relative viscosities of those samples (15), the change induced is far smaller than the more than 50% change anticipated (16).

The fact that the water nuclides share a common frequency dependence indicates that a single relatively slow process determines the rate of longitudinal relaxation of  $^{17}\text{O}$ ,  $^2\text{D}$ , and  $^1\text{H}$ . This rate-limiting process might be the slow rate of tumbling of water immobilized to the surface of macromolecules in rapid exchange with a larger fraction of free molecules; this mechanism appears responsible for the enhanced longitudinal relaxation of  $^1\text{H}$  and  $^{17}\text{O}$  in diamagnetic protein solutions (17, 18).<sup>1</sup> Alternatively, the rate-determining step might be the relatively slow rate of movement of the water molecules about some single axis characterizing the putative ordering of bulk muscle water. Either mechanism could be responsible for the observed enhance-

<sup>1</sup>Koenig, S. H., and M. Shporer. In preparation.

ment of the spin-lattice relaxation for the three nuclei. However, other of the data presented suggest that ordering of the intracellular water is unlikely to be of significance in determining the rate of longitudinal relaxation.

In any given isotopic medium, the ratios  $(T_1)_{2D}/(T_1)_{17O}$  and  $(T_1)_{1H}/(T_1)_{17O}$  reflect only the relative magnitudes of the basic interactions responsible for the spin-lattice relaxations of the three nuclides; these interactions are quadrupolar for  $^2D$  and  $^{17}O$ , and dipolar for  $^1H$ . If ordering effects of the medium contribute to the relaxation processes, these ratios will be determined by the same relaxation factors, multiplied by the ratio of some geometrical or ordering parameters; the ordering factor is determined by the angle between the axis of interaction of each nuclide and the axis of orientation of the water molecule. The precise analytic expression for the ordering factor depends upon the specific model under consideration (19–21). The present results (Table I) indicate that the quotient  $(T_1)_{2D}/(T_1)_{17O} = 79\text{--}80$  in muscle water, not significantly different from the value of 77 in pure water. Therefore, the ratio of ordering factors for  $^2D$  and  $^{17}O$  is one, i.e. the two nuclides share the same angular dependence in the hypothetically ordered medium.

In an ordered phase of water molecules, it is most reasonable to expect that the axis of alignment would be along the bisector of the  $H-O-H$  angle parallel to the molecular dipole moment. Therefore, the electrical gradient operating on  $O$  would make an angle of approximately  $0^\circ$  with the molecular axis of alignment. On the other hand, the electrical gradient operating on  $^2D$  should act at an angle of approximately  $52^\circ$ . Therefore, were geometric factors of importance,  $(T_1)_{2D}/(T_1)_{17O}$  would be expected to be much greater in muscle water than in pure water. In fact, the experimental value is, within the limits of error, identical with that in bulk water.

It should be emphasized that this experimental observation indicates that significant ordering of the major phase of muscle water is unlikely to be responsible for the enhanced longitudinal relaxation, but does not entirely preclude this possibility. For example, the ratio of the quadrupolar coupling constant for  $^{17}O$  to that of  $^2D$  might have decreased in muscle water, offsetting the increase in the geometric factor, so that the product of these two factors remained fortuitously unchanged. Such a possibility seems remote. The quadrupolar coupling constant for  $^2D$  has been calculated to be approximately 230 kHz in water (22) on the basis of measurements of  $T_1$ , and has been found to be 215 kHz in ice (23) on the basis of more direct measurements. The quadrupolar coupling constant for  $^{17}O$  in water is approximately 7.9 MHz calculated from measurements of  $T_1$  (10), and in ice is 6.5 MHz obtained directly from pure quadrupole resonance (24). Thus, even on the basis of this extreme comparison between liquid and solid phases of water, the quadrupolar coupling constants for  $^2D$  and  $^{17}O$  are unlikely to be much changed within the tissue.

It is also possible, but unlikely, that the axis of molecular orientation is not along the axis of symmetry, but rather along some other axis, so that the identical angle is fortuitously formed with the interaction axis of  $^2D$  and with that of  $^{17}O$ .

The value for  $(T_1)_{1H}/(T_1)_{17O}$  in muscle water in the present study has been found to be considerably smaller than that in pure water. In addition, partial replacement of

the muscle protons with deuterons did not alter the longitudinal relaxation of the  $^1\text{H}$  as expected (16).

The spin-lattice relaxation of protons in pure water arises both from intramolecular dipolar interactions and from intermolecular dipolar interactions of  $^1\text{H}$  with other molecules of water. The magnetic dipole moment of  $^2\text{D}$  is considerably smaller than that of  $^1\text{H}$ . Therefore, substitution of deuterons for protons produces an approximately proportional reduction in the measured rate of longitudinal relaxation of  $^1\text{H}$  in pure water (16). No such effect was noted in muscle water, even when 49% of the muscle protons were replaced with  $^2\text{D}$ . A similar phenomenon has been noted in protein solutions (25, 26).

The absence of a marked effect on the  $T_1$  of  $^1\text{H}$  following substitution with  $^2\text{D}$  cannot be simply explained by a hypothetical ordering of the bulk muscle water. On the other hand, this phenomenon is entirely consistent with the concept of a rapid exchange between a small fraction of immobilized and a much larger fraction of free water molecules. As long as the major determinant of the proton relaxation was a dipolar interaction of the immobilized fraction with the macromolecular substrate, the substitution of  $^2\text{D}$  for  $^1\text{H}$  in the water molecules would not necessarily induce a measurable change in the  $T_1$  of  $^1\text{H}$ .

In summary, the measurement of the frequency dependence of the longitudinal relaxation rates of  $^{17}\text{O}$ ,  $^2\text{D}$ , and  $^1\text{H}$  in the same samples of frog striated muscle provides further constraints on any model which may be invoked to describe the NMR properties of muscle water. The results suggest that the enhanced relaxation rates of muscle water arise from a rapid exchange between a small fraction of immobilized and a large fraction of free water. These data render alternate interpretations based on ordering of the intramuscular water unlikely.

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## REFERENCES

1. BRATTON, C. B., A. L. HOPKINS, and J. W. WEINBERG. 1965. *Science (Wash. D.C.)*. **147**:738.
2. BELTON, P. S., R. R. JACKSON, and K. J. PACKER. 1972. *Biochim. Biophys. Acta*. **286**:16.
3. HAZLEWOOD, C. F., D. C. CHANG, B. L. NICHOLS, and D. E. WOESSNER. 1974. *Biophys. J.* **14**:583.
4. CIVAN, M. M., and M. SHPORER. 1972. *Biophys. J.* **12**:404.
5. THOMPSON, R. T., R. R. KNISPEL, and M. M. PINTAR. 1973. *Chem. Phys. Lett.* **22**:335.
6. KNISPEL, R. R., R. T. THOMPSON, and M. M. PINTAR. 1974. *J. Magn. Resonance*. **14**:44.
7. FUNG, B. M., and T. W. MCGAUGHY. 1974. *Biochim. Biophys. Acta*. **343**:663.
8. CIVAN, M. M., and M. SHPORER. 1974. *Biochim. Biophys. Acta*. **343**:399.
9. CIVAN, M. M., and R. J. PODOLSKY. 1966. *J. Physiol. (Lond.)*. **184**:511.
10. HINDMAN, J. C., A. SVIRMICKAS, and M. WOOD. 1970. *J. Chem. Phys.* **74**:1266.
11. WOESSNER, D. E. 1964. *J. Chem. Phys.* **40**:2341.
12. KRYNICKI, K. 1966. *Physica (Utrecht)*. **32**:167.
13. HARDY, R. C., and R. L. COTTINGTON. 1949. *J. Chem. Phys.* **17**:509.
14. KUDISH, A. I., D. WOLF, and F. STECKEL. 1972. *J. Chem. Soc. Faraday Trans. I*. **68**:2041.

15. BAKER, W. N., and V. K. LA MER. 1935. *J. Chem. Phys.* **3**:406.
16. SMITH, D. W. G., and J. G. POWLES. 1965. *Mol. Phys.* **10**:451.
17. KOENIG, S. H., and W. E. SCHILLINGER. 1969. *J. Biol. Chem.* **244**:3283.
18. KOENIG, S. H., and W. E. SCHILLINGER. 1969. *J. Biol. Chem.* **244**:6520.
19. MCBRIERTY, V. J., and D. C. DOUGLASS. 1970. *J. Magn. Resonance.* **2**:352.
20. DONG, R. Y. 1972. *J. Magn. Resonance.* **7**:60.
21. WOESSNER, D. E., and B. S. SNOWDEN, JR. 1973. *Ann. N.Y. Acad. Sci.* **204**:113.
22. POWLES, P. G., M. RHODES, and J. H. STRANGE. 1966. *Mol. Phys.* **11**:515.
23. WALDSTEIN, P., S. W. RABIDEAU, and J. A. JACKSON. 1964. *J. Chem. Phys.* **41**:3407.
24. EDMONDS, D. T., and A. ZUSSMAN. 1972. *Phys. Lett.* **41A**:167.
25. KIMMICH, R., and F. NOAK. 1970. *Z. Naturforsch.* **25a**:1680.
26. KIMMICH, R., and F. NOAK. 1971. *Ber. Bunsenges. Phys. Chem.* **75**:269.