PULSED NUCLEAR MAGNETIC RESONANCE STUDY OF ^{17}O FROM $H_2{}^{17}O$ IN RAT LYMPHOCYTES

MORDECHAI SHPORER, MARTIN HAAS, *and* MORTIMER M. CIVAN

From the Departments of Isotope Research and Cell Biology, The Weizmann Institute of Science, Rehovot, Israel, and the Departments of Physiology and Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19174

ABSTRACT Lymphocytes obtained from thymus glands of normal rats and culture lines of malignant rat thymocytes were enriched with $H_2{}^{17}O$. The longitudinal and transverse relaxations of the ^{17}O were determined separately in samples of packed cells and supernatant solutions. The longitudinal relaxation of intracellular ^{17}O of fresh viable lymphocytes was nonexponential, becoming simply exponential with eventual necrosis. The rate of spin-lattice relaxation ($1/T_1$) was fitted by a sum of two exponentials. The average mole fraction of the molecules subject to the slower relaxation rate ($1/T_{1s}$) was two-thirds of the total water. Lowering the Larmor frequency (ω) from 7.72 to 4.36 MHz increased the faster component ($1/T_{1f}$) by 12% without altering ($1/T_{1s}$). The value of the single exponential decay of the nonviable cells was not appreciably different from the initial rate of relaxation of the fresh cells. Similar results were obtained in studies of the transverse relaxation rates. The simplest interpretation is that two-thirds of the cell water is located within the nucleus and is characterized by a slower rate of relaxation than the one-third of the cell water in the cytoplasm because of the different macromolecular compositions of the two subcellular compartments. The malignant lymphocytes were characterized by prolonged values for the slow and fast components of both the longitudinal and transverse relaxations of ^{17}O.

INTRODUCTION

Variations in the nature and composition of the intracellular fluids may be of considerable physiologic and pathophysiologic importance (1). Application of the techniques of nuclear magnetic resonance (NMR) to this problem has already provided considerable information.

The magnetic relaxation of water protons in diamagnetic protein solutions is strongly dependent upon the proton Larmor frequency (2–9). The nature of this dispersive effect is a function of the size and concentration of the protein in solution (2–9). Water deuterons and ^{17}O undergo similar relaxation dispersions in such protein solutions (10). Recently, we have found that the longitudinal relaxation times (T_1) for the three water nuclides exhibit the same frequency dependence in frog striated muscle, and that this dependence is similar to that in cell-free protein solutions (11).

Study of intracellular $H_2{}^{17}O$ has been particularly advantageous since the magnetic relaxation times for ^{17}O are far faster than those for ^1H and ^2D. Because of the

presence of subcellular organelles, intracellular water must be characterized by some degree of compartmentalization. If the relaxation rates of the water nuclide under study are slower than exchange rates of water among the different compartments, only average NMR values will be detected. Use of the ^{17}O from $H_2\ ^{17}O$ permits far greater resolution among the different populations of intracellular water. For example, study of ^{17}O has permitted distinction between the intracellular and extracellular water of erythrocyte suspensions (12); a similar resolution is impossible for water protons and deuterons unless paramagnetic substances such as Mn^{++} are added (13, 14).

The longitudinal relaxation rate of ^{17}O from intracellular $H_2\ ^{17}O$ in human erythrocytes is purely exponential (12); these cells are relatively simple, lacking both nuclei and mitochondria. On the other hand, the rate of longitudinal relaxation in frog striated muscle is nonexponential until necrosis supervenes (15). Striated muscle is clearly more complex than such erythrocytes, containing, e.g., nuclei, mitochondria, the sarcoplasmic reticulum, the transverse tubular system, and ordered arrays of proteins. It has been unclear whether the nonexponential character of the magnetic relaxation of ^{17}O is unique to striated muscle, and to what extent the phenomenon reflects the physiologic state of the tissue.

In order to examine this problem further, we have turned to another cell type, the small lymphocyte of the rat, for at least two reasons. First, most of the cell volume consists of nucleoplasm, in striking contrast to striated muscle where the ratio of nuclear to cytoplasmic volume is very low. Study of the lymphocyte then permits us to assess the role of the nucleus without resorting to techniques of cell fractionation inevitably resulting in some degree of nuclear damage. Second, it has been possible to develop two types of malignant lymphocytes from the same organ in the same species; this has provided a further opportunity to examine the thesis that changes in the relaxation rates of water nuclides are characteristic of the cancer state (16, 17).

METHODS

Thymus glands were excised from inbred male Lewis rats, 2.5–3-mo old (Animal Breeding Center, The Weizmann Institute of Science, Rehovot, Israel). Suspensions of normal lymphocytes were obtained by pressing the thymus glands through a fine wire mesh into phosphate-buffered saline (NaCl, 113.9 mM; KCl, 2.7 mM; $CaCl_2$, 0.9 mM; Na_2HPO_4, 8.1 mM; KH_2PO_4, 1.5 mM; $MgCl_2$, 0.5 mM; pH 7.2–7.4). Four suspensions of normal cells were collected from two sets of five to eight rats.

Two types of malignant thymocytes (K127T and K127R) were also studied. Thymomas were induced in 2-mo old female Lewis rats injected intrathymically with mouse Radiation Leukemia virus and subsequently given an immunosuppressive dose of X rays (400 rads). Large thymomas appeared in all rats approximately 60 days after injection and were excised and grown in culture 10 or more days later. The cells were grown in suspension in a Dulbecco-modified Eagle's medium poor in Ca^{++} and Mg^{++} and supplemented with 10% fetal calf serum and 50 μg/ml Gentamycin (18). Cells in culture were freely passaged back by subcutaneous injection into 2-mo old rats

and passaged once again into culture. A palpable tumor appeared 10 days after subcutaneous injection of 10^6 cells, reaching a diameter of 15–20 cm or more and thus becoming as large as the rat itself. This type of tumor (K127T) killed the rats within 3–4 wk after injection.

In addition, a spontaneous cell line (K127R) was found which also produced a palpable tumor 10 days after subcutaneous injection, but which reached a maximum diameter of only 3–5 cm some 7–10 days later. Subsequently, the tumor regressed spontaneously, disappearing completely within 4 wk after the initial implantation. The immunological basis for the difference in behavior of the two tumor lines is unknown.

In culture, both cell lines grew to concentrations of 2×10^7 cells/ml and produced ample C-type RNA virus identified as mouse Radiation Leukemia virus, without any evidence of rat virus. The cells of both types of thymomas are morphologically indistinguishable from typical lymphoblasts, the nucleus occupying nearly the entire cell (18); their total cell volume is three to four times larger than that of normal small lymphocytes. In the current study, both types of malignant thymocytes were harvested into phosphate-buffered saline from the in vitro cultures.

The suspensions of normal and malignant cells were centrifuged for 5 min at about 150 *g*. 1 vol of the packed cells was then suspended in 1–2 vol of modified Eagle's medium (Grand Island Biological Corp., Grand Island, N.Y.) supplemented with 100 U/ml penicillin G and 100 μg/ml dihydrostreptomycin sulfate, and containing 5%–18% $H_2\,{}^{17}O$. After incubation at 4°C for 1.5–2 h the suspension was centrifuged at approximately 100 *g* for 5 min at 4°C. The supernate and pellet were retained for study; the interface material found at the junction between the pellet and supernate was rejected. Viability was defined by exclusion of the dye eosin Y (19).

Measurements were performed with a Bruker Pulsed NMR B-KR 320 Spectrometer (Bruker Physik AG, Karlsruhe, Germany) at frequencies (ω) of 7.72 and 4.36 MHz; the strength (H_o) of the steady magnetic field provided by a 12-in model V 4012 A-HR electromagnet system of a Varian (Varian Associates, Instrument Div., Palo Alto, Calif.) DP-60 spectrometer was adjusted appropriately. The temperatures of the samples were controlled by flowing nitrogen gas, thermally regulated by a Bruker B-ST 100/700 heating unit around the sample. The temperatures of the samples were measured by means of a thermocouple introduced through a glass capillary immersed in the sample materials. Measurements were made only after thermal equilibrium had been reached (at 26.5 ± 1°C and 4.5 ± 0.5°C), at which point the thermocouple was removed.

As previously described in detail (15), T_1 (the spin-lattice relaxation time) was measured by standard techniques. The *z* component of the magnetization was studied after paired pulses of 180° and 90°. In each case, the intensity was sampled within 200 μs after the 90° pulse. The free-induction decay after the 90° pulse was time-averaged in order to improve the signal to noise ratio.

T_2 was obtained directly from the free-induction decay. The contribution of field inhomogeneity had been shown to be negligible by comparing results obtained by the

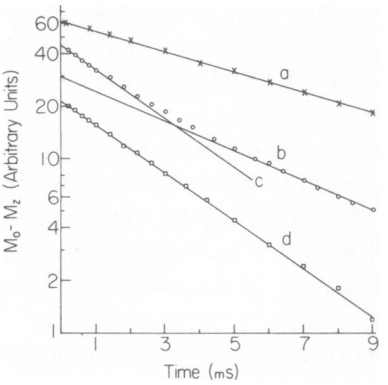

FIGURE 1

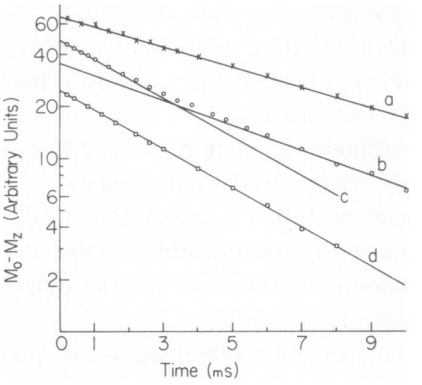

FIGURE 2

FIGURE 1 Longitudinal relaxation of ^{17}O in supernate, pellet of fresh cells, and pellet of nonviable cells from suspension of normal lymphocytes. The ordinate is the $\log_{10}$ of the difference between M_o (the full magnetization) and M_z (the z component of the residual magnetization) in arbitrary units, after a pulse of 180° and of 90°. The abscissa is the time interval after the end of the 90° pulse. All data were obtained at 26.5°C and at a Larmor frequency of 7.72 MHz. (*a*) The data points obtained with the supernatant solution exhibit a single exponential decay. (*b*, *c*) The data obtained from fresh viable cells exhibit markedly nonexponential behavior. (*d*) The data points obtained with nonviable cells from the same sample as *b* and *c* 32 h later now exhibit simple exponential behavior. The initial rate of relaxation of the viable cells is similar to the relaxation rate of the necrotic cells (*d*).

FIGURE 2 Effect of temperature on the longitudinal relaxation of ^{17}O in normal viable lymphocytes. The coordinates are defined in the legend to Fig. 1. The data points of *a* and *b* obtained at 26.5°C have been reproduced from Fig. 1 *b*, *c* for purposes of reference. The data points of *c* and *d* were obtained from the same sample at 4.5°C.

Carr-Purcell sequence with those obtained from the free-induction decay for ^{17}O in pure water.

RESULTS

Fig. 1 presents the recovery with time of the z component of the magnetization for ^{17}O from H_2 ^{17}O in the supernatant sample (*a*), pellet of normal fresh lymphocytes (*b*, *c*), and in the same pellet after 32 h had elapsed (*d*). All measurements were obtained at 26.5°C, at a Larmor frequency of 7.72 MHz. Some two-thirds of the cells studied still excluded eosin Y even 3 h after the data of curves *b* and *c* were obtained, whereas none of the cells excluded dye when the data of curve *d* were obtained.

In the fresh state (*b*, *c*) the longitudinal relaxation of ^{17}O was distinctly nonexponential. The data could be fitted by the sum of two exponentials. From the initial slope of the longitudinal relaxation of these data points (fitted by line *c*) the initial longitudinal relaxation time (T_{1o}) was calculated to be 3.1 ms. From line *b*, fitting the final rate of longitudinal relaxation, the spin-lattice relaxation time (T_{1s}) characterizing the slower component was found to be 5.1 ms. The ratio of the intercepts of lines *b* and *c* with the *y* axis was 0.68.

The rate of longitudinal relaxation of ^{17}O in the supernate (Fig. 1 *a*) was appreciably slower than that of line *b*, corresponding to a value of T_1 equal to 7.5 ms. With death of the cells, the rate of longitudinal relaxation (line *d*) was characterized by a single relaxation time of 3.2 ms, not appreciably different from the initial relaxation time of the fresh pellet.

The data of Fig. 1 could reflect the existence of two or more anatomically distinct populations of water, each characterized by a different relaxation time. However, the data points could reflect not only the intrinsic relaxation rates of the two sites, but also the processes of exchange of water between the two populations. In order to examine this possibility, the cell pellet was examined at two different temperatures.

Fig. 2 *a*, *b* presents the data points of Fig. 1 *b*, *c* for reference, as well as the data points obtained with the same pellet at 4.5°C. The longitudinal relaxation remained nonexponential. At the lower temperature T_{1o} and T_{1s} were 1.8 and 2.8 ms, respectively. The ratio of the intercepts of lines *c* and *d* with the y axis was unchanged from that at 26.5°C.

The effect of temperature on the relaxation of ^{17}O in the supernate was also measured. At 4.5°C, T_1 was 3.8 ms.

From the observations that temperature did not affect P_{1s} appreciably, but did reduce T_{1o} and T_{1s} of the pellet and T_1 of the supernate approximately proportionately, we conclude that the rate of exchange of water between the two populations must be slow in comparison to both $1/T_{1s}$ and $1/T_{1f}$. Under these conditions, the slopes of curves *a* and *c* should directly reflect the relaxation time of the slow component, and P_{1s} should equal the mole fraction of the population of water undergoing the slower relaxation.

In order to reduce the uncertainty associated with data reduction, the following iterative approach was taken. Repeated measurements of the longitudinal relaxation of the fresh pellet should have led to precisely the same estimate of P_{1s}. Any variation in the estimates of P_{1s} must have reflected random scatter in the data rather than true variation in P_{1s}. Therefore, after the initial stage of fitting the data with two exponentials, the mean value of P_{1s} was calculated for all the measurements of longitudinal relaxation of the fresh pellet. This value was then used as an added constraint in the final choice of a sum of two exponentials to best fit the data. Using this approach, the means ± SD of the values for T_{1o}, T_{1f}, and T_{1s} under base-line conditions (fresh pellet of viable cells 26.5°C, ω = 7.72 MHz) were found to be 3.12 ± 0.07, 1.69 ± 0.06, and 5.18 ± 0.08 ms, respectively (Table I). The average value of P_{1s} was 0.68 and changed very little (0.68–0.71) under the different experimental conditions of the current study (Table I).

In order to examine the effect of frequency on the normal lymphocytes, the longitudinal relaxation was studied at 4.36 MHz, as well as at the base-line frequency of 7.72 MHz. Lowering the Larmor frequency by 41% reduced T_{1f} by 11% without appreciably changing T_{1s} (Table I).

The effect of temperature was more striking. Averaging the experimental results, a lowering of the temperature to 4.5°C reduced T_{1f} by 37% and T_{1s} by 45% (Table

TABLE I

EFFECTS OF LARMOR FREQUENCY, TEMPERATURE, VIABILITY, AND MALIGNANT STATE ON THE LONGITUDINAL AND TRANSVERSE RELAXATION RATES OF INTRACELLULAR ^{17}O

The normal and malignant thymocytes were initially studied as fresh pellets at 26.5°C, with ω = 7.72 MHz (base-line conditions). The normal cells were subsequently examined at 26.5°C with ω = 4.36 MHz, and at 4.5°C with ω = 7.72 MHz. After each perturbation, the pellet was restudied under base-line conditions. The values entered in the first line are means ± SD. Values entered in the rows labeled "nonviable" were obtained many hours after initially transferring the cells to the test tube, at a time when none of the cells excluded eosin Y. The subscripts *o*, *f*, and *s* refer to values of the longitudinal (T_1) and transverse (T_2) relaxation times derived from the initial rates of relaxation, fast component of the relaxation, and slow component of the relaxation, respectively. P_{1s} and P_{2s} are the average mole fractions of the slow relaxation components of the longitudinal and transverse relaxations, respectively.

Sample	Conditions	T_1				T_2			
		T_{1o}	T_{1f}	T_{1s}	P_{1s}	T_{2o}	T_{2f}	T_{2s}	P_{2s}
			ms				*ms*		
Normal thymocytes	Base line	3.12 ± 0.07	1.69 ± 0.06	5.18 ± 0.08	0.68	1.52 ± 0.06	0.83 ± 0.04	2.46 ± 0.04	0.69
	4.36 MHz	2.90	1.51	5.14	0.68	1.30	0.66	2.36	0.69
	4.5°C	1.84	1.06	2.83	0.68	1.10	0.58	1.80	0.69
	Nonviable	3.19	—	—	—	1.86	—	—	—
K127T	Base-line	3.75	1.91	6.18	0.71	2.08	0.96	4.02	0.71
	Nonviable	3.91	—	—	—	2.17	—	—	—
K127R	Base-line	3.62	1.97	5.83	0.69	2.04	1.04	3.61	0.69
	Nonviable	3.42	—	—	—	2.07	—	—	—

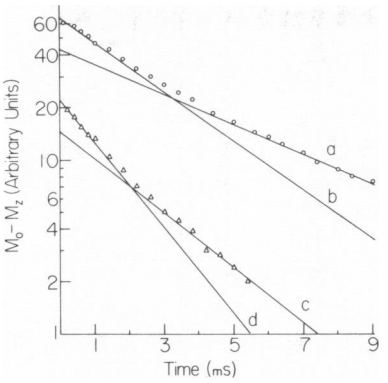

FIGURE 3 Longitudinal relaxation of ^{17}O in supernate, pellet of fresh cells, and pellet of nonviable cells from suspensions of malignant lymphocytes (K127T). The coordinates and symbols are defined in the legend to Fig. 1. The nonviable cells studied in *d* were the same as those examined in *b* and *c* after a period of 43 h had elapsed, when none of the cells excluded eosin Y.

I). Given the standard deviations cited for these measurements, we consider that temperature affects both the fast and the slow components of the spin-lattice relaxation similarly.

Qualitatively, the longitudinal relaxation of the ^{17}O of the two types of malignant thymocytes was similar to that of the normal cell pellet. The relaxation of the fresh pellets was nonexponential, but became simply exponential with the death of the cells (Fig. 3). The values for T_{1o} of the fresh pellet were similar to the values of T_1 for the nonviable cells. T_{1s} was appreciably shorter than the T_1 of the supernatant solution. No qualitative or quantitative differences were noted between the K127T and K127R cells.

The average values for the estimates of the longitudinal relaxation times of the tumors are presented in Table I. Although there was no appreciable shift in P_{1s} from the base-line mean, the tumors were characterized by an increase of 13–19% in T_{1f} and T_{1s}.

The transverse relaxation of ^{17}O was also studied in all the samples under all the experimental conditions described above. Qualitatively, the results are entirely similar to those noted for the longitudinal relaxation, both of the normal cells (Fig. 4) and of the malignant cells (Fig. 5). The average values for the analogous spin-spin parameters are entered in Table I.

DISCUSSION

The current results demonstrate that the longitudinal relaxation of ^{17}O from the extracellular $H_2\,^{17}O$ of rat lymphocytes is nonexponential. Like frog striated muscle (15), and unlike human erythrocytes (12), the spin-lattice relaxation must be fitted by the sum of two or more exponentials. The time constants of the slower (T_{1s}) and faster (T_{1f}) components are some 1.5 and 4.5 times shorter, respectively, than the T_1 of

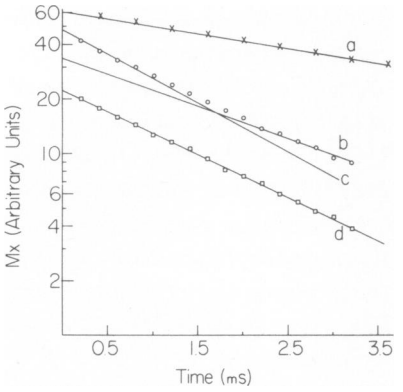

FIGURE 4

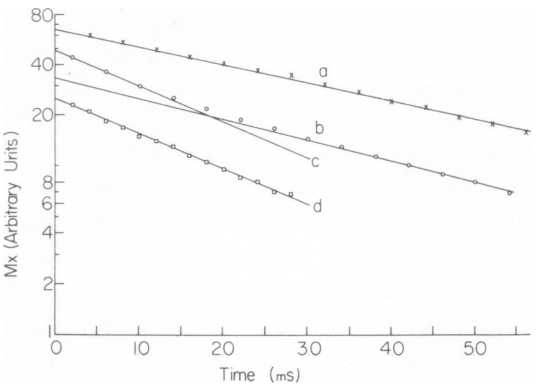

FIGURE 5

FIGURE 4 Transverse relaxation of ^{17}O in supernate, pellet of fresh cells, and pellet of nonviable cells from suspensions of normal lymphocytes. The ordinate is the $\log_{10}$ of the transverse component (M_X) of the residual magnetization after a pulse of 90°. The abscissa is the time interval after the end of the pulse. The data were taken from the same sample under the same conditions as those of Fig. 1. The symbols are defined in the legend to Fig. 1.

FIGURE 5 Transverse relaxation of ^{17}O in supernate, pellet of fresh cells, and pellet of nonviable cells from suspensions of malignant lymphocytes (K127R). The ordinates are defined in the legend to Fig. 4, and the symbols in the legend to Fig. 1. The nonviable cells studied in *d* were the same cells as those examined in *b* and *c* after a period of 42 h had elapsed, when none of the cells excluded eosin Y.

^{17}O in the supernatant solution. The mole fraction (P_{1s}) of the molecules subject to the slower rate of relaxation is approximately two-thirds of the total water content.

Reduction of the sample temperature from 26.5°C to 4.5°C resulted in proportional shortening of T_1 in the supernatant solution. Thus, the data were not measurably influenced by exchange of water between the different populations; the rate constant (k_X) for exchange must be considerably less than $1/T_{1s}$ (i.e., $k_X < 193\ s^{-1}$).

The anatomic site of the two populations of water cannot be rigorously established from the data presented. However, it is likely that the slower component reflects the nuclear water, and the faster component the cytoplasmic water, of the lymphocytes. At the angular velocities used for centrifugation, the cells appear nearly fused into a single mass; the extracellular interstitial space is therefore likely to be less than that of erythrocyte pellets, which amounts to only a few percent of the total pellet volume (20). Both the normal and malignant lymphocytes studied consist primarily of nuclei surrounded by narrow rims of cytoplasm. Furthermore, the water contents per dry weight of the cellular nucleus and cytoplasm of another rat tissue (normal liver) may be calculated to be similar, within some 8% (21); in the amphibian oocyte, where the water contents do differ markedly, it is the nucleus which is richer in water (22). Therefore, the large relative volumes of the nucleus to cytoplasm are likely to reflect a similar subcellular distribution of water. The simplest interpretation is to ascribe the larger, more slowly relaxing population to the nucleus, and the smaller, more rapidly relaxing population to the cytoplasm.

The difference in the longitudinal relaxation of ^{17}O within the two subcellular compartments could reflect a different dispersive effect or an increased ordering of the cytoplasmic water. Recent studies of intracellular water protons, deuterons, and ^{17}O indicate that ordering of cytoplasmic water in striated muscle, and presumably in other cells, is highly unlikely (11). On the other hand, Koenig and his colleagues (2, 10) have demonstrated that at any given frequency, the enhanced relaxation of water nuclides in protein solutions is critically dependent upon the concentration and nature of the protein. Insofar as the macromolecular compositions of the nucleoplasm and cytoplasm are very different (e.g., in terms of DNA, RNA, and histone content), different dispersive effects are to be anticipated in the two subcellular compartments. The data obtained at the two Larmor frequencies are consistent with this concept.

Reduction of ω from 7.74 to 4.36 MHz modestly shortened T_{1f} without appreciably changing T_{1s}. This suggests that over the frequency range examined, the average size of the nuclear macromolecules was too great to permit significant enhancement of the spin-lattice relaxation. On the other hand, the average size of the cytoplasmic molecules was sufficiently small to permit expression of the dispersive effect under these conditions. The difference in dispersive effect may, of course, reflect not simply the size but also the chemical nature of the intracellular macromolecules; differences in the magnitude of the effect may also reflect differences in concentration.

The persistence of the nonexponential behavior of the longitudinal relaxation was correlated with the viability of the lymphocytes. During the course of the present study, the nonexponential character of the spin-lattice relaxation was apparent in pellets containing at least 30–50% of cells capable of excluding eosin Y. With progressive necrosis of the sample, the longitudinal relaxation became simply exponential, and was characterized by a value of T_1 not appreciably different from the value (T_{1o}) characterizing the initial relaxation rate of the fresh pellet. The loss of nonexponential behavior and the constancy of the initial slope during the course of deterioration of the sample suggest that necrosis leads to mixing of the different compartments of water within the tissue, yielding a single population of water, in terms of NMR parameters. The mixing may occur between nuclear and cytoplasmic water, as we presume, or possibly between other physical or functional subcellular compartments of water.

Measurements of the transverse relaxation of ^{17}O of the fresh and nonviable cell pellets, both of the normal and malignant lymphocytes, have led to qualitatively and quantitatively similar results (Figs. 4, 5; Table I). However, measurements of the T_2 characterizing nuclides, such as ^{17}O, with a spin number of 5/2 are less open to ready interpretation than those of T_1. Therefore, we consider the spin-spin relaxation data to be of value primarily in providing a parallel confirmation of the effects noted for the spin-lattice relaxation.

Both types of malignant rat thymocytes exhibited an NMR behavior qualitatively similar to that noted for the normal thymocytes. In addition, P_{1s} was not measurably changed. However, the slow and fast components of the longitudinal and transverse relaxation times were prolonged by 13–19% and by 16–63%, respectively, consistent with previous studies of other cancer preparations (16, 17). The values of T_1 and T_2

of the nonviable malignant cells were also prolonged in comparison with those of the normal lymphocytes. In principle, these changes could reflect a decrease in the size or content per milligram dry weight of the macromolecules, or an increase in the water content of the subcellular compartments.

Although the biological behaviors of the two thymomas are very different, the cells are morphologically indistinguishable. The intracellular ^{17}O of both cell types is also characterized by quantitatively similar NMR parameters.

The authors are grateful to Dr. Irun R. Cohen for providing Lewis rats and for placing his laboratory facilities at their disposal, and to Mr. Zion Gadassi for his skillful technical assistance.

This study was supported in part by Research Grants from the National Science Foundation (BMS 73-01261 A01), the United States-Israel Binational Science Foundation (No. 366), and the University of Pennsylvania Cancer Center Core Grant (CA 16520-01). Dr. Civan is an Established Investigator (70-148) of the American Heart Association.

Received for publication 3 December 1975 and in revised form 11 March 1976.

REFERENCES

1. CIVAN, M. M. 1974. Active sodium transport and the sodium pool. *In* Recent Advances in Renal Physiology. L. G. Wesson and G. M. Fanelli, Jr., editors. University Park Press, Baltimore, Md. 27.
2. KOENIG, S. H., and W. E. SCHILLINGER. 1969. Nuclear magnetic relaxation dispersion in protein solutions: I. Apotransferrin. *J. Biol. Chem.* **244**:3283.
3. FABRY, M. E., S. H. KOENIG, and W. S. SCHILLINGER. 1970. Nuclear magnetic relaxation dispersion in protein solutions. IV. Proton relaxation at the active site of carbonic anhydrase. *J. Biol. Chem.* **245**:4256.
4. KIMMICH, R., and F. NOACK. 1970. Kernmagnetische Relaxation in Protein-lösungen. *Z. Naturforsch. Teil. C Biochem. Biophys. Biol. Virol.* **25a**:299.
5. KIMMICH, R., and F. NOACK. 1970. Zur Deutung der kernmagnetischen Relaxation in Proteinlösungen. *Z. Naturforsch. Teil. C Biochem. Biophys. Biol. Virol.* **25a**:1680.
6. KIMMICH, R., and F. NOACK. 1971. Nuclear magnetic relaxation in solutions of proteins and peptides. *Ber. Bunsenges. Phys. Chem.* **75**:269.
7. KOENIG, S. H., and R. D. BROWN. 1973. Anomalous relaxation of water protons in solutions of copper-containing proteins. *Ann. N.Y. Acad. Sci.* **222**:752.
8. KOENIG, S. H., R. D. BROWN, III, and C. F. Brewer. 1973. Solvent proton magnetic relaxation dispersion in solutions of Concanavalin A. *Proc. Natl. Acad. Sci. U.S.A.* **70**:475.
9. LINDSTROM, T. R., and S. H. KOENIG. 1974. Magnetic-field-dependent water proton spin-lattice relaxation rates of hemoglobin solutions and whole blood. *J. Magn. Resonance.* **15**:344.
10. KOENIG, S. H., K. HALLENGA, and M. SHPORER. 1975. Protein-water interaction studied by solvent ^{1}H, ^{2}H, and ^{17}O magnetic relaxation. *Proc. Natl. Acad. Sci. U.S.A.* **72**:2667.
11. CIVAN, M. M., and M. SHPORER. 1975. Pulsed nuclear magnetic resonance study of ^{17}O, ^{2}D, and ^{1}H of water in frog striated muscle. *Biophys. J.* **15**:299.
12. SHPORER, M., and M. M. CIVAN. 1975. NMR study of ^{17}O from $H_2\,^{17}O$ in human erythrocytes. *Biochim. Biophys. Acta.* **385**:81.
13. CONLON, T., and R. OUTHRED. 1972. Water diffusion permeability of erythrocytes using an NMR technique. *Biochim. Biophys. Acta.* **288**:354.
14. FABRY, M. E., and M. EISENSTADT. 1975. Water exchanges between red cells and plasma: a measurement by nuclear magnetic relaxation. *Biophys. J.* **75**:1101.
15. CIVAN, M. M., and M. SHPORER. 1974. Pulsed NMR studies of ^{17}O from $H_2\,^{17}O$ in frog striated muscle. *Biochim. Biophys. Acta.* **343**:399.
16. DAMADIAN, R. 1971. Tumor detection by nuclear magnetic resonance. *Science* (*Wash. D.C.*). **171**: 1151.

17. WEISMAN, I. D., L. H. BENNETT, L. R. MAXWELL, SR., M. W. WOODS, and D. BURK. 1972. Recognition of cancer in vivo by nuclear magnetic resonance. *Science (Wash. D.C.)*. **178**:1288.
18. HAAS, M. 1974. Continuous production of radiation leukemia virus in C57BL thymoma tissue culture lines: Purification of the leukemogenic virus. *Cell*. **1**:79.
19. BLACK, L., and M. C. BERENBAUM. 1964. Factors affecting the dye exclusion test for cell viability. *Exp. Cell Res.* **35**:9.
20. GIBERMAN, E. 1973. Determination of the trapped volume in a pellet of red blood cells. *Experientia (Basel)*. **29**:1083.
21. SCHIEMER, H. G., G. GÜNTHER, and D. SINA. 1967. Die Wirkung des Cytostaticum Methotrexat auf den Wassergehalt und das Trockengewicht von Kern und Cytoplasma der Leberzellen der Ratte bei der Regeneration nach Teilhepatektomie. *Frankf. Z. Pathol.* **76**:427.
22. CENTURY, T. J., I. R. FENICHEL, and S. B. HOROWITZ. 1970. The concentrations of water, sodium and potassium in the nucleus and cytoplasm of amphibian oocytes. *J. Cell Sci.* **7**:5.