PULSED NUCLEAR MAGNETIC RESONANCE STUDY OF ^{39}K IN FROG STRIATED MUSCLE

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ABSTRACT Samples of 1 M KCl solution and 10 samples of intact frog striated muscle were studied at 4–7°C and/or at 21–22°C. Field inhomogeneity was minimized by using small sample volumes and by using a superconducting magnet designed specifically to provide highly homogeneous fields. In the present experiments, magnetic field inhomogeneity was measured to contribute < 15% to the free induction decay observed for intracellular ^{39}K. The signal-to-noise ratio of the measurements was enhanced by means of extensive time-averaging. The rates of nuclear relaxation for ^{39}K in aqueous solution were 22 ± 3 (mean ± 95% confidence limits) s^{-1} at 4–7°C and 15 ± 2 s^{-1} at 21–22°C. For intracellular ^{39}K, $(1/T_2)$ was measured to be 327 ± 22 s^{-1} and 229 ± 10 s^{-1} at the lower and higher temperatures, respectively. The corresponding values for $(1/T_1)$ in the same muscle samples were 198 ± 31 s^{-1} and 79 ± 15 s^{-1} at 4–7°C and at 21–22°C, respectively. These results for ^{39}K are similar to those previously obtained for intracellular ^{23}Na. Since < 1% of the intracellular ^{23}Na has been estimated to be immobilized, fractional immobilization of intracellular ^{39}K is also likely to be insubstantial.

INTRODUCTION

From a comparison of the values estimated for the electrochemical activity coefficients of Na^+ and K^+ within a large number of cells (1), it has seemed likely that compartmentalization and/or immobilization partially determine the electrochemical activity of the alkali cations within the intracellular fluids. By the term "compartmentalization," we refer to the nonuniform distribution of the free form of ions between the cytoplasm and subcellular organelles. By "immobilization," we refer to a period of residence by an ion at a binding site which is several orders of magnitude larger than the ion's correlation time in dilute aqueous solution.

Early results obtained by techniques of nuclear magnetic resonance (NMR) were interpreted to reflect the presence of substantial intracellular immobilization of Na^+ (2). This initial conclusion reached by a number of investigators was based upon the assumption that the relative intensity of the visible spectral line for ^{23}Na was directly dependent upon the concentration of free intracellular Na^+. Subsequent work has demonstrated that this basic assumption was incorrect (3–5). More recent studies of the effect of magnetic field strength upon the nuclear magnetic relaxation times suggests that < 1% of the intracellular Na^+ of frog striated muscle is immobilized.

By exclusion, compartmentalization is presumably playing a significant role in determining the electrochemical activity of Na^+ within the cytoplasm.

Far fewer NMR studies have been devoted to an examination of intracellular K^+, in part because of its less favorable relative sensitivity. Magnuson et al. (6) have reported that the ^{39}K accumulated by etiolated pea stem is in free form. However, as they pointed out, most of the intracellular fluid of their preparation was present within vacuoles, which may not be representative of the intracellular fluids within animal cells.

More recently, Damadian and Cope (7) and Cope and Damadian (8) have found that the longitudinal relaxation time (T_1) was shortened by a factor of 5–8 for ^{39}K within the intracellular fluids of rat muscle and brain. On the other hand, these authors claimed that the transverse relaxation time (T_2) for intracellular ^{39}K is some 200 times shorter than that for ^{39}K in dilute aqueous solution. Their data were interpreted to indicate substantial fractional immobilization of intracellular K^+. However, the apparently marked shortening of T_2 could also have primarily reflected inhomogeneity of their applied magnetic field.

In order to examine this possibility in greater detail, the following study of ^{39}K within frog striated muscle was performed. As discussed in the Methods, there are considerable technical difficulties in properly adjusting the Carr-Purcell sequence of pulses when applied to samples (such as this) with very low signal-to-noise ratios. Therefore, considerable care was taken to minimize magnetic field inhomogeneity. Sample volumes were reduced to a value seven times smaller than that used by Cope and Damadian (8). In addition, the superconducting magnet used was part of a high resolution NMR system designed specifically to provide highly homogeneous fields.

METHODS

Samples

Male and female specimens of the frog *Rana pipiens* were obtained from West Jersey Biological Farm, Wenonah, N.J. As previously described (9), gastrocnemius, sartorius, and semitendinosus muscles were excised intact from the doubly pithed animals, and bathed for at least 5 min in a standard aerated Ringer's solution (10) (NaCl, 115.5 mM; KCl, 2.5 mM; $CaCl_2$, 1.8 mM; Na_2HPO_4, 2.5 mM; NaH_2PO_4, 0.5 mM; and *d*-tubocurarine chloride, 9 mg/liter). The muscles were subsequently removed from solution, blotted dry with filter paper, and gently packed into the sample chamber.

A sufficient number of whole muscles was introduced to fill the chamber volume (0.43 cm^3). In all, 10 preparations were studied either at 4–7°C, the equilibrium temperature within the base of the magnet, and/or at 21–22°C by flowing air at ambient temperature around the sample.

Since the muscles were studied under anerobic conditions for periods as long as 8 h, it was of interest to have some index of their physiologic state at the conclusion of each experiment. For this reason, after the NMR measurements, the muscles were removed from the sample tube, washed with fresh Ringer's solution and stimulated electrically with a pair of platinum electrodes. All muscle samples which had been studied at 4–7°C were still able to respond to electrical stimuli, albeit less vigorously, after the NMR measurements were

completed. Tissue preservation was less satisfactory at 21–22°C, but contractile responses were observed in one or more muscles in each of the five samples studied at that temperature.

NMR Techniques

All NMR measurements were obtained with a pulse spectrometer (Model CPS-2, Spin-Lock Instruments, Ltd., Port Credit, Ontario, Canada) operating at 10.2 MHz. A magnetic field of 51.7 kG was generated by the superconducting magnet of a high resolution spectrometer (Varian HR 220; Varian Associates, Palo Alto, Calif.). In practice, the sample chamber was inserted into a radio frequency (RF) coil (10 mm in inner diameter and 15 mm in length), which in turn was introduced into the clear bore of the magnet with its principal axis oriented perpendicular to the superconductor solenoid. The chamber consisted of a hollow cylinder constructed from Lucite, whose inner diameter was 8 mm and whose inner length was 8.5 mm.

The apparent transverse relaxation time T_2^* was measured directly from the free induction decay (FID) following a pulse 50–60 μs in duration; this duration was adjusted to produce a 90° pulse in an aqueous standard containing 4 M KCl. As will be discussed in the Results, field inhomogeneity provided only a small contribution in the present study to the observed FID of the intracellular ^{39}K.

Under many circumstances, it is possible to reduce this effect of field inhomogeneity by using either the Carr-Purcell sequence or the Meiboom-Gill modification of that technique to measure T_2. However, success with the first technique is critically dependent upon application of precisely 180° pulses after the initial 90° pulse. The latter modification requires that the initial 90° pulse be precisely 90° out of phase with the subsequent train of pulses. For such requirements to be met, it is necessary to tune the spectrometer while directly observing the output from the receiving coil. This approach was not practicable with the equipment used in the present studies of intracellular ^{39}K. Because of the unfavorable signal-to-noise ratio, its signal was visible only after prolonged averaging. It was, of course, possible to directly observe the ^{39}K spectrum from solutions containing very high concentrations of potassium salts. However, the spectrometer tuning appropriate for such highly concentrated solutions of electrolytes would be inappropriate for the biological preparations, since the quality factor (Q) of the coil would be different.

T_1 was measured by studying the free induction decay following pairs of pulses of approximately 90°.

The shape of the FID at the Larmor frequency (ω_o) is exquisitely sensitive to slight deviations from the resonance condition. Because of finite inevitable drift occurring over the many hours of measurement, the pulsed studies were performed at a frequency ω displaced approximately 1 kHz from ω_o. Under these conditions, the output from the receiver coil appears in the form of damped oscillations at the frequency of approximately 1 kHz. The envelope of this signal expresses the correct form of the FID, and is now insensitive to small shifts in frequency.

The output from the detector was filtered with a resistance-capacitance (RC) network whose time constant was 10 μs.

Data Acquisition

A Varian 620i computer, equipped with a 13-bit 50 kHz conversion rate analog-to-digital converter (ADC) and with 9- and 14-bit digital-to-analog converters (DAC), was used for noise reduction and data display. A command pulse from the computer was transmitted to the spectrometer, initiating a single pulse train which was then under the control of the pulse programmer. The programmer signaled the computer which would then begin data acquisition after an optimal acquisition delay period. Following the data acquisition, a pulse

delay period ensued; the magnitude of that delay was chosen, so that the total duration of each transient study was four to five times longer than the value of T_1 for the sample.

Noise was reduced by means of a double-precision (30-bit) summation type program. The program provided a base-line correction by sampling the receiver output in the absence of pulses. Bias in the ADC and the DC offset in the receiver output were subtracted from the observed signals. As many as 380,000 free induction decays were sampled in a single measurement of T_2. Therefore, a secondary base-line correction was included after every hundredth FID, and a tertiary base-line correction after every thousandth FID, to deal with the small round-off error in the original correction factor. The program provided a continuous normalized display during data accumulation, and permitted control of the parameters such as sampling rate, repetition rate, number of free induction decays to be summed, number of data points to be taken, and delay time before beginning data acquisition. The maximum sampling rate was limited by the software used to 180 μs per point.

Immediately after an applied pulse, a signal artifact was recorded even in the absence of any sample. Without signal averaging, this artifact appeared to decay to the background noise level within 50 μs. When, however, the signal was averaged over tens of thousands of pulse sequences, the pulse artifact was still clearly detectable 400 μs after the end of the 90° pulse. For this reason, data were analyzed only after 600 μs had elapsed.

Data Reduction

The data obtained were fitted with a modified version of a standard multiexponential optimization program (available at the Medical School Computer Facility) to the following functional forms:

$$M_x = M_z e^{-t/T_2} \cos(p + \omega t) + A, \tag{1}$$

and

$$M_x = (M_z e^{-t/T_2} + M_z' e^{-t/T_2'}) \cos(p + \omega t) + A, \tag{2}$$

where p is the phase angle and A is a constant.

In studies of T_1, a series of free induction decays was observed after the second of paired pulses of approximately 90°, separated by a variable interval τ. The values obtained for M_z were weighted according to their estimated variances, and were fitted by the multiexponential optimization program to the form:

$$M_z = M_o(1 - e^{-\tau/T_1}) + A, \tag{3}$$

and

$$M_z = M_o(1 - e^{-\tau/T_1}) + M_o'(e^{-\tau/T_2}) + A. \tag{4}$$

In the event that the applied pulses produced rotations of the magnetization vector through an angle other than precisely 90°, a fraction of the observed signal would decay by a transverse relaxation process. Form 4 was specifically applied to examine this contribution, which was found to be negligible in the present experiments.

The measurements of the longitudinal relaxation process were subjected to a slightly modified version of the data analysis described above for the muscle samples, because of the relatively greater importance of field inhomogeneity in studying the standard solutions. Specifically, field inhomogeneity caused a spin echo, modifying the shape of the free induction decays after the second pulse. For each value of τ, the effect of the echo was negligible for points taken before

0.6 τ. Therefore, data were analyzed only from the time period 0.6 ms to 0.5 τ ms after the end of the second pulse. In estimating M_z after each pulse separation τ, the value of the relaxation rate in function 3 was permitted to vary. Unlike the analysis of the muscle samples, the relaxation rate was not constrained to be the weighted mean for the whole series of experiments. The program used Fletcher and Powell's (11) modification of Davidon's function minimization method in the form of a locally improved version of that available in the IBM Scientific Subroutine Package. In addition to estimating the parameters, this procedure estimates the variance-covariance matrix of the optimization; the latter was used to calculate confidence limits for the parameter estimates. When derived parameters were used for further calculations, as in functions 3 and 4, their estimated variances were used to develop weighting functions according to the methods described by Wilkinson (12).

The values of the individual parameters, or the relationships between any of the parameters (such as the ratio M_z/M_z') could be specified and fixed by the program. When a series of measurements at various delays was analyzed for the muscle samples, the data were first fitted to function 1, allowing all parameters to vary. The weighted mean was then calculated for the values of $1/T_2$ obtained, and the data were refitted, using this value; this procedure minimized errors in M_z due to the covariance cov $(M_z, 1/T_2)$. These final estimates of M_z were then used in function 3. Other values imposed on the parameters are presented in the Results. In general, p and ω were allowed to vary in functions 1 and 2. However, in series of determinations from the same muscle sample, the values for ω were extremely consistent, and those for p reasonably consistent. Each value calculated with the computer program is presented in the Results section in the form of a mean ± the 95% confidence limits.

RESULTS

Fig. 1 presents a representative free induction decay for ^{39}K in 1 M KCl solution at 6°C. From the data analysis described above, the transverse relaxation behavior could

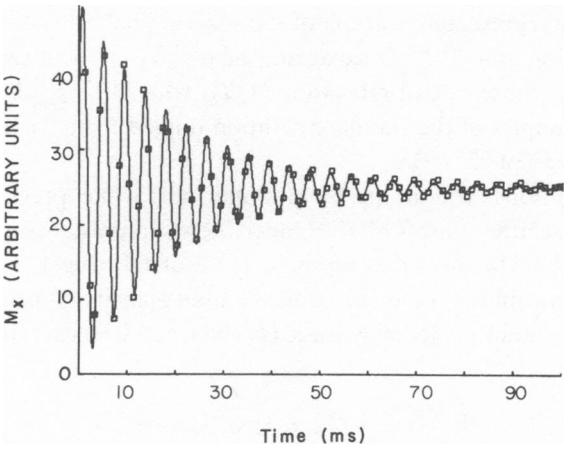

FIGURE 1 Transverse relaxation of ^{39}K in aqueous solution containing 1 M KCl at 6°C. The ordinate is the x-component of the magnetization (M_x), in arbitrary units following a single pulse of approximately 90°; ; the abscissa is time (t), in milliseconds. These and all subsequent data points were obtained off resonance in order to minimize the effect of slight drifts with time. The data have been fit to the function, presented as the uninterrupted curve: $M_x = (24.9)e^{-0.0488t} \cos(5.110 + 1.467t) + 25.0$.

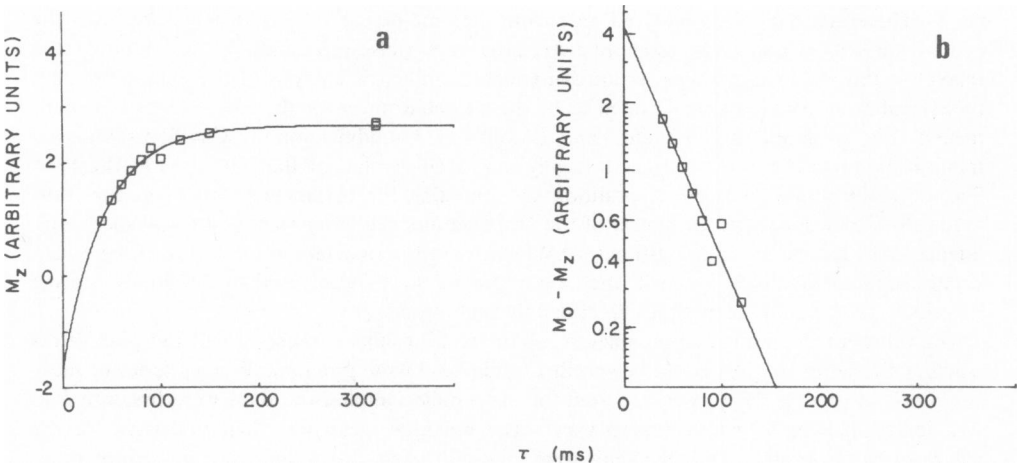

FIGURE 2 Longitudinal relaxation of ^{39}K in aqueous solution containing 1 M KCl. Data were obtained by measuring the magnetization after applying pairs of pulses separated by various time intervals (τ). Function 1 was applied to the data in order to estimate M_z, the transverse component of the magnetization immediately following the second pulse. The ordinate of (*a*) is M_z, in arbitrary units, and of (*b*) is log ($M_o - M_z$); the abscissa in each case is τ, in milliseconds. The data points were obtained at 4–7°C and have been fitted to the function: $M_z = (4.31)(1 - e^{-0.0237\tau}) - 1.65$.

be fitted with a single exponential, whose apparent rate of relaxation ($1/T_2^*$) was 48.8 s^{-1}. The mean value for $1/T_2^*$ varied from 46 ± 3 to 48 ± 5 s^{-1} in different samples at temperatures of 4–7°C, with an overall mean of 47 ± 2 s^{-1}.

Fig. 2 presents a representative graph of the longitudinal relaxation behavior of ^{39}K in 1 M KCl solution at 4–7°C. Once again, the data could be fitted with a single exponential process, whose rate of relaxation ($1/T_1$) was 23.7 s^{-1}. The mean value for ^{39}K in different samples of the standard solution ranged from 21 ± 4 to 24 ± 5 s^{-1}, with an overall mean of 22 ± 3 s^{-1}.

In aqueous solutions, conditions of motional narrowing prevail; i.e., $\omega_o\tau_c \ll 1$, where τ_c is the correlation time for ^{39}K. Under these circumstances, $(1/T_1) = (1/T_2)$. The observation that the measured estimate $1/T_2^*$ of the true $1/T_2$ was greater than $1/T_1$ reflected a contribution of magnetic field inhomogeneity. Assuming that this contribution is simply additive, its magnitude (k_I) can be estimated from these relationships:

$$1/T_2^* = 1/T_2 + k_I = 1/T_1 + k_I. \quad (5)$$

Introducing from the data cited above the mean values of 47 and 22 s^{-1} for ($1/T_2^*$) and ($1/T_1$), respectively, into Eq. 5, k_I is calculated to be approximately 25 s^{-1}.

Since the properties of intracellular ^{39}K were studied both at 21–22°C and at 4–7°C, the longitudinal relaxation of ^{39}K in the standard solution was also studied at the higher temperature. The value of $1/T_1$ for different samples of solution ranged from

13 ± 7 to 17 ± 2 s^{-1}, with an overall mean of 15 ± 2 s^{-1}. The mean of the ratio of $1/T_1$ measured at low temperature to that at high temperature was 1.4 ± 0.3. As expected, $1/T_2^*$ was found to be very little different (45 ± 2 s^{-1}) at the higher temperatures, because of the large constant contribution of the field inhomogeneity. Introducing these values obtained at 21–22°C into Eq. 5, k_f is calculated to be 30 s^{-1}. As will become apparent later in the text, from these estimates of k_f, we can conclude that field inhomogeneity contributed less than 15% of the observed value of $(1/T_2)$ for the muscle samples.

Fig. 3 presents the raw data obtained during a free induction decay of ^{39}K within frog muscle studied at 5.5–7°C. Initially, attention was devoted to the question of whether only one or two exponential processes contributed to the observed transverse

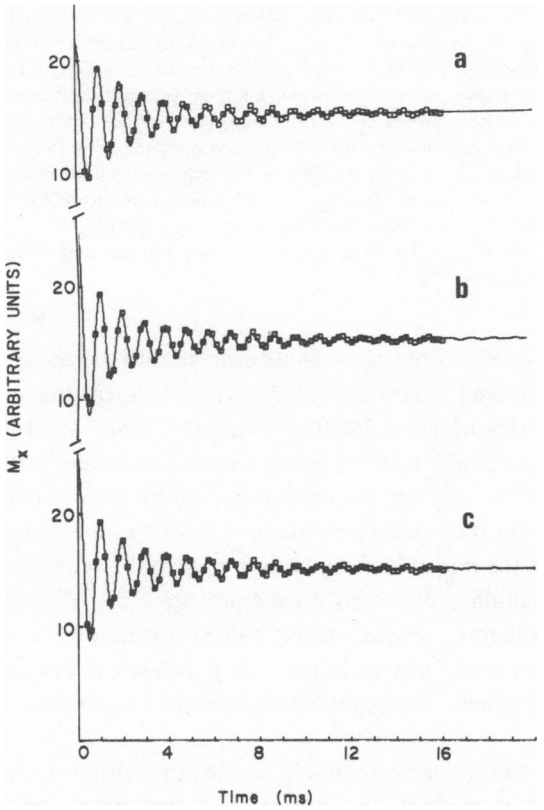

FIGURE 3 Transverse relaxation of ^{39}K in frog muscle at 5.5–7°C. The magnetization (M_x) is presented as a function of time (t) following a single pulse of approximately 90°. The same data points (obtained after accumulation of 383,293 transients) are entered in all three panels, but have been fitted to the different functions: (*a*) $M_x = (6.73)e^{-0.398t}\cos(6.040 + 6.534t) + 15.0$; (*b*) $M_x = (7.33\,e^{-1.48t} + 3.32\,e^{-0.222t})\cos(5.993 + 6.544t) + 15.0$; (*c*) $M_x = 1.00(6\cdot e^{-1.57t} + 4\cdot e^{-0.258t})\cos(6.003 + 6.542t) + 15.0$. The sums of the squares of the deviations from the functions used in *a*, *b*, and *c* were 3.94, 0.60, and 0.80, respectively.

TABLE I

RATES OF TRANSVERSE RELAXATION ($1/T_2^*$) MEASURED AFTER LONG ACCUMULATIONS AND FITTED EITHER WITH ONE OR TWO EXPONENTIALS

Experiment	Temperature	Single Exponential ($1/T_2^*$)	Two exponentials		
			($1/T_2^*$)	($1/T_2^*$)′	d^2
		s^{-1}	s^{-1}	s^{-1}	
1	Low	320 ± 35	1,550 ± 550	276 ± 35	0.53
2	Low	283 ± 37	1,690 ± 500	306 ± 45	0.52
3	Low	302 ± 22	1,570 ± 250	258 ± 15	0.20
4	Low	239 ± 27	1,670 ± 550	250 ± 41	0.44
	Room[n]	208 ± 18	—	—	~ 1
5	Low[n]	321 ± 37	—	—	~ 1
	Room[n]	251 ± 27	—	—	~ 1

After extended periods of data accumulation, free induction decays have been analyzed either in terms of a single, or of a double, exponential relaxation. All tabulated values are presented as means ± the 95% confidence limits. In each case, the measured value ($1/T_2^*$) of the rate of transverse relaxation is a falsely high estimate of the true rate of transverse relaxation ($1/T_2$) because of a contribution of 25–30 s^{-1} by field inhomogeneity. The degree of the improvement of fit by using two exponentials has been quantified by the parameter d^2, the ratio of the sum of the squares of deviations obtained with two exponentials to that obtained with a single exponential. In fitting the data to two exponentials, the relative intensity of the faster relaxing fraction to that of the slower relaxing fraction has been taken to be 6:4, for the reasons discussed in the text. In the case of the experiments identified with the superscript n, $d^2 \sim 1$, so that analysis in terms of two exponentials was not meaningful. The entries "low temperature" and "room temperature" refer to 4–7°C and to 21–22°C, respectively.

relaxation. In order to improve the signal-to-noise ratio, prolonged signal averaging was carried out with five preparations (Table I); some measurements were performed after accumulation of as many as 380,000 transients. Analysis of the data was limited by events occurring early and late in each sequence. As noted in the Methods section, because of an early pulse artifact, data points appearing before 0.6 ms had elapsed were routinely ignored. On the other hand, there was little point in analyzing the extreme terminal portions of the free induction decays. Late in the sequence, the small fraction of ^{39}K in the extracellular fluid (constituting perhaps 0.5% of the total ^{39}K) would be expected to contribute to the signal; indeed, some evidence of this contribution was observed. As a result of these very early and late processes, it was not possible to determine conclusively whether more than one exponential was necessary to characterize the intracellular ^{39}K.

In the case of several measurements in the lower temperature range, the fit with two exponentials was significantly better than that with one, although the confidence limits of the parameters obtained were substantially broader (Table I). The degree of the improvement of fit was estimated from the ratio (d^2) of the sum of the squares of the deviations calculated for the data fit with two exponentials to that fit with one; a value of $d^2 < 1$ implies an improved fit with two exponentials. A representative example of this improved fit is presented in Fig. 3, where the same data have been fitted by three different functions. In the top panel, the data were fitted to a

single exponential. In the two lower panels, two exponentials were used. In the middle panel, the ratio of the intensities (M_z/M_z') was permitted to vary. In the lowest panel, the ratio was fixed at 6:4, the larger intensity being assigned to the more rapidly relaxing fraction. This specific ratio was chosen to conform to the theoretical ratio expected if intracellular ^{39}K undergoes a first-order nuclear quadrupolar interaction, as seems to be the case for intracellular ^{23}Na (3–5).

The functions of the middle and lowest panels of Fig. 3 appeared to improve the fit to approximately the same degree; d^2 was 0.15 and 0.20 for the former and latter functions, respectively. Thus, although two exponential processes appear to have affected the data of Fig. 3, the precise ratio of the intensities of the two relaxing fractions cannot be specified. This observation held for the other curves, as well, where the fit with two exponentials was significantly better than that with one. In such cases, when permitted to vary, the ratios for M_z/M_z' averaged approximately 2:1. In no case did the imposition of a ratio of 6:4 significantly reduce the goodness of fit, nor however did it tighten the confidence bounds of the parameters. In summary, the data can be fit well by a single exponential. However, some of the results suggest that two exponentials may play a role, with the faster relaxing fraction accounting for some $\frac{2}{3}$ of the total signal; a precise definition of its relative intensity is not possible at present.

In those cases where fitting the data with two exponentials was meaningful, the measured value of $(1/T_2)$ for the more rapidly relaxing fraction ranged from 1,000 to 2,000 s^{-1} at 4–7°C (Table II). In those cases, the slower component of $(1/T_2)$ ranged from 250 ± 41 to 306 ± 45 s^{-1}. In the same group of preparations, fitting the data to a single exponential led to an estimated rate of transverse relaxation ranging from 239 ± 27 to 321 ± 37 s^{-1} at the same temperatures.

In six preparations, both $(1/T_1)$ and $(1/T_2)$ were measured in the same muscles (Table II). In studying $(1/T_2)$ in these cases, points up to 1.0 ms were deleted. If two exponentials were present, this would introduce some distortion. Comparing the constants obtained from those curves that could be fitted to both functions 1 and 2, it

TABLE II

RATES OF TRANSVERSE $(1/T_2^*)$ AND LONGITUDINAL $(1/T_1)$ RELAXATION SIMULTANEOUSLY MEASURED IN THE SAME MUSCLE SAMPLES

Experiment	Temperature	$(1/T_2^*)$	$(1/T_1)$
		s^{-1}	s^{-1}
6	Low	362 ± 53	198 ± 46
7	Low	308 ± 22	197 ± 58
2	Low	311 ± 44	200 ± 104
8	Room	230 ± 22	79 ± 61
9	Room	223 ± 26	78 ± 22
10	Room	234 ± 17	79 ± 41

The values for $(1/T_2^*)$ have been calculated by fitting the data to a single exponential. The initial data points up to 1.0 ms were omitted from this analysis.

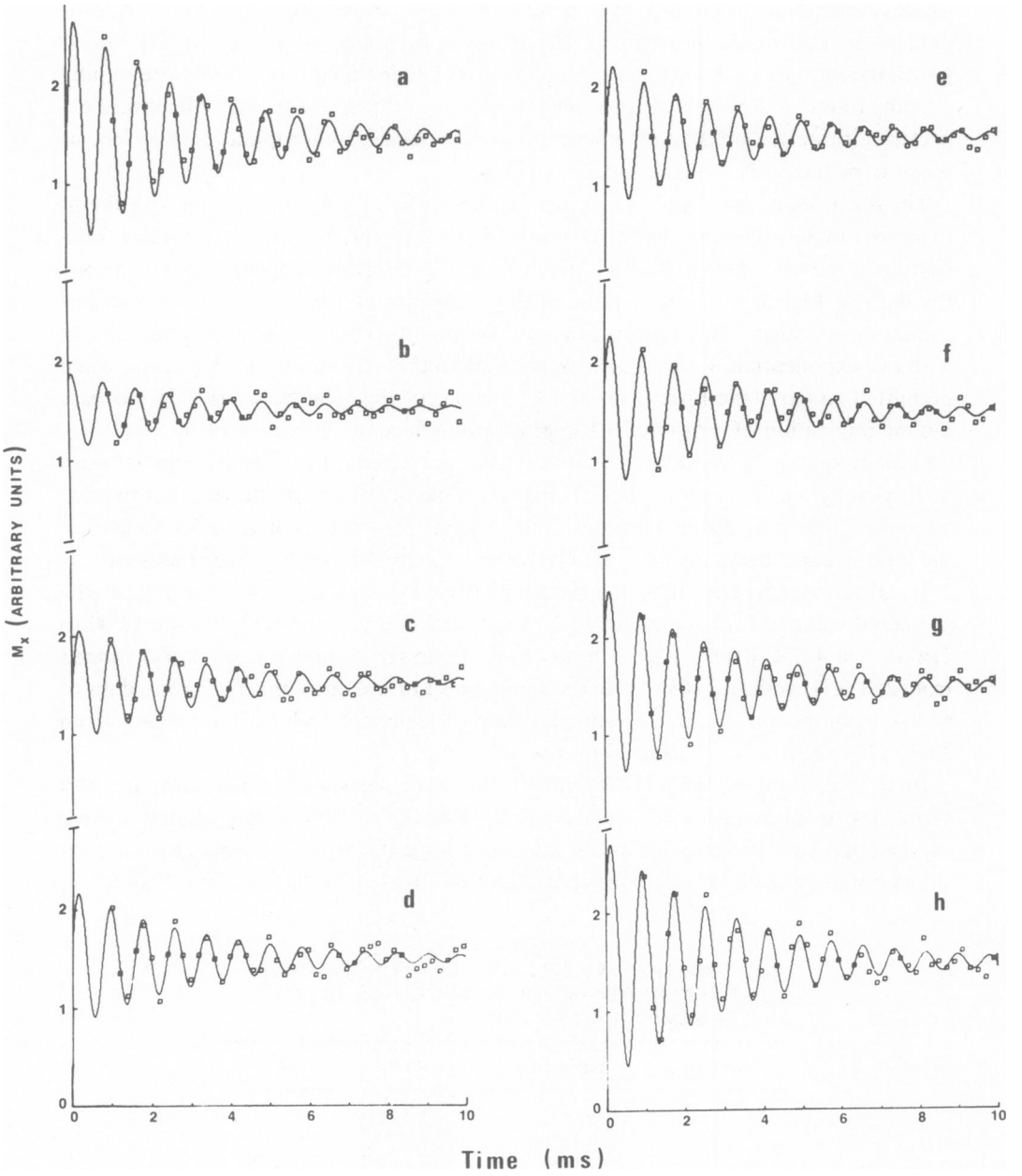

FIGURE 4 Longitudinal relaxation of ^{39}K in frog muscle at 6°C. The x-component of the magnetization (M_x), in arbitrary units, is presented as a function of the time (t), in milliseconds, following the second of a pair of pulses separated by an interval τ. All of the data have been fitted to the function: $M_x = M_z e^{-0.308t} \cos(p + 7.83t) + 1.5$. The values of M_z and p are different for the various values of τ. (*a*) τ = 30 ms, M_z = 1.21, p = 4.54; (*b*) τ = 3 ms, M_z = 0.39, p = 5.72; (*c*) τ = 4 ms, M_z = 0.59, p = 4.62; (*d*) τ = 5 ms, M_z = 0.70, p = 5.09; (*e*) τ = 6 ms, M_z = 0.75, p = 4.72; (*f*) τ = 7 ms, M_z = 0.81, p = 5.10; (*g*) τ = 9 ms, M_z = 1.02, p = 5.22; (*h*) τ = 30 ms, M_z = 1.24, p = 5.26. The data were obtained in the chronological order: *a*, *b*, *e*, *g*, *c*, *d*, *f*, *h*.

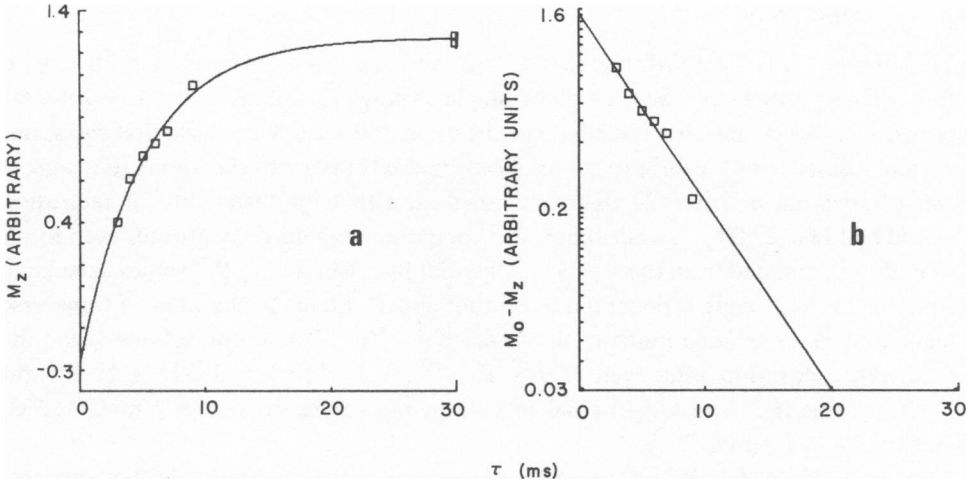

FIGURE 5 Longitudinal relaxation of ^{39}K in frog muscle. The values of M_z obtained from the data of Fig. 4 are presented as a function of τ, in milliseconds. The data have been fitted by the function: $M_z = (1.53)(1 - e^{-0.197\tau}) - 0.26$. The ordinate of (*a*) is M_z, in arbitrary units, and of (*b*) is $\log(M_o - M_z)$.

appears that deletion of the first 1.0 ms leads to an approximately 20% overestimate of $(1/T_2)$. Using this approach, the values of $(1/T_2)$ measured for the three different preparations studied at 4–7°C ranged from 308 ± 44 to 362 ± 53 s^{-1}; the overall mean was 327 ± 22 s^{-1} (Table II).

In order to measure $(1/T_1)$, series of experiments with various values of τ were fitted to function 1. Since the fractional distortion of the values for M_z resulting from deletion of the first 1.0 ms should be constant, the estimates of $(1/T_1)$ obtained with function 3 should not be affected.

Fig. 4 presents a representative series of determinations at various intervals τ. Fig. 5 presents the values of M_z derived from the data of Fig. 4 as a function of time, in semilog form; the value of $1/T_1$ calculated for these data was 197 s^{-1}. The mean values for $1/T_1$ for the three separate muscle samples studied at 4–7°C ranged from 197 ± 58 to 200 ± 104 s^{-1} (Table II); the overall mean was 198 ± 31 s^{-1}.

The intracellular ^{39}K was also studied at 21–22°C. The form of the free induction decays following single or paired pulses was unchanged at the higher temperature, although the rates of both transverse and longitudinal relaxation were slowed (Tables I–II). Both $(1/T_2)$ and $(1/T_1)$ were studied in three separate muscle samples at 21–22°C. In these samples, the mean value for $(1/T_2)$ ranged from 223 ± 26 to 234 ± 17 s^{-1}, with an overall mean of 229 ± 10 s^{-1} (Table II). The mean values for $1/T_1$ in the same samples of Table II, and at the same temperatures, ranged from 78 ± 22 to 79 ± 41 s^{-1}, with an overall mean of 79 ± 15 s^{-1}

It should be noted that in studying the longitudinal relaxation, the fits to function 3 were uniformly good. In no case was it possible to fit the data with function 4, imposing the value obtained for $1/T_2$ from function 1.

DISCUSSION

The present study demonstrates that the relaxation rates of intracellular ^{39}K within frog striated muscle can be well fit by single exponential processes. A second exponential process may also contribute to the measured transverse relaxation rates; our current results do not resolve this issue. Fitting the transverse relaxations to a sum of two exponential processes leads to the conclusion that the faster rate of relaxation would be 1,000–2,000 s^{-1}, while the slower component would relax at rates even lower than those estimated from the single exponential fits. Therefore, the values calculated from the fits to a single exponential constitute upper bounds to the rates of transverse relaxation characterizing much of the intracellular ^{39}K. These upper bounds for the transverse relaxation rates were 229 ± 10 s^{-1} (at 21–22°C) and 327 ± 22 s^{-1} (at 4–7°C) (Table II). The longitudinal relaxation rates were 79 ± 15 s^{-1} (at 21–22°C) and 198 ± 31 s^{-1} (at 4–7°C).

From studies of 1 M KCl at the same temperatures, the longitudinal relaxation rate for ^{39}K in aqueous solution was 15 ± 2 s^{-1} (at 21–22°C) and 22 ± 3 s^{-1} (at 4–7°C); the true rates of transverse relaxation are expected to assume the same values under these conditions. From the same studies of ^{39}K in aqueous solution, the field inhomogeneity of the current study was estimated at the lower and higher temperatures to contribute 25 and 30 s^{-1}, respectively, to the observed rate of transverse relaxation. Thus, the above estimates for the maximum transverse relaxation rates characterizing much of the intracellular ^{39}K should be revised downward even further. Applying these corrections, the maximum possible values for the rates of transverse relaxation may be estimated to be 302 s^{-1} at 4–7°C and 199 s^{-1} at 21–22°C.

Damadian and Cope (7) and Cope and Damadian (8) have claimed that the values at 25°C for $1/T_1$ and $1/T_2$ of the intracellular ^{39}K of rat striated muscle are 141 s^{-1} and 2,857 s^{-1}, respectively. Although somewhat higher, their estimate of $1/T_1$ is in basic agreement with that of the present study, within a factor of two. On the other hand, their estimate of $1/T_2$ was 14 times larger than ours, undoubtedly reflecting the greater inhomogeneity of their magnetic field. Although Cope and Damadian (8) considered this possibility, they rejected it in the belief that the free induction decay used to measure $1/T_2$ necessarily assumes a gaussian shape when limited by field inhomogeneity. Since the shape of their free induction decays was gaussian for ^{39}K in aqueous solution, and exponential for ^{39}K in muscle, they concluded that field inhomogeneity played a role in their studies of solution, but not of muscle.

Actually, field inhomogeneity may cause the FID to assume a variety of different shapes. For example, in the present study, the form of the FID for ^{39}K in aqueous solution could be well fitted by a single exponential, despite the fact that field inhomogeneity accounted for $\frac{1}{2}$–$\frac{2}{3}$ of the observed rate of transverse relaxation. We conclude, therefore, that the correct ratios for $[(1/T_2)/(1/T_1)]$ are those given by the present study, and are no greater than about 1.5 at 4–7°C and 2.5 at 21–22°C.

The results obtained for the intracellular ^{39}K of frog striated muscle are similar to those obtained for the intracellular ^{23}Na of the same tissue (4, 5). Both nuclides have approximately the same relaxation rates in aqueous solution. At 22–24°C and at a

Larmor frequency (ω_o) of 15.7 MHz, the mean value of $1/T_1$ for ^{23}Na was 41 s^{-1}; this value was not significantly changed by reducing ω_o to 7.8 MHz (5). Under the same conditions, $1/T_2$ for ^{23}Na was 73 s^{-1}, and was slightly increased to approximately 92 s^{-1} by reducing ω_o to 7.8 MHz (5); interpolating the data to the Larmor frequency of the current study, $(1/T_2)$ for ^{23}Na is approximately 86 s^{-1} at 10.2 MHz. Thus, the relaxation rates of ^{39}K are enhanced only two to three times more than for ^{23}Na within striated muscle.

The temperature dependences of the relaxation rates of intracellular ^{39}K and ^{23}Na are also qualitatively similar. For both nuclides, $1/T_2$ rose less sharply than did $1/T_1$ as the temperature was reduced from room temperature to 4–7°C. At a Larmor frequency of 15.7 MHz, the ratio $[(1/T_2)/(1/T_1)]$ for intracellular ^{23}Na was approximately 1.3 at 3°C and 1.8 at 23–24°C (5). As noted above, at the current Larmor frequency of 10.2 MHz, we estimate the same ratio for intracellular ^{39}K to be 1.5 at 4–7°C and 2.5 at 21–22°C.

The observations that the relaxation rates for ^{39}K are increased only two to three times more than for ^{23}Na, and that the ratio $[(1/T_1)/(1/T_2)]$ is similar for the two nuclides suggest that a common mechanism determines the NMR properties for both intracellular ions. Less than 1% of the intracellular Na^+ of frog striated muscle is likely to be immobilized (5). Therefore, fractional immobilization of K^+ is also likely to be negligible within striated muscle. Rather, the enhancement of the relaxation rates for both nuclides most likely reflects the presence of a condensed phase of cations associated with the surface of charged macromolecules within the cell (13). This condensation does not immobilize Na^+; its freedom of motion remains very large. Presumably, the same conditions prevail for K^+.

Within this framework, the two- to three- fold greater enhancement for ^{39}K than for ^{23}Na would arise from the greater number of electrons and, therefore, greater value of its Sternheimer antishielding factor (14). Such a mechanism also appears responsible for the greater enhancements of the relaxation rates of ^{87}Rb over those of ^{23}Na by aqueous solutions of DNA (15); ^{87}Rb is characterized by a larger antishielding factor than either ^{23}Na or ^{39}K (14). The larger the alkali cation, the more effectively will its electronic cloud be polarized by a given electrostatic field, resulting in a larger electric field gradient. Thus, nonspecific electrostatic interactions between these ions and polyelectrolytes should be stronger for the larger cations, resulting in greater enhancements of the nuclear relaxation rates.

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