# THE STATE OF WATER IN POLARIZED AND DEPOLARIZED FROG NERVES

## A PROTON MAGNETIC RESONANCE STUDY

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ABSTRACT The high resolution proton magnetic resonance spectrum of the sciatic nerve of the frog was studied in both the polarized and depolarized states. Paramagnetic salts were introduced into the system in order to separate the signals from the intra- and extracellular environments. It was determined that about 65% of the proton signal from the nerve trunk was accounted for by the intracellular environment in the polarized nerve trunk and that this percentage decreased to about 34% in the depolarized case.

The temperature dependence of the line widths of the intracellular proton signal was studied. The enthalpy and entropy of activation for proton exchange between the intra- and extracellular environments were found to be 11.1 kcal/mole and −17.1 cal/deg-mole respectively. The pseudo-first-order rate constant for proton exchange between the intra- and extracellular environments was determined at 20°C and shown to agree with the measured permeability coefficients of similar cells.

Data are presented which indicate that the pseudo-first order rate constant for proton exchange between the two environments decreases upon depolarization of the nerve trunk and that the proton spin-spin relaxation time of the protons of intracellular water decreases significantly with depolarization.

These results indicate a possibly quite important role of water in neural phenomena.

## INTRODUCTION

Theories of the mechanism by which a living cell maintains its ionic gradients can be classified according to two fundamentally different concepts (1). One, the membrane theory, considers the cell interior to be an aqueous electrolytic solution separated from its environment, another aqueous electrolytic solution, by a membrane. The ionic gradients are maintained by the selective permeability of the membrane to various cations and by the expenditure of metabolic energy (2–5). The other, the association theory, regards the cell interior as a gel separated from its environment by an interface. The ionic gradients are maintained by the selective

adsorption of the ions to the framework of the gel. The degree of association of an ion to the gel is determined by the state of the water in the cell interior (6).

An expression for the transmembrane potential has been derived for each of these models (6 [chapter 10], 7). In the general case the two expressions are quite different but in the special case which represents physiological conditions they reduce to the same form. Thus results of measurements of the transmembrane potentials as a function of ion concentration are consistent with either model.

The fundamental difference between the two models lies in their requirements concerning the state of intracellular ions and intracellular water. Contained in the membrane theory is the assumption that ions and water molecules behave in essentially the same fashion in both the internal and external environments. This is implied when it is assumed that the partition functions between the extracellular solution and the membrane are the same as the partition functions between the intracellular environment and the membrane (6). Any changes in the composition of the intracellular environment accompanying polarization or depolarization are considered to be results of new equilibrium conditions defined by a change in the permeability of the membrane. This theory has substantial experimental support from the voltage clamp experiments (3, 7, 8). The association theory, however, requires a considerable fraction of the intracellular cations to be associated with other charged entities. Changes in the composition of the internal environment accompanying polarization or depolarization are attributed to alterations in the degree of association between these intracellular cations and the intracellular anionic sites. The degree of association between these two entities is determined by the state of the water in the gel. The association theory has received some experimental support recently through the determination of mean activities for the intracellular sodium and potassium salts in certain cells (9). The activities are considerably lower than in simple aqueous solutions of the same salt concentrations.

Recent proton spin-echo measurements have shown that the proton spin-spin relaxation time of water in the muscle tissue of the *Rana pipiens* frog differs significantly between the relaxed and the stimulated states (10). In both states the spin-spin relaxation time is considerably shorter than expected for an electrolytic solution. This fact was interpreted by the authors (10) as an indication that a certain percentage of the intracellular water in the muscle exists in a state characterized by a relatively long proton correlation time. This water is considered to be exchanging very rapidly with the rest of the water in the cell. The change in the proton relaxation time with stimulation was interpreted as arising from a change in the relative concentrations of this "bound" water and the rest of the intracellular water. This is not the only reasonable explanation for their results but it is an interesting possibility and one that deserves future examination.

One important source of ambiguity in the interpretation of these results (10) is that the signal observed in the spin-echo measurements is a composite of the signals from both intracellular and extracellular water. This ambiguity is potentially remov-

able through the use of high resolution proton magnetic resonance which, hopefully, could permit the observation of separate resonance signals for the intracellular and extracellular water.

A highly suitable biological system for this purpose is the nerve. High resolution proton magnetic resonance studies can be performed on large sgements of both polarized and depolarized nerves and the results of these studies are the subject of this paper.

## EXPERIMENTAL

The system chosen for study was the sciatic nerve of the frog; both bullfrog (*Rana catesbiana*) and grassfrogs (*Rana pipiens*) were used with similar results. In most of the experiments reported here the bullfrog was used because of the larger size and ease of handling.

A Varian A60A high resolution proton magnetic resonance spectrometer was used throughout the experiments to obtain proton spectra. The V-6040 temperature control unit was used to vary the temperature of the nerve between $-11°$ and 30°C. Special sample tubes were obtained from the Wilmad Glass Co., Inc., Buena, N.J. These tubes were 16.5 cm in length and had an outside diameter of 0.5 cm. The inside diameter was 0.1 cm. The inside of the sample tubes was coated with mineral oil and the nerve trunk was carefully inserted. The nerve trunk extended at least 1 cm above and below the receiver coil to ensure that there was no distortion. It had been previously determined that filling the sample tube beyond 1 cm above the receiver coil did not affect the signal. This was shown by filling the sample tube with water to various heights and recording the spectrum. Sample tubes filled to much less than 1 cm above the receiver coil produced grossly distorted signals, e.g., spurious peaks, broadened lines, etc. Sample tubes filled to at least 1 cm above the coil with water produced proton resonance signals of the order of 1 cps wide (full width at half maximum amplitude) and slightly shifted to high fields from the water signal observed in the normal Varian sample tube.

The absolute value of the line width and shift for the water signals was slightly different from tube to tube. This was attributed to the fact that each tube sampled a slightly different region of the applied magnetic field, i.e., they were not all perfectly concentric. However, since only changes in line widths within a given sample tube are considered in this paper these slight differences were not considered important.

All the spectra compared in this study were obtained with the same sample tube. For a given sample tube the spectra were reproducible with respect to line widths, chemical shifts, and signal intensity.

It proved necessary to add a small amount of a paramagnetic salt to the Ringer's solution bathing the axons in order to separate the extracellular water signal from the intracellular water signal (11, 12). Two different paramagnetic salts were used: $CoCl_2$ and $MnCl_2$. When the cobalt salt was used the Ringer's solution was made 0.04 M in cobalt; manganous ion was used in a concentration of 0.01 M.

Nerve trunks with the muscle attached were equilibrated for several hours in Ringer's solution containing 0.04 M $Co^{2+}$ or 0.01 M $Mn^{2+}$ and then tested to see if they were still functional. A contraction of the muscle following electrical stimulation of the nerve was considered evidence of a functional nerve. It was demonstrated that the nerves remained functional in these paramagnetic Ringer's solutions. The action potentials were not measured to see if various parameters such as the pulse amplitude, propagation velocity, refraction time, etc. had changed owing to the paramagnetic ions.

Prior to insertion in the sample tube, the nerve trunk was equilibrated with the para-

magnetic Ringer's solution for several minutes. It is quite possible, even likely, that some of this Ringer's solution adhered to the nerve when it was inserted in the sample tube. This Ringer's solution external to the nerve trunk would not be expected to produce a signal different from the solution internal to the nerve trunk but external to the nerve cells.

Great care was taken to ensure there were no air spaces in the sample volume since it had been observed that the presence of such air spaces caused signal distortion. The entire sample volume was filled with the nerve trunk quite possibly surrounded by a thin surface layer of paramagnetic Ringer's solution.

The nerves were depolarized in one of two ways: by immersion in a Ringer's solution containing 0.133 M excess KCl (13) or by applying a constant current through the axons (14). Both methods gave the same results. Usually the electrical stimulation was used since it required less handling of the nerves and the interpretation of results was not complicated by possible effects due to the additional electrolyte. The NMR spectra of depolarized nerves were obtained either while the current was being applied or after it had been applied in an anaerobic environment. The anaerobic environment was a result of the fact that operation of the V-6040 temperature control unit requires a constant flow of nitrogen around the sample tube. In addition, the sample was encased in a snuggly fitting glass tube. The top of the tube was plugged by Ringer's solution and the bottom was plugged by a portion of the nerve trunk itself. This portion of the nerve trunk quickly dried in the nitrogen flow and formed a hard plug in the bottom of the tube.

It was felt that the flow of nitrogen was not essential to the anerobic condition once the hard plug had been formed in the bottom of the sample tube and as long as the Ringer's solution plug in the top of the sample tube remained undisturbed. Thus the sample tube could be removed from the instrument, the nerve trunk depolarized, and the sample tube replaced without destroying the anaerobic conditions. It needs to be emphasized that the nerve trunk was not removed from the sample tube during this process. Thus it was ensured that the same region of the nerve trunk was within the signal coil while both the polarized and depolarized spectra were obtained. The nature of the A60A probe insert arrangement ensured that the same region of the magnetic field was being sampled each time.

The actual method used for the data reported in this paper was to remove the sample tube from the apparatus with the nerve intact, depolarize it, and replace it in the probe insert. This method was shown to yield the same result as depolarization within the spectrometer and it is much more convenient.

The spectrum obtained from any nerve which is depolarized electrically varies as a function of the strength of the depolarizing current and the length of time for which it is applied. The degree of depolarization was considered complete when no further change in the spectrum was observed following additional application of current. The degree of depolarization depends both on the strength of the applied current and on the duration of its application; low current applied for long periods of time produced the same results as a high current applied for a short period of time. This is compatible with the known depolarizing effect of a constant current (14).

A convenient choice of the depolarizing current was 50 $\mu$amp for 10 min. It must be kept in mind that not all this current passed through the axons but it is divided in some unknown way between the extracellular and intracellular conduction paths.

The possibility that the phenomena observed upon electrical depolarization of the nerve trunk were caused in part by an electrolysis effect was considered. However, since the same phenomena were observed in chemically depolarized nerve trunks, any electrolysis effect was considered to be negligible. Platinum electrodes were used to ensure that no new metallic cations would be added to the system. Also, the observed phenomena were independent of the

direction of the depolarizing current as they should be if they are related to the depolarization of the membrane and not some electrolytic reaction occurring under the influence of the applied potential.

All the data reported in this paper for depolarized nerves were obtained from completely depolarized nerves as defined above.

It was determined that the changes in the NMR spectrum of the nerve trunks was reversible by the following test. The spectrum was obtained for a polarized nerve trunk in the usual fashion. The nerve was then depolarized according to the method described above and its spectrum recorded. The nerve was removed from its special sample tube and placed in a well aerated paramagnetic Ringer's solution where it was allowed to remain for at least 1 hr. It was then replaced in the special sample tube and the spectrum recorded. This spectrum agreed both in line width and amplitude with the spectrum of the polarized nerve.

It was also observed that a nonfunctional nerve trunk, i.e. one for which the muscle did not contract after the nerve was stimulated, gave only a single proton resonance signal when it was doped with either $Co^{2+}$ or $Mn^{2+}$. In the case of the $Co^{2+}$ doping, the single signal corresponded to peak *B* of Fig. 1 and 2; for $Mn^{2+}$ the single signal corresponded to the broad line on which the signals shown in Fig. 3 *a* and 3 *b* are superimposed. Thus the presence of two proton signals appeared to be related to the functionality of the nerve trunk.

Hence the reversibility of the NMR spectrum was assumed to imply reversibility of the depolarization.

## RESULTS AND DISCUSSION

The proton resonance spectrum of a polarized nerve consists of a single relatively sharp signal (total line width at half-maximum intensity is 2.7 cps) located at 2.9 cps to the higher field from the water proton signal of Ringer's solution. The area under this signal was obtained with a planimeter and compared to the area under the signal from pure water in the same sample tube. The result showed that the nerve signal is 80% as intense as the pure water signal. This number agrees very well with the reported percentage of water in similar systems (9, 10).

If the nerve is bathed in Ringer's solution made with $D_2O$, the strong proton resonance signal no longer appears, and it is concluded that this signal arises from the water protons of the nerve and all protons which can exchange with the water protons within a few minutes.

Some small changes are observed in the nerve resonance signal upon electrical or chemical depolarization. The total line width becomes 3.3 cps and the chemical shift to the high field side of Ringer's solution is 2.1 cps. These changes are undoubtedly significant but no unequivocal interpretations could be given without further experimentation.

We first investigated the possibility that the "single" signal is really the sum of two or more separate signals in the case of either polarized or depolarized nerves or both. Separate signals are potentially resolvable if there are two or more nuclear environments in the system which contain comparable numbers of protons and if the rates of proton exchange between these environments are sufficiently slow.

A commonly employed means for the separation of such potentially resolvable

signals involves the introduction of paramagnetic ions (11, 12) into the system studied. Two ions were shown to be applicable in the studies reported here, $Co^{2+}$ and $Mn^{2+}$. The nerve was shown to remain functional in the presence of $Co^{2+}$ and $Mn^{2+}$ at the concentrations employed through the experiments mentioned previously. The proton spectrum of a $Co^{2+}$-doped nerve trunk is shown in Fig. 1. The chemical shift of signal *B* is a linear function of the $Co^{2+}$ concentration within experimental uncertainty while that of signal *A* is independent of $Co^{2+}$ concentration.

Hence there are indeed two nuclear environments containing comparable numbers of protons in a polarized nerve trunk, and the proton exchange rate between these environments is relatively slow. The actual exchange rate was determined in the experiments involving $Mn^{2+}$ discussed below. The protons in only one of these

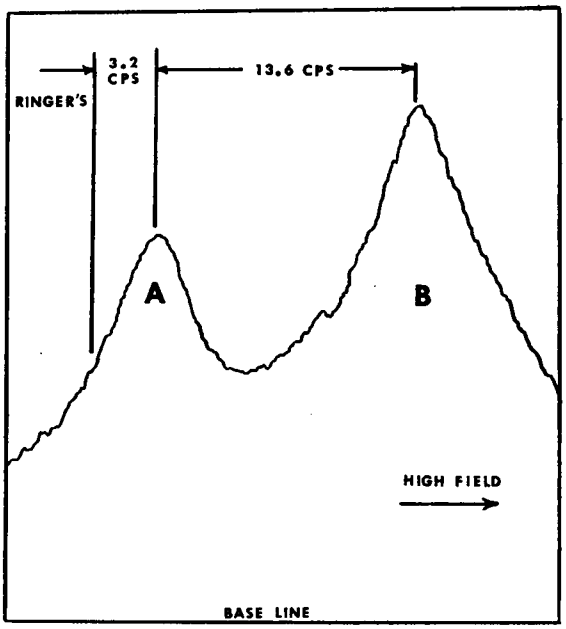


FIGURE 1 Proton magnetic resonance spectrum for a $Co^{2+}$-doped polarized nerve trunk. Temperature 20°C. Gain: 63.

two environments appear to exchange rapidly with the protons of the primary solvation sphere of $Co^{2+}$. Based on these results the most straightforward assignment of the two signals of Fig. 1 is that signal *B* arises from extracellular water and signal *A* from intracellular water.

Either chemical or electrical depolarization of the $Co^{2+}$ doped nerve trunks produces the proton spectrum shown in Fig. 2. It must be emphasized that this quite dramatic change is observed even when the nerve trunk is depolarized electrically within the NMR spectrometer and the spectra of the polarized and depolarized nerve trunks are recorded within minutes of each other. The line shape change

between Figs 1 and 2 may reflect changes in one or all of at least three quantities. These are the relative concentrations of protons in the two nuclear environments, the spin-spin relaxation times in the two environments, and the exchange rate between environments.

Unfortunately the signals of Figs. 1 and 2 are not sufficiently well resolved to permit the analysis of line shape changes in terms of these or other factors. This difficulty was overcome through use of $Mn^{2+}$.

The proton spectrum of a $Mn^{2+}$-doped polarized nerve trunk consists of the sharp peak given in Fig. 3 *a* superimposed on a broad signal of a total line width of about 200 cps. The line width of a Ringer's solution which contains 0.01 M $Mn^{2+}$ is also approximately 200 cps at 17°C. Therefore one of the two types of water protons in

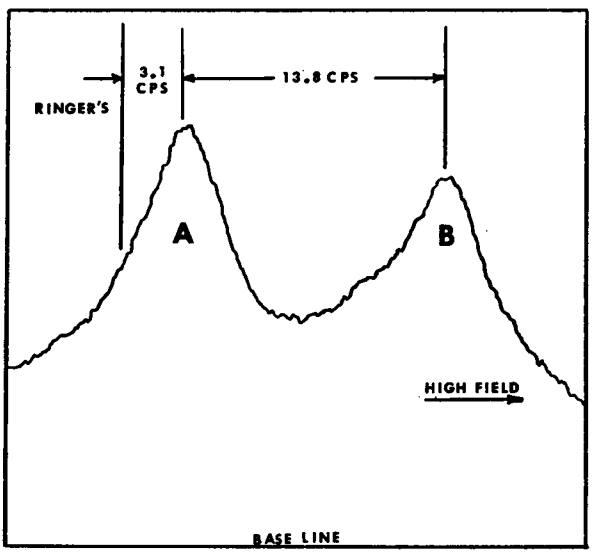


FIGURE 2 Proton magnetic resonance spectrum for a $Co^{2+}$-doped depolarized nerve trunk. Temperature 20°C. Gain: 80.

the nerve exchanges rapidly with the primary solvation sphere protons of the manganous ion. This is essentially the same situation encountered with $Co^{2+}$-doped nerve trunks. The sharp signal of Fig. 3 *a* is assigned as arising from the intracellular water and it corresponds to signal *A* of Figs. 1 and 2.

The presence of this sharp line in the $Mn^{2+}$-doped nerve trunks also is a strong reinforcement of the conclusion that signals *A* and *B* arise from intracellular and extracellular water rather than from some other division. It is not necessary for protons to enter the first solvation sphere of a paramagnetic ion for signal broadening to occur although signal broadening is considerably greater when such rapid exchange can occur. The dominant broadening mechanism at distances beyond the first coordination sphere arises from a dipole-dipole interaction through space

between the magnetic nucleus and the unpaired electrons of the paramagnetic ion. This broadening has been shown (15) to be proportional to $r^{-6}$ where $r$ is the separation between the two magnetic dipoles. The width of the sharp line observed for a polarized $Mn^{2+}$-doped nerve trunk was found to be independent of $Mn^{2+}$ concentration when the $Mn^{2+}$ concentration was changed from 0.01 M to 0.02 M. Since a change in the full line width as small as 0.5 cps could have been detected, the dipole-dipole broadening must be less than 0.5 cps. Using this value as an upper limit for the dipolar contribution to the line width a minimum average value of $r$ can be computed from the relation (15).

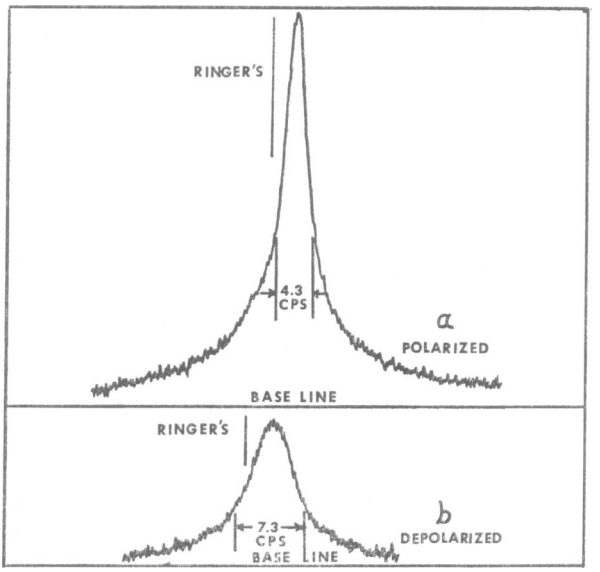


FIGURE 3 (*a*) Proton magnetic resonance spectrum for a $Mn^{2+}$-doped polarized nerve trunk. Temperature 17°C. Gain: 40. (*b*) Proton magnetic resonance spectrum for a $Mn^{2+}$-doped depolarized nerve trunk. Temperature 13.5°C. Gain: 40. Both spectra are 3.2 cps to the high field from the Ringer's line.

$$\left(\frac{\tau N T_2}{\tau' N' T_2'}\right)^{1/6} r' = r, \tag{1}$$

or, since $T_2 = (\pi W)^{-1}$,

$$\left(\frac{\tau N/\pi W}{\tau' N' T_2'}\right)^{1/6} r' = r \tag{2}$$

where $N$ is the molar concentration of the manganous ion, $1/T_2$ is the dipolar contribution to the spin-spin relaxation, and $\tau$ is the proton correlation time. The primed quantities refer to a known standard and the unprimed quantities refer to

the intracellular neural environment. The study of Bloembergen and Morgan (16) of proton relaxation by $Mn^{2+}$ in aqueous solution provides a standard and $N'T_2' = 8.5 \times 10^5$ at 20°C and $r' = 2.8$ A. Thus

$$r = 2\left(\frac{\tau}{\tau'}\right)^{1/6} r' \text{ A}$$

$$= 5.6\left(\frac{\tau}{\tau'}\right)^{1/6} \text{A}. \tag{3}$$

It is highly likely that $\tau$ is at least as large as $\tau'$ and hence 5.6 A is a quite reasonable lower limit for $r$, and, in fact, $r$ may be much larger. As is mentioned below more than 50% of the water molecules are restricted to this distance (twice the diameter of a water molecule) from the manganous ions.

This result is easily explained if the manganous ions are confined to the extracellular environment, and it is very difficult to explain if the manganous ions are free to enter and diffuse through the intracellular environment.

The proton spectrum of a depolarized $Mn^{2+}$-doped nerve trunk is shown in Fig. 3 *b*. This signal is also superimposed on a very broad signal.

There is obviously a sizeable change in line width between Figs. 3 *a* and 3 *b*. There is also a relatively large difference in peak areas. The area of the signal in Fig. 3 *a* corresponds to 65% of the total water of a polarized nerve trunk while that of the signal in Fig. 3 *b* corresponds to only 43% of the total water of a depolarized nerve. From integrations of the signals from undoped nerve trunks it was shown that no significant changes in total water concentration accompanied depolarization. The conclusion is that a rather large shift in the ratio of intracellular to extracellular water accompanies depolarization.

This is one of the factors listed above as possible sources of the spectral changes observed between Figs. 1 and 2. The other two factors listed are reflected in line width changes in Fig. 3. This change is separable into a spin-spin relaxation time contribution and an exchange-rate contribution (17).

In the case of the spectra of Fig. 3 the total line width at half-maximum intensity is simply given by

$$\pi W = \frac{1}{T_2} + k_{AB} \tag{4}$$

where $T_2$ is the spin-spin relaxation time of intracellular water protons and $k_{AB}$ is the pseudo-first order rate constant for exchange of protons from the $A$ to $B$ environments.

It is possible to separate $1/T_2$ from $k_{AB}$ experimentally through a temperature study of $W$ (18, 19). Fig. 4 shows log $\pi W$ as a function of $1/T$ for both a polarized and a depolarized $Mn^{2+}$-doped nerve trunk. Line *a* in Fig. 4 is for the polarized

nerve trunks and line $b$ is for the depolarized. The temperature dependence of $\pi W$ for the polarized nerve trunk indicates a sizeable contribution from chemical exchange at the higher temperatures, while at lower temperatures $1/T_2$ appears to dominate. The values of $\pi W$ as a function of temperature were fit as the sum of the two straight lines shown in Fig. 4. The line $a$ drawn through the experimental points represents the sum of the straight lines. The value of $k_{AB}$ at 20°C is 6.8 $sec^{-1}$ and the activation parameters are 11.1 kcal/mole for $\Delta H^*$ and $-17.1$ cal/deg-mole for $\Delta S^*$.

The value of the exchange rate can be related to the permeability of the cell by

$$k_{AB}P^{-1} = f^{-1}(s/v) \tag{5}$$

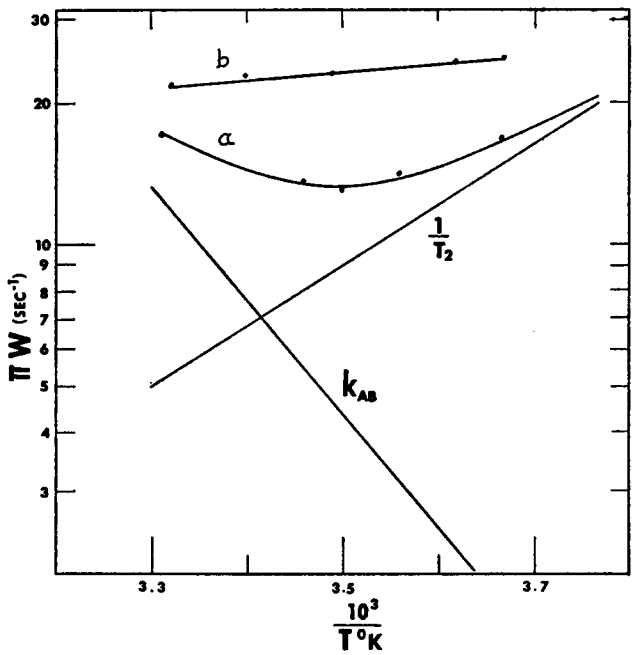


FIGURE 4 Temperature dependence of the line width of the proton magnetic resonance spectra of $Mn^{2+}$-doped nerve trunks. Line $a$: Polarized; Line $b$: Depolarized.

where $P$ is the permeability coefficient of the cell in cm/sec, $f$ is the ratio of the total volume of intracellular water to the total volume of the nerve, and $(s/v)$ is the surface to volume ratio of the cell. If cylindrical geometry is assumed for the cell then $(s/v) = 2r_a^{-1}$ where $r_a$ is the radius of the cell. Thus

$$k_{AB}P^{-1} = 2f^{-1}r_a^{-1} \tag{6}$$

or, for a composite system such as a nerve trunk,

$$k_{AB}\langle P^{-1}\rangle = 2f^{-1}\langle r_a^{-1}\rangle \tag{7}$$

where $\langle P \rangle$ and $\langle r_a \rangle$ represent the average permeability and radius of all the axons in the trunk. If a figure of $1.0 \times 10^{-3}$ cm/sec is assumed for the average permeability (20, 21) then

$$\langle r_a \rangle = 5.65 \times 10^{-4} \text{ cm} \tag{8}$$

or, in the sciatic nerve trunk of the frog, the average diameter of the axons is 11.3 $\mu$. Optical birefringence measurements (22) yield an average diameter of 10 $\mu$ for the same neural system. This result shows that transport of water across the membrane is not affected detectably by the presence of the $Mn^{2+}$.

When the nerve is depolarized in the $Mn^{2+}$-Ringer's solution the area under the resonance signal decreases by 34% and the line width increases by about 70% at 20°C. Fig. 4, line *b*, shows log $\pi W$ as a function of $1/T$ for a depolarized nerve trunk. There is no evidence of exchange rate–controlled broadening in the depolarized nerve as there was for the polarized nerve. There are several factors which might be proposed to explain the behavior observed in line *b*.

First it is possible that depolarization has allowed some $Mn^{2+}$ to penetrate into the intracellular environment. The arguments against this are that the process is reversible and that the line widths given in line *b* are independent of $Mn^{2+}$ concentration in the concentration range studied.

A second possibility is that depolarization produces rapid exchange of the intracellular and extracellular water. Such exchange must produce a very broad line with a width of the order of 100 cps. This is clearly not the case and the exchange between the two environments must remain relatively slow and equation (1) must still be applicable.

Line *b* shows no indication of a bend and therefore it is concluded that either $1/T_2$ or $k_{AB}$ dominates in equation (1) over the temperature range studied. The temperature dependence is quite unlike that of a typical rate process and is much more like that of a spin-spin relaxation process, although the slope does differ markedly from that of the spin-spin relaxation rate of line *a*.

The correlation time of the protons of the intracellular water would appear to have been significantly increased upon depolarization and the temperature dependence of the correlation time decreased.

In addition, the apparent lack of a contribution to line *b* from $k_{AB}$ indicates that this rate constant is smaller for the depolarized nerve than for the polarized nerve. It should be noted that this conclusion does not eliminate the possibility of a transient increase in this rate constant when the depolarizing current is first applied.

The conclusions concerning the changes in correlation time and exchange rate with depolarization coupled with the very straightforward conclusion concerning the change in relative water proton concentrations form the principal results of this investigation.

The spectra on which these conclusions are based were obtained in the presence of paramagnetic ion concentrations beyond the normal physiological range. There

is, of course, the possibility that the paramagnetic ion altered the behavior of the nerve trunk. However, the observations that the nerve could still propagate an action potential, that the process of depolarization was reversible, that the exchange rate calculated for the polarized nerve is compatible with the known permeability coefficients, that the total water concentration and the ratio of intracellular to extracellular water for the polarized nerve agrees with data obtained under more physiological conditions, and that the data obtained are quantitatively independent of the paramagnetic ion concentration and qualitatively independent of the type of paramagnetic ion represent good evidence that the presence of paramagnetic ions and the prolonged periods of depolarization did not fundamentally alter the behavior of the nerves.

In addition, the line width change observed upon depolarization of an undoped nerve trunk is qualitatively in agreement with the changes observed with the doped nerve trunks. This change for the undoped nerve trunk offers a possibility that magnetic resonance experiments can be performed on undoped nerves and that the results can be interpreted quantitatively.

It is clear from the results presented here that very marked changes in the state of the intracellular water accompany nerve depolarization. If this change does occur in undoped nerves it is of great potential significance in the theory of neural phenomena and further experiments are being pursued.

*Note Added in Proof* Chapman and McLauchlan (1967. *Nature.* **215**:391) have recently reported PMR spectra for rabbit sciatic nerves as a function of orientation. Two types of water protons present in comparable concentrations are clearly indicated by the authors' results in their study of a neural system which contained no added paramagnetic ions.

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