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*NUCLEAR MAGNETIC RESONANCE EVIDENCE FOR COMPLEXING OF SODIUM IONS IN MUSCLE**

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Most investigators have assumed that the Na^+ of the cell is largely in free solution in intracellular water. The opposite conclusion was drawn from the application of a kinetic theory proposed by Cope[1, 2] to van der Kloot's data on Na^+ leakage from muscle.[3] On other grounds, Troshin[4] and Ling[5, 6] previously had deduced that a large fraction of intracellular Na^+ existed in a complexed state. To test the prediction of Cope's kinetic theory, and because a knowledge of the extent of complexing of Na^+ in the cell is of general importance for the derivation of theories of ion transport and nerve conduction, a new experimental approach to cellular Na^+ complexing was sought. Nuclear magnetic resonance (NMR) analysis of muscle Na^+ proved to have the required sensitivity, specificity, and clarity of interpretation.

A large bullfrog (*Rana catesbeiana*) was killed by decapitation, the muscle was cut off in fairly large pieces from the upper portion of the hind leg, and was blotted to remove blood and extracellular fluid insofar as possible. Pieces of muscle then were packed tightly up to an 8-ml mark in a Pyrex test tube that had been lined with

petroleum jelly (Vaseline) to prevent complexing of Na^+ with the glass wall of the tube. The NMR spectra of Na^+ were obtained in a conventional manner on a Varian wide-line NMR spectrometer with a setting of approximately 8900 gauss modulated sinusoidally at 80 cycles per second, and with a radio frequency field of 10 megacycles. Four repetitions of the NMR spectrum of the muscle were recorded within about 10 min after decapitation of the frog. The mean peak height of the four NMR spectra was computed, thus reducing errors due to instrumental noise.

The NMR spectrum of a standard solution of 0.1 NaCl in a tube also lined with petroleum jelly was recorded before and after each set of muscle measurements. Peak height of the NMR spectrum of Na^+ was shown by Jardetzky and Wertz[7] to be proportional to Na^+ concentration, provided that ions complexing Na^+ were absent. These findings were confirmed in the present study. Therefore, calculations of Na^+ concentrations in muscle were based on the assumption of direct proportionality between concentration and the peak height of the NMR spectrum. The standard contained also 0.1 *N* KCl, corresponding approximately to the cellular concentration of K^+, because careful measurement showed that 0.1 *N* K^+ changed the shape of the NMR spectrum of Na^+ slightly, resulting in a change in peak height of about 5 per cent. These observations were consistent with conductivity measurements[8] and with other NMR studies[9] which indicated small but significant interactions between pairs of mixed inorganic cations in aqueous solutions. Total Na^+ was determined on the same muscle sample by NMR analysis after ashing followed by dilution in 0.1 *N* HCl. Ashing was carried out in platinum crucibles for 12–15 hr at 700°C, preceded by drying under a heat lamp. When 0.5 ml of 0.4 *N* NaCl plus 1 ml of 1 *N* KCl were carried through the ashing procedure and NMR analysis, recoveries of Na^+ of 94, 93, 89, 107, and 96 per cent (mean 95) were obtained in six studies, indicating that the ashing and analytical procedures gave reasonable accuracy and reasonable reproducibility.

Table 1 summarizes the results of NMR experiments on six different frogs. First, it is evident that the mean value of 28.5 mM for total muscle Na^+ concentration shown in Table 1 agrees well with the mean value of 27.4 mM for collected data of other investigators using different methods (ref. 5, p. 217) (computed on basis of specific gravity of 1.1 for muscle). Thus the validity of NMR analysis after ashing as a method for determination of total tissue Na^+ concentration is confirmed. Second, Table 1 shows that much less Na^+ was visible to NMR in fresh muscle than was present in the same muscle samples after ashing. For each individual muscle sample, the Na^+ concentration visible to NMR was subtracted from the total Na^+ concentration to give the concentration of Na^+ that was invisible to NMR. It is apparent from the data in Table 1 that, on the average, 72 per cent of total muscle Na^+ is invisible to NMR.

The NMR spectrum of Na^+ in free solution consists of a single line of about

TABLE 1

	Frog Number						
	1	2	3	4	5	6	Mean
Total muscle Na^+ (mM)	27.3	33.0	29.6	26.2	25.1	29.4	28.5
NMR-invisible Na^+ as % of total muscle Na^+	72	66	75	78	60	78	72

Values of total muscle Na^+ represent concentrations of Na^+ in mmoles *per liter of fresh muscle* as determined by NMR analysis after ashing. Values of NMR-invisible Na^+ were determined by subtraction of the concentrations of Na^+ that were visible to NMR in fresh muscle from total Na^+ concentrations in the same muscle samples after ashing.

32 milligauss in width[10] which becomes broadened when the solution contains anions that complex with Na^+.[7] Complexation of Na^+ with ion exchange resin broadens the NMR spectrum of Na^+ so greatly that it becomes invisible.[7] It therefore seems probable that the invisibility of 72 per cent of muscle Na^+ to NMR is due to complexing by macromolecules, which causes the spectrum of that fraction of Na^+ to be broadened beyond detection. Another less likely possibility is that complexation may produce a chemical shift of the spectrum of 72 per cent of muscle Na^+ to another position along the magnetic field (H) axis. This possibility was tested experimentally on one sample of fresh muscle. The H-axis was scanned 100 parts per million (ppm) above, and 100 ppm below, the position of the visible Na^+ spectrum (which was observed to be at the same position as the Na^+ spectrum of the standard NaCl solution). No additional Na^+ spectrum could be observed, providing no support for the possibility of disappearance due to a chemical shift.

The NMR evidence that 72 per cent of muscle Na^+ exists in a complexed form correlates well with the cation-sensitive microelectrode studies of Lev[12, 13] on individual muscle fibers, which indicated that 70 per cent of intracellular Na^+ was in some state that excluded it from contact with the microelectrode. The NMR evidence supports the theoretical and experimental work of Ling,[5, 6] Troshin,[4] and Hechter and Lester,[11] which indicated that a large fraction of intracellular cation concentration probably exists in a complexed form. Also, the NMR studies partially verify Cope's theory of ion transport[1] based on analogies of ions in cells to electrons in solids[2, 14]

Summary.—NMR spectra of fresh frog muscle compared with spectra of the same samples after ashing show that approximately 70 per cent of total Na^+ of fresh muscle gives no detectable NMR spectrum. This is probably due to complexation of Na^+ with macromolecules which causes a broadening of the NMR spectrum beyond detection. An analogous effect on the NMR spectrum is observed when Na^+ interacts with ion exchange resin. The data supports a theory of ion transport based on analogies of the cell to a semiconductor solid.

A more detailed presentation of the work in this preliminary report, together with additional related NMR studies, is in preparation.

* Opinions and conclusions contained in this report are those of the author. They are not to be construed as necessarily reflecting the views or endorsement of the Navy Department.

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