Sodium and Potassium Activities in Normal and "Sodium-Rich" Frog Skeletal Muscle

Abstract. *In frog sartorius muscles immersed for 2 hours at 26°C in normal Ringer solution, the intrafiber potassium concentration, C_K (in millimoles per liter), was 123 ± 2 (mean value plus or minus standard error), and the potassium activity, a_K (in millimoles per liter), was 90 ± 1.0. The corresponding sodium concentration and activity were 20 ± 1 and 6.5 ± 0.4, respectively. After overnight immersion in K+-free Ringer solution the values were: C_K, 97 ± 2; a_K, 81.5 ± 1.6; C_{Na}, 47 ± 2; and a_{Na}, 11.2 ± 0.6. The changes in a_K and a_{Na} during storage were not consistent with an exchange between predominantly "free" fiber K+ and external Na+. These results suggest that the Na+ taken up during overnight immersion largely replaced adsorbed or sequestered K+ in the fibers.*

Evidence that intrafiber Na^+ in muscle (and intracellular Na^+ in other cells) is not uniformly distributed in an osmotically active form throughout the cytoplasm has been accumulating for some years. The conclusion that a large part of the fiber Na^+ is "bound" or sequestered in one or more states of relatively low thermodynamic activity originally emerged indirectly from kinetic studies of Na^+ exchange in muscle (*1, 2*). More recently, direct support for this conclusion has been obtained from techniques such as the determination of intracellular Na^+ and K^+ activities with cation-selective microelectrodes (*3–6*), nuclear magnetic resonance (NMR) spectroscopy (*7, 8*), tissue press experiments (*9*), and electron microscopy (*10*).

Current views concerning the physical state of intracellular K^+ are more controversial. Because the apparent intracellular activity coefficient for this ion (calculated as the ratio of the measured activity to the concentration, a_K/C_K) is usually close to the mean activity coefficient of normal Ringer solution, some workers (*1, 4, 6*) have concluded that the traditional concept of cytoplasmic K^+ as an essentially "free" solution separated from the medium by a limiting membrane not possessing any significant capacity for absorbing ions is essentially correct. However, as these workers have pointed out, this conclusion rests on a number of unproven assumptions, including the assumption that ionic activity coefficients in cytoplasm are similar to those in Ringer solutions of similar composition.

On the other hand, the idea that cell K^+ is mainly in a "bound" or associated state has been vigorously championed (*7, 11, 12*), although, here again, most of the supporting evidence cited is indirect. Of particular relevance to the present study are the results of NMR investigations of cell Na^+. Cope (*7*) showed that the Na^+ content of frog muscle consists of an NMR-visible and an NMR-invisible fraction and suggested that these fractions corresponded to free and complexed Na^+ ions, respectively. The existence of these two fractions has been confirmed by other workers (*8*).

On the basis of these findings, Ling and Cope (*12*) compared the NMR spectra of frog sartorius muscles containing normal amounts of Na^+ with those of muscles that had been allowed to accumulate extra Na^+ (and lose corresponding amounts of K^+) by storage in media low in K^+. They reported that the greater part of the extra Na^+ taken up during storage entered the NMR-invisible fraction, and concluded that, since this Na^+ is presumably bound, the cell K^+ with which it exchanged was also bound. Hence, they inferred that most of the intracellular K^+ in frog muscle is adsorbed and that Na^+-K^+ exchange in this tissue takes place by adsorption of Na^+ on sites vacated by K^+ ions lost from the fibers.

This interpretation is in sharp contrast to the widely accepted Na^+ pump hypothesis which envisages Na^+-K^+ exchange as taking place across a membrane separating two electrolyte solutions and controlled essentially by mechanisms located in this membrane. Because of this differing interpretation and in order to gain more insight into the physical state of intracellular K^+, we have studied net Na^+-K^+ exchange in isolated sartorius muscles of *Rana pipiens*, using cation-selective microelectrodes. Adult frogs were pithed and both sartorii were rapidly removed. The animals were then decapitated. One muscle was immersed for 2 hours at 26°C in a phosphate Ringer solution containing 105 m*M* Na^+, 2.5 m*M* K^+, and 111 m*M* Cl^- (*13*). The medium also contained 5 m*M* sulfate to which a tracer amount of ^{35}S-labeled sulfate had been added. The companion muscle was stored for 24 hours at 5°C in a K^+-free Ringer solution containing 120 m*M* Na^+. The *p*H of these solutions was 7.2.

After the control muscles had been immersed for 2 hours, we recorded their membrane potentials with conventional open-tip microelectrodes filled with 3*M* KCl (tip resistances, 20 to 50 megohms; tip potentials, < 5 mv). These potentials ranged from 80 to 90 mv. Muscles that consistently gave readings below 80 mv were discarded. During the same time penetrations on different fibers of the muscle were also made with a pair of sealed cation-selective glass microelectrodes (*14*). These were made from NAS 27-05 glass (Na_2O, 27 moles percent; Al_2O_3, 5 moles percent; SiO_2, 68 moles percent) (*14*) by the method of Lev (*4*), and had tip diameters of < 1.5 μm. The length of the exposed cation-sensitive glass tip was 3 to 5 μm. The electrodes were calibrated before and after the experiment with test solutions containing NaCl and KCl (1 m*M* to 100 m*M*). Electrodes were chosen for use only if the calibration curves (potential in millivolts as a function of the natural logarithm of a_i) for Na^+ and K^+ were linear throughout the activity range tested, if the selectivity cofficient $k_{K\text{-}Na}$ (*4*) remained constant over this range, and if neither of these parameters changed significantly during the experiment. In individual experiments advantage was taken of the fact that different microelectrodes made from the same glass can have widely different selectivity coefficients (*4*) and, for each muscle used, a pair of electrodes with coefficients differing by 30 to 60 percent was chosen. The mean values (± S.E.) for these constants were 0.246 ± 0.012 for the 16 more K^+-selective electrodes and 0.333 ± 0.011 for the less K^+-selective electrodes.

An average of eight penetrations with the open-tip electrode and with each of the cation-selective electrodes were made on different fibers in each muscle used. Using Nicolsky's equation (*15*), we calculated intrafiber Na^+ and K^+ activities (a_{Na} and a_K) from the recorded potentials (*4*). The muscles were then dried to constant weight at 105°C to determine their total water content. We estimated the total muscle

Table 1. Sodium and potassium activities (a) and concentrations (C) in frog sartorius muscle fibers. Mean value ± standard error for eight muscles are shown for each of the parameters listed, except a_{Na} and a_K ($n = 64$ for these). Group I, control muscles immersed for 2 hours at 26°C in Ringer solution containing 2.5 mM K^+; group II, companion muscles stored for 24 hours at 5°C in K^+-free Ringer solution.

Total H_2O (g/g dry weight)	Fiber H_2O (g/g dry weight)	Total Na^+ (μmole/g wet weight)	C_{Na} (mM)	a_{Na} (mM)	Total K^+ (μmole/g wet weight)	C_K (mM)	a_K (mM)
			Group I				
3.93 ± 0.07	2.91 ± 0.08	33 ± 1	20 ± 1	6.5 ± 0.4	73 ± 2	123 ± 2	89.9 ± 1.0
			Group II				
3.85 ± 0.09	2.80 ± 0.11	52 ± 1	47 ± 2	11.2 ± 0.6	57 ± 1	97 ± 2	81.5 ± 1.6

Na^+ and K^+ and the extracellular (^{35}S-labeled sulfate) space, using aliquots of a 0.1N HNO_3 extract of the dried tissue as described elsewhere (*16*). Fiber water was taken as the difference between the total tissue water and the fraction of tissue water occupied by labeled sulfate.

After storage at 5°C the companion muscles and their bathing media were allowed to warm to room temperature (26°C). They were then treated in a manner identical to that described for the control muscles. Thus one member of each pair of companion muscles used served as a control for its contralateral partner.

Table 1 presents a summary of the results obtained. The control data (group I) are in close agreement with those previously reported for muscles treated under similar conditions by Lev (*4*). Specifically, the relatively low a_{Na} and relatively high a_K values shown in Table 1 are in agreement with observations of Lev and other workers recorded by similar techniques (*3–6*). Thus our results further support the conclusion that most of the fiber Na^+ in skeletal muscle is normally in a bound or sequestered condition.

The salient point of interest in the study reported here, however, emerges from examination of the data obtained with the "sodium-rich" muscles (group II of Table 1). At first glance, the fact that the apparent molar activity coefficient for K^+ (expressed as the ratio a_K/C_K) for the muscles in group I (0.73) is reasonably close to that predicted for this ion in free solution under similar conditions (*17*) might be taken as evidence that little if any K^+ is bound in myoplasm. However, this conclusion, which, as pointed out by McLaughlin and Hinke (*5*), is subject to relatively large uncertainties concerning the proportion of "bound" water within the fibers and the essential equality of intra- and extracellular ionic activity coefficients under conditions of similar ionic strength, is not, in our view, borne out by the data presented here.

During overnight immersion in K^+-free media the average amount of K^+ (in millimoles per liter) lost by these muscles (26 ± 2) was replaced by an identical amount ($P > .5$) of Na^+ (27 ± 2). Similarly, there was no significant change ($P > .5$) in the amount of cell water (in grams per gram of dry tissue weight). The corresponding changes (in millimoles per liter) in a_K (−8.4 ± 1.3) and a_{Na} (4.7 ± 0.4), although significantly different from zero ($P < .005$), did not differ significantly from each other ($P > .1$).

These activity changes are not consistent with a loss of approximately 26 millimoles per liter of osmotically active K^+ from the fiber water and its replacement by an equivalent amount of osmotically active Na^+. The observed decrease in a_K is smaller and the concomitant increase in the a_K/C_K ratio is larger than one would predict on this basis (*17*). These results strongly suggest that a considerable part of the K^+ lost during overnight immersion was initially adsorbed. In the case of Na^+, the evidence is even more striking. The observed increase in a_{Na} is much less than would be predicted from the entry of 27 millimoles per liter of osmotically active Na^+ into the fibers, and the fact that the a_{Na}/C_{Na} ratio decreased (from 0.32 to 0.24) during storage overnight strongly indicates (unless one makes the unlikely assumption that Na^+-K^+ exchange under these conditions was associated with a large change in the fraction of "bound" fiber water) that most of the extra Na^+ taken up entered a region of low activity in the fibers.

Quantitatively, therefore, these results are at variance with the hypothesis (*1*) that Na^+-K^+ exchange in muscle, to the extent observed in our experiments, occurs predominantly by direct exchange of these ions between phases in which they exist at relatively high electrochemical potentials. On the contrary, these results lend partial support to the contention of Ling and Cope (*12*) that such exchange involves occupation by Na^+ of adsorption sites vacated by K^+, and, in fact, can be interpreted as providing direct evidence for the existence of the "bound" K^+ postulated by these authors. However, the activity data shown in Table 1 indicate that, although some of the fiber K^+ is probably bound, under normal conditions the greater part of it is not. Also the increase in a_{Na} and the concomitant decrease in a_K during overnight immersion indicates that replacement of at least part of this "free" K^+ by "free" Na^+ does occur.

As a working hypothesis we suggest that our results so far can best be explained in terms of a model similar to that proposed by Harris and Sjodin (*18*) to account for the kinetics of K^+ exchange in muscle. These authors suggested that movement of K^+ into and out of the cytoplasm is mediated by a mechanism in an outer region of the fiber in which K^+ exists predominantly in an adsorbed state and which has a finite capacity for ions. The existence of a region possessing such properties, possibly including the membrane itself together with the sarcoplasmic reticulum, could account for our findings. Thus, intrafiber Na^+ and K^+ could exist in dynamic equilibrium between this region and the cytoplasm, and external Na^+ would have to traverse this region before being released to a high-activity cytoplasmic compartment of the cell interior. Clearly, asymmetry of the binding affinities for Na^+ and K^+ between those adsorption sites directly accessible to external cations and those accessible to cytoplasmic cations is a necessary condition if such a model is to account for the known specificities of the inwardly and outwardly directed components of Na^+ and K^+ transport in muscle.

At first glance, the low values of a_{Na} in frog sartorius muscle found by Lev (*4*) and by us may seem inconsistent with earlier studies such as that of Desmedt (*19*), who reported a linear relationship (slope, 58 mv) between the logarithm of C_{Na} and the height of the overshoot in the action potentials of Na^+-rich muscles prepared by a technique essentially similar to ours. This finding would appear to require a close correspondence between the activity coefficients of Na^+ in the fiber and in the external medium. Inspection of figure 5 of Desmedt's paper (*19*)

reveals that this inconsistency is more apparent than real. First, the linear relationship he reported was obtained only with muscles that had been subjected to prolonged (30 to 60 hours) soaking in media low in K^+ and in which the estimated C_{Na} was relatively high (30 mM to 70 mM). Second, when C_{Na} was between 10 mM and 30 mM, this variable had little effect on the height of the overshoot. If we take into account possible discrepancies between our estimates of the extracellular space and those of Desmedt, this finding is entirely consistent with our results (Table 1). Third, in interpreting Desmedt's experiments, the possibility of a local increase in a_{Na} at the fiber membrane during depolarization should be considered. Determinations of intrafiber activity in muscles immersed for longer times in K^+-free media than those used in the present study should provide further information about this question.

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References and Notes

1. E. J. Conway, *Physiol. Rev.* **37**, 84 (1957).
2. S. E. Simon, in *Membrane Transport and Metabolism*, A. Kleinzeller and A. Kotyk, Eds. (Academic Press, New York, 1961), p. 148.
3. J. A. M. Hinke, *Nature* **184**, 1257 (1959); *J. Physiol. (London)* **156**, 314 (1961); *J. Gen. Physiol.* **56**, 521 (1970).
4. A. A. Lev, *Nature* **201**, 1132 (1964).
5. S. G. A. McLaughlin and J. A. M. Hinke, *Can. J. Physiol. Pharmacol.* **44**, 837 (1966).
6. D. A. T. Dick and S. G. A. McLaughlin, *J. Physiol. (London)*, **205**, 61 (1969).
7. F. W. Cope, *J. Gen. Physiol.* **50**, 1353 (1967); *Biophys. J.* **10**, 843 (1970).
8. D. Martinez, A. A. Silvidi, R. M. Stokes, *Biophys. J.* **9**, 1256 (1969); J. L. Czeisler, O. G. Fritz, Jr., T. J. Swift, *ibid.* **10**, 260 (1970).
9. J. D. Robertson, *J. Exp. Biol.* **38**, 707 (1961).
10. J. A. Zadunaisky, *J. Cell. Biol.* **31**, C11 (1966).
11. A. S. Troschin, in *Membrane Transport and Metabolism*, A. Kleinzeller and A. Kotyk, Eds. (Academic Press, New York, 1961), p. 45; G. N. Ling, *A Physical Theory of the Living State: The Association-Induction Hypothesis* (Blaisdell, New York, 1962).
12. G. N. Ling and F. W. Cope, *Science* **163**, 1335 (1969).
13. R. H. Adrian, *J. Physiol. (London)* **151**, 154 (1960).
14. G. Eisenman, D. O. Rudin, J. U. Casby, *Science* **126**, 831 (1957).
15. B. P. Nicolsky, *Zh. Fiz. Khim.* **10**, 495 (1937).
16. W. McD. Armstrong, D. Lurie, M. Burt, J. R. High, *Amer. J. Physiol.* **217**, 1230 (1969).
17. J. Kielland, *J. Amer. Chem. Soc.* **59**, 1675 (1937).
18. E. J. Harris and R. A. Sjodin, *J. Physiol. (London)* **155**, 221 (1961).
19. J. E. Desmedt, *ibid.* **121**, 191 (1953).
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Development of Excitability in Embryonic Muscle Cell Membranes in Certain Tunicates

Abstract. *During the course of development of muscle cells in certain tunicates, a sign of regenerative membrane response appears in the gastrula stage. In the early tadpole larva, the action potential consists of a spike followed by a plateau. The latter disappears in fully differentiated cells, conceivably in association with the establishment of delayed rectification.*

The initiation of action potentials is one of the unique features of nerve and muscle cell membranes. It would, therefore, be of great interest if one could follow up the appearance and establishment of the electrical excitability of the cell membrane throughout various stages of embryonic development. For this type of study it is desired that the preparation have the following properties: (i) the destination of each blastomere should be fully determined at the very early stage of development, so that one can follow all stages of development up to fully differentiated cells; and (ii) the cells should have diameters large enough for intracellular microelectrode techniques to be applied throughout the course of cleavage. Certain tunicates (*Halocynthia roretzi* Drashe and *Halocynthia aurantium* Pallas) have large eggs (270 μm in diameter; Fig. 1A) and specific blastomeres are destined in the course of development to give rise to specific parts of the larva (*1, 2*). Moreover, the cells destined to be muscle cells remain large throughout cleavage. In fully differentiated larva there are six relatively large chains of striated muscle cells arranged longitudinally in the tail of the tadpole larva, their diameter being 15 to 20 μm (*3*) (Fig. 1A). Each chain consists of seven to eight mononuclear cells connected along the long axis of the chain (*2, 4*).

Matured eggs and sperms for fertilization were obtained from *Halocynthia roretzi* or *Halocynthia aurantium* captured in the northern part of Japan. Fertilized eggs were cultured under constant aeration at temperatures of 6° to 7°C in natural seawater. The total time of the development from the first cleavage to the hatching was about 70 hours. For the peneration of micropipettes, the follicular envelope and the chorion around the egg or the tadpole before hatching were removed by fine needles. The tunic coat of the hatched tadpole was also taken off by the needles. For the tadpole, pronase was applied for 10 minutes (at 10°C) at a concentration of 0.1 percent in the external solution to loosen the intercellular contact within the epithelium. The enzyme was washed away with a large amount of fresh artificial seawater (ASW) cooled to 0°C. Then the preparation was kept in ASW cooled to about 5.0° to −0.5°C by thermoelectric device to decrease the rate of development during the experiment. The ASW used had the composition of 452 mM NaCl, 9.8 mM KCl, 10.6 mM $CaCl_2$, and 48 mM $MgCl_2$, buffered at pH 8.0 by 10 mM tris (hydroxymethyl) aminomethane HCl. Glass microelectrodes filled with a solution of 2M potassium citrate saturated with methyl blue for marking the penetrated cell (*5*) and having a resistance of 20 to 40 megohms were used as recording electrodes. Those filled with 3M KCl and having a resistance of 5 to 10 megohms served as reference electrodes. So that we might observe the electrical responses from two cells simultaneously, we introduced a second electrode filled with potassium citrate. For checking the value of resting pitentials, we used recording electrodes filled with 3M KC1. To record potential changes while a current pulse was applied through the recording micropipette, we used the input stage of the modified bridge circuit (*6*) improved by application of FET operational amplifiers (143B, Analog Devices).

The development of the excitable membrane in the embryo consisted of the following four successive stages, tentatively called A, B, C, and D. They were characterized by unique responses to depolarizing current pulses (Fig. 2) and by the amount of the resting potential.

At stage A, from the period of the first cleavage to the initiation of invagination (128 cell stage), all cells examined showed the same amount of resting potentials, -19.4 ± 7.1 mv (standard deviation, $n=9$) on the average, irrespective of whether their cell types were presumptive muscle cells or the other cells. No regenerative responses were induced when the cells were depolarized by current pulse (Fig. 2A). In the blastula or the gastrula at the next stage (B) an electrotonic