STUDIES ON ION PERMEABILITY. I. WHAT DETERMINES THE RATE OF NA^+ ION EFFLUX FROM FROG MUSCLE CELLS?

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SUMMARY

The study of Na^+-ion efflux from single muscle fibers and multiple muscle fiber preparations led to the conclusion that both the fast and slow fraction originate from within. the same cells. The rate-limiting step for the slow fraction is shown, not to be membrane permeability but the rate of desorption from intracellular adsorption sites. The other fraction which is 4 to 8 times faster represents the rate of exchange between intracellular free Na^+ ion and the external medium. The significance of this finding in relation to the minimally needed energy for the Na pump is discussed.

Since Levi and Ussing's study, it has been well known that the efflux from isolated frog sartorius muscle consists of one fast fraction and one or more slow fractions.' Mod investigators have regarded the fast fraction as representing Na^+ ion in the extracellular space and perhaps also Na^+ ion loosely adsorbed on connective tissues: and the slow fraction as representing membrane-limited transport of Na^+ ion from the muscle cells.

This widely accepted interpretation demands a large volume for the extracellular space. Thus, Johnson gave the volume of extracellular space estimated from the fast fraction of Na^+-ion efflux as 37.2% ± 2.9% (S.E.) of the muscle? in contrast to the once widely accepted value of 13% introduced by Boyle et al?

Recently, however, three lines of independent experimental evidence have suggested that the extracellular space proper (space filled with plasma or Ringer solution), may be even less than 13%. From the distribution of poly-L-glutamate, Ling and Kromash estimated a ceiling value of only 8.9%.[5] The total sucrose and D-mannitol space is about 25%; of this, at least 15% has been shown in single muscle fiber studies to be intracellular, leaving an extracellular space of no more than 10%.[6] The total free Na^+ ion in frog muscles measured with the nuclear magnetic resonance (NMR) technique is 10 mmoles/kg.' This concentration is about 10% of that in the surrounding medium (102 mM).

tion and to suction, respectively. The entire apparatus is placed in a constant-temperature room set at 25°C unless otherwise noted. After an initial count the tissue is washed by a constant flow of solution (a minimum of 3 liters per hour providing renewal of the solution around the muscle every 0.6 seconds) and the radioactivity remaining in the tissue is continuously monitored on the scaler.

To study the efflux of labeled Na^+ ion from single muscle fibers or small fiber bundles we used a special U-tube in which the two limbs were separated by a coarse-grade scintered glass disc. The fiber preparation was placed on a small "'boat'' made from a piece of folded celluloid (1 cm x 0.2 cm), and inserted into the straight limb of the U-tube. Studies showed that such celluloid does not take up radioactive Na^+ ion. Immediately after the commencement of washing, the muscle-fiber preparation became dislodged from the boat and came to rest on the surface of the scintered glass partition where it remained for the duration of the experiment.

The Na^+-ion concentrations in frog muscle before and after 4.5 hours of vigorous washing at 25°C were analyzed using the method described earlier.[16] The total Na^+-ion content of the muscles was unchanged by this washing procedure. Thus, from 17 sets of experiments, the unwashed control muscles contained 30.1 ± 2.02 μmoles of Na^+ ion per gram of fresh tissue while the washed muscle contained 29.9 ± 1-14.

Figure 1 shows 4 examples of Na^+-ion efflux curves from single and multiple fiber preparations. In general, the data on the single muscle fiber study confirms our earlier report on the Na^+-ion efflux from single isolated muscle fiber (15, p. 293). The solid line in Figure 1 represents the corrected Na^+-ion efflux curve. It is obtained after a correction was made, based on the assumption that these fibers contain 5% connective tissue. I chose this somewhat lower figure than that for whole sartorius muscle (9.09%, see 15, p. 210) because the isolated muscle fibers are devoid of the thick covering of fascia present in the sartorius. It should be pointed out that using a correction as high as 10% would not materially change the efflux curve (the top line marked 'a' represents the single fiber efflux obtained after a 10% correction; the lower line marked 'b', a 5% correction).

As one reduces the number of muscle fibers and hence the time for diffusion from the extracellular space, there is no observable trend toward a rectilinear efflux curve or the elimination of the fast fraction. Indeed, all curves shown in Figure 1 exhibit the general shape shown for the whole sartorius muscle.[2,15] The corrected curve can be resolved into two fractions. The average half-time ($t_{½}$) of the fast component of Na^+-ion efflux is 3.69 ± 0.57 (S.E.) minutes; that of the slow fraction, 2.5 ± 2.6 minutes from 8 experiments on muscle preparations incubated (4°C) and washed (25°C) in a normal Ringer solution.

DISCUSSION

Data of Single Fiber Na^+-Ion Efflux from Another Laboratory

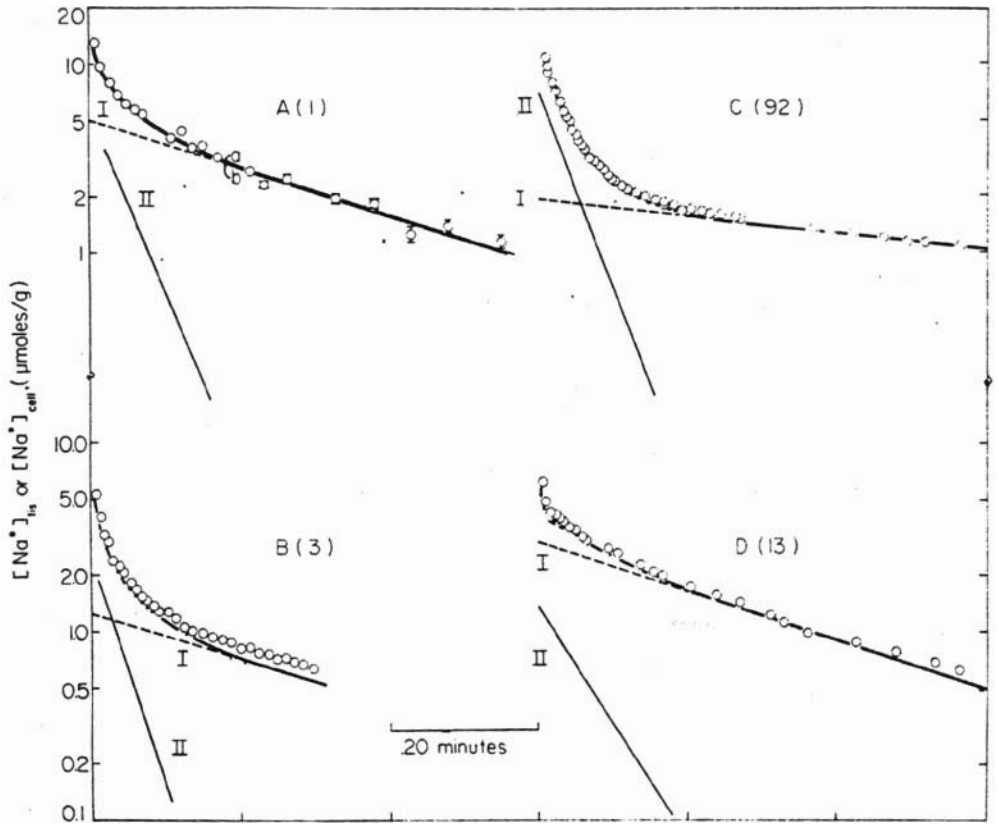

Figure 1. Time course of labeled Na^+-ion efflux from a single fiber and small multiple fiber bundles. Number of fibers indicated on figure. All fibers were washed in normal Ringer phosphate at 25°C except B, which was washed at 0°C. Experimental points are in units of μmoles per gram of fresh muscle tissue. Correction for connective tissue was made on the basis of a composite curve of Na^{22}-ion efflux from similarly incubated connective tissues from 3 frogs. The corrected curve is in units of μmoles per gram of fresh muscle cell. In A, curve a was obtained on a basis of a 5% correction for the connective tissue as was the case for B, C, and D. Line b in A, was on the basis of a 10% connective tissue correction. Experiment designations for A, 6H1; B, 5K12; C, 6K2G4 and D, 6G27.

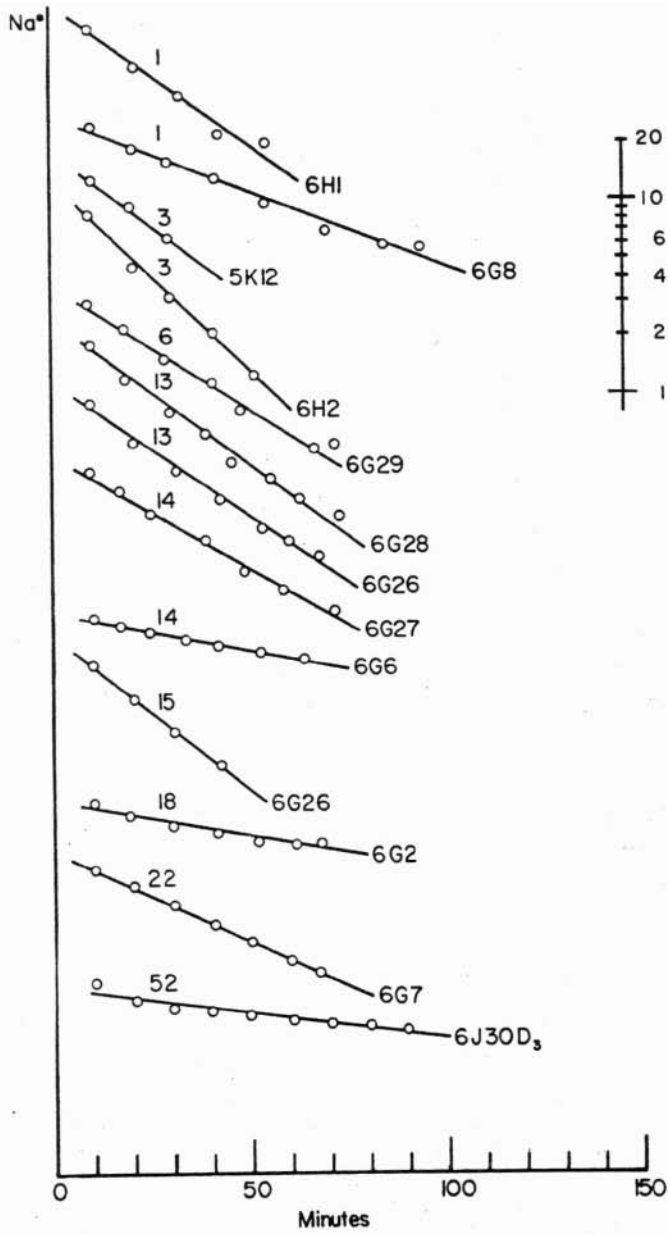

Figure 2. Replot of experimental data. The graph shows the data from a random collection of experiments, including those shown in Fig. 1, all exhibiting pronounced curvature, replotted to correspond to time intervals used by Hodgkin and Horowicz in similar studies.[23]

In 1959, Hodgkin and Horowicz concluded from their findings on Na^+-ion efflux from single muscle fiber preparations (which contains no extracellular space) that the semi-logarithmic plot of the efflux is a straight line." These authors were careful in pointing out the lack of data in the initial 10 minutes of washing.

In Figure 2, I have replotted a randomly chosen group of my own data of Na^+-ion efflux from intact sartorius muscle and as well as from single and multiple muscle fiber preparations. All of these curves originally show pronounced curvatures as in the cases shown in Figure 1, which are also included. In these replots all exponential points are deleted except those corresponding to the time intervals used by Hodgkin and Horowicz. Using these time intervals one can no longer recognize the pronounced curvature that is clearly shown when more points were present. The data can now all be fitted with straight lines, which in general resemble the data of Hodgkin and Horowicz.

Two Efflux Mechanisms

In 1947, Ussing suggested that only a part of the Na^+-ion efflux is due to the Na pump: The remainder may represent efflux via an "exchange diffusion mechanism."[1,19] However, results of most experimental studies have not supported this theory[2*,"] (see also 23, 24).

Above all, Ussing's theory was not proposed to explain, nor can it explain, the existence of two fractions of solute efflux under conditions where the total Na^+-ion concentration (labeled and unlabeled) is constant throughout the experiment (see Methods). Under these conditions any combination of carrier and "leak" pathways would produce a single rate constant, and a simple exponential efflux curve would be seen.

Two Compartment Interpretation

A number of investigators have suggested that each muscle cell may have more than one separate compartment.[25,26] Conway and his co-workers suggested that 8 mmoles of Na^+ ion per kg of cell water belong to the sarcolemna.[25] On the other hand, however, Rosenthal et al.[27] gave the thickness of the sarcolemna as 0.1 μ.* Using this later estimate one can calculate that the sarcolemna occupies about 0.3% of the muscle volume. In order for it to contain an 8 mmole fraction of the Na^+ ion in muscle, it would have to contain Na^+ ion at a concentration of $\frac{.008 \text{ moles}}{.003}$ or 2.7 moles of Na^+ ion/kg of sarcolemna.

The sarcolemna contains, as its major component, collagen and ground substances common to connective tissues. Most likely it resembles loose connective tissue. Its labeled Na^+-ion content must therefore be included in the connective tissue correction already made.

*We have made electron micrographs of frog muscles showing the width of the sarcolernna in this animal to be of the same order of magnitude as that in the rat.[36]

Another compartment that could be considered as the origin of the 11-fraction is the tubular system. However, the transverse tubules (T-system) are the only part confluent with the external solution.[28] It occupies no more than 0.4% of the tissue volume[29,30] and is already included in the correction for the extracellular space.

Another possibility is that the entire sarcoplasmic reticulum is tubular in structure and is filled with a simple aqueous solution like the tissue fluid. If the barrier between the sarcoplasmic reticulum and the T-system were of a specialized kind, more permeable than the-rest of the plasma membrane, loss of labeled Na^+ from this "compartment" might account for the 11-fraction. Weighing against this interpretation, however, is the finding reported earlier[31] that, in an experiment roughly equivalent to that shown in Figure 1, short dipping of the sartorius muscle in a Ringer solution containing 100 mM labeled K^+ ion did not reveal a large 11-fraction for K^+ ion as is the case with Na^+ ion.

Further, Chambers and Hale[32] found that when an ice-tipped micropipet is pushed into the cell, abnormally shaped ice spikes grow exclusively in the ***longitudinal direction*** (Normal ice forms hexagonal structures, see 33 also.) The sarcoplasmic reticulum runs both longitudinally and horizontally. The fact that ice crystals in resting muscle cells can grow only longitudinally in the direction of the myofilaments, and never horizontally, is evidence that the sarcoplasmic reticulum is not filled with tissue fluid but, like the rest of the cytoplasm, contains polarized oriented water whose freezing properties are different from those of a dilute salt solution.

Other anatomical subdivisions of muscle cells include such structures as the nuclei and the mitochondria. Since these are internally placed, they cannot be the source of the fast fraction of Na^+-ion efflux. They can, however, be the source of the slow fraction (Fraction I) if the rate of efflux from these subcellular compartments is slower than the rate of efflux through the cell surface. This possibility is ruled out, however, by the fact that by lowering the external K^+-ion concentration the slow fraction can be made to increase in size until it corresponds to the bulk of the whole cell (Ling, to be published), and that there is no existing evidence that the nuclei and mitochondria can enlarge to such sizes as to occupy the whole cell.

The Identification of the Slow and Fast Fractions

Within the last ten years it has been established from three different lines of mutually supporting data that the muscle proteins have the capability of adsorbing Na^+ ion. Thus, Lewis and Saroff[8] demonstrated, with the aid of a permaselective membrane electrode, that Na^+ ion adsorbs onto actomyosin (as well as myosin). This conclusion was confirmed by Cope[13] using the technique of nuclear magnetic resonance.

Cope's NMR studies have left little doubt that a major fraction of the Na^+ ion in ***normal, uninjured*** bullfrog muscle is adsorbed, a conclusion later extended to leopard frogs.'

Now, the question is: What efflux profile would be expected from a cell in which a

major part of the intracellular Na^+ ion is in an adsorbed state, the remainder being free?

The pattern of labeled Na^+-ion efflux would be determined by two rate processes: (1) the rate of desorption from the adsorption sites, and (2) the rate of permeation through the cell surface (or of diffusion through the cytoplasmic water, see below). If desorption is faster than or equal to permeation, the efflux curve would be indistinguishable from a simple surface-limited diffusion. In a semilogarithmic plot, a straight line should be obtained as we have shown for the efflux of K^+ ion (ref. IS, p. 292). If, on the other hand, desorption is slower than permeation, the efflux curve will no longer be represented by a single straight line. Instead it will appear as two fractions. Since this is exactly what we have observed, we must conclude that the desorption rate is indeed slower than the permeability (or bulk-phase diffusion) rate.

If this conclusion is correct, one significant fact to emerge is that ***in the bulk of published work what has been considered as the rate constant of permeability is in fact the rate constant of desorption.*** This desorption is considerably slower than the true rate constant for the exchange between the cell and its environment as seen in the fast fraction of Na^+-ion efflux. This view was suggested by Ling in 1953[34] and further developed in 1962.[15] Recently, Jones came to the same conclusion from his studies of mammalian smooth muscles.[35]

The average $t_{½}$ for the fast fraction is 3.68 minutes from all 8 experiments on single and multiple fiber preparations. The rapid rate of exchange of the fast fraction has many implications, not the least of which is that relating to the rate of Na^+-ion efflux used in calculating the energy need of the Na pump.

As mentioned above, the conventional procedure has been to use the rate of slower exchanging fraction as the permeability rate with a $t_{½}$ of about 30 minutes. It was on the basis of this figure that the conclusion was reached that the Na pump would consume at least 16 to 30% of the total resting energy output of the resting muscle cell.[1,3,36] The fast fraction now has a $t_{½}$ of only 3.69 minutes, which is from 4 to 8 times faster than the values these authors used. Thus, if they had used what now appears to be the more correct value for the rate of Na^+-ion efflux, the minimum energy would not be 16 to 30% but 128 to 240%.

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