Permeability of Alkali Metal Cations in Lobster Muscle

A comparison of electrophysiological and osmometric analyses

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ABSTRACT Single muscle fibers from lobster walking legs are effectively impermeable to Na, but are permeable to K. They shrink in hyperosmotic NaCl; they swell in low NaCl media which are hyposmotic or which are made isosmotic with the addition of KCl. In conformity, the membrane potential is relatively insensitive to changes in external Na, while it responds according to the Nernst relation for changes in external K. When the medium is made isosmotic or hyperosmotic with RbCl the volume and membrane potential changes are of essentially the same magnitudes as those in media enriched with KCl. The time courses for attaining equilibrium are slower, indicating that Rb is less permeant than K. Substitution of CsCl for NaCl (isosmotic condition) produces no change in volume of the muscle fiber. Addition of CsCl (hyperosmotic condition) causes a shrinkage which attains a steady state, as is the case in hyperosmotic NaCl. Osmotically, therefore, Cs appears to be no more permeant than is Na. However, the membrane depolarizes slowly in Cs-enriched media and eventually comes to behave as an ideal Cs electrode. Thus, the electrode properties of the lobster muscle fiber membrane may not depend upon the diffusional relations of the membrane and ions, and the osmotic permeability of the membrane for a given cation may not correspond with the electrophysiologically deduced permeability. Comparative data on the effects of NH_4 and Li are also included and indicate several other degrees of complexity in the cell membrane.

INTRODUCTION

The idealized model to describe the resting potential of a cell (12, 20, 23) asserts that the potential is a function of the permeability of the various ions

that are distributed on both sides of the cell membrane, as well as of the electrochemical (Nernst) emf's of the respective ionic batteries. Thus, increasing the concentration of a given cation species in the external medium should depolarize the membrane, if the latter is permeable to that species. Conversely, if the cell depolarizes when the external concentration of a given cation is increased, the membrane must be permeable to that ion. This electrophysiological evidence for permeability can be checked by the classical methods of osmometry, examining the volume changes that occur when the bathing medium is made hyper- or hyposmotic with appropriate changes of the ionic constituents. This paper reports such a comparison with respect to Na, K, Rb, NH_4, and Cs. A preliminary account of some of the results has been published (9).

MATERIALS AND METHODS

Bender muscles from the walking legs of *Homarus americanus* were generally used in this study. The experiments were done from 1961 to 1965 on lobsters that had been obtained from dealers in New York City, Woods Hole, and Washington, D. C. No significant differences were found in the membrane potentials and osmotic properties of control preparations used in the different seasons, years, and geographic locations.

The stock bathing solution was a K-free saline containing 455 mM NaCl, 24 mM $CaCl_2$, 8 mM $MgCl_2$, and 5.8 mM H_3BO_3, and adjusted with NaOH to a pH of 7.4 ± 0.1. The Cl salt of K, Rb, NH_4, or Cs was added to the K-free stock saline to make the experimental bathing solutions. Propionate was used as an impermeant anion substitute for chloride (29). The various changes in ionic composition of the salines will be described in the text in connection with specific experiments. Saline solutions containing 15 mM K will hereafter be referred to as standard saline, since they most closely approximated that proposed by Cole (4). When the preparations were to be soaked in the experimental saline for more than 6 hr, they were kept at 6°C, then brought to room temperature before the experimental measurements were made.

In some of the experiments in which only potentials were measured under various conditions, whole muscle preparations were studied, made as described previously (19). Membrane potentials were recorded from the surface fibers of the muscles using techniques that are standard for the laboratory (11, 19). When volumetric studies were done they were carried out on single muscle fibers dissected from the bender muscle by a procedure similar to that described in earlier work on crayfish muscle fibers (11). The average diameter of the muscle fibers was 157 μ with a range between 65 and 312 μ. All the fibers had sarcomere lengths between 9 and 10 μ. Both the single fiber and whole muscle preparations appeared to be in good condition when kept for as long as 12 hr at room temperature, and for more than 24 hr at 6°C. Synaptic activity was studied in many of these preparations and electrophysiological data regarding the augmenting effects of Cs have been published (10).

Single fibers that were used for osmometric experiments were dissected out while the muscle was bathed in the standard saline. The chamber containing the fiber was

then flushed with 50 ml of the saline, in order to insure that K leaking out from the cut cells and discarded fibers was removed. The fiber was allowed to equilibrate in 10 ml of the standard saline. Photomicrographs were made at intervals to check constancy of volume. Changes in the saline to induce osmotic challenges were always accompanied by a 50 ml flush of the chamber with the experimental saline. The final volume of the experimental saline of the chamber equalled 10 ml.

Osmotic strengths of the solutions are expressed as "osmotic pressures." Due to the uncertainty involved in determining osmotic and reflection coefficients in mixed solutions, the term osmotic pressure (P) denotes osmotic strengths calculated, assuming no interactions, from the molal concentrations of the constituents, and molal osmotic coefficients, measured in pure solutions (32). The reflection coefficients for the Na, Ca, and Mg salts as well as sucrose appear to be unity, since a steady displacement of fiber volume occurred with the hyperosmotic addition of these substances to the standard saline. The invariant, osmotically active constituents of the experimental media were the Ca and Mg salts, and the borate buffer.

Osmotic responses of the muscle fibers were measured from dimensional changes in enlargements of photomicrographs that were made at chosen intervals during the experiment. The fibers are essentially cylindrical or only slightly elliptical over most of their length. Errors introduced by nonuniformity of structure were minimized by measuring the diameter at a single topographically identified region of the fiber. The diameter could be read from the enlarged records with an accuracy of $\pm 2\%$. The preparation was discarded if a change in sarcomere length occurred during the experiment. If the fibers retain the same cross-sectional shape, as they appear to do, and their length remains constant, then their relative volumes in different solutions are given by the relation $V_2/V_1 = (D_2/D_1)^2$ where D_1 is the initial diameter, D_2 is the new value, and V_1 and V_2 are the corresponding volumes. The error in the calculated volume was of the order of 5%. For experiments like those shown in Figs. 3 and 4 the osmotic challenges were limited to the linear range of the P-V relation of Fig. 2.

RESULTS

The Fiber As an Osmometer for Na

The first requirement of the present experiments was to establish whether or not lobster muscle fibers behave as osmometers for Na. Two sample experiments are shown in Fig. 1. One is for the fiber exposed to a hypotonic medium (filled circles, solid lines), made hyposmotic by reducing the NaCl concentration by 197 mM/liter. The fiber swelled to a steady volume somewhat more than 50% above the control volume within about 20 min. The fiber shrank rapidly, although incompletely, when it was returned to the control medium. Crayfish muscle fibers also remain swollen, and to a somewhat greater degree, after being returned from a hyposmotic solution to the control medium (29).

The second fiber of Fig. 1 was exposed to a medium made hypertonic by the addition of 262 mM/liter NaCl to the standard saline (open circles, broken line). Both the shrinkage and the subsequent swelling, when the fiber was

returned to the control medium, were somewhat more rapid than the volume changes in the hyposmotic experiment. However, the fiber had not returned to its original volume after some 30 min in the control medium. Different types of cells exhibit different degrees of reversibility from osmotic challenges. In crayfish fibers, the volume change induced by increasing NaCl is fully reversible (29). In lobster axons the reversals from hyposmotic and hyperosmotic challenges are not quite symmetrical, but the volume changes of squid giant axons are fully reversible (8).

The results of a series of experiments with various osmotic challenges are shown in Fig. 2. Each point represents an experiment on one fiber. The

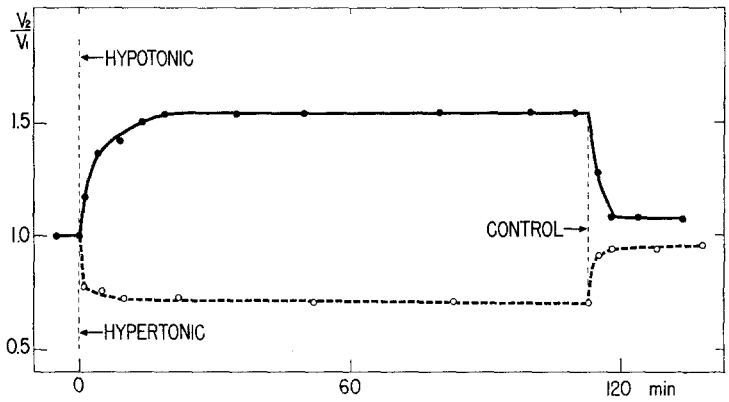

FIGURE 1. Effects of changes in osmotic pressure on muscle cell volume. The upper curve shows the effect of placing a muscle fiber in a saline that was made hypotonic by the removal of 197 mM/liter of NaCl from the standard medium. The lower curve is the result of placing another cell in a hypertonic saline (+262 mM/liter NaCl). The results are plotted as relative volume (V_2/V_1) against time, where V_1 represents the initial volume in the standard saline and V_2 represents the volume of the cell in the experimental saline.

relative volumes in the steady state (V_2/V_1) are plotted against the reciprocals of the relative osmotic pressures (P_1/P_2) of the medium. The linear (van't Hoff) relation held over a fairly wide range, but for values of P_1/P_2 greater than 2, the cells were unable to swell much further. The open circles represent experiments made with additions of sucrose to increase the osmotic pressure, since the increase in ionic strength that resulted from increasing the NaCl in the medium had secondary, detrimental effects on the cell's membrane. Such effects were not observed in crayfish muscle (29) or lobster axons (8).

The swelling of frog muscle fibers is limited to about 60% above the control volume (30), while crayfish fibers can swell to at least three times their normal volume (29). The extrapolated intercept on the ordinate (b in the van't Hoff relation) was 0.1, suggesting that 10% of the cell's volume was osmotically

inactive. The linear regression line showed a correlation coefficient (r) of 0.92 ($P < 0.001$). In similar experiments on single muscle fibers of crayfish (29) and frog (30) the intercept on the ordinate was of the order of 0.4, indicating that the osmotic dead spaces are different in different muscles. In lobster axons the intercept is at about 0.20 (8).

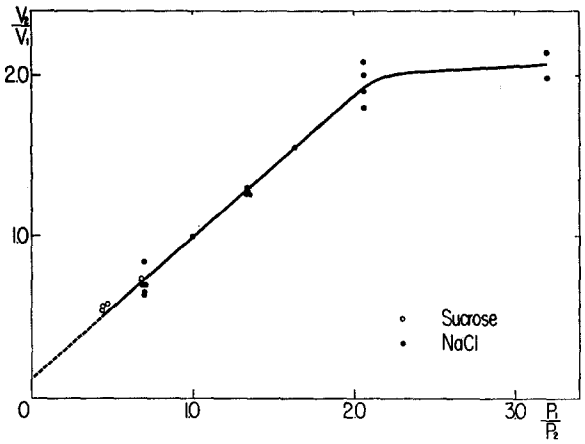

FIGURE 2. Relative volumes of single muscle fibers in media of different osmotic pressures. Each point represents an experiment on a separate muscle fiber. Ordinate, ratio of the final (steady-state) volume (V_2) to the initial (steady-state) volume (V_1). Abscissa, ratio of the initial osmotic pressure (P_1) to the final osmotic pressure (P_2). Changes in osmotic pressure were made by increasing or decreasing the NaCl content of the media (filled circles), except for P_1/P_2 less than 0.60 when sucrose was used as the added impermeant substance (open circles). The solid line is the calculated regression of P_1/P_2 on V_2/V_1. The regression line shows a strong correlation (correlation coefficient, r = 0.92; $P < 0.001$), and extrapolates (dotted line) to an intercept of 10% on the ordinate. The V_2/V_1 values obtained when P_1/P_2 was 3.0 were not used in the regression calculation.

Osmometric Data on Relative Permeabilities of Different Cations

Since lobster muscle fibers appear to be fairly good osmometers for Na over a wide range of osmotic pressures, it appears valid to infer relative permeabilities from the kinetics of redistribution of permeant cation species (5). As already noted, this series of experiments was confined to osmotic challenges that were within the linear range of the P-V relation of Fig. 2.

Fig. 3 depicts a group of four osmotic experiments that were performed in order to test the relative permeabilities of alkaline metal cations. In these experiments, the standard saline was made hyperosmotic by a 262 mM/liter increase in external concentration of KCl, RbCl, CsCl, or NaCl. The upper curves in Fig. 3 show the changes in volume of the fibers placed in salines made hyperosmotic with either KCl or RbCl. These cells underwent a rapid

transient shrinkage, and then returned practically to their initial volumes as the permeant salt entered. The reverse processes occurred when the cells were returned to the standard saline. The rates of return from either direction for the initial volume should be specific functions of the specific cation permeabilities (5). The volume changes induced by the hyperosmotic challenges differed to some degree from those of crayfish muscle fibers (29) or lobster axons (8). In both these latter tissues the cells were markedly swollen in the steady state, whereas the lobster muscle fibers in the steady state were nearly at their original volume.

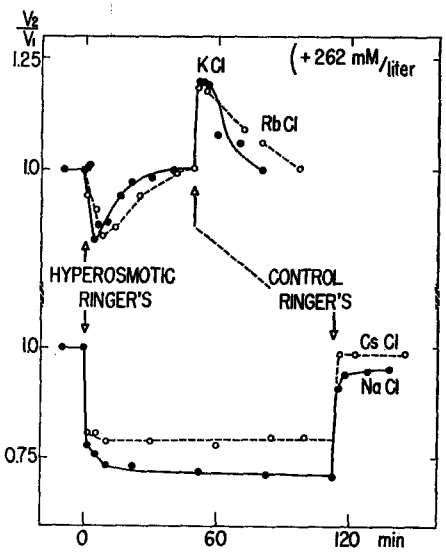

FIGURE 3. Changes in relative volume (V_2/V_1) in four single muscle fibers which were exposed for a time to a medium made hyperosmotic by the addition of 262 mM/liter of the following: KCl, RbCl (upper graphs), NaCl, or CsCl (lower graphs). Further description in the text.

The lower curves in Fig. 3 show the changes in cell volume in response to solutions made hyperosmotic with NaCl or CsCl. In both cases the cells shrank to new steady-state volumes, indicating that the cells were effectively as impermeable to Cs as to Na. The cells placed in hypertonic Cs saline consistently appeared to shrink less than those in the equivalent hypertonic NaCl. However, measurements of the relative steady-state volume (V_2/V_1) in five cells in hypertonic CsCl and five cells in hypertonic NaCl salines gave average values of 0.78 ± 0.03 (SE) and 0.71 ± 0.04 (SE), respectively. A Fisher *t*-test of the data indicated that the difference between Cs and Na was not statistically significant ($P > 0.05$). Nevertheless, it may be noted (Fig. 3) that after the fiber was caused to shrink by addition of Cs it returned promptly to the original volume when the hyperosmotic challenge was removed.

Fig. 4 presents typical results from another series of experiments in which changes in membrane potential as well as changes in volume were measured on the same muscle fibers. In the upper portion of Fig. 4, the changes in rela-

tive volume (V_2/V_1) for the given changes in the external medium were plotted against time. In each case the fiber was first equilibrated in standard saline. It was then challenged with a saline in which the NaCl was reduced by 262 mM/liter. In the experiment labeled "hypotonic NaCl," the Na that was removed was not replaced by another cation. The cell swelled to the new steady-state volume rapidly, consistent with the high permeability for water. The transient peak response was typical for large hyposmotic changes, and was also observed in frog muscle fibers (30). In the other experiments the Na that was removed was substituted for by an equivalent amount of K, Rb, NH_4, or Cs. The fibers exposed to the first three of these salts swelled toward

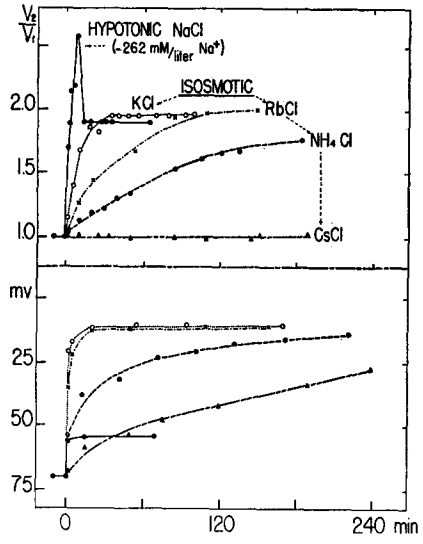

FIGURE 4. Correlation of changes in relative volume (V_2/V_1) and membrane potential (millivolts) in five muscle fibers, each of which was exposed to a medium in which the NaCl was reduced by 262 mM/liter. In four of these experiments the NaCl removed was replaced by an equimolar amount of KCl, RbCl, NH_4Cl, or CsCl. See text for further details.

the same steady-state volume, but the fiber exposed to the isosmotic medium containing Cs did not swell. In four experiments fibers were kept for 20 hr in the isosmotic CsCl medium with no observable change in volume. Thus, even with so long an exposure the cells appeared to be effectively impermeable to Cs.

The relative permeabilities may be calculated from the osmotic data on the basis of the time required to reach 50% of the steady-state volume in a given osmotic experiment. For example, the average half-time for the fibers to reach a swollen steady state in isosmotic K-enriched media was 13.4 min. In similar experiments with NH_4-enriched solutions, the average half-time was 86.7 min. Thus, relative to the K permeability that for NH_4 was 0.15. Data averaged from sets of experiments on five muscle fibers each gave permeabilities, relative to K (=1), as follows: Rb, 0.53; NH_4, 0.15; Cs and Na, 0. The standard error of the mean in these measurements was ± 2.3 min.

In the lower part of Fig. 4 are shown the membrane potentials of each of the fibers that had been subjected to the experiments described in the upper portion of the figure. The rapid, 17 mv depolarization in the hypotonic saline was consistent with the Nernst prediction (1, 29), since K_i had decreased to about half, due to the increase in intracellular water, but the overshoot observed in the volumetric data was not reflected in the electrical measurements. The isosmotic solutions, including the Cs-enriched saline, all produced much larger depolarizations, those caused by K and Rb developing much more rapidly than the depolarizations induced by NH_4 or Cs. However, the

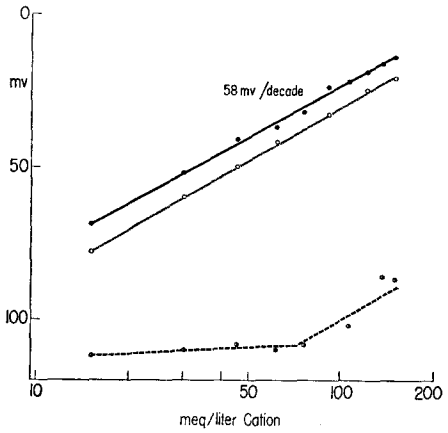

FIGURE 5. Membrane potentials are plotted against the logarithms of the external K(●), Rb(○), or NH_4(◑) concentrations. Before the initial potential measurements were made, in each experiment the muscle was equilibrated for 20 min in a K-free saline to which 15 mM/liter of the specific cation under study had been added. Increases in the external cation content of the saline were by fixed increments, and 20 min were allowed after each increment before the membrane potentials were measured. Each of the points is an average for 10 surface fibers from the bender muscle. The maximum standard errors of the means found for all the points on the K, Rb, and NH_4 curves were ±1.06 mv, ±0.96 mv, and ±2.03 mv, respectively.

fact that Cs did induce depolarization indicates, by electrophysiological evidence, that the membrane is permeable to Cs, whereas the osmometric evidence indicates that the cell was effectively impermeable to Cs.

Changes in Membrane Potential with Concentration of the "Critical" Cation

Further electrophysiological measurements were carried out by studying the relation between membrane potential (E_m) and the concentrations of the various cations (X_o) in the external medium. For these experiments whole muscle preparations were used. They were initially equilibrated for about 1 hr in the K-free stock saline and the resting potentials averaged about −110 mv (10, cf. also Figs. 9, 11, 13). A given concentration of the critical ion was

added as the Cl salt and the membrane potentials of at least 10 superficial muscle fibers were measured. In the experiments of Fig. 5, the resting potentials were determined after the preparations had been equilibrated in the new medium for at least 20 min. The depolarization induced by adding 15 mM K brought the resting potential to −70 mv (filled circles). This was also the average resting potential of fibers in all preparations that were equilibrated in 15mM K_o (Table I, and Figs. 7–12, 14, 15). Further increase in K_o depolarized the fibers at a rate of about 58 mv/decade increase in K.

The introduction of 15 mM Rb (open circles) caused about 10 mv less depolarization than did the addition of the same amount of K. However,

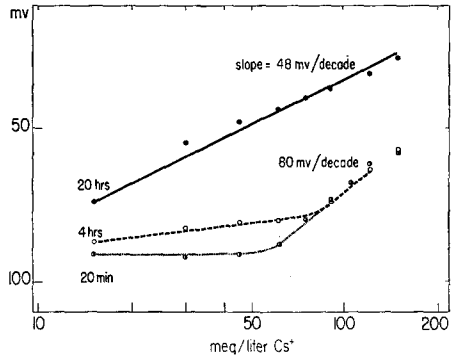

FIGURE 6. Effects of Cs on the resting potentials of lobster muscle fibers. The lowest curve (20 min) is identical in conditions with the experiments on K, Rb, and NH_4 described in Fig. 5; i.e., these cells were soaked for 20 min in 15 mM/liter Cs saline before the start of the experiment. Two other series of measurements were made on preparations exposed to the indicated concentrations of Cs for 4 and 20 hrs, respectively. Each point is the average of the potentials of 10 superficial muscle fibers. The maximum standard errors of the means for all the points on the 20 min, 4 hr, and 20 hr curves were ±2.46 mv, ±1.56 mv, and ±1.47 mv, respectively.

the changes in potential induced by further increase of Rb also had a slope of 58 mv/decade. In contrast, the addition of 15 mM NH_4 (half-filled circles) caused no appreciable depolarization of the fiber. Further increase in NH_4, up to about 75 mM, also caused only little change in the potential. Thereafter, however, further addition of NH_4 depolarized the fiber, the rate of change likewise being close to 58 mv/decade.

The effects of adding Cs to the K-free stock solution are shown in Fig. 6. Measurements comparable to those of Fig. 5, i.e. made 20 min after the solution was changed, beginning with introduction of 15 mM Cs into the K-free stock saline, are shown by the half-filled circles, and the dotted line. The addition of 15 mM Cs caused the fiber to depolarize to about −90 mv, but subsequent additions of Cs, up to about 60 mM, caused little or no change in the membrane potential during the 20 min equilibration intervals. Further

increase of the Cs, however, caused marked depolarization with a slope of about 80 mv/decade increase in Cs.

Similar measurements, but made after the preparations had been initially equilibrated for 4 hr in 15 mM Cs, are represented by the open circles of Fig. 6. During the 4 hr exposure to 15 mM Cs the fiber depolarized further by less than 5 mv. When the Cs was raised to about 60 mM and the preparation was allowed to equilibrate for 20 min, there was further depolarization by 15 or 20 mv. Still larger increase in the Cs caused depolarization with a rate of about 80 mv/decade increase in Cs. The filled circles in Fig. 6 represent still another series of measurements that were made on muscles that had been equilibrated in 15 mM Cs for 20 hr. The presence of 15 mM Cs caused the fibers to depolarize to about −80 mv, or to about the same value as was attained after 20 min exposure to 15 mM Rb (Fig. 5). The relation between E_m and log Cs concentration was linear in these preparations, but the rate of the depolarization was only about 48 mv/decade increase in Cs (Fig. 6).

The linear relations of the data in Figs. 5 and 6 permit an approximation at an electrophysiological measurement of the relative permeabilities of K, Rb, and Cs. The constant field equation (12, 23) reduces to the form

$$-E_M = 58 \text{ mv} \log \frac{P_K(K_i) + P_{Cl}(Cl_o)}{P_x(X_o) + P_{Cl}(Cl_i)}$$

if it is assumed that K_i remains constant and X_i = (Cs or Rb) is zero. The first assumption is supported by the findings in Fig. 5, that the membrane behaves as an ideal K electrode even in the presence of hyperosmotic K saline during the first 20 min of exposure to the K-enriched media. Experiments on the exchange rates of ^{42}K for bender muscles[1] showed that in standard saline the K exchange was less than 1%/hr. The electrophysiological calculation for P_{Rb} was made on preparations that had been exposed to Rb for only 20 min. On the basis of the foregoing, only negligible amounts of Rb could have exchanged with internal K. In the calculation of P_{Cs}, it is true, the fibers had been exposed to 15 mM Cs for 20 hr, but the osmotic data indicated that Cs had not entered the cell. Furthermore, a cell soaked in Cs for 20 hr still behaved as an ideal electrode for K and the calculated value for K_i is the same as in the case of the data in Fig. 5, 240 mM (Gainer, unpublished data).[2]

The "permeability coefficient" for Cl (P_{Cl}) was taken as 0.25 on the basis of data shown in Fig. 7 (cf. also Fig. 8). The "transport number" (t_{Cl}) for

[1] Dunham, P. B., and H. Gainer. 1968. The distribution of inorganic ions in lobster muscle. *Biochim. Biophys. Acta.* In press.

[2] Inasmuch as the calculations are based on the linear portion of the constant field equation, which reduces in this case to a form resembling the Nernst relation, the value of K_i (240 mM) used is that estimated from the electrophysiological data of Figs. 5 and 11. Measurements by flame photometry indicate that K_i is considerably smaller: ca. 155 mM/kg cells or about 180 mM/kg cell water.[1]

Cl was determined electrophysiologically from the instantaneous change in E_m when Cl_o was changed (21). The calculated value of t_{Cl} (0.21) was the same after the muscle had been soaked for 20 hr in 15 mM K (Fig. 7 B) or in 15 mM Cs (Fig. 7 C) as in the preparation which had been kept for only 1

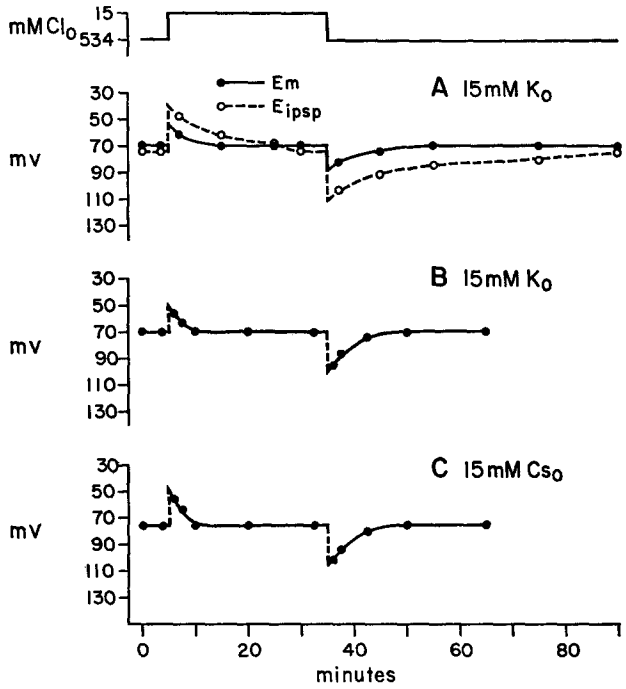

FIGURE 7. Transient changes in E_M and in the equilibrium value of the inhibitory postsynaptic potential (E_{IPSP}) resulting from changes in Cl_o indicated on the uppermost line. Propionate was substituted for Cl stoichiometrically. A, stretcher muscle equilibrated in the standard saline for 1 hr. Filled circles and solid line, E_M, open circles and broken line, the maximally summated IPSP's evoked by repetitive stimulation of the inhibitory axon. B and C, two bender muscles equilibrated for 20 hr respectively in the standard saline (B) and in the Cs saline (C). In the bender preparations only E_M was determined. Each point in the graphs represents the average for 10 superficial muscle fibers. The maximum standard error of the means was ±0.79 mv for all values of E_M, and ±0.95 mv for E_{IPSP}.

hr in the standard saline (Fig. 7 A). Furthermore, Cl appears to be redistributed passively in the lobster muscle fibers (Fig. 7 A) as it also is in muscles of frog (21) and crayfish (28, 29). Thus, Cl_i could be calculated from the Nernst relation

$$E_M = 58 \text{ mv} \log \frac{534}{Cl_i}$$

The correlation of such electrophysiological calculations for Cl_i with analyti-

cal measurements will be described elsewhere.[1] Implicit in all the calculations is the assumption of equal activity coefficients inside and outside the cell.[3]

On the basis of the foregoing, the permeability coefficient for Rb relative to K was $P_{Rb} = 0.72$. Since the slope of the linear relation E_m vs. log Cs_o was only about 48 mv/decade (Fig. 6), different values of P_{Cs} could be calculated for different values of Cs_o. When $Cs_o = 15$ mM the calculated value $P_{Cs} = 0.85$. With Cs_o increased to 60 mM P_{Cs} was 0.75 and for 150 mM it was 0.54. It will be recalled that the osmotically determined permeability coefficients were 0.58 for Rb and 0 for Cs.

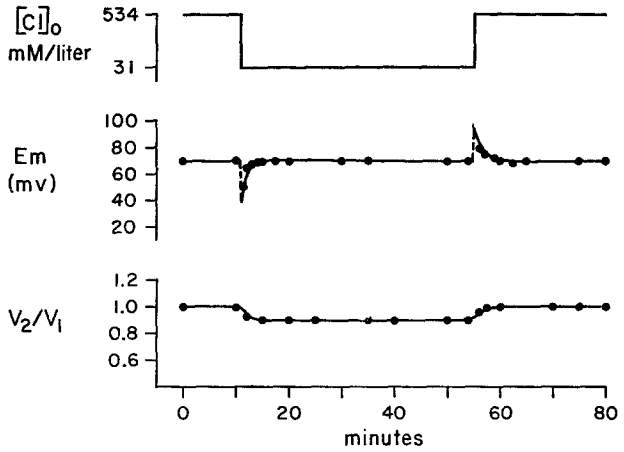

FIGURE 8. Correlation of changes in relative volume (V_2/V_1) and membrane potential (E_M) in a single muscle fiber when the Cl_o was reduced from 534 to 31 mM by substitution of propionate for Cl. Depolarization is shown downward in this figure. K_o was constant (15 mM) throughout the experiment. The effects on E_M and relative volume of the lobster muscle fibers resulting from changing Cl_o are discussed in the text.

The discrepancy between osmometric and electrophysiological data might be explained within the framework of the idealized diffusion model, if it is assumed further that the cell is compartmentalized, so that the compartment reflected in the electrophysiological data is small in volume relative to the volume of the fiber. Compartmentalization has been found in crayfish muscle fibers (29). An experiment attempting to estimate the size of the assumed electrophysiological compartment in lobster muscle fibers is shown in Fig. 8.

An isolated muscle fiber was first equilibrated in the standard saline, which contained 534 mM Cl. It was then placed in a saline in which Cl had been reduced to 31 mM by substitution of propionate for Cl. The membrane potential and fiber volume were followed with time. When the fiber appeared to be in a steady state with regard to these parameters it was returned to the

[3] The "passive" distribution of Cl is probably subject to the activity of a Cl pump, since the IPSP's of lobster muscle fibers are normally hyperpolarizing with respect to the resting potential (10,19; see Fig. 7 A).

standard saline. As in crayfish muscle fibers (29) and lobster axons (8) the transient depolarization and transient hyperpolarization of the membrane caused by changes in the gradient for Cl were accompanied by changes in volume (Fig. 8). The fiber shrank on exposure to the low Cl saline and the volume attained a steady state at about the time that the membrane potential had returned to its resting value.[4] The fiber remained shrunken until the Cl was restored, the subsequent return to the original volume also coinciding in time with the duration of the transient hyperpolarization. The sustained shrinkage is larger than is to be expected from changes in the osmotic strengths of the two media: that of the control saline was 978 milliosmols/liter while that of the low Cl saline was 1070 milliosmols/liter, so that the ratio P_1/P_2 was 0.92. In five experiments like that of Fig. 8 the average decrease in relative volume was to 0.88 $\pm$ 0.02, or a shrinkage of 12%. The excess shrinkage therefore was of the order of 3.5%. The intracellular Cl is of the order of 30 mM/liter and complete loss of this as KCl would have reduced the intracellular pressure by some 60 milliosmols/liter. Thus, the intracellular "mobile Cl" compartment must be nearly or fully coextensive with the osmotically active space of the fiber. If Cs could have diffused into the Cl compartment the muscle fibers should have swollen to about the same extent as did fibers in the presence of K, Rb, or NH_4 (Fig. 4).

The steady-state shrinkage of the lobster muscle fibers (Fig. 8) is itself of interest. In crayfish muscle only transient changes in volume were observed under similar experimental conditions (29), while in lobster axons the volume change had two components, a rapid transient phase as in crayfish muscle and a smaller maintained shrinkage (8). These differences indicate some, as yet unanalyzed, differences among the different tissues. One possible factor might be a difference in the effectiveness of electroosmotic forces which are known to affect cell volume (29).

The Time Course of Depolarizations Induced by K, Rb, or Cs

The depolarizations induced by challenges with large amounts (262 mM) of the cations are shown in the lower part of Fig. 4. When smaller amounts were added rather similar relations were also obtained. Fig. 9 shows experiments on preparations that had been equilibrated for 20 hr in the K-free stock solution (A) and in a K-free solution with Cl reduced to only 15 mM (substitution with propionate). The initial resting potentials were comparable in all six preparations. At time zero of the graphs 15 mM of K (filled circles), Rb (triangles), or Cs (open circles) was added. Each point represents an average of measurements on 10 muscle fibers.

The steady state of the membrane potential (-70 mv) in the presence of K was attained within a few minutes in the standard saline. The depolariza-

[4] The amplitudes of the transient changes of E_M are larger in Fig. 8 than in Fig. 7 and the time courses are briefer because the experiment of Fig. 8 was done on a single isolated fiber. These differences have also been observed in crayfish (28, and unpublished data).

tion was more rapid in the presence of only 15 mM Cl_o. In fact, the initial depolarization was somewhat larger than that of the steady state, as had also been observed under Cl-free conditions in crayfish muscle fibers (29). The depolarization caused by Rb was also speeded up, but it still was slower than that caused by K. However, the depolarization caused by Cs was slowed somewhat when the Cl of the medium was reduced to 15 mM.

Further evidence of this difference is presented in the experiments of Fig. 10 A and B. The preparations had been equilibrated in salines containing

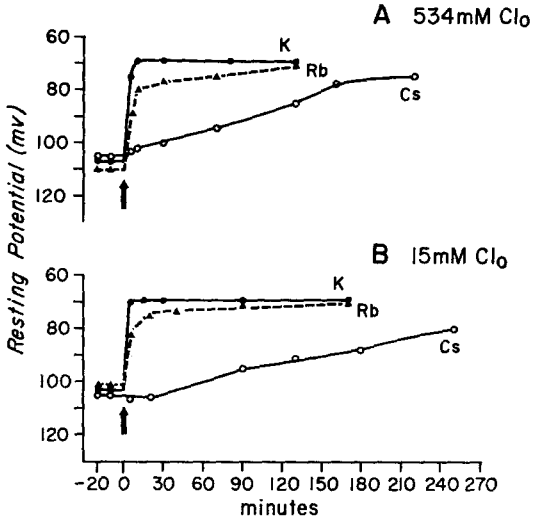

FIGURE 9. Time course of depolarization induced by challenging the muscle fibers with 15 mM of K (filled circles), Rb (triangles), or Cs (open circles). All the preparations were equilibrated for 20 hr in a K-free solution. Arrows indicate the introduction of the cation. A, the equilibration medium was the stock K-free saline containing 534 mM Cl. B, Cl was reduced in this medium to 15 mM, with substitution of propionate. The points represent averages of measurements on 10 muscle fibers. The maximum standard error was ±1.34 mv.

15 mM K_o, but the Cl_o was reduced to 15 mM in the experiments of Fig. 10 B. At zero time the muscle fibers were challenged with the addition of 30 mM K, Rb, or Cs. The depolarizations that were induced by K or Rb were more rapid in the low Cl media and the initial overshoot of the K-induced depolarization was marked (Fig. 10 B, filled circles). The depolarizations caused by 30 mM Cs, however, developed more slowly in the low Cl medium (open circles).

The kinetics of depolarizations caused by the three cations under still other conditions are also shown in Fig. 10 C and D. The preparations used in these experiments were equilibrated for 20 hr in the presence of 15 mM of the critical cation. The concentration of the latter ions was raised to 45 mM in

the experiments of Fig. 10 C. The relative effectiveness of the cations was still in the order of K > Rb > Cs, but the membrane potentials attained after about 2 hr tended toward the same level.

The preparations used in Fig. 10 D were challenged by the addition of 30 mM K. Thus, the final compositions of the bathing solutions were 45 mM K; 30 mM K + 15 mM Rb; and 30 mM K + 15 mM Cs. The fibers that had been equilibrated in Rb depolarized more slowly to the steady-state level.

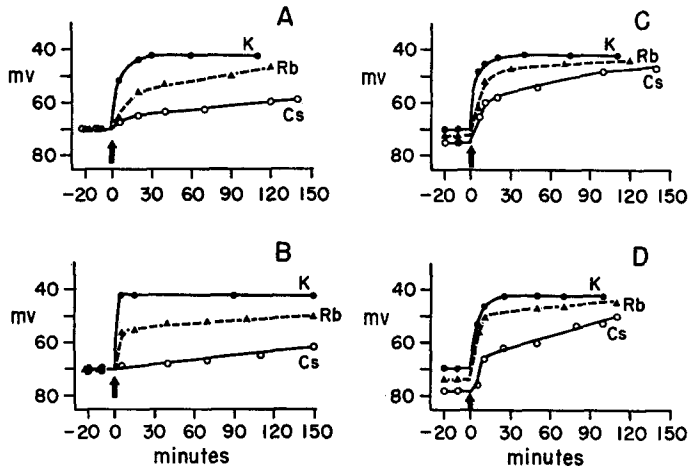

FIGURE 10. Changes in the kinetics of depolarization under various conditions. A, preparations were equilibrated for 20 hr in 15 mM K. At arrow 30 mM of additional cation was introduced. B, same, except that the bathing medium contained only 15 mM Cl. C, these preparations were equilibrated for 20 hr in saline containing 15 mM of the critical cation. At arrow 30 mM of this ion was added. D, equilibration as in C, but at arrow 30 mM K was added. All the points represent averages on 10 superficial muscle fibers of each preparation. The maximum standard errors were ±1.65 mv (A); ±0.87 mv (B); ±1.15 mv (C); and ±1.02 mv (D). Note that the graphs described by the filled circles in A, C, and D represent identical conditions, 15 mM K initially and 45 mM K finally in the presence of full Cl. Further description in text.

The same challenge to fibers that had been equilibrated in 15 mM Cs caused a smaller initial depolarization and this was followed by depolarization at the rate of 10 mv/hr. The depolarization attained after about 2 hr (ca. −50 mv) was of the same order as that which would have been attained in a few minutes by the addition of 30 mM K in the absence of Cs (Fig. 5). Thus, the presence of Cs caused marked impairment of the K electrode characteristic of the lobster muscle fiber membrane. Cs causes pharmacological K inactivation in the membrane of eel electroplaques (25) and lobster and crayfish muscles (10, 26), and pharmacological K inactivation is associated with impairment of the membrane as a K electrode (13, 17). While Rb can also induce pharmacological K inactivation in lobster muscle fibers (cf. Fig. 14) and in other

cells (17), it has relatively little effect on the K electrode characteristic of the lobster muscle fiber membrane.

It will be noted that in all the experiments with K as the critical cation (Fig. 10, filled circles) the initial condition was the presence of 15 mM K and the final condition was the presence of 45 mM K. Thus, the comparisons of the time courses of depolarization in all the experiments of Fig. 10 contain internal controls.

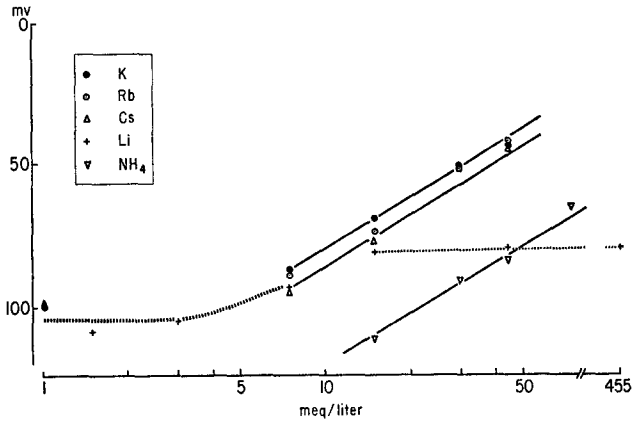

FIGURE 11. Steady-state E_M—log X_o relations for different monovalent cations. All measurements were on preparations that had been soaked for 20–24 hr in a saline containing the indicated concentration of cation. The points are averages of measurements for 10–80 muscle fibers. At low concentration (<7.5 mM) K and Cs had little effect on the potential (thick dotted line). Higher concentrations of K, Rb, or Cs depolarized the cells at a rate approximately 58 mv/decade (upper solid lines). NH_4 also depolarized the cells at the same rate (lowest solid line), but only when the concentration was >15 mM. Li in concentrations >15 mM has no effect on the membrane potential (thin dotted line).

Changes in Membrane Potential Induced by Li

Although we have not done osmometric measurements with substitution of Li for Na the volume of crayfish and frog muscle fibers is not altered by replacing all Na with Li (27, 30, and unpublished data). Nevertheless, Li does have some effect on the membrane potential of lobster muscle fibers (Fig. 11). In this series of experiments a large number of whole muscle preparations were equilibrated for 20 to 24 hr in salines which contained between 1 mM and 75 mM of the various cations. The points are averages obtained on at least 10 muscle fibers and in some cases on as many as 80 fibers. The results with the different cations appear to fall into three groups. In the presence of 1 mM of K or Cs and also Rb (10), the membrane potential was about −100 mv and the fiber depolarized somewhat when the concentration was raised to 7.5 mM. Increasing the level of K, Rb, or Cs to which the preparations

were exposed caused depolarization at a rate of approximately 58 mv/decade. Long exposure to a concentration of 15 mM NH_4 did not cause significant depolarization of the muscle fibers, which had resting potentials in the vicinity of −110 mv. This confirmed earlier observations by Reuben and Grundfest (unpublished). Equilibration in higher concentrations of NH_4 resulted in depolarization at the same rate as in K, Rb, or Cs. The addition

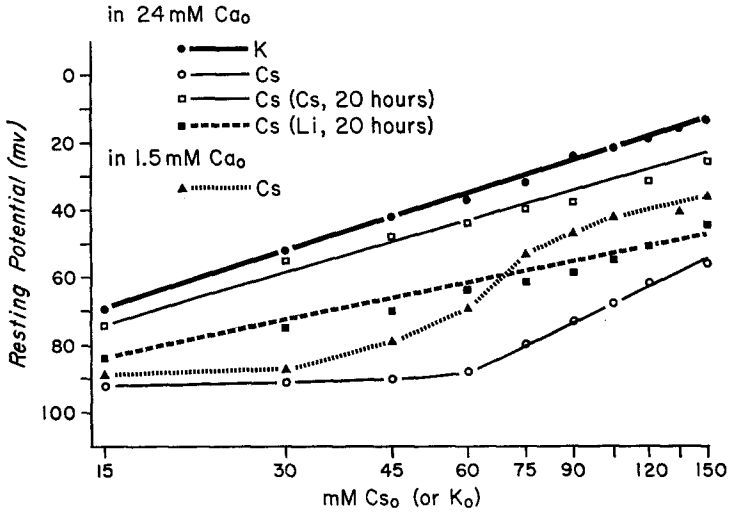

FIGURE 12. E_M—log K_o and E_M—log Cs_o characteristics, the latter under various experimental conditions. Filled circles, measurement on 10 muscle fibers 20 min after introducing the concentration of K_o indicated on the abscissa. Open circles, similar measurements, but with increasing concentrations of Cs. Triangles, similar data as in the last, but with Ca of the bathing medium reduced to 1.5 mM. Open squares, this preparation was equilibrated for 20 hr in a saline containing 15 mM Cs. Subsequent measurements made at 20 min intervals after increasing concentrations of Cs. Filled squares, this preparation had been equilibrated for 20 hr in a saline containing 15 mM Li. The first point represents the membrane potentials observed 20 min after addition of 15 mM Cs. Subsequent measurements also made 20 min after increasing concentration of Cs as shown on abscissa. The maximum standard error of all the points in this figure was ±2.4 mv. Further description in text.

of Li in low concentration (1.5 mM) also caused no appreciable depolarization. However, further increase did cause significant depolarization, up to a point. When the concentration was 15 mM the membrane potential (−80 mv) was about the same as in 15 mM Cs. However, further increase in Li, including complete replacement of all the Na with 455 mM Li, did not change the membrane potential any more.

When muscle fibers are equilibrated for a long time in 15 mM Li their responsiveness to an increase in Cs improves considerably. The filled squares (and broken line) in Fig. 12 represent measurements on muscle fibers of a

preparation that had been soaked for 20 hr in a saline containing 15 mM Li. Each point is the average of measurements on 10 muscle fibers, made 20 min after the addition of Cs to the bathing medium in the concentration indicated on the abscissa. The slope of about 35 mv/decade is less than that (52 mv) obtained on fibers that had been equilibrated for 20 hr in 15 mM/liter Cs (open squares), but the response of the membrane to Cs after equilibration in Li (filled squares) was considerably larger than that of fibers that were exposed to Cs for only 20 min (open circles). Companion experiments with changing K (filled circles) yielded a slope of 58 mv.

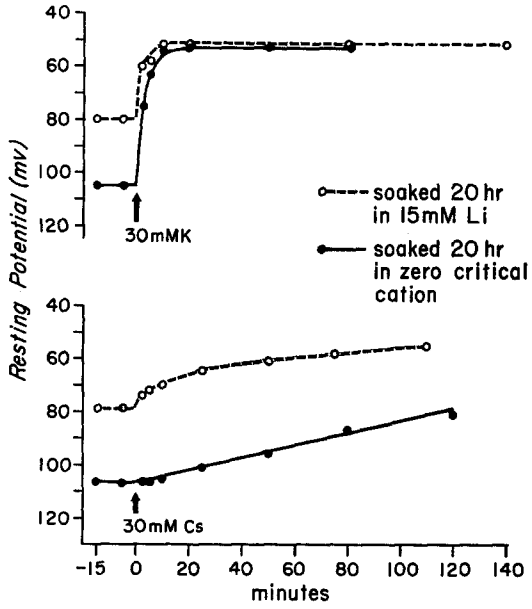

FIGURE 13. Time course of depolarizations induced by 30 mM K or Cs in muscle fibers that had been equilibrated for 20 hr either in 15 mM Li or in the K-free stock solution. Each point is an average of measurements on 10 fibers. The maximum standard error in this experiment was ±1.42 mv. Further description in text.

The data represented by the open circles and by the filled triangles of Fig. 12 also show that a decrease in the level of Ca affects the time variance of the relation E_M log Cs_o. These measurements were made on preparations that had been initially equilibrated in the K-free stock solution, but in one case (triangles), with Ca reduced to 1.5 mM. Changes of E_M for the indicated increments of Cs were measured after 20 min. In the low Ca medium large depolarization with increase of Cs occurred at a lower level than in the presence of 24 mM Ca and for concentrations of Cs > 75 mM the potential changed with a slope of approximately 50 mv/decade.

The presence of Li also markedly affected the time course of the depolarization that was induced by Cs. The lower part of Fig. 13 shows the responses to 30 mM Cs of muscle fibers in two preparations, one of which had been soaked for 20 hr in the K-free stock solution (filled circles). The second prepa-

ration (open circles) had been equilibrated in the same solution, but with 15 mM Li added.

In the Li-treated preparation the Cs caused more rapid depolarization. Although the initial membrane potentials differed by almost 30 mv, the more rapid depolarization cannot be entirely ascribed to the resting potential which was lower in the presence of Li than in the K-free stock saline. In the experiment of Fig. 10 A, the initial potential was still lower (−70 mv). The

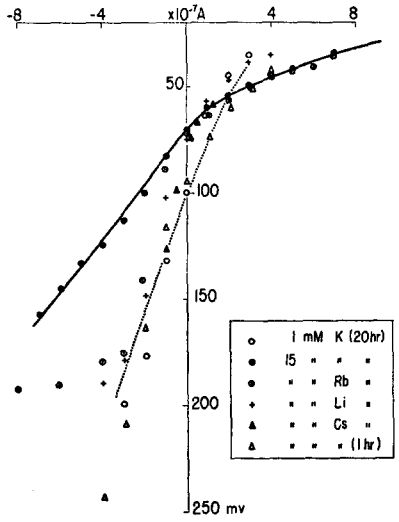

FIGURE 14. *I-E* characteristics of muscle fibers in the presence of different critical cations. Filled circles and solid line represent measurements on a fiber bathed in the standard saline for 20 hr. The resting potential was −70 mv. Note that the relation is linear for a range of 75 mv in the hyperpolarizing quadrant, while depolarizations greater than about 10 mv cause marked rectification. Fibers exposed to 1 mM K for 20 hr or to 15 mM Cs for 1 hr had resting potentials of about −100 mv and the slope resistance was high. However, the resistance was also high in fibers that had been soaked for 20 hr in 15 mM Rb, Li, or Cs, although the resting potentials ranged between −70 and −75 mv.

addition of 30 mM Cs depolarized the fibers to about −60 mv within 2 hr in both cases, but in the experiment of Fig. 13 the depolarization began from a resting potential of −80 mv.

Similar measurements of responses to a challenge with 30 mM K are shown in the upper part of Fig. 13. The depolarization that was induced in the presence of Li (open circles) occurred about as rapidly as in its absence (filled circles). This is in contrast to the very slowed depolarization caused by the equilibration in 15 mM Cs (Fig. 10 D, open circles) and the somewhat slower depolarization in the presence of Rb (triangles). Thus, Li does not

appear to have an effect on the K electrode characteristics of the muscle fiber membrane whereas Rb, and particularly Cs, do affect this property.

Effect of the Cations on Membrane Resistance

Cs increases the resistance of lobster and crayfish muscle fibers for inward currents (10, 26). However, this property cannot account for the anomalous relation between the osmotic and electrophysiological criteria for permeability of this cation. Fig. 14 shows the steady-state current-voltage characteristics

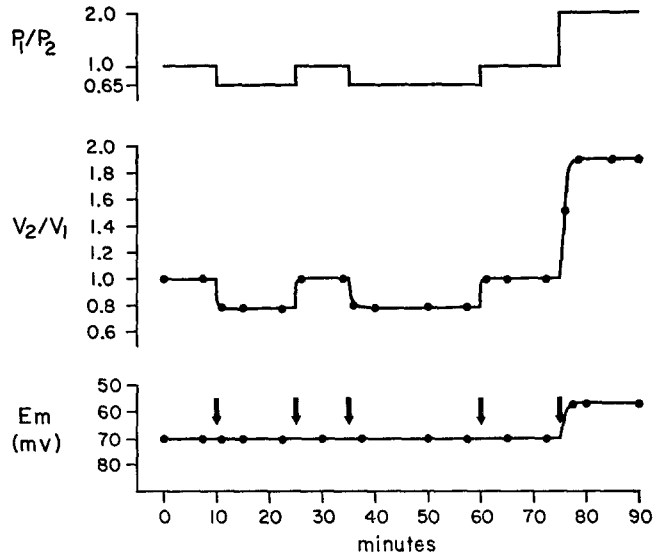

FIGURE 15. Changes in relative volume (V_2/V_1) and membrane potential (E_M) in a single muscle fiber in response to changes in osmotic pressure (P_1/P_2) in the external medium. The osmotic changes were made by varying the NaCl content of the medium. The arrows on the lowest curve (E_M vs. time) indicate times of the osmotic changes.

of fibers in a series of bender muscles that were exposed to various cations. The slope resistance as measured in the vicinity of the resting potential increased to about the same degree in the presence of 15 mM Rb, Cs, or Li in fibers that had been equilibrated in the respective media for 20 hr. Lobster muscle fibers also have a high resistance in the presence of 15 mM NH_4 (Reuben and Grundfest, unpublished data). Thus, the very different electrophysiological effects of the different cations cannot be correlated with changes in membrane permeabilities for current carriers. This is also emphasized further by the fact that the resistance was the same in muscle fibers that were equilibrated in 15 meq/liter Cs for 1 hr or for 20 hr, whereas the behavior of the fiber membrane as an electrode for Cs is changed markedly by prolonged exposure to this cation (Fig. 6).

Electrophysiological Asymmetry of the Cell Membrane

In the experiments described above the increases in X_o were made under hyperosmotic conditions, by addition of the Cl salt without removal of a corresponding amount of NaCl. The entry of KCl or RbCl into the cell (Fig. 3) therefore might be expected to change the ratio X_i/X_o. Particularly in the case of $X_o = K_o$, entry of KCl would be expected to reduce the slope of the E_M—log K_o relation to about 46 mv/decade. In fact, however, the relation

TABLE I

EFFECTS OF WATER MOVEMENT ON MEMBRANE POTENTIAL

	Conditions			No.		Potential: Observed		Potential: Calculated	
(1) Experiment series	(2) P_1/P_2	(3) mM K_o	(4) Exposure	(5) Cells	(6) Legs	(7) Mean	(8) Range	(9) Mean	(10) Difference
			hr			*mv*	*mv*	*mv*	*mv*
1	1	15	1	20	2	70	67–74	—	—
2	2	15	1	20	2	53	46–60	52	+1
3	0.65	15	1	20	2	71	68–74	81	−10
4	2	30	1	10	1	34	25–37	35	−1
5	1	45	1	20	2	42	37–49	41	+1
6	0.65	45	1	20	2	43	38–45	53	−10
7	1	15	20	10	1	71	65–80	—	—
8	0.65	15	20	10	1	69	68–71	81	−12

Muscles were exposed to control saline ($P_1/P_2 = 1$), to saline made hyposmotic by removal of NaCl ($P_1/P_2 = 2$), or hyperosmotic, by addition of NaCl ($P_1/P_2 = 0.65$). Number of cells examined is given in column 5. The measured potentials (mean and range) are shown in columns 7 and 8. Column 9 shows the potential expected for an initial concentration of $K_i = 240$ mM, on the basis of the volume changes observed in Figs. 1 and 2. Column 10 shows the difference between columns 7 and 9.

was surprisingly close to 58 mv/decade (Figs. 5 and 11), as was also observed by Werman and Grundfest (34) and by Dunham and Gainer.[1]

Two series of experiments were done to examine this matter further. Fig. 15 shows an experiment in which a single fiber was twice subjected to a medium made hyperosmotic by addition of NaCl ($P_1/P_2 = 0.65$) and then to a hyposmotic medium ($P_1/P_2 = 2.0$). During the hyperosmotic challenges the fiber shrank ($V_2/V_1 = 0.78$), but the membrane potential was unchanged from the control level (−70 mv). The hyposmotic challenge, which caused a swelling ($V_2/V_1 = 1.9$), also caused a depolarization of about 13 mv. About 16 mv depolarization is the calculated value, based upon the dilution of K_i through the entry of water (1, 29). In the experiment of Fig. 4, 17 mv depolarization was observed under closely similar conditions.

A larger series of experiments was carried out measuring only the membrane potentials of superficial fibers of muscles that were exposed to the same osmotic challenges. The concentration of K in the medium was altered from 15 to 30 mM or 45 mM in different experiments (Table I, lines 1 to 6 inclusive). In one preparation the hyperosmotic challenge was maintained for 20 hr (line 8). Fibers of the preparations that were exposed to the hyposmotic medium (lines 2 and 4) showed average membrane potentials of −53 mv (15 mM K_o) and 34 mv (30 mM K_o), while the calculated values were 52 and 35 mv, respectively. Fibers that were challenged with hyperosmotic media (lines 3, 6, and 8) did not show the theoretically expected hyperpolarizations, not even when the fibers were initially depolarized to about −42 mv by the presence of 45 mM K_o (lines 5 and 6).

These findings are at variance with the results of similar studies on frog muscle fibers (1, 21), in which the membrane potential appears to depend primarily on the ratio of internal and external activities of K. In crayfish muscle the response to a hyperosmotic challenge depends upon the level of K in the bathing medium (Reuben, Girardier, and Grundfest, unpublished data). When the fibers are bathed in 20 mM K shrinkage is accompanied by hyperpolarization that approximates rather closely the theoretically expected values (29). When the bathing medium contains only 5 mM K the hyperosmotic challenge induces depolarization. Presumably, there would be no change of the potential at some intermediate level of K, but this has not been examined further, as yet. The lobster fibers, however, did not hyperpolarize even in the presence of 45 mM K_o (Table I, lines 5 and 6). Long equilibration of crayfish muscle fibers in the challenging media in the presence of 20 mM K causes the membrane potential to approach the expected values very closely (29, Fig. 7). In the lobster the maintenance of a hyperosmotic challenge for 20 hr did not bring the membrane potential any nearer to the theoretical level (Table I, lines 7 and 8).

In their insensitivity to an elevation of internal K lobster muscle fibers appear to resemble squid axons (2, 18). With K_o at 10 mM the increase of K_i from 150–600 mM in the perfused axons caused an increase in membrane potential of only 5–10 mv (2), while a change of 35 mv is expected for an ideal K electrode. It was suggested (2) that the deviation arises because the K permeability of the squid axon falls as the membrane potential approaches −60 mv. This explanation is not applicable to the lobster muscle fiber. In the standard bathing medium the membrane resistance of these fibers is not increased appreciably by hyperpolarization with intracellularly applied currents until the membrane potential exceeds about 150 mv (10, 19, 26, 31; cf. also Fig. 14). For larger hyperpolarizations the resistance increases drastically, causing the regenerative hyperpolarizing inactivation response (31). It may be argued, in fact, that the depolarizations induced by hypos-

motic challenges (Figs. 4 and 15) are related to an improvement of the K electrode characteristic of the membrane that probably accompanies the marked increase in conductance which occurs when the fibers are depolarized (Fig. 14).

DISCUSSION

We cannot at the present time offer an adequate explanation of the foregoing "anomalous" observations and measurements. Assuming that osmometric data are the more direct measure of permeability, it is obvious that conclusions as to permeability that are based on electrophysiological data can be far off the mark. Part of the difficulty may reside in a deficiency in the theoretical treatment of diffusional emf's in a heterogeneous system (7). At rest a heterogeneous membrane may behave as a mixed or multielectrode system, but can become a good electrode for single ions (3, 13, 14, 17). However, even when the lobster muscle fiber becomes an electrode for Cs (Figs. 6, 11, and 12) it is still effectively impermeable to this cation (Figs. 3 and 4). A second discrepancy that has been observed is in the behavior of Li. At low concentrations this cation can influence the membrane potential (Fig. 11), but increasing the concentration above 15 mM does not have any further effect.

Removal of all or most of the Cl from the bathing medium speeded up the depolarizations that were evoked by challenges with K or Rb (Figs. 9 and 10). Similar findings in frog (21) and crayfish (29) have been interpreted as indicating that Cl is normally redistributed across the membrane along with the cation, the transient change in emf of the Cl battery opposing the change in emf of the cation (K) battery. The time course of depolarizations induced by increasing Cs was slowed, however, when Cl was removed from the bathing medium (Figs. 9 and 10). This difference may provide some clues as to the anomalous properties of Cs, but the data presently available are inadequate for further analysis of the findings.

The data of Figs. 5, 6, 11, and 12 reflect the two extremes of the effects of time variance in the E_M—log X_o relation. Brief exposure of the cell to low concentrations of NH_4 or Cs does not affect E_M and the curve is essentially flat. At some concentrations, however, there occurs a change in the electrode properties which is perhaps related to some cumulative effect of the depolarization. This is evidenced particularly when the cation is Cs (Figs. 6 and 12). When the fiber depolarized to about −80 mv there appeared a tendency for the membrane potential to "catch up" with the larger depolarizations that are evoked by long exposures to the cation. Thus, the slope of the E_M—log X_o relation is greater than the theoretical value. The effect is particularly marked in low Ca media (Fig. 12). Similar observations have been made for the E_M—log K_o relation in muscle fibers of the crayfish *Orconectes* (cf. 16,

Fig. 8). In this case E_M changes very little until K_o exceeds three or four times the normal concentration, when the slope of the relation becomes 100 mv/decade K_o.

The data with respect to NH_4 (Figs. 5 and 11) indicate that a regenerative increase in membrane permeability is not the sole factor. For brief exposures to NH_4 (Fig. 5) E_M remains strongly inside negative until the concentration of NH_4 is raised above about 75 mM, but with higher concentrations the slope of the relation is nearly 58 mv/decade. However, even after exposures to 15 mM NH_4 for 24 hr, E_M remains strongly negative, while depolarization with increase of NH_4 also develops at a rate of 58 mv/decade (Fig. 11).

Li provides still another variation of the E_M—log X_o relation (Fig. 11). While small additions of this cation change E_M almost in the same way as do additions of comparable amounts of K, Rb, or Cs, E_M no longer changes when the concentration of Li exceeds 15 mM. We are unaware of comparable experiments on other cell membranes. Such measurements might give information as to whether or not the "cut off" value of 15 mM is related to the level of K that is in the standard saline which was the original equilibration medium for the lobster muscle fibers.

The cell membrane includes not only what appear to be permselective channels for different ions, but also a bulk (lamellar) phase, the surface of which is probably very much larger than that of the channels (15, 17). Thus, relative differences even in a relatively low permeability for different ions through this bulk phase might contribute significantly to determining the membrane potential. The role of the large surface, which may be the site of the leak resistance that was postulated by Hodgkin and Huxley (22), has been discussed in connection with data on pharmacological K inactivation by Cs and Rb in eel electroplaques (25). Low concentrations (5–25 mM) of Cs or Rb completely shut off the electrogenically reactive K channels of the electroplaques. The cells, nevertheless, depolarize when the concentrations of these cations are increased further (25). However, no osmometric or chemical data are as yet available with respect to the permeability of the electroplaques to these cations.

Some of the data presented above undoubtedly could be accounted for by a formal analogy with the analyses of several types of model charged membranes. In general, these are described by combining interfacial forces which induce phase boundary potentials with a diffusional regime in a homogeneous charged membrane (6, 24, 33). The data presented above on the membrane of lobster muscle fibers, however, exhibit three varieties of complexity. Foremost is the discrepancy between osmometric and electrophysiological indices of the permeability of the membrane to Cs. Furthermore, the electrophysiological effects of Cs exhibit an extremely prolonged time variance. Finally, the electrophysiological effects of Cs, NH_4, and Li differ markedly one from

the other. It seems likely, therefore, that several additional parameters must be inherent in the processes which determine the E_M—log X_o relations of the cell membrane.

Additional parameters also appear to be necessary to account for different varieties of electrophysiological data (Fig. 14). The membrane potential was about −100 mv and the effective resistance was higher in fibers that were equilibrated in 1 mM K_o as compared with 15 mM K, where the potential was about −75 mv. Fibers that were exposed to Cs for 1 hr also had high resting potentials and high resistance. Only the membrane potential changed to the lower value when the fibers were equilibrated for 20 hr with 15 mM Cs. Likewise, in the presence of 15 mM Rb or Li the membrane potential was about −75 mv, but the effective resistance was about as high as in 1 mM K.

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