# Mechanisms of Anion and Cation Permeations in the Resting Membrane of a Barnacle Muscle Fiber

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ABSTRACT The resting membrane of a barnacle muscle fiber is mostly permeable to cations in a solution of pH 7.7 whereas it becomes primarily permeable to anions if the pH is below 4.0. Mechanisms of ion permeation for various monovalent cations and anions were investigated at pH 7.7 and 3.9, respectively. Permeability ratios were obtained from the relationship between the membrane potential and the concentration of the test ions, and ionic conductances from current-voltage relations of the membrane. The permeability sequence for anions ($SCN > I > NO_3 > Br > ClO_3 > Cl > BrO_3 > IO_3$) was different from the conductance sequence for anions (Br, $Cl > ClO_3$, $NO_3 > SCN$). In contrast, the permeability and conductance sequences were identical for cations ($K > Rb > Cs > Na > Li$). The results suggest that anion permeation is governed by membrane charges while cation permeation is via some electrically neutral mechanism.

## INTRODUCTION

The resting membrane of the giant fiber of a barnacle is permeable mainly to K ions in solutions of pH 7.7; the Cl permeability is negligible. The Cl permeability undergoes a drastic increase if the pH of the solution is reduced below 4.0, while the K permeability decreases with decreasing pH. Thus, in acid solutions, the Cl permeability becomes much greater than the K permeability and the membrane permeability is predominantly determined by anions (Hagiwara et al., 1968). It is, therefore, possible to separate the anion permeability characteristics from the cation permeability characteristics by studying the preparation in acid and mildly basic solutions.

Various mechanisms have been proposed to explain ion permeation through biological membranes (Eisenman, 1968). When the membrane is predominantly permeable to either cations or anions, the solutions of a number of different membrane models predict a Goldman, Hodgkin, and Katz type

relationship between the membrane potential, $V$, at zero membrane current and the concentrations of ions in the solutions.

$$V = \frac{RT}{F} \ln \frac{[C_1^+]_o + \frac{P_{C_2}}{P_{C_1}}[C_2^+]_o + \frac{P_{C_3}}{P_{C_1}}[C_3^+]_o + }{[C_1^+]_i + \frac{P_{C_2}}{P_{C_1}}[C_2^+]_i + \frac{P_{C_3}}{P_{C_1}}[C_3^+]_i + } \tag{1}$$

Here $[C_1^+]$, $[C_2^+]$ . . . . are the concentrations of cations in the solutions; $P_{C_2}:P_{C_1}$ . . . . are ionic permeability ratios; the subscripts $o$ and $i$ denote the outside and the inside of the cell, respectively. The permeability ratio, $P_{C_2}:P_{C_1}$, is given by the product of the mobility ratio of the ions within the membrane and the ratio of their partition coefficients between the membrane and the solution.

The permeability ratio, however, is related to the membrane conductance in different ways depending on the mechanism of ion permeation. If $G_{C_1}$ (or $G_{C_2}$) represents the membrane conductance at zero membrane current when the membrane is interposed between identical solutions of $C_1^+$ (or $C_2^+$) at a concentration of $[C_1^+]$ (or $[C_2^+]$), $G_{C_1}:G_{C_2}$ is equal to the mobility ratio of $C_1^+$ and $C_2^+$ within the membrane and independent of either $[C_1^+]$ or $[C_2^+]$ in charged membranes (Conti and Eisenman, 1965, 1966). In other words, the conductance ratio, $G_{C_2}:G_{C_1}$, could be very different from $P_{C_2}:P_{C_1}$. In fact, Eisenman has shown that in a certain fixed charge membrane $P_K \gg P_{Na}$ but $G_K \ll G_{Na}$ (Eisenman, 1965). For the neutral membrane, however,

$$\frac{G_{C_2}}{G_{C_1}} = \frac{P_{C_2}[C_2^+]}{P_{C_1}[C_1^+]}. \tag{2}$$

When $[C_2^+] = [C_1^+]$, the conductance ratio and permeability ratio become identical (Hodgkin and Horowicz, 1959; Eisenman, Ciani, and Szabo, 1968). This suggests that different mechanisms of ion permeation can be distinguished by comparing the permeability ratio and the conductance ratio.

In the present work the mechanisms of ion permeation in the resting membrane of a barnacle muscle fiber were studied for various monovalent cations ($Li^+$, $Na^+$, $K^+$, $Rb^+$, and $Cs^+$) as well as for anions ($Cl^-$, $Br^-$, $I^-$, $NO_3^-$, $ClO_3^-$, $SCN^-$ . . .). Equation (1) suggests that the permeability ratio, $P_{C_2}:P_{C_1}$, can be obtained by observing bi-ionic potentials. If we suppose that the external solution contains only a salt of $C_1^+$ and that a change in the membrane potential, $\Delta V$, is observed when $C_1^+$ is replaced with an equivalent amount of $C_2^+$, then

$$\Delta V = \frac{RT}{F} \ln \frac{P_{C_2}}{P_{C_1}}. \tag{3}$$

If the counterion permeability is not negligible, this type of experiment gives only the permeability sequence but not actual values of permeability ratios. The actual values were then obtained from changes in the membrane potential with alteration of the ion concentration. Even when the membrane is permeable to counterions, the membrane potential should still be a function of only $([C_1^+]_o + P_{c_2}/P_{c_1}[C_2^+]_o)$ if $[C_1^+]_o$ and $[C_2^+]_o$ are altered by replacement with equivalent amounts of impermeant ions and if the concentrations of other ion species, including those of the internal ions, are kept constant. When a change in the membrane potential from $V_1$ to $V_2$ occurs with the alteration of $[C_1^+]_o$ by $\Delta[C_1^+]_o$ ($[C_2^+]_o$ kept unaltered) as well as with the alteration of $[C_2^+]_o$ by $\Delta[C_2^+]_o$ ($[C_1^+]_o$ kept unaltered)

$$\frac{\Delta[C_2^+]_o}{\Delta[C_1^+]_o} = \frac{P_{c_1}}{P_{c_2}}. \tag{4}$$

Current-voltage relations of the membrane were observed at various external ionic compositions and $G_c$'s were estimated from those results. The experimental results show that the permeability ratios differ markedly from the conductance ratios for anions whereas they are nearly identical for cations. This suggests that anion permeation occurs via a charged system such as mobile or fixed positive sites in the membrane and that cation permeation is via an electrically neutral system: carriers or pores. A preliminary note concerning this work has been published (Hagiwara, Hayashi, and Toyama, 1969).

## MATERIALS AND METHODS

*Material* Giant muscle fibers (0.5–2.0 mm in diameter) of a barnacle, *Balanus nubilus* Darwin, were used. Specimens were obtained from the coast of California.

*Preparation and Recording* The preparation and recording technique were similar to those described in a previous paper (Hagiwara et al., 1968). Changes in membrane potential were recorded as a potential difference between a longitudinal internal glass pipette, the tip of which was located inside the fiber close to the tendinous end, and a 3 M KCl- or 8 M CsCl-filled glass microelectrode placed just outside the fiber. The longitudinal pipette was usually filled with isotonic K citrate. When internal injection was done, the injection pipette served as the internal electrode. The ground electrode in the external saline was a glass pipette of about 1 mm tip diameter filled with the saline most frequently used in each experiment. The absolute value of the membrane potential was checked frequently by introducing the 3 M KCl- or 8 M CsCl-filled micropipette into the fiber. The resting potential of a fiber immersed in the normal barnacle saline at pH 7.7 was −70 to −75 mv when observed with transmembrane micropipettes. The introduction of a longitudinal internal pipette shifted the potential in the positive direction by about 10 mv. Hoyle and Smyth (1963) have described numerous invaginations in the surface membrane of a barnacle muscle fiber.

The insertion of a longitudinal electrode may destroy some of the membrane in these invaginations, resulting in a reduced amplitude of the resting potential. The resting potential observed with a transmembrane micropipette was not altered significantly when the pH of the normal saline was reduced from 7.7 to 3.9. No appreciable reduction of the amplitude of the resting potential was found when a longitudinal electrode was inserted in the low pH normal saline (pH 3.9). This is probably due to a high membrane conductance at low pH (Hagiwara et al., 1968). In the present experiments longitudinal electrodes were used mainly to observe the membrane potential continuously during rapid exchange of the external solution. In a few experiments similar recordings were attempted with transmembrane micropipettes. The tip of the electrode very often came out of the fiber and it was difficult to obtain successful recordings, especially when the effects of a number of different solutions were examined in the same fiber. The volume of the solution surrounding the fiber was about 1 ml. The first 20 ml of a test solution were applied with a flow rate of about 1 ml/sec allowing the complete exchange of saline outside the fiber within a few seconds. The flow rate was then reduced. Potential changes were recorded on a chart recorder.

The current-voltage relation of the fiber was measured by using a double wire electrode. The electrode consisted of two silver wires cemented together along their longitudinal axes. One wire, used for current injection, had a diameter of 200 $\mu$ and was insulated except for a final stretch of 1.5 cm, which was platinized. Current pulses were applied between this electrode and a large chlorided silver plate in the saline. The other wire, used for potential measurement, was 60 $\mu$ in diameter and was insulated except for a stretch of 2 mm centered at the platinized region of the first wire. This bare region of the fine wire was chlorided. The external saline was kept at the ground potential level during polarization as well as during alteration of the external saline by using a feedback circuit (Fig. 1). The circuit was arranged to maintain the potential at the tip of the external KCl- or CsCl-filled micropipette at the ground potential. The intensity of the applied current was observed as an IR drop across a resistor (20 K$\Omega$) inserted between the output of the pulse generator and the internal current electrode.

*Saline Solutions* The composition (mM) of the normal barnacle saline was NaCl, 466; KCl, 8; $CaCl_2$, 20; $MgCl_2$, 12; and Tris-HCl buffer (pH 7.7), 10. The composition of the low pH normal saline was the same as that of the normal saline except that the pH was adjusted to 3.9 with 10 mM of Na–hydrogen phthalate and an appropriate amount of HCl. Compositions of the modified external solutions are shown in Tables I and II. Ca salts were eliminated when sulfate was used as an impermeant substitute for chloride. In order to prevent the deterioration of the membrane in the absence of ionized Ca, a large amount of $Mg^{++}$ (80–100 mM at final concentration) was added to these solutions. Comparison of the results obtained in normal Mg and Ca media and in 100 mM Mg and zero Ca media indicates that this does not alter monovalent ion permeability significantly.

In most of the present experiments the junction potential between the external solution and 3 M KCl or 8 M CsCl micropipettes was ignored since changes in the junction potential with the change of the external solution seems to be much smaller than those in the membrane potential. In anion permeability experiments, however,

observed membrane potential changes were often small and, therefore, it was necessary to examine this point. Potential differences ($E$'s) between a glass electrode filled with Cl solution A (or C) and that filled with 3 M KCl were measured in various other anion solutions A (or C). $E$ in Cl solution A (or C) was taken as zero. If differences among junction potentials between 3 M KCl and those test solutions are negligible, $E$ should be equal to the junction potential between the Cl solution and the test solution, $E_{Cl-X}$. $E_{Cl-X}$ in Table III was calculated by using the Henderson-Planck equation

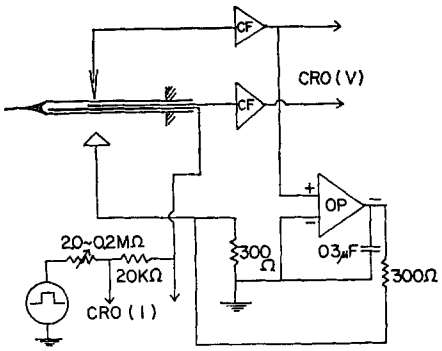


FIGURE 1. General arrangement of experiment. See text.

TABLE I

COMPOSITIONS OF EXTERNAL SOLUTIONS FOR ANION EXPERIMENTS

| Amion solution | Na$X$ | $NaSO_4$ | K$X$ | $K_2SO_4$ | $Ca(MS)_2$ | $Mg(MS)_2$ | $MgSO_4$ | TOH | T. P. | Sucrose | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM | mM | mM | mM | mM | mM | mM | mM | mM | mM | mM |
| A | 466 | — | 8 | — | 20 | 12 | — | — | 10 | — | 3.9 |
| B | 466 | — | 8 | — | 20 | 12 | — | 10 | — | — | 7.7 |
| C | 300 | — | 150 | — | — | — | 80 | — | 20 | — | 3.9 |
| D | — | 150 | — | 75 | — | — | 80 | — | 20 | 225 | 3.9 |
| E | — | — | 300 | 75 | — | — | 80 | — | — | 75 | 3.9 |

$X$ = Cl, Br, $ClO_3$, I, $NO_3$, and SCN for A-C and Cl, $BrO_3$, $IO_3$, methanesulfonate, and $p$-toluenesulfonate for E. MS = methanesulfonate. T.P. = Tris-hydrogen phthalate. TOH = Trizma base. pH in A-D was adjusted by adding an appropriate amount of methanesulfonic acid. pH of solution E was adjusted with 20 mM of K hydrogen phthalate and an appropriate amount of $H_2SO_4$.

(MacInnes, 1961, equation 21, p. 232). Observed $E$'s are very close to the calculated $E_{Cl-X}$. This indicates that any possible error which might be introduced by the above assumption should be within 0.5 mv. Some of the micropipettes show tip potentials and may not behave as simple KCl or CsCl electrodes. In order to avoid these the ground electrode was filled with Cl saline A (or C) and the change in the potential difference between the ground electrode and the micropipette was frequently monitored when the change in the membrane potential during the alteration of the external solution was observed. Usually the former agreed with those observed with large 3 M KCl electrodes. If the deviation was greater than 1 mv, micropipettes were discarded.

For the analyses of anion permeability and conductivity at low pH, internal injection was done to raise the internal Cl concentration. The composition (mM) of the high Cl-injected solution was Tris (hydroxymethyl)aminomethane hydroxide, 604; HCl, 450; phthalic acid, 100; and the pH was adjusted to 4.7 by adding a small amount of

TABLE II

COMPOSITIONS OF EXTERNAL SOLUTIONS FOR CATION EXPERIMENTS

| Cation solution | $X_2SO_4$ | $K_2SO_4$ | $H_2SO_4$ | KOH | $MgSO_4$ | TOH | T.M. | Phth. | Glycine | Sucrose | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM | mM | mM | mM | mM | mM | mM | mM | mM | mM | |
| A | 237 | — | — | — | 80 | 10 | — | — | — | 169 | 7.7 |
| B | — | — | 237 | — | 80 | 617 | — | — | — | 46 | 7.7 |
| C | 237 | — | — | — | 80 | 10 | — | 10 | — | 169 | 5.0 |
| D | — | — | 237 | — | 80 | 484 | — | 10 | — | 169 | 5.0 |
| E | — | 225 | — | 15 | 100 | — | — | — | 50 | 150 | 9.0 |
| F | — | — | — | 12 | 100 | — | — | — | 50 | 764 | 9.0 |
| G | 225 | — | 45 | — | 80 | 142 | 30 | — | — | — | 7.7 |
| H | — | — | 233 | — | 80 | 623 | 30 | — | — | — | 7.7 |

$X$ = K, Rb, Cs, Na, and Li. TOH = Trizma base. T.M. = Tris-maleate. Phth. = phthalic acid. pH was adjusted by adding an appropriate amount of $H_2SO_4$ for A and B, TOH for C, D, G, and H, and KOH for E and F.

TABLE III

JUNCTION POTENTIALS

| $X$ | Anion solutions A | | Anion solutions C | |
|---|---|---|---|---|
| | $E_{Cl-X}$, calculated | $E_{observed}$ | $E_{Cl-X}$, calculated | $E_{observed}$ |
| | mv | mv | mv | mv |
| Cl | 0 | 0 | 0 | 0 |
| Br | +0.56 | +0.5 | +0.50 | +0.5 |
| I | +0.55 | +0.5 | +0.50 | +0.5 |
| $ClO_3$ | −2.46 | −2.5 | −2.25 | −2.5 |
| $NO_3$ | −1.51 | −2.0 | −1.36 | −2.0 |
| SCN | −1.48 | −2.0 | −1.47 | −2.0 |

$E_{Cl-X}$ was calculated by the Henderson-Planck equation with the use of ion activities instead of concentrations. The activity of a given salt in the solution was estimated from the table at a concentration which gives the ionic strength as the same as that of the solution. Cl or SCN solution A indicates that $X$ in anion solution A is Cl or SCN. Positive sign indicates that the 3 M KCl electrode becomes more positive.

$H_2SO_4$. A small amount of phenol red was added to the solution to monitor the internal pH. The injection was made over the entire length of the fiber until the diameter became 1.5 to 2.0 times the original diameter. On a few occasions, fibers were injected with high $K^+$ internal solution for studying cation permeation. Its composition (mM) was KOH, 450; $H_2SO_4$, 225; and Tris, 190; Tris-maleate buffer (pH 7.7), 150; and sucrose, 40. Isolated muscle fibers were often immersed in a high K solution

(NaCl in the normal saline had been replaced with KCl) prior to the experiment. This resulted in a contraction which lasted approximately 5–10 min. Fibers were used for recording after complete relaxation. This procedure prevented any vigorous shortening either during the injection or the application of some of the external test solutions.

All experiments were carried out at room temperature (22°–23°C).

## RESULTS

### I. *Anion Permeability*

#### PERMEABILITY SEQUENCE AT LOW pH (3.9)

The resting potential of the fiber in the low pH normal saline was −70 to −75 mv. In order to study the permeability sequence among different anion species, changes in the membrane potential were observed following the replacement of external Cl ions with various test anions. Salts of relatively impermeable anions such as methanesulfate ions (anion solution A) were substituted for calcium and magnesium salts of Cl in the low pH normal saline. This substitution itself did not alter the resting potential. The remaining Cl in the solution was in the form of either Na or K salt. These Cl ions were then replaced with either $NO_3$, I, Br, $ClO_3$, or SCN ions (anion solution A). In all cases the replacement resulted in a negative shift of the membrane potential. The change became maximal within a half-minute and then the potential slowly returned toward the original Cl potential level. The amplitude of the potential change, $\Delta V$, from the original Cl potential level was measured for each test anion at its maximum. The results obtained with five different fibers are summarized in Table IV A. Although $\Delta V$ for a given ion species varies from fiber to fiber, the sequence of $\Delta V$ for different anion species is identical among different fibers. Upon returning to the Cl solution from the test solution, the membrane potential temporarily became more positive than the original Cl potential level and this was, as a rule, followed by a slow return to the original level. Fig. 2-I shows a record obtained with the same fiber when potential changes for $ClO_3$, Br, and SCN were compared with that for $NO_3$. The foregoing result suggests that the permeability ratio, $P_A:P_{Cl}$, for anion species $A^-$ has the following sequence:

$$SCN > I > NO_3 > Br > ClO_3 > Cl.$$

If the membrane is permeable exclusively to monovalent anions, actual values of permeability ratios can immediately be obtained from $\Delta V$ by using the equation for anions corresponding to equation (3) for cations. If this is the case, the membrane potential should change with a slope of 58 mv for a 10-fold change in Cl concentration when the external Cl is replaced with impermeant anions. As will be shown below, the rate of change is slightly

but significantly smaller than that expected in a Cl electrode. Therefore, the actual values of permeability ratios were obtained through the equation for anions corresponding to equation (4) for cations by observing relationships between the membrane potential and the concentration of various test anions. In order to obtain relationships accurately, the experimental condition was slightly modified. As already described, the change in the membrane potential for the replacement of Cl with test anion becomes maximal within a short time after the replacement and then slowly declines; i.e., it shows a

TABLE IV

ΔV'S FOR DIFFERENT ANION SPECIES

| | A | | | | | |
|---|---|---|---|---|---|---|
| | Resting potential in Cl solution | Br | $ClO_3$ | $NO_3$ | SCN | I |
| | *mv* | *mv* | *mv* | *mv* | *mv* | *mv* |
| Fiber 1 | −70 | −12 | −6 | −17 | −37 | |
| 2 | −68 | −9 | −3 | −13 | −31 | |
| 3 | −75 | −12 | −5 | −16 | −34 | |
| 4 | −74 | −8 | −4 | −12 | −31 | −21 |
| 5 | −71 | −7 | −3 | −11 | −28 | −16 |
| | B | | | | | |
| Fiber 1 | −9 | −7 | −5 | −9 | −35 | |
| 2 | −6 | −7 | −4 | −9 | | |
| 3 | −10 | −8 | −5 | −10 | −35 | |
| 4 | −12 | −10 | −3 | −14 | −27 | −19 |
| 5 | −18 | −11 | −5 | −14 | −28 | −22 |
| | C | | | | | |
| Fiber 1 | −60 | −1.5 | +5 | −2 | −13 | |
| 2 | −65 | −2.5 | +4 | −8 | −27 | −15 |
| 3 | −60 | −1 | +2.5 | −6 | −17 | −12 |

transient peak. This seems to be due to the exchange between the internal Cl and the external test anions while the fiber is in the test solution. Since the normal internal Cl concentration of the fiber is low (about 30 mM [HAGIWARA, Chichibu, and Naka, 1964; Gayton and Hinke, 1968]) even a small amount of exchange may result in a significant change in the membrane potential. In order to minimize this effect, the internal Cl concentration was artificially increased by intracellular injection. Since the Cl concentration of the injected solution was 400 mM and since the injection was continued until the fiber diameter became 1.5–2.0 times the original diameter, the Cl concentration should have been raised by a factor of 10. After this treatment, the

transient peak of potential change almost disappeared (compare Fig. 2-I with II and III). Another advantage of working with injected fibers is that the external test anion concentration can be reduced without producing contraction. The membrane potential of an uninjected fiber was usually more negative than −70 mv in the present anion solutions. If the concentration of the external test anion was reduced under this condition, the depolarization often caused a vigorous contraction which disturbed the potential measurement. When the internal Cl concentration was raised, the initial resting potential became close to zero. This might initially produce a con-

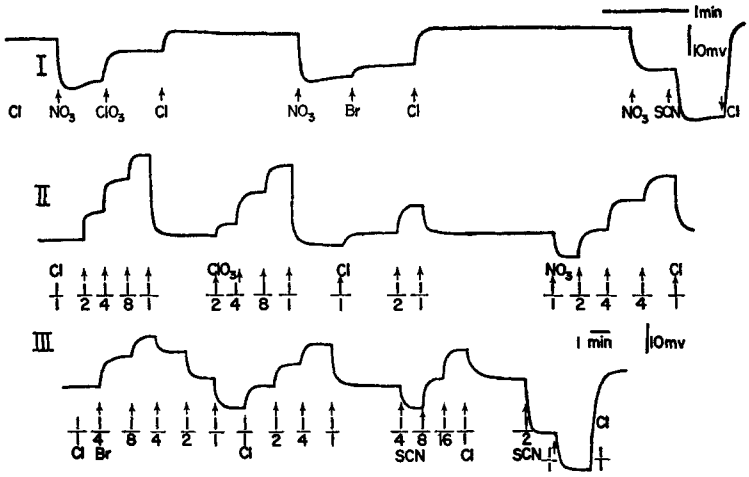


FIGURE 2. Membrane potential changes in a barnacle muscle fiber for different anion species at pH 3.9. I, normal uninjected fiber. The 474 mM Cl in anion solution A is first replaced with $NO_3$ and then $ClO_3$, Br, and SCN, respectively. The resting potential in Cl solution was −70 mv. II and III, the fiber had been injected with the Cl-rich solution. The concentration (450 mM) of the test anion species in anion solution C was reduced to ½, ¼, ⅛, or 1/16 with anion solution D. The resting potential in 450 mM Cl was −5 mv. The upward direction of the trace corresponds to the positivity of the internal potential.

traction. After the complete relaxation was obtained, however, additional shift of the membrane potential in the positive direction did not produce contraction.

The relationship between membrane potential and the concentration was then examined for each test anion species. Sulfate ions were used as substitutes for test anions and, therefore, anion solutions C and D were used. Each test solution contained 150 mM of K salt and 300 mM of Na salt of the test anion species. When the injected fiber was immersed in anion solution C ($X$ = Cl), the resting potential was −5 to −20 mv. Records II and III of Fig. 2 were obtained in a typical experiment. The Cl concentration was first reduced from 450 to 225, 112, and 56 mM and then returned to 450 mM. The

membrane potential shifted in the positive direction with an approximately exponential time course, reaching a final level for a given concentration within 1–2 min. In this experiment, a similar measurement was performed successively with $ClO_3$, $NO_3$, Br, and SCN. Occasionally potential changes for given decreases in the Cl concentration were observed to see whether any systematic change had occurred in the fiber membrane during a prolonged measurement. The membrane potential at the final steady level in each test solution was measured from the membrane potential at $[Cl]_o$ = 450 mM prior to the application of each test solution. The latter sometimes showed a gradual change during a prolonged experiment, probably due to a change in the internal ion concentrations. In spite of this gradual change of the membrane potential at 450 mM Cl, the amplitude of potential change for any given decrease of the Cl concentration was unaltered. If any change occurred in the latter, the measurement was discontinued. Fig. 3-I shows the relations between the potential change and the logarithm of concentrations obtained for Cl, $ClO_3$, Br, $NO_3$, and SCN with the same muscle fiber at pH 3.9. Permeability constant ratios were obtained from these sets of relationships, as described below.

In the present experiment, the concentrations of the external Na and K are kept constant. The permeability of sulfate ions is neglected and the internal ionic composition is assumed to be unaltered for a few minutes during the application of each test solution. Therefore, the permeability ratio, $P_A:P_{Cl}$, can be obtained from an equation similar to equation (4). If the membrane potential found in the solution of test anion species, $A^-$, at a concentration, $[A^-]_o$, is equal to that found in the Cl solution at $[Cl^-]_o$ :

$$\frac{P_A}{P_{Cl}} = \frac{[Cl^-]_o}{[A^-]_o}.$$

Several pairs of $[A^-]_o$ and $[Cl^-]_o$ satisfying this condition were found for each test anion species (Fig. 3-I) and such $[A^-]_o$'s are plotted against $[Cl^-]_o$ in Fig. 3-II. The fact that the plot for each test anion species approximates a straight line which intersects the origin indicates that the permeability constant ratio, $P_A:P_{Cl}$, is constant and independent of the concentration of the test anion concentration. Permeability constant ratios calculated from the slope of the straight line relationship are presented in Table V. In a few cases, a similar observation was made in K-free media; i.e., 150 mM of K salt in the external solution was replaced with Na salt and the result was essentially the same. Average values of permeability ratios were $P_{Cl}(1.0)$: $P_{ClO_3}(1.3):P_{Br}(1.8):P_{NO_3}(2.0):P_{SCN}(4.8)$. In two fibers $P_I:P_{Cl}$ was examined and these were 2.3 and 2.5.

Potential changes for the replacement of 450 mM Cl with an equivalent amount of test anion species obtained with five injected fibers in anion solu-

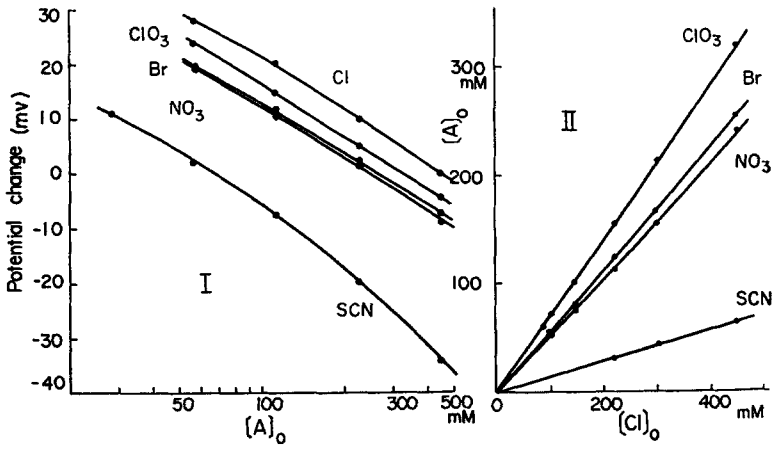


FIGURE 3. I, relationships between the membrane potential and the concentration of test anions at pH 3.9. The membrane potential was measured from that at 450 mM Cl. Anion solutions C and D were used. II, relationships between the concentrations of Cl and test anion species giving equal membrane potentials. They were obtained from the result in Fig. 3-I.

TABLE V

PERMEABILITY AND CONDUCTANCE RATIOS AMONG DIFFERENT ANION SPECIES AT pH 3.9

| | $P_A:P_{Cl}$ | | | |
|---|---|---|---|---|
| | Br | $ClO_3$ | $NO_3$ | SCN |
| Fiber 1 | 1.8 | 1.4 | 2.0 | 5.0 |
| 2 | 1.8 | 1.3 | 2.2 | 4.0 |
| 3 | 1.8 | 1.4 | 1.9 | 6.6 |
| 4 | 1.6 | 1.2 | 1.7 | 4.1 |
| 5 | 1.8 | 1.2 | 2.0 | 4.4 |
| Average | 1.8 | 1.3 | 2.0 | 4.8 |
| ±SD | 0.1 | 0.1 | 0.2 | 1.1 |
| | $\bar{g}_A$ | | | |
| Fiber A | 1.00 | 0.75 | 0.64 | 0.60 |
| B | 1.05 | 0.77 | 0.70 | 0.69 |
| C | 1.00 | 0.82 | 0.74 | 0.62 |
| D | — | 0.82 | — | 0.57 |
| E | — | 0.84 | 0.72 | — |
| Average | 1.02 | 0.80 | 0.70 | 0.62 |
| ±SD | 0.03 | 0.03 | 0.04 | 0.05 |

tions C are summarized in Table IV B. These values are not much different from those obtained with uninjected fibers in anion solution A and at least their sequences are identical. This suggests that the permeability constant ratios are not significantly altered either by the internal injection or by replacing the external Ca with a large amount of Mg.

The permeability sequence for anion species less permeable than Cl was also studied at pH 3.9. The fiber was first immersed in Cl saline E and then the Cl in the saline was replaced with either $BrO_3$, $IO_3$, methanesulfonate (MS), or *p*-toluenesulfonate (PTS). This resulted in a positive shift of the membrane potential. The amplitude of the positive shift had the following order:

$$IO_3 > MS, PTS > BrO_3.$$

This gives the permeability sequence:

$$Cl > BrO_3 > PTS, MS > IO_3.$$

MEMBRANE CONDUCTANCE AT LOW pH (3.9)

Fig. 3-I shows that the slope of the relationship between the change in the membrane potential and the concentration of anion species examined ranges between −35 and −45 mv for a 10-fold increase in the concentration. This suggests that the major fraction of membrane current should be carried by anions if current pulses are applied to the membrane immersed in a solution containing 450 mM of one of these anion species. Constant current pulses of about 100 msec duration were applied through one of the wires of the double-wire electrode, and the potential change was obtained at the time just before the termination of the pulse. Fig. 4 shows current-voltage relations obtained with the same fiber in 450 mM Cl and SCN (anion solution C). The membrane potential at 450 mM Cl was taken as the reference membrane potential. The fiber had been injected with the high Cl solution and the reference membrane potential was −6 mv. The current-voltage relationship in the Cl solution was linear over a relatively wide range of membrane potential (±40 mv). The replacement of Cl with SCN in this fiber resulted in a negative shift of the membrane potential by 31 mv. A linear current-voltage relation was also found in the SCN solution in the range of ± 30 mv from this membrane potential. The slope tended to become larger for membrane potentials more positive than this range and smaller for those more negative than this range. Membrane conductance in the SCN solution calculated from the slope at zero membrane current was significantly smaller than that in the Cl solution.

In order to compare the membrane conductance in solutions of different anion species, inward and outward constant current pulses of a fixed small amplitude were applied to the membrane alternately at 5 sec intervals during replacement of 450 mM Cl (anion solution C, injected fibers) with an

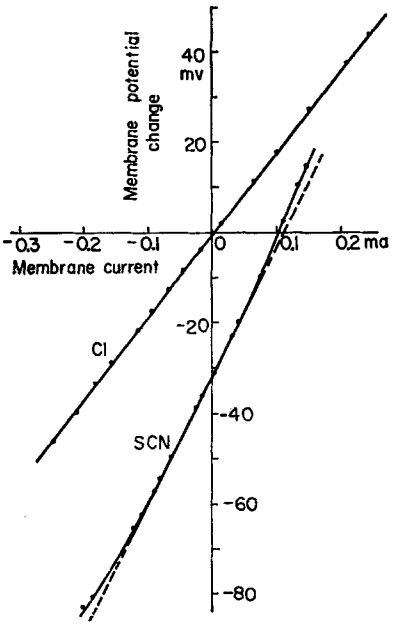


FIGURE 4. Current-voltage relations obtained from the same membrane at pH 3.9 in Cl solution and SCN solution C. The membrane potential was measured from that in Cl solution.

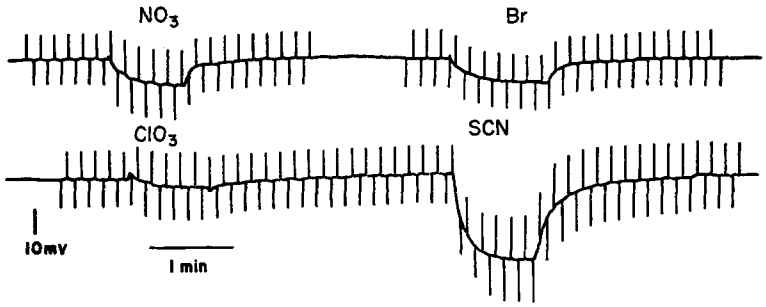


FIGURE 5. Constant current pulses of different polarities were applied alternately to the membrane at pH 3.9 during the replacement of 450 mM Cl with various different test anion species in anion solution C. The duration of the pulse was about 100 msec.

equivalent amount of test anion species (Fig. 5). The membrane conductance at zero membrane current, $g_A$, in the solution of anion species A, was obtained at the steady state of the potential change. The conductance in Cl solution was observed before applying each test solution and after returning to Cl solution from the test solution, and their average value, $g_{Cl}$, was then obtained. Finally, the relative conductance, $\bar{g}_A$, for anion species $A^-$ to $Cl^-$

was calculated as a ratio between $g_A$ and $g_{Cl}$. The results are summarized in Table V which shows that the sequence of $\bar{g}_A$ is:

$$\text{Br, Cl} > \text{ClO}_3, \text{NO}_3 > \text{SCN}^-.$$

Average values of $\bar{g}_A$'s are Br (1.02), Cl (1.00), $ClO_3$ (0.80), $NO_3$ (0.70), and SCN (0.62). This sequence does not coincide with the one obtained for the permeability ratio. For example, $SCN^-$ shows the highest permeability ratio to $Cl^-$ among these four species but the lowest relative conductance. The replacement of $Cl^-$ with $NO_3^-$ resulted in a hyperpolarization and the permeability ratio, $P_{NO3}:P_{Cl}$, was 1.8–2.2. This hyperpolarization was, however, associated with a decrease in membrane conductance. The results indicate, therefore, that high permeability of an anion is associated with low conductivity. The specific membrane resistance of an uninjected fiber immersed in the normal saline at pH 7.7 was about 1–3 KΩ-cm², if invaginations of the membrane were ignored (see Brinley, 1968). The reduction of pH together with internal injection lowers the membrane resistance by a factor of 8–10. This indicates that the resistance of the solution outside the fiber may not be totally negligible with the present technique of measurement. In a few experiments, potential changes for current pulses of a given intensity were observed before and after the rupture of the fiber membrane by forceps or the treatment of the fiber with 50% methyl alcohol in normal saline. More than 90% of the potential change due to a current pulse disappeared.

The discrepancy between the permeability sequence and the conductivity sequence can be explained if the anion permeation through the barnacle muscle fiber membrane at pH 3.9 is via fixed positively charged pores (Conti and Eisenman, 1965; Tasaki, 1968). In such a membrane, the permeability ratio, $P_A:P_{Cl}$, should be given by:

$$\frac{P_A}{P_{Cl}} = \frac{u_A}{u_{Cl}} \cdot K_{Cl}^A$$

$K_{Cl}^A$ represents the ion-exchange equilibrium constant or binding constant of the membrane for $A^-$ and $Cl^-$. When the mobility ratio between anion species $A^-$ and $Cl^-$, $(u_A:u_{Cl})$, within the membrane is assumed to be constant and independent of the concentrations of $A^-$ and $Cl^-$ in the external as well as the internal solutions, $u_A:u_{Cl}$ can be calculated from $\bar{g}_A$ as in a positive fixed-charge membrane (Conti and Eisenman, 1965). When the membrane separates an internal Cl solution from an external solution of ion species $A^-$, the membrane conductance, $g_A$, at zero membrane current is given by (from equation (74) in Conti and Eisenman, 1965):

$$g_A = \frac{F \cdot X \cdot u_{Cl} \cdot u_A \cdot \ln (u_{Cl}/u_A)}{\delta(u_{Cl} - u_A)} \tag{5}$$

where $\delta$ and $X$ are the thickness and the fixed-charge density of the membrane, respectively. When the external anion species is also $Cl^-$,

$$g_{Cl} = \frac{F \cdot X \cdot u_{Cl}}{\delta} \tag{6}$$

therefore, the relative conductance, $\bar{g}_A$, is given by,

$$\bar{g}_A = \frac{g_A}{g_{Cl}} = \frac{\ln\left(\frac{u_{Cl}}{u_A}\right)}{\left(\frac{u_{Cl}}{u_A}\right) - 1} \tag{7}$$

Since the cation conductance is not totally negligible in the present case, equation (7) may not be applicable in a strict sense. However, it suggests the following order of $u_A:u_{Cl}$ among different anion species:

$$Br^-, Cl^- > ClO_3, NO_3 > SCN^-.$$

This, in turn, suggests that the ion-exchange equilibrium constants of the membrane for anion species $A^-$ have the following sequence:

$$SCN^- > NO_3^- > ClO_3^- > Br^- > Cl^-.$$

The results, therefore, indicate that an anion with a higher binding constant tends to have a lower mobility in the membrane, as one would intuitively expect.

In two fibers the membrane conductances at zero membrane current were examined in mixtures of Cl and $NO_3$ solutions at various ratios. The fiber was first immersed in 450 mM Cl solution which was then replaced with a solution containing Cl and $NO_3$. After the membrane potential reached a steady level the fiber was brought back to 450 mM Cl solution. This was done with various ratios of $[Cl]_o$ and $[NO_3]_o$, keeping $[Cl]_o + [NO_3]_o = 450$ mM. The relative conductance ($g:g_{Cl}$) for the test solution to that for 450 mM Cl solution was obtained as before. Fibers had been injected with the Cl-rich solution. The result (Fig. 6) shows that the conductance decreases from the pure Cl medium to the pure $NO_3$ medium monotonically and this is expected in a fixed positively charged membrane if the mobility ratio, $u_{NO3}:u_{Cl}$, is constant and independent of the concentration of either Cl or $NO_3$ (from equation (74) in Conti and Eisenman, 1965).

In one experiment membrane conductances were compared between muscle fibers internally injected with Cl and $NO_3$ solutions. Two homologous muscle bundles were isolated from the same specimen. One was soaked several hours in a KCl solution (the solution was obtained by replacing Na

in the normal saline with K) and the other in a $KNO_3$ solution (this solution was obtained by replacing Cl in the above KCl solution with $NO_3$) at pH 7.7. Muscle fibers from the Cl-soaked bundle were injected with the Cl-rich internal solution (pH 4.7) and the membrane conductance was observed in 450 mM external Cl and $NO_3$ (anion solution C) successively at pH 3.9. A similar experiment was performed with fibers from the $NO_3$-soaked muscle bundle after the injection of $NO_3$ internal solution (the solution was obtained by replacing Cl with $NO_3$ in the Cl-rich internal solution). The ratio between membrane conductances of the Cl-loaded fiber in $NO_3$ and Cl external solutions ranged between 0.62 and 0.65 while that of the $NO_3$-loaded fiber was between 0.72 and 0.77. If it is assumed that the conductance of the membrane separating the internal Cl from the external $NO_3$ is equal to that of the same membrane which separates the internal $NO_3$ from the external Cl, then the

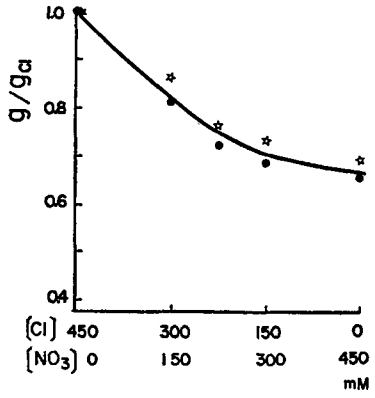


FIGURE 6. The membrane conductance relative to the one obtained at 450 mM Cl in anion solution C at pH 3.9. The concentrations [Cl] and [$NO_3$] were varied but [Cl] + [$NO_3$] was kept at 450 mM Solid circles and stars show results obtained from two different fibers.

membrane conductance found when solutions on both sides of the membrane are Cl should be 2.0–2.2 times that found when both sides of the membrane are exposed to $NO_3$. This suggests that $u_{Cl}:u_{NO_3}$ within the membrane is 2.0–2.2. The value of ratio 0.62-0.65 obtained in Cl-loaded fibers represents $\bar{g}_{NO_3}$ given by equation (7). Calculation by the equation gives 2.2–2.4 for $u_{Cl}:u_{NO_3}$ and this is in good agreement with the value mentioned above. Since $P_{NO_3}:P_{Cl}$ found in these fibers was about 1.8, a $u_{Cl}:u_{NO_3}$ of 2.0–2.4 gives 3.6–4.3 for the ion-exchange equilibrium constant $K_{Cl}^{NO_3}$.

### ANION PERMEABILITY SEQUENCE AT pH 7.7

The anion permeability sequence of the membrane was obtained at pH 7.7 by using a technique similar to that used at pH 3.9, i.e. the Cl salts of Ca and Mg in the normal saline (pH 7.7) were replaced with methanesulfonate salts and membrane potential changes associated with the replacement of the remaining Cl ions with various test anions were then observed (anion solution B). Because of the low permeability of anions relative to that of cations

at pH 7.7, amplitudes of changes were much smaller than those observed at pH 3.9. Potential changes observed with three fibers are summarized in Table IV C. Although the amplitudes are small these changes indicate the following permeability sequence at pH 7.7:

$$SCN > I > NO_3, Br > Cl > ClO_3.$$

There is an inversion of the sequence between $ClO_3$ and Cl for the change of external pH from 3.7 to 7.7.

### II. *Cation Permeability*

#### CATION PERMEABILITY SEQUENCES

It has been shown in previous work (Hagiwara et al., 1964) that the permeability sequence among alkali cations in the barnacle muscle fiber membrane at pH 7.7 is:

$$K > Rb > Cs > Na.$$

In the present experiment, an attempt was made to obtain actual values of permeability ratios through equation (4) by observing relationships between the membrane potential and the concentration of test cations. When a muscle fiber was immersed in K solution A (cation solution A for $X$ = K) which contained 474 mM K at pH 7.7, the resting potential was about +10 mv. In order to reduce the effect of anions the external Cl was removed. The major external anion species was $SO_4$. The trace shown in the lower part of Fig. 7-III was obtained when the K concentration of 474 mM was reduced to 236, 79, and 26 mM by replacement with Tris solution at pH 7.7. The membrane potential changed in the negative direction with decreasing K concentration. The time course of the potential change was relatively slow and a final steady level for a given concentration was reached 5–8 min after the application of the test solution. The membrane potential at the final level was measured at each K concentration from the potential level at $[K]_o$ = 474 mM. Potential changes of the same fiber were observed for other cation species (Rb, Cs, Na, and Li) by changing their concentrations from 474 to 158 and 53 mM and by measuring also from the potential level at $[K]_o$ = 474 mM. Fig. 7-I (upper) shows relationships between the membrane potential and the logarithm of concentration obtained for K, Rb, Cs, Na, and Li. The permeability constant ratios were then obtained from this set of relationships by neglecting the permeability of Tris ions. If the membrane potential in the solution of test cation species C at a concentration $[C]_o$ is equal to that in the K solution at $[K]_o$,

$$P_K \cdot [K]_o = P_C \cdot [C]_o$$

when $X$ and $Y$ represent 474 mM/[C]$_o$ and 474 mM/[K]$_o$, respectively

$$\frac{P_C}{P_K} = \frac{X}{Y}.$$

$Y$'s for $X$ = 1 and 2 were found for Rb, Cs, and Na as shown in Fig. 7-I

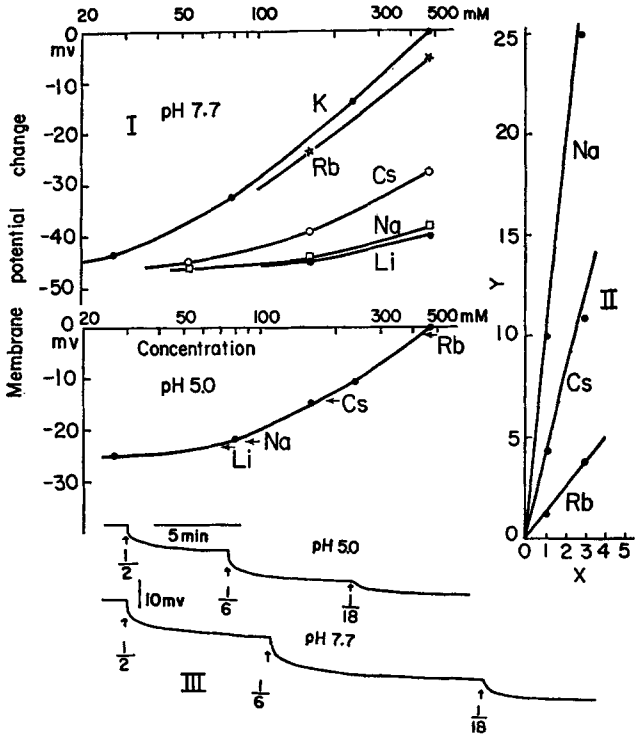


FIGURE 7. I, relationships between the membrane potential and the concentration of the test cation species obtained at pH 7.7 (upper) and 5.0 (lower). Cation solutions A and B at pH 7.7 and C and D at pH 5.0. Membrane potentials were measured from the potential at 474 mM K. II, relationships between $X$ (474 mM/[Cl]) and $Y$ (474 mM/[K]) obtained from the result in I (upper). III, membrane potential changes at pH 5.0 and 7.7 when the K concentration was reduced to ½, ⅙, and 1/18. The downward direction corresponds to the inside negativity.

(upper) and plotted against $X$ in Fig. 7-II. The fact that the extension of the straight line passing the two points for each cation species approximately intersects the origin suggests that the permeability constant ratio is independent of the cation concentration. No plot was made for Li since the data obtained were not significant for concluding that the permeability ratio was independent of the concentration. Permeability constant ratios, ($P_C$:$P_K$), obtained from the slope of the straight line relationship are shown in Table

VI. ($P_{Li}:P_K$ was obtained from the data at $X = 1$). A few experiments were performed after injecting high K internal solution and the results obtained with those fibers were essentially the same as those obtained with untreated fibers.

Potential changes shown in Fig. 7-III (upper) were obtained during the alteration of the K concentration at pH 5.0 (cation solutions C and D). The amplitude of potential change for a given change in the concentration was small at pH 5.0 as compared with that obtained at pH 7.7. This was due to the decrease of the cation permeability relative to the anion permeability at lower pH (Hagiwara et al., 1968). Membrane potentials at different K concentrations were measured from the potential level at $[K]_o = 474$ mM

TABLE VI

PERMEABILITY RATIOS AMONG DIFFERENT CATIONS

| | Rb | Cs | Na | Li |
|---|---|---|---|---|
| pH 7.7 | | | | |
| Fiber 1 | 0.71 | 0.17 | 0.11 | 0.07 |
| 2 | 0.78 | 0.13 | 0.10 | 0.07 |
| 3 | 0.77 | 0.23 | 0.09 | 0.08 |
| 4 | 0.80 | 0.15 | 0.09 | 0.08 |
| 5 | 0.80 | — | — | — |
| 6 | — | 0.27 | — | — |
| Average | 0.77 | 0.19 | 0.096 | 0.075 |
| ±SD | 0.04 | 0.06 | 0.009 | 0.006 |
| pH 5.0 | | | | |
| Fiber A | 0.91 | 0.33 | 0.17 | 0.13 |
| B | 0.91 | 0.37 | 0.15 | 0.11 |

and plotted against log $[K]_o$. Membrane potentials of the same fiber at 474 mM of Rb, Cs, Na, and Li (cation solutions C and D) were also measured from the level at $[K]_o = 474$ mM. Their values are indicated by arrows in Fig. 7-III (lower). Permeability constant ratios, ($P_C:P_K$), were obtained from these values by assuming a constant permeability ratio. The result is shown in Table IV. The results show that the permeability sequence, K > Rb > Cs > Na > Li, is common for pH 7.7 and 5.0. However, differences among permeability ratios of different cations were reduced at a lower pH. In other words, the cation selectivity of the membrane was slightly reduced by lowering the pH of the medium.

### MEMBRANE CONDUCTANCE FOR DIFFERENT CATION SPECIES

In order to compare membrane conductances for different cation species, current-voltage relations of a fiber membrane were obtained when the fiber

was immersed in salines containing 450 mM of K, Rb, Cs, Na, or Li at pH 7.7 (cation solution G). As shown by Fig. 7-I (upper), the slope of the relationship between the membrane potential and the logarithm of the external K or Rb concentration at pH 7.7 is close to the Nernst slope when their concentrations are high. Therefore, a current crossing the membrane in 450 mM K or Rb should be carried mainly by K or Rb ions. This, however, may not be true for less permeable cation species such as Na and Li. Therefore, it is necessary to consider the contribution of anion species to the membrane current. In some cases fibers were injected with the high K internal solution. The result obtained with those fibers was essentially the same as that obtained with uninjected fibers. Constant current pulses of about 100 msec duration were applied and potential changes at the time just before the termination of the current pulse were measured and plotted against the current intensity in Fig. 8. The abscissa in the figure does not represent the absolute value of the membrane potential, but the change from the membrane potential at zero membrane current for the respective test solutions. All the relations were obtained from the same fiber. The relation labeled "Tris" was obtained in cation saline H. The current-voltage relation for K was obtained at the beginning as well as at the end of the series of measurements in order to see whether there was any progressive change in the membrane. The relation was linear in the vicinity of the origin but the linear range of the potential change is generally much smaller than that found for anion species at low pH. The membrane conductance at zero current was

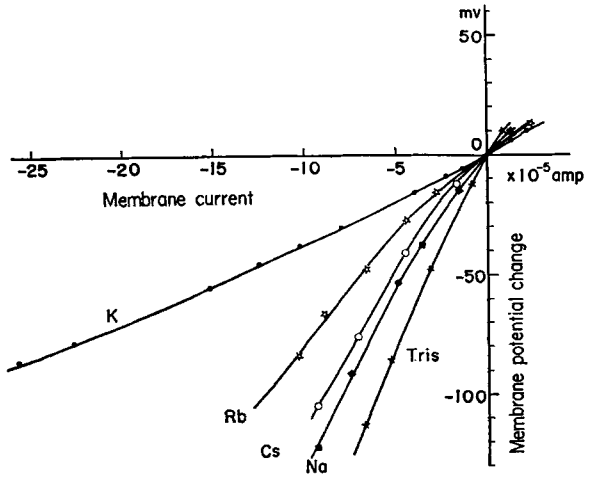


FIGURE 8. Current-voltage relations obtained in the same muscle fiber membrane at pH 7.7 when 450 mM of K in cation solution G was replaced with various test cation species. The relation labeled Tris was obtained in cation solution H. The ordinate represents the potential change from the membrane potential at zero membrane current found in each test solution. The fiber was injected with the K-rich solution.

estimated from the slope of the linear relation. The order of the conductance found in different cation species at 450 mM was:

$$K > Rb > Cs > Na, Li > Tris$$

and is, therefore, the same as the permeability sequence. Their values relative to that found in the K solution in the case shown in Fig. 8 were K (1.00), Rb (0.63), Cs (0.50), Na (0.36), and Li (0.34). The permeability constant ratios found in the same fiber membrane were K (1.00), Rb (0.78), Cs (0.13), Na (0.10), and Li (0.07). In order to compare the permeability ratio and the conductance ratio it is desirable to obtain the conductance of the membrane at a symmetrical condition; i.e., when the solutions on both sides of the membrane contain 450 mM of test cation species. When the membrane separates an internal K solution from an external solution of cation species C, the net membrane current always represents the sum of the outward current carried by K ions and the inward current carried by C ions if current carried by anions is neglected. Therefore, the membrane conductance at zero membrane current is not only dependent on the external cation species but also on the internal cation species. When the membrane potential is altered in the negative direction, the outward K current decreases and the inward C current increases. If the negative shift of the membrane potential becomes very large, the outward movement of K tends to vanish so that the total applied current will be carried by external cation species C. Thus, the limiting slope of the current-voltage relationship for a large negative potential shift should be approximately proportional to the conductance when both sides of the membrane are exposed to the 450 mM C solution. This limiting conductance was estimated for each cation species from the slope of the corresponding relationship between $-50$ and $-70$ mv (the reference was the membrane potential at zero membrane current) and the ratios obtained for the case shown in Fig. 8 were:

K (1.00), Rb (0.32), Cs (0.24), Na (0.20), Li (0.16), and Tris (0.12).

These ratios are not much different from the permeability ratios. In the Tris solution the membrane gave a small but finite limiting conductance. If the Tris permeability can be neglected this conductance presumably is due to the contribution of the outward flow of the internal anions. If this is taken into consideration the similarity between the permeability ratios and the conductance ratios becomes even closer.

### MEMBRANE CONDUCTANCE AND EXTERNAL K CONCENTRATION

The foregoing result shows that the permeability and the conductance ratios are nearly identical for monovalent cations. This suggests that the cation

permeation is via electrically neutral mechanisms. If this is the case, the conductance of the membrane separating two identical solutions of cation $C^+$ at a concentration $[C^+]$ should be proportional to $[C^+]$. In order to examine this, the relationship between the limiting conductance and the external K concentration was studied. In order to minimize the anion permeability the external pH was raised to 9.0 (K solution E) since the K permeability relative to the anion permeability increases with increasing pH of the medium (Hagiwara et al., 1968). The resting potentials found in K solution E containing 465 mM K were +6 to +10 mv. The K concentration was then reduced by substituting an equimolar amount of sucrose (cation solution F) for $K_2SO_4$ in K solution E. This resulted in a negative shift of the membrane potential. Fig. 9-II shows relationships between the membrane potential and the logarithm of the external K concentrations obtained with three different fibers. The membrane potential at 465 mM K was taken as the reference

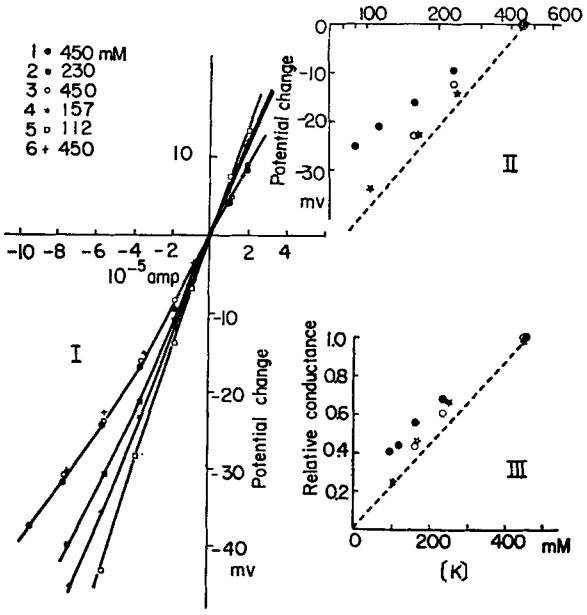


FIGURE 9. I, current-voltage relations obtained in the same fiber membrane at pH 9.0 obtained when the K concentration in cation solution E was altered. The symbols for each K concentration applied are listed to the left. The concentrations were changed in the numerical order given. The ordinate represents the potential change from the membrane potential at zero current found in each test solution. II, relationships between the membrane potential and the K concentration (on the abscissa in logarithmic scale). The membrane potential at 450 mM was the reference membrane potential for each fiber. Cation solutions E and D. III, relations between relative membrane conductance and the K concentration at pH 9.0. The conductance at 450 mM K was taken as a unity for each fiber. Three different symbols in II and III indicate results obtained from three different fibers.

membrane potential. The result shows that the relations are almost linear in the range of concentrations between 465 and 100 mM and their slopes are close to the Nernst slope (58 mv for a 10-fold increase in the concentration, indicated by a broken line in Fig. 9-II). This result suggests that the membrane current is almost exclusively carried by K ions in this range of K concentrations; i.e., the observed membrane conductance should represent the conductance of the membrane for K ions. Current-voltage relations for the same fiber membrane were obtained at various K concentrations as before (Fig. 9-I). Limiting conductances were then estimated at about −40 mv from the membrane potential at zero current. They are plotted against the external K concentration in Fig. 9-III, the conductance at 465 mM K being taken as unity for each fiber. The result shows that the membrane conductance is roughly proportional to the K concentration and agrees with expectations from electrically neutral permeation mechanisms.

## DISCUSSION

The permeability sequence of monovalent anions for the resting barnacle muscle fiber membrane at pH 3.9 was:

$$SCN^- > I^- > NO_3^- > Br^- > ClO_3^- > Cl^- > BrO_3^-$$

$$> p\text{-toluenesulfonate, methanesulfonate} > IO_3^- .$$

Conductance measurements for the species more permeant than Cl suggest that the conductance sequence among different anions is very different from the permeability sequence. The discrepancy can be explained by assuming competition among anions in occupying positively charged sites in the membrane (Conti and Eisenman, 1965). The calculation shows that species having higher permeability tend to show lower mobility. This indicates that the anion permeability sequence follows the binding sequence of the respective ion to the membrane, rather than its mobility sequence.

According to Eisenman (1965) and Diamond and Wright (1969), the sequence of anion (or cation) binding to a membrane site can be explained by considering the interactions of the ions with the membrane positive (or negative) charges on the one hand and with water molecules on the other. When the site has a high electric field strength, free energies of interaction between the anion and the positive site and their differences should dominate over hydration energies of anions and their differences. Therefore, among different anion species the relative affinities to the site would be controlled by the ion-site interaction. In contrast, the affinity would be governed by the hydration energy of anions for weakly positive charges; i.e., a higher affinity is found for anion species of a lower hydration energy. This permeability sequence coincides with the sequence called *lyotropic series* (Voet, 1937). Charges

of intermediate strength yield a transition sequence. Hydration energies for anion species examined in the present work show the following order:

$$IO_3^- > BrO_3^- > Cl^- > ClO_3^- > Br^- > NO_3^- > I > SCN^-.$$

This represents exactly the reverse order of the permeability sequence observed. The evidence, therefore, suggests that the positively charged site in the barnacle muscle fiber membrane should have a low electric field strength. The permeability sequence of anions found at pH 7.7 was the same as that found at pH 3.9 except for the revision between Cl and $ClO_3$. If the permeation occurs through charged membrane and if the charges originate from amphoteric compounds of the membrane, the alteration of the pH should change the field strength of the membrane charges, so that the permeability sequence may change. At present, however, it is not likely that inversion between Cl and $ClO_3$ can be explained only by the field strength. Although the discrepancy between the permeability and conductance sequences is successfully explained with a positively charged membrane, it should be mentioned that the same result could also be explained by assuming some sort of interaction among anions in the neutral membrane, for example, external SCN ions reduce the Cl permeability (Adrian, 1961).

Hutter and Noble (1961) studied anion permeabilities in cardiac muscle fibers and found the sequence:

$$I^- > NO_3^- > Br^- > Cl^-.$$

Muscle fibers of a certain group of marine elasmobranch fish show very high anion compared with cation permeability (Hagiwara and Takahashi, 1967). At pH 7.7 the permeability sequence of this preparation is:

$$SCN^- > NO_3^- > Br^- > Cl^- > ClO_3^-.$$

Del Castillo, de Mello, and Morales (1964) have shown that the permeability sequence in *Ascaris* muscle is $I^- > Br^- > Cl^-$. These sequences resemble the sequences found in barnacle muscle fiber membrane.

The result obtained for the alkali cation permeation shows (*a*) the permeability ratios among different species are nearly identical to their ratios of limiting membrane conductances, and (*b*) the limiting membrane conductance obtained in the K solution is roughly proportional to the K concentration. These suggest that the cation permeation occurs in the barnacle muscle fiber membrane via electrically neutral mechanisms, such as via neutral molecular carriers (Pressman, Harris, Jagger, and Johnson, 1967; Eisenman et al., 1968; Finkelstein and Cass, 1968) or fixed neutral sites in the membrane (Barry and Diamond, personal communication).

In the present study, the experimental results show that the membrane can be considered as a system having constant permeability ratios; i.e., the permeability constant ratios, $P_A : P_{Cl}$ or $P_C : P_K$, are independent of the concentrations of $A^-$ and $Cl^-$ or $C^+$ and $K^+$ in the external as well as in the internal medium. Furthermore, some of the results were discussed by assuming constant mobility ratios among different anion species since the experimental results do not conflict with this assumption. However, the possibility exists that this is not strictly the case (Ilani, 1966; Sandblom, 1967). The anion mobility sequence as estimated from the assumption of constant mobility ratios is:

$$Br^-, Cl^- > NO_3^-, ClO_3^- > SCN^-.$$

This sequence is similar to the one obtained in frog skeletal muscle fibers by Hutter and Padsha (1959) ($Cl^- > Br^- > NO_3^- > I^-$), but slightly different from the one found in the postsynaptic membrane of the inhibitory neuromuscular junction in crayfish (Takeuchi and Takeuchi, 1967; $Br^- > Cl^- > SCN^- > I^- > NO_3^-$). Harris (1958) showed that the Cl outflux of frog skeletal muscle was slowed in external solutions in which a proportion of the $Cl^-$ had been replaced by $Br^-$, $NO_3^-$, $I^-$, or $SCN^-$ in order of increasing effect. If a constant mobility ratio is assumed the result suggests that the mobility in the membrane has an order, $Br^- > NO_3^- > I^- > SCN^-$, and this is similar to the one found in barnacle muscle fiber.

In the present work, the membrane potential for a given test solution was obtained at a steady state, reached a few minutes after the application of the test solution. The membrane potential may show a much slower change after reaching this steady state; thus, different membrane potentials would be obtained for a given test solution if the membrane potential were observed over a very long period; e.g., several hours or a day after the immersion of the fiber in the test solution (Gainer and Grundfest, 1968). If the barnacle muscle fiber membrane is composed of two or more different components with different properties of ion permeation, such as are found in the surface and tubular system membranes in frog skeletal muscle (Eisenberg and Gage, 1967), the present results should indicate an average of the permeability characteristics of these parts of the membrane. Giradier et al. (1963) suggested different permeabilities between surface and tubular membranes in crayfish muscle fibers.

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