Chloride Fluxes in Isolated Dialyzed Barnacle Muscle Fibers

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ABSTRACT Chloride outflux and influx has been studied in single isolated muscle fibers from the giant barnacle under constant internal composition by means of a dialysis perfusion technique. Membrane potential was continually recorded. The chloride outfluxes and influxes were 143 and 144 pmoles/cm²-sec (mean resting potential: 58 mv, temperature: 22°-24°C) with internal and external chloride concentrations of 30 and 541 mM, respectively. The chloride conductance calculated from tracer measurements using constant field assumptions is about fourfold greater than that calculated from published electrical data. Replacing 97% of the external chloride ions by propionate reduces the chloride efflux by 51%. Nitrate ions applied either to the internal or external surface of the membrane slows the chloride efflux. The external pH dependence of the chloride efflux follows the external pH dependence of the membrane conductance, in the range pH 3.9-4.7, increasing with decreasing pH. In the range pH 5-9, the chloride efflux increased with increasing pH, in a manner similar to that observed in frog muscle fibers. The titration curve for internal pH changes in the range 4.0-7.0 was quantitatively much different from that for external pH change, indicating significant asymmetry in the internal and external pH dependence of the chloride efflux.

INTRODUCTION Considerable knowledge has been obtained on the mechanisms underlying transport of ions across the membrane of giant nerve fibers, following the control of the internal medium by intracellular perfusion techniques. The application of those methods to the study of ion transport in muscle fibers has been difficult because of the small size of most vertebrate single muscle fibers. In the present work the intracellular microdialysis technique has been applied to single giant barnacle muscle fibers, to permit study of certain features of chloride transport across the muscle membrane under conditions of well-defined constant internal concentration and membrane potential.

Experiments will be described concerning the effect of permeant and im-
permeant anions on the chloride efflux and membrane potential. The results suggest that, since chloride ions contribute but little to the membrane conductance at the resting potential (Hagiwara et al., 1968), the magnitude of the unidirectional fluxes is much too high to consider all the chloride ions crossing the membrane as electrical charge carriers.

Experiments in which the external chloride was replaced by impermeant anions such as propionate or methanesulfonate reduced the chloride efflux by about 50% and suggest that much of the chloride flux is of an exchange type and does not contribute to the membrane conductance. The presence of nitrate ions on either side of the membrane was found to reduce chloride efflux by about 30–40%.

In a second series of experiments, the chloride fluxes were studied as a function of both internal and external pH. The results confirmed the previous work in both barnacle (Hagiwara et al., 1968) and crayfish muscle fibers (Reuben et al., 1962; De Mello and Hutter, 1966) which showed that chloride conductance increases in acid solutions (pH 3.9–4.7), but also indicated that the conductance varied in a rather complicated manner with external pH change, showing a minimum at pH 5.5 with a rather sharp rise in flux at more acid pH and a significant though smaller rise in the range pH 5–9. Internal pH changes in the acid range also produced marked increases in chloride efflux, but the effects of internal and external pH were not symmetrical.

**METHODS**

*Experimental Material*

Two species of barnacles were used in this study: *Balanus nubilis* and *Balanus aquila*. All material was collected in Monterey Bay, Calif., by the Pacific Biomarine Supply Co. (Venice, Calif.) and transported by air to Baltimore, Md. Specimens were kept in a cold room in aquaria supplied with recirculated filtered artificial seawater at 8°–12°C. Under these conditions the barnacles survived for several months.

*Dissection of Single Fibers*

Preparation of muscle bundles was according to Hoyle (Hoyle and Smyth, 1963). The depessor scutum rostralis was used most frequently because these fibers are more nearly circular in cross-section. Each bundle was isolated with a fragment of scutum attached to the tendinous end and with a piece of shell on the other end. Single muscle fibers were dissected out in standard saline by cutting the tendon from the scutum and tearing gently the strands of connective tissue with the tip of a pair of scissors. The fiber was lifted with forceps and cut in the air at the end closest to the shell. Cutting the fiber usually resulted in a slight shortening. In order to avoid further spreading of the contraction along the length of the fiber, the cut end was blotted by touching it briefly to filter paper and then immersed to a depth of a few millimeters in mineral oil. Finally, a fiber from 3.5 to 4.5 cm in length was floated on a
piece of filter paper well covered with saline. The ends of the fiber were insulated from the saline by being surrounded by a blob of Vaseline. After isolation the fiber was left undisturbed for about 5 min before cannulation.

**Dialysis Procedure**

(A) **DIALYSIS CAPILLARIES** The porous glass capillaries used in the present studies were supplied by Corning Glass Works, Corning, N.Y. The dimensions were as follows: length 12–14 cm, diameter 150–175 μm, wall thickness 50–75 μm, and porous region 1.5–2.0 cm. Although the porosity of the capillaries as supplied by the manufacturer was not adequate for the present purpose, it could easily be increased by soaking the capillaries in 60 mM Na-ethylenediaminetetraacetic acid (EDTA) (pH 6.9) for 10–40 hr at room temperature. The degree of porosity was checked using phenol red as a marker. Porous capillaries were mounted in Lucite holders and stored in distilled water containing 20% ethanol. The permeability of these capillaries as judged by their ability to pass a standard test dye, phenol red, was comparable to capillaries previously evaluated in detail for use in squid dialysis studies (Brinley and Mullins, 1967). The half time for isotope equilibration within the fibers (10–30 min) was appropriate for the assumed porosity of the capillaries and dimensions of the barnacle muscle fibers.

(B) **DIALYSIS CHAMBER** The efflux-influx chamber was essentially that described for squid axons by Brinley and Mullins (1967). Significant modifications in the design are as follows: (1) the dimensions of the chamber were adjusted on the basis of minimum dead space critical for certain electrophysiological techniques, and on the fiber diameter (1-2 mm); (2) two air gaps were created to avoid shunting of the membrane potential by the damaged cannulated regions; (3) the vertical motion of a micromanipulator holding the entire chamber was used to position the fiber in the central slot. Fig. 1 shows a schematic drawing of the apparatus used to hold the cannulated fiber during dialysis.

(C) **EXPERIMENTAL PROCEDURE** A single muscle fiber was placed in the dialysis chamber and cannulated at both ends using glass capillaries of 450 μm diameter (end cannulas). A few microliters of a calcium binding solution (tris(hydroxymethyl)-aminomethane-ethylene glycol bis(β-aminoethyl ether) N,N',N''-tetraacetic acid, tris-EGTA) was injected into the cut ends through the end cannulas. The blocking of the contraction mechanism in these regions permits the subsequent immersion of the whole fiber in saline during the insertion of the porous capillary without shortening of the fiber. The porous capillary was introduced through the left-hand end cannula and slowly steered under microscopic control through the fiber, then through the right-hand end cannula until the porous region lay in the center of the collection chamber. Because the internal structure of the fiber offers some resistance during the impalement, it was occasionally found helpful to inject a few microliters of fluid per centimeter of travel into the center of the fiber. This injection seemed to spread the sarcoplasm along the track of the impalement and to facilitate further insertion of the capillary. The volume injected was always less than 5% of the fiber volume and did not seem to affect the membrane properties of the fibers, at least as compared with similar fibers not injected.
Figure 1. Schematic drawing of an apparatus used to hold the muscle fiber during dialysis. (A) is a top view and (B) is a cross-section. The whole chamber is mounted in a vertical manipulator; after the fiber has been positioned into the slot, the Lucite pieces are advanced with the thumbscrew to form part of the guard chamber.
Following the positioning of the porous capillary, a second glass capillary used to measure membrane potential was introduced through the right-hand end cannula until the tip reached the middle of the porous region. After the fiber had been positioned in the slot, a system of greased blocks converted the guard compartments into watertight channels (see Fig. 1).

Recording of Membrane Potential

The membrane potential of nondialyzed fibers was recorded with standard glass micropipettes filled with 3 M KCl having resistances in the range 5–10 MΩ. Micropipettes with more than a 5 mV tip potential were discarded. The membrane potential was obtained as a potential difference between the micropipette inside and a small polyethylene tube containing 3 M KCl-1% agar just outside the fiber. Silver-silver chloride electrodes were used as reversible electrodes for measuring membrane potential. For transmembrane potential measurements the fiber was tied at both ends with fine string and placed in the slot of the chamber; the cut end was isolated from the center compartment by an air gap. The insertion of the microelectrode was done in the area close to the tendon.

Membrane potentials in dialyzed fibers were recorded using a longitudinally inserted glass capillary of 90–100 μ diameter filled with 0.5 M KCl. The same reference system was used for recording the membrane potential.

Efflux Experiments

Effluxes were measured by perfusing a radioactive species through the porous capillary and collecting the radioactivity which crossed the membrane. The internal perfusing solution was delivered to the porous capillary by a motor-driven syringe at a rate of 2–2.5 μl/min. The external solution was pumped into the center compartment by means of a peristaltic pump at a rate of 1–3 ml/min. Efflux samples were collected by suction at regular intervals (2, 3, or 5 min). Temperature was controlled at ±0.5°C by passing the fluid delivered by the pump through a thermoelectric immersion cooler system.

Washout of the chamber was about 95% completed in 1 min. Efflux samples were collected in planchets, dried in an oven, and counted in a low background counter.

Influx Experiments

For influx experiments the same chamber was used except that the guard system was inactivated and the entire porous length of the capillary was used to collect the isotope. The portions of the fiber beyond the porous region were covered by thick grease to avoid contact with the radioactive solution. The solution in the center compartment was replaced by a radioactive one and the perfusate was collected from the tip of the porous capillary by capillary action using glass tubes attached to a manipulator. Collection samples were usually taken every 5 min. Temperature control was achieved by circulating a mixture of ethylene glycol-water underneath the chamber from a thermal bath.
External Solutions

The composition of the various solutions used is given in Table I. Strophanthidin (Sigma Chemical Co., St. Louis, Mo.) was added directly to the saline solution to yield final concentrations of from 1 to 20 μM. Radioactive seawater was made by adding solid ^22NaCl or Na^36Cl directly to the saline. The increase in Na or Cl content after the addition of the radioactive isotope was less than 5%.

Internal Dialysis Solutions

The standard dialysis solution used had the following composition (in millimoles per liter): K-isethionate, 170 or 190; KCl, 25 or 0; Na-TES, 15 (TES: N-tris(hydroxymethyl) methyl-2-aminoethane sulfonic acid); Tris-EGTA, 10; MgSO₄, 4; sucrose, 550. In preliminary experiments a number of dialysis solutions were used which approximated to varying extents the constituent analysis of barnacle sarcoplasm. None of them evidenced any obvious advantages over the simpler solution listed above, which contains sodium, potassium, and chloride in the concentrations determined by analytical means in this and other laboratories, and sucrose in amounts osmotically equivalent to the sum of several nonelectrolytes present in sarcoplasm. Neither adenosine triphosphate (ATP) nor phosphoarginine was added to the dialysis solutions. The ATP concentration in dialyzed unpoisoned barnacle fibers falls extremely slowly with a time constant of several hours (Brinley, unpublished), presumably because much ATP is bound tenaciously to sarcoplasmic proteins and more is generated continuously from intramitochondrial energy stores not removed by dialysis. For the relatively short-term dialysis reported here, it was thought the ATP level would not drop sufficiently to reduce the activity of a chloride pump if such should exist. Furthermore the sodium outflux from barnacle muscle fibers dialyzed with fuel-free solutions is near normal levels (Brinley, 1968). The pH was adjusted to 7.0. Radioactive solutions were made by adding solid ^22KCl or Na^36Cl.
solutions at different values of pH were buffered by 5 mM Tris-hydrogen phthalate and the pH was adjusted to the range 3.9–5.0 by adding an appropriate amount of methanesulfonic acid. Internal nitrate solution was prepared by replacing 25 mM K-isethionate with KNO₃. All solutions were stored at -90°C.

Isotopes

Radioactive isotopes were obtained from New England Nuclear Corp., Boston, Mass., with the following specific activities (in Curies per mole): ³²NaCl, 60; Na⁺Cl, 0.2 or 5.0; ⁴²KCl, 60 (on arrival).

Calculations

(a) Efflux was calculated according to the following formula

\[ M_e(P/CS) = \frac{\text{cpm collected in efflux sample}}{\text{specific activity of dialysis solution} \times \text{surface area} \times \text{time}}. \]

(b) Influx was calculated according to the following formula

\[ M_i(P/CS) = \frac{\text{cpm collected in the perfusate}}{\text{specific activity of soak solution} \times \text{surface area} \times \text{time}}. \]

For the calculations the fiber was assumed to be a cylinder and no corrections were made for the well-known invaginations of the sarcolemma (Selverson, 1967).

RESULTS

Membrane Potential in Dialyzed and Nondialyzed Fibers

The membrane potential recorded with transmembrane microelectrodes from fibers bathed in standard saline was 70 ± 2 mv (n = 10) at 24°C. The potential was stable for long periods of time, and no signs of inhomogeneity were observed along the length of the fiber. The average membrane potential measured in dialyzed fibers by the longitudinal insertion of a glass capillary was 58 ± 2 mv (n = 55) at 24°C. This value is about 12 mv less than that recorded with standard microelectrodes. The presence in these fibers of deep invaginations of the outer membrane or sarcolemma, which can be damaged by the introduction of a capillary in the interior of the fiber, is probably the cause of this discrepancy. It is also possible that electrotonic conduction of spread of depolarization from the cut ends of the fiber reduced the potential somewhat. Even though the cut ends were kept in air to minimize this error, a certain amount of current leakage through the saline-filled clefs is unavoidable. However, fibers perfused continuously with the porous capillary were able to maintain constant membrane potentials for 3–6 hr.

¹ P/CS = pmole/cm² per sec.
Evidence that Dialysis of the Fiber Does Not Alter Ion Fluxes Across the Membrane

Selverson (1967) has shown that the outer membrane or sarcolemma, instead of forming a cylindrical covering around the fiber, actually invaginates deeply into the interior forming a series of clefts several hundred microns deep. It seemed important to determine what effect damage of this membrane network caused by insertion of the dialysis capillary might have upon the ionic fluxes measured across the whole fiber. This was done by comparing published data on the passive sodium and potassium fluxes in intact or injected fibers with comparable fluxes obtained with the dialysis technique.

A value of 120 P/CS was obtained for potassium efflux from fibers dialyzed with 195 mM K at 22°C. This resting efflux, referred to the same range of internal concentration and temperature used by Brinley (1968), corresponds to a value of 54 ± 5 P/CS \( (n = 4) \), which is not too different from the value of 60 P/CS obtained in intact and microinjected fibers by that author. Similarly, the sodium influx in dialyzed fibers, 37 ± 7 P/CS \( (n = 4) \), agrees reasonably well with the value of 48 P/CS reported by Brinley for intact fibers under similar conditions of external concentration and temperature. These results, in connection with collateral information including high membrane resistance obtained in injected fibers (Hagiwara et al., 1964) and presence of high temperature coefficients for the fluxes of some of these ions (DiPolo and Latorre, 1972), suggest that, although damage to the cleft system may occur along the track of the capillary, it does not seem to affect the absolute magnitude of the ionic fluxes.

CHLORIDE FLUXES

Absolute Size of the Resting Chloride Fluxes

The resting chloride fluxes estimated in dialyzed fibers are summarized in the first six columns of Tables II and V. The chloride efflux at 24°C was found to be 143 ± 19.6 P/CS \( (n = 12) \) and 18.4 ± 4 P/CS \( (n = 31) \) for internal chloride concentrations of 30 and 5 mM, respectively. The mean resting potential in these fibers was 58 mV. 30 mM chloride was selected as the standard internal chloride concentration on the basis of analysis of fresh intact fibers (Hagiwara et al. 1964; Brinley, unpublished). As was pointed out before, in computing the magnitude of the chloride efflux no correction was made for the extra membrane area resulting from the sarcolemma invaginations. Resting chloride influxes measured in dialyzed fibers are listed in Table III. A value of 144 ± 21 P/CS \( (n = 6) \) was obtained which is almost the same
as the chloride efflux under the same conditions of membrane potential and temperature. This close agreement between the unidirectional fluxes indicates

that, under the standard conditions reported here, the net flux of chloride is nearly zero.

Fibers dialyzed continuously were able to maintain constant fluxes and membrane potential for long periods of time (3–4 hr) without showing signs of deterioration. See Fig. 4.
Effect of Temperature, Cardiac Glycosides, and Metabolic Inhibitors on the Chloride Efflux

When the temperature of the external medium decreases, a reduction in both chloride efflux and membrane potential is observed which is much greater than that predicted by the constant field equation. Table IV summarizes the effect of temperature on the chloride efflux. In agreement with previous work (DiPolo, Latorre, 1972), a high activation energy is found for the chloride efflux. Fig. 2 shows that a drop of 10°C in the external medium reduces the chloride efflux to 65% of its control value associated with a decrease of 9 mv in the resting membrane potential. The cardiac glycoside, strophanthin, which is known to produce a substantial reduction of the

### TABLE III

**CHLORIDE INFLUXES IN BARNACLE MUSCLE FIBERS**

| Fiber reference | Diameter | T °C | (Cl)i mM | (Cl)o mM | -E_m mv | M^Cl_s P/CS |
|-----------------|----------|------|----------|----------|---------|-------------|
| BCLI2           | 1249     | 24   | 30.0     | 541.0    | 55      | 139.0       |
| BCLI3           | 1221     | 23   | 30.0     | 541.0    | 52      | 126.0       |
| BCLI4           | 1203     | 24   | 30.0     | 541.0    | 58      | 141.0       |
| BCLI9           | 1274     | 21   | 30.0     | 541.0    | 63      | 135.4       |
| BCLI10          | 1408     | 22   | 30.0     | 541.0    | 50      | 130.0       |
| BCLI11          | 1091     | 22   | 30.0     | 541.0    | 55      | 191.0       |
| **Mean±SEM**    | 1241±94  | 23±1 | 30.0     | 541.0    | 56±4    | 144±21      |
| n               | 6        | 6    | 6        | 6        | 6       | 6           |

### TABLE IV

**EFFECT OF TEMPERATURE ON CHLORIDE EFFLUX AND MEMBRANE POTENTIAL**

| Fiber reference | (Cl)i mM | M^Cl_s P/CS | -E_m mv | T °C | M^Cl_s P/CS | -E_m mv | T °C | QO | n |
|-----------------|----------|-------------|---------|------|-------------|---------|------|----|---|
| BCLI1A          | 4.7      | 23.0        | 54      | 25.0 | 9.0         | 45      | 15.5 | 2.8 | 6 |
| BCLI8A          | 4.7      | 18.3        | 59      | 25.0 | 3.3         | 45      | 13.0 | 3.2 | 6 |
| BCLI12          | 4.7      | 30.2        | 59      | 25.0 | 7.5         | 51      | 12.0 | 2.4 | 6 |
| BCLI34          | 31.0     | 126.0       | 57      | 25.0 | 33.1        | 44      | 15.0 | 3.2 | 6 |
| BCLI63A         | 30.0     | 160.0       | 58      | 21.0 | 53.0        | 40      | 11.0 | 3.0 | 6 |
| BCLI64          | 30.0     | 143.0       | 59      | 21.0 | 48.3        | 49      | 10.5 | 3.0 | 6 |
| **Mean±SEM**    | 58±2     | 24±2        | 46±4    | 13±2 | 2.9±27      |         |      |    |   |
| n               | 6        | 6           | 6       | 6     | 6           |         |      |    |   |
sodium efflux in these barnacle fibers (Brinley, 1968), at concentrations of
$10^{-8}$--$10^{-4}$ M has no significant effect on chloride efflux or on the resting mem-
brane potential (see Fig. 3). The well-known metabolic inhibitor, cyanide, at
external concentrations of 2 mM was also ineffective in changing the efflux
level even over periods of 3–4 hr.

**Effect of Foreign Anions on the Chloride Efflux**

Table II represents the summary of a series of experiments in which the effect
of permeant and impermeant anions on the chloride efflux was studied. The
dependence of the $^{36}$Cl efflux on the external chloride concentration was first
investigated by replacing 97% of the external chloride by an impermeant
anion (methanesulfonate or propionate). This low chloride solution causes a
mean reduction of 51% ($n = 8$) in the chloride efflux level and a depolariza-
tion of about 10 mV.

Figs. 3 and 4 show the reversible drop in the Cl efflux in the presence of
a low external chloride solution. Although the maintained depolarization
could have been considered to result from a constant internal chloride con-
centration during dialysis, it was also seen in intact fibers in which the
potential was recorded with microelectrodes. This result is in contrast to the
situation in frog muscle in which depolarizations in low chloride solution
are only transitory, presumably reflecting relatively rapid shifts in chloride concentration. (Hodgkin and Horowicz, 1959). Two factors probably explain the constant membrane potential in barnacle muscle: first, the large sarcoplasmic volume in barnacle fibers reduces the rate of change of internal chloride concentration, and second, the comparatively low chloride fluxes are further reduced in chloride-free solutions.

It has been shown conclusively by conductance and tracer methods that nitrate reduces the fluxes of chloride across the membrane of vertebrate muscle fibers. In view of one report, that nitrate had no effect on chloride efflux in a crustacean muscle (Maia squinado, Richards, 1969), the effect of this ion on chloride movements in barnacle muscle fibers was investigated. Table II summarizes the effect of external and internal nitrate ions on the chloride efflux of barnacle muscle fibers. A 49% ($n = 15$) decrease in the steady-state level of the chloride efflux was observed when the external chloride was replaced by nitrate. In agreement with Hagiwara et al. (1971), a mean increase in the resting membrane potential of 5 mv was also observed in nitrate solutions, which indicates that in these fibers nitrate ions are more permeable than chloride ions. Fig. 2 shows an experiment demonstrating the effect of different proportions of external nitrate and chloride on the chloride

Figure 3. Effect of low chloride saline and external cardiac glycosides on resting level of the chloride efflux and membrane potential.
efflux. It is clear that the greater the proportion of external nitrate, the greater the degree of inhibition of the Cl efflux.

The effect of internal nitrate ions on the chloride efflux has been difficult to analyze in small vertebrate muscles because of the difficulty of maintaining a constant internal nitrate concentration during the time required for tracer measurements of efflux. Since diffusion of nitrate out of the porous capillary is much faster than across the sarcolemma, the dialysis technique permitted measurement of chloride efflux in the presence of various steady-state concentrations of internal nitrate. In these experiments the internal chloride concentration was set at 5 mM and 25 mM nitrate ion was added where indicated. Fig. 5 shows the results of one such experiment in which the fiber was first perfused with 5 mM internal chloride followed by a dialysis with 5 mM chloride plus 25 mM nitrate ion. The procedure produced a marked reversible decrease in efflux, in which the presence of 80% of the permeant anions as nitrate in the internal medium causes a reduction of the outflux of chloride to 60% of its control value. The magnitude of this inhibition is close to that observed for the same external nitrate proportion. The membrane potential changes are in the direction to be expected for the replacement of chloride by a more permeant ion.

**Effect of External and Internal pH on the Cl Fluxes**

It has been shown by electrical measurements that decreasing the external pH enhances Cl conductance in barnacle muscle fibers (Hagiwara et al.,
This finding is confirmed in the following series of experiments which also investigated the effects of different values of external and internal pH on the chloride fluxes directly.

**Figure 5.** Effects of external and internal nitrate on chloride efflux and membrane potential. The upper graph shows the drop in the efflux in the presence of external NO$_3$ ions. The bottom graph shows the inhibition of the Cl efflux when NO$_3$ constitutes 80% of the permeant anions in the internal perfusion solution (NO$_3^-$, 25 mM; Cl$^-$ = 5 mM).

(A) **EXTERNAL PH** Fig. 6 illustrates a typical experiment in which a dialyzed fiber was exposed to solutions of different external pH. It is clear from this figure that the fiber can tolerate moderate deviations from its normal pH for long periods of time as demonstrated by the reversibility of
the Cl efflux and membrane potential at pH 5.5 and 9.0. However, experiments done at very low values of pH 3.9-4.2 were only reversible when the external solution was applied for short periods of time; the maximum level of the efflux as well as the hyperpolarization of the membrane which accompanies the increase in the efflux shows a progressive decay with time.

Table V and Fig. 11 summarize behavior of the chloride efflux in response to changes in the external and internal pH of the fiber. When the external hydrogen ion concentration decreases below pH 5.0, an increase in the chloride efflux is always observed. In the extreme pH range studied, 3.8-4.0, the chloride efflux increases by an average factor of five from its control level at pH 7.0. In this pH range, the membrane potential shows a hyperpolarization attaining values closer to the equilibrium potential for chloride. In the range pH 4.2-4.5 the chloride efflux increases only by a factor of three.

A different behavior was observed at more alkaline pH (5.0-9.0). Table V shows that the 36Cl efflux decreases 59% at pH 5.5, while at pH 9.0 it shows an increase of 46%. A similar relation between efflux and pH in the range 5.0-9.0 has been reported for frog muscle by Hutter and Warner (1967) and Moore (1969). The extent to which the effects of pH on chloride efflux
### Table V

**CHLORIDE EFFLUX AT DIFFERENT EXTERNAL AND INTERNAL pH**

*(a) External pH*

| Fiber reference | Diameter | T (°C) | (Cl)\textsubscript{i} | -Em | $\Delta M$ | Test/control | $-E_m$ | Test/control | $-E_m$ | Test/control | $-E_m$ | Test/control |
|-----------------|----------|--------|----------------|-----|----------|--------------|--------|--------------|--------|--------------|--------|--------------|
| BCL14A          | 1532     | 25     | 4.7 56        | 15.0 | 71       | 6.0          |        |              |        |              |        |              |
| BCL15           | 1416     | 24     | 4.6 56        | 17.1 | 64       | 4.2          |        |              |        |              |        |              |
| BCL16           | 1168     | 24     | 5.2 56        | 17.0 | 77       | 4.0          |        |              |        |              |        |              |
| BCL29           | 1031     | 25     | 5.2 60        | 25.7 | 70       | 4.0          |        |              |        |              |        |              |
| BCL31           | 1256     | 23     | 30.0 59       | 132.0 65 | 8.5 |        |              |        |              |        |              |        |              |
| BCL36B          | 1230     | 23     | 30.0 59       | 20.0 | 71 | 7.0 |              |        |              |        |              |        |              |
| BCL41           | 1239     | 23     | 5.0 63        | 20.0 | 76 | 7.0 |              |        |              |        |              |        |              |
| BCL22           | 1362     | 25     | 5.2 62        | 16.9 | 71 | 3.0 |              |        |              |        |              |        |              |
| BCL11           | 1292     | 25     | 4.6 58        | 19.2 |        | 60 | 2.2 |              |        |              |        |              |        |              |
| BCL24           | 1026     | 25     | 5.2 60        | 16.4 |        | 64 | 3.0 |              |        |              |        |              |        |              |
| BCL28           | 943      | 25     | 5.2 60        | 20.7 |        | 61 | 2.9 |              |        |              |        |              |        |              |
| BCL44A          | 1097     | 24     | 30.0 59       | 131.0 |        | 63 | 3.6 |              |        |              |        |              |        |              |
| BCL32           | 1327     | 25     | 5.2 58        | 22.3 |        |              |        |              |        |              |        |              |
| BCL36           | 1230     | 23     | 30.0 58       | 135.0 |        | 52 | 0.8 |              |        |              |        |              |        |              |
| BCL42           | 1150     | 23     | 30.0 53       | 140.0 |        | 47 | 0.6 |              |        |              |        |              |        |              |
| BCL43           | 1019     | 23     | 30.0 57       | 110.0 |        | 52 | 0.5 |              |        |              |        |              |        |              |
| BCL47A          | 1132     | 23     | 5.0 62        | 29.5 |        | 53 | 0.6 |              |        |              |        |              |        |              |
| BCL44B          | 1097     | 24     | 30.0 58       | 130.0 |        |              |        |              |        |              |        |              |        |              |
| BCL45           | 1044     | 24     | 30.0 57       | 154.0 |        | 53 | 1.4 |              |        |              |        |              |        |              |
| BCL64           | 1190     | 21     | 30.0 59       | 115.0 |        | 52 | 1.3 |              |        |              |        |              |        |              |
| BCL65           | 1200     | 22     | 5.0 57        | 20.0 |        |              |        |              |        |              |        |              |        |              |
| **Mean±SEM**    | **1189±140** | **58±2** | **5.1±1.8** | **2.9±0.5** | **0.58±0.03** | **1.43±0.6** |        |              |        |              |        |              |        |              |
| **n**           | **21**   |        |                |        |        |              |        |              |        |              |        |              |

*(b) Internal pH*

| Fiber reference | Diameter | T (°C) | (Cl)\textsubscript{i} | -Em | $\Delta M$ | Test/control | $-E_m$ | Test/control | $-E_m$ | Test/control | $-E_m$ | Test/control |
|-----------------|----------|--------|----------------|-----|----------|--------------|--------|--------------|--------|--------------|--------|--------------|
| BCL25           | 1175     | 24     | 30.0 58        | 120.0 | 50 | 3.4 |              |        |              |        |              |        |              |
| BCL26           | 1346     | 25     | 5.2 62        | 15.2 | 54 | 3.0 |              |        |              |        |              |        |              |
| BCL35           | 1345     | 25     | 5.2 55        | 24.5 | 48 | 2.9 |              |        |              |        |              |        |              |
| BCL37           | 1239     | 24     | 5.2 65        | 31.5 | 57 | 3.5 |              |        |              |        |              |        |              |
| BCL46           | 1250     | 23     | 5.2 64        | 20.3 |        |              |        |              |        |              |        |              |
| BCL47B          | 1132     | 23     | 5.0 61        | 30.0 |        |              |        |              |        |              |        |              |
| BCL54           | 1300     | 23     | 30.0 60       | 143.0 |        | 53 | 2.1 |              |        |              |        |              |        |              |
| BCL55           | 1250     | 23     | 30.0 52       | 160.0 |        | 47 | 4.0 |              |        |              |        |              |        |              |
| BCL56           | 1150     | 24     | 30.0 58        | 150.0 |        | 51 | 3.7 |              |        |              |        |              |        |              |
| **Mean±SEM**    | **1243±74** | **24±7** | **59±3** | **3.2±0.3** | **5.5±0.5** |        |        |              |        |              |        |              |        |              |
| **n**           | **9**    |        |                |        |        |              |        |              |        |              |        |              |        |              |
in the barnacle might be secondary to changes in ionized calcium was not studied in the present series of experiments. A rather extensive investigation of this point by Hutter and Warner for frog muscle indicated that even large changes in concentrations of ionized calcium did not influence chloride efflux. In order to check the reversibility of the low (3.9–4.2) external pH experiments, the resting membrane potential was measured in seven different fibers using standard microelectrodes. Low external pH solutions were applied for different short times. The usual increase in the resting membrane potential produced by an increase in the Cl conductance was found to be completely reversible within periods not longer than 10 min. Therefore the reported increase in the chloride efflux found at this low pH was always taken within the period of time when the effect was still reversible.

The effect of low pH on influx of chloride is illustrated in Fig. 7, where a change of external pH from 7.0 to 4.0 can be seen to produce a large non-sustained increase of influx about four to five times larger than the control level.

(b) Internal pH In order to study the effect of changing the internal hydrogen ion concentration on the chloride efflux, fibers were dialyzed internally against solutions buffered at different values of internal pH. One of these experiments is illustrated in Fig. 8, which shows the increase in the

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**Figure 7.** Effect of low external pH solution on chloride influx in a dialyzed barnacle muscle fiber.
chloride efflux when the internal pH of the fiber is changed from pH 7.0 to 3.9. Contrary to the prompt increase in the chloride efflux produced by the external pH experiments, a time delay in the onset of the efflux increase was observed in these experiments. The half time of the slow onset of the internal pH effect was appropriate for the permeability properties of the dialysis capillaries used and the dimensions of the fiber, and is taken as evi-

dence that (a) the dialysis solution was in fact changing the internal pH of the fiber, and (b) when a steady-state level of efflux was finally obtained, the internal pH was, in fact, at or near the pH of the dialyzing solution. Fig. 8 also shows that the effect of low internal pH is reversible, in contrast to the effect of external pH.

The data at hand indicate four main differences between the effects of internal and external pH changes: (1) a reduction in external pH to the region of 4.0 increases the efflux about five times, whereas the same reduction in internal pH augments the efflux only threefold. (2) A qualitative difference between the external and internal pH experiments was obtained in the pH range 5.0–6.0. Lowering the external pH to 5.5 decreases the chloride efflux
by 59%, although lowering the internal pH to 5.5 increases the chloride efflux by a factor of three. (3) Lowering the internal pH of the fiber from 7.0 to 3.9 fails to produce a hyperpolarization similar to that observed in the same range of external pH. (4) All the internal pH experiments were completely reversible, even after long periods of time with low pH solutions.

The asymmetrical effect of internal and external pH on chloride efflux is clearly shown in Fig. 9, which also shows that the membrane undergoes a reversible depolarization when exposed to low internal pH.

![Figure 9. Asymmetric behavior observed at pH 5.0 on chloride efflux for external and internal change. This effect is shown to be completely reversible.](image)

**Membrane Potential and Chloride Efflux**

In view of the fact that in some experiments simultaneous changes in the chloride efflux and membrane potential were observed, it is necessary to know how sensitive the chloride efflux is to changes in the electrical part of its electrochemical potential gradient. The resting membrane potential of dialyzed fibers was modified by two different methods: first, by changing the potassium concentration in the standard external solution and, second, by the application of a current pulse through a platinum-iridium wire located inside the porous capillary and connected to a current source via a high resistance element. No effect on the resting Cl$^-$ efflux was observed by raising the external K$^+$ to 20 or 40 mm. Fig. 4 shows that the presence of a 10
mv depolarization produced by a 10 mM increase in the external potassium concentration has no effect on the resting level of chloride efflux. The same figure illustrates the drop in the efflux in low chloride solutions. This type of experiment suggests that the decrease in the membrane potential observed in low chloride solutions is not the cause of the decrease in the chloride efflux found in such solutions.

The effect of depolarizing and hyperpolarizing current pulses on the chloride efflux is shown in Fig. 10. The chloride efflux was found to be essentially unaffected by a decrease of 20 mv in the resting membrane potential. Contrary to the absence of a significant change in the chloride efflux during a depolarizing current pulse, a significant small increase in the efflux was observed as a result of inward hyperpolarizing current pulse. Quantitatively, this increase in efflux is more pronounced than the rectification predicted by the constant field equation, although it is in the right direction. Hagiwara et al. (1964) have found an increase in the chloride conductance for hyperpolarizing currents in KCl-treated fibers and a similar result has been obtained for crayfish muscle fibers by Grundfest (1962).

![Figure 10](image-url)

Figure 10. Effect of long current pulses on chloride efflux and membrane potential.
DISCUSSION

Absolute Size of the Resting Chloride Fluxes

The experimental chloride fluxes measured in giant barnacle muscle fibers are given in Tables II, III, and V.

The measured value of 144 P/CS for the chloride efflux is much lower than that found in another crustacean muscle fiber, *Maia squinado* (Richards, 1969). The presence of a low membrane resistance in crab muscle fiber seems to be the reason for the nearly 20-fold difference in the magnitude of the chloride efflux between the two preparations. In the case of the barnacle, if the extra area due to the presence of membrane infoldings is taken into account, the chloride flux would be reduced by a factor of 10 (Selverson, 1967) yielding a value of 14 P/CS, which is of the same order as that found in some other single cells: frog muscle fibers, 50 P/CS (Adrian, 1961); Homarus giant axon, 9 P/CS (Brinley, 1965); squid giant axon, 20–28 P/CS (Keynes, 1963). In frog muscle fibers a value of about $4 \times 10^{-4}$ cm/sec has been obtained for the chloride permeability (Hodgkin and Horowicz, 1959); if a similar calculation is carried out for the total Cl⁻ flux in barnacle muscle fibers a value of about $1.9 \times 10^{-4}$ cm/sec is obtained. Such similarity, however, is only apparent since the presence of membrane infoldings should be taken into account. In this case the chloride permeability would be reduced by about 10-fold (see above).

There are two quantitative difficulties with the data as it stands: (a) The experimental flux ratio is very close to one. For an intact fiber this would be a satisfactory result, since the chemical gradient of 73 mv (based on an internal chloride concentration of 30 mm) is very nearly equal to the membrane potential of 70 mv.

Actually however, for dialyzed fibers, the membrane potential averaged 58 mv and in this case the ratio influx/efflux should be about 1.8 rather than 1.0 as found experimentally. This result could of course indicate a component of active outward chloride transport. If such occurs, it probably is not related to the sodium or potassium transport system, since the chloride efflux was not affected by 0.1 mm strophanthin, which markedly reduces the sodium outflux or potassium influx in barnacle muscle. Such a chloride transport system might be related to one found for squid axons by Keynes (1963), who found that the chloride influx in squid axons, presumably active on energetic grounds, was not affected by strophanthin although it was reduced by cyanide. No effect of cyanide was found for the chloride fluxes in barnacle, but probably none should have been expected because of the more active glycolytic metabolism in muscle as well as the relatively large amounts of poorly dialyzable ATP contained in the fibers.
It is also possible that the experimental flux ratio is brought close to one by the occurrence of a relatively large percentage of chloride-chloride exchange. In the present case, a chloride exchange component amounting to about 70% of the total, coupled with the usual experimental error in measuring fluxes, could permit the experimental ratio to appear reasonably close to one.

The present data are compatible with a component of chloride exchange, since replacement of 97% of the external chloride reduced the efflux by about one-half. In the case of external chloride replacement with relatively impermeant anions, the reduction in efflux could be due to the fact that, under these circumstances, chloride cannot leave the fiber unless it is accompanied by a cation, such as potassium. However a similar reduction was also seen when chloride was replaced by nitrate which Hagiwara (1971) has shown to be at least as permeable as chloride.

(b) A second quantitative difficulty with the data results from the poor correlation between the chloride conductance calculated from the tracer data and that estimated from electrical measurements.

Estimates of the chloride conductance calculated from the measured flux of 144 P/CS using either the equilibrium or nonequilibrium form of the constant field conductance equation (Brinley, 1965) give a value for chloride conductance of about 540 \( \mu \text{mhos/cm}^2 \).

Recent unpublished experiments (Caputo and DiPolo) indicate a total membrane resistance in dialyzed barnacle fibers of about 1000 ohm-cm\(^2\) (in fair agreement with Hagiwara’s (1968) value of 1500 ohm-cm\(^2\) for fibers which have received less manipulation). Assuming that the ratio potassium conductance/chloride conductance in dialyzed fibers is the same as injected fibers, i.e. about 6, then the chloride conductance would be about 135 \( \mu \text{mhos/cm}^2 \). To reconcile the tracer and electrical measurements of chloride conductance on the basis of an exchange diffusion contributing to the tracer flux but not to the electrical conductance requires about 70–75% of the efflux to be carrier mediated. This value is somewhat larger than the percentage reductions in efflux actually observed, but the external solutions were not completely chloride free. It is also possible that the chloride concentration in the deep clefts of the fibers was even higher.

It should be noted in passing that the chloride outflux was roughly proportional to the internal chloride concentration (see Table II) under circumstances in which the membrane potential was essentially unchanged. This result is of course consistent with passive diffusion, but can also be seen in other circumstances. For example, the sodium outflux in squid axons is proportional to internal sodium concentration (Brinley and Mullins, 1967; Baker et al., 1969), although the outflux is unequivocally carrier mediated. It is interesting to note that Venosa et al. (1970) have postulated the possibility of a chloride exchange diffusion in frog striated muscle.
Another explanation for the discrepancy between tracer and electrical methods may be the presence of ion pair diffusion (Shanes, 1958) whereby oppositely charged ions, by virtue of the low effective dielectric constant of the membrane, are undissociated and diffuse independently of the electric field and simply as a function of concentration from the side on which they are formed.

Chloride Efflux and Nitrate Ions

The effect of nitrate ions on the chloride efflux of barnacle muscle fibers is in accordance with that found in frog muscle fibers by Harris (1958) and Adrian (1961). Hagiwara et al. (1971) have recently reported the permeability sequence of single barnacle fibers to different anions, as determined by electrical means, showing that the permeability order for anions follows the lyotropic series, which corresponds to the absorbability of the ions (Weiser 1949). The presence of a mobility ratio, $u_{\text{NO}_3}/u_{\text{Cl}}$, constant and independent of the concentration of either Cl or NO$_3$ (Hagiwara et al., 1971), suggests that the inhibitory effect of nitrate on the chloride efflux is not due to a change in the chloride mobility within the membrane, but to a decrease in the partition coefficient membrane-solution for chloride ion as consequence of an ion-exchange equilibrium constant $K_{\text{NO}_3}^a$ greater than one, implying that nitrate ions compete with chloride for the same binding sites in the membrane.

An apparent asymmetry of the effect of the foreign anions on the frog muscle membrane has been reported by Harris (1958) and Spurway (1965), but more recently Moore (1969) has explained such apparent asymmetry by a hypothesis which takes into account the fact that the internal nitrate concentration is not constant during the experiments.

The present experiments concerning the effect of external and internal nitrate in dialyzed barnacle fibers, in which the internal nitrate concentration can be maintained, are not subject to the nonsteady-state complication. The data indicate that the inhibition of the chloride efflux by nitrate ions is symmetric with respect to the relative amounts of nitrate and chloride ions present on either side of the membrane.

Effect of External and Internal pH on the Chloride Fluxes

The pH dependence of the chloride efflux in barnacle muscle fibers is summarized in Fig. 11 by plotting the ratios of the efflux at different values of pH relative to the efflux at pH 7. Although the data will be discussed provisionally in terms of a model assuming fixed membrane charges, possible effects of the hydrogen ion on other forms of chloride transport have not been ruled out, and, in fact, the efflux changes observed at alkaline pH are not well explained by fixed charge models.

The form of the external pH curve in the range pH 3.9–5.5 is obviously
similar to the titration curve of an amphoteric substance, consistent with the presence of ionizable fixed charge groups in or on the membrane. From the theory of fixed charge membranes (Teorell, 1953), a decrease in pH is expected to increase the ratio of positively to negatively charged groups, raising the permeability ratio of chloride to potassium. The data shown in Fig. 11 give an apparent acidic isoelectric point of about 4.4 for the external membrane fixed charges. This result is consistent with the isoelectric point found by Hagiwara et al. (1968) in a study of the pH dependence of the total membrane conductance of barnacle muscle fibers. Observations made in other preparations: crustacean (Astacus) muscle fibers (DeMello and Hutter, 1966), frog nerves (Hille, 1968), and gallbladder epithelium (Wright and Diamond, 1968), also suggest the presence of fixed negative charges which are neutralized at acidic pH.

The internal pH dependence of the chloride efflux is also shown in Fig. 11 in the range pH 3.9–7.0. The shape of the curve for internal pH is considerably different, suggesting that the pK of the sites affected by internal pH changes is at a considerably less acid pH than those affected by change of external pH. Since the maximum increase in efflux is less with internal pH changes, it seems probable that the density of titratable sites is less. Several explanations may account for this asymmetric behavior: first, a model in
which the same ionogenic groups are responsible for the fixed charges on both sides of the membrane, but in which the relative amounts of acidic and basic groups are different on the two sides. For example, if the external surface of the membrane contains more acidic than basic groups the isoelectric point of the fixed charge matrix will be toward the acid side. On the other hand the presence of more basic groups in the inner face of the membrane will shift the isoelectric point to a higher value. Second, completely different ionogenic groups on the two sides of the membrane may exist, those located on the outer surface having a more acidic pK than the ones on the internal surface. Third, the electrical field profile across the membrane may not be symmetrical. As a consequence of differences in local field strength, the ionization constants of the same phosphate or carboxyl groups could vary on either side of the membrane.

Although the behavior of the efflux in highly acid solutions (pH 4–5) in terms of fixed membrane charges is plausible, it is hard to explain the flux changes seen in less acid or alkaline solutions, i.e. pH 5–9, on that basis. In this range, the chloride efflux increases although there are no known ionizable groups which could, by liberating H\(^+\) ions, increase the positive charge of the membrane. Between pH 5 and 9 the nature of the efflux change in barnacle is similar to that found in frog muscle (Hutter and Warner, 1967; Moore, 1969). In this latter tissue, the pH dependence of the resting conductance in the range pH 5–9 is qualitatively similar to that of the efflux, as might be expected since the chloride conductance represents most of the membrane conductance. However, in barnacle muscle, there is little change of the membrane conductance which is mostly potassium in this pH range (Hagiwara et al. 1968), although a significant increase in the chloride efflux is observed. A mechanism which operates through an increase in positive charge, despite the consequent accumulation of anions, has been proposed in frog muscle fibers by Hutter and Warner (1967) to account for the pH behavior of the chloride efflux in the pH range 5.0–9.0. In this model the chloride permeability depends on the fraction of sites unoccupied by anions which, in turn, is greater at high than at low pH.

The similarity of the pH dependence (pH 5.0–9.0) of the chloride efflux found in both frog and barnacle muscle fibers considered in connection with the similar inhibitory effects of nitrate on the chloride efflux suggests that, at pH 5–9 part of the mechanism of chloride permeation is common to both preparations.

In summary, from the data presented in this paper we may suggest two possible mechanisms which are involved in the chloride permeability of barnacle muscle fibers: first, exchange diffusion accounting for somewhat

\(^1\) Although it is intuitive to think that changing the internal pH affects only the inner face of the membrane and conversely for external pH changes, obviously this need not be so.

\(^2\) Note Added in Proof I am grateful to Dr. L. E. Moore for pointing out to me that more crucial
more than half of the efflux, at neutral pH. Secondly, there may be a negative ion exchange matrix, responsible for the low permeability ratio $P_{CI}/P_K$ at neutral pH and the higher ratio at values of pH lower than 4.5. At higher pH, the data are more compatible with the site occupation scheme suggested by Hutter and Warner (1967). The asymmetrical titration curves for chloride efflux may indicate structural asymmetry in the membrane as well.

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REFERENCES

ADRIAN, R. H. 1961. Internal chloride concentration and chloride efflux of frog muscle. J. Physiol. (Lond.). 156:623.

Baker, P. F., M. P. Blaustein, A. L. Hodgkin, and R. A. Steinhardt. 1969. The influence of calcium on sodium efflux in squid axons. J. Physiol. (Lond.). 200:421.

Brinley, F. J., Jr. 1963. Sodium, potassium and chloride concentrations and fluxes in the isolated giant axon of Homarus. J. Neurophysiol. 28:742.

Brinley, F. J., Jr. 1968. Sodium and potassium fluxes in isolated barnacle muscle fibers. J. Gen. Physiol. 51:445.

Brinley, F. J., Jr., and L. J. Mullins. 1967. Sodium extrusion by internally dialyzed squid axons. J. Gen. Physiol. 50:2303.

DE Mello, W., and O. F. Hutter. 1966. The anion conductance of crustacean muscle. J. Physiol. (Lond.). 183:11P.

DePolo, R., and R. Latorre. 1972. Effect of temperature on membrane potential and ionic fluxes in intact and dialyzed barnacle muscle fibres. J. Physiol. (Lond.). In press.

Grundfest, H. 1962. Ionic Transport Across Neural and Non-Neural Membranes in Properties of Membranes and Diseases of the Nervous System. M. D. Yahr, editor. Springer Publishing Company, New York.

Hagiwara, S., S. Chichibu, and K. Naka. 1964. The effects of various ions on resting and spike potentials of barnacle muscle fibers. J. Gen. Physiol. 48:163.

Hagiwara, S., R. Gruner, H., H. Hayashi, H. Sakaja, and A. D. Grinnell. 1968. Effect of external and internal pH changes on K and Cl conductances in the muscle fiber membrane of a giant barnacle. J. Gen. Physiol. 52:773.

Hagiwara, S., K. Toyama, and H. Hayashi. 1971. Mechanisms of anion and cation permeations in the resting membrane of a barnacle muscle fiber. J. Gen. Physiol. 57:408.

Harris, E. J. 1958. Anion interaction in frog muscle. J. Physiol. (Lond.). 141:351.

Hille, B. 1968. Charges and potentials at the nerve surface: divalent ions and pH. J. Gen. Physiol. 51:221.

Hodgkin, A. L., and P. Horowicz. 1959. The influence of potassium and chloride ions on the membrane potential of single muscle fibers. J. Physiol. (Lond.). 148:127.

Hoyle, G., and T. Smyth. 1963. Neuromuscular physiology of giant muscle fibers of a barnacle, balanus nubilus Darwin. Comp. Biochem. Physiol. 10:291.

Hutter, O. F., and A. E. Warner. 1967. The effect of pH on the $^{36}Cl$ efflux from frog skeletal muscle. J. Physiol. (Lond.). 189:427.

Keynes, R. D. 1963. Chloride in the squid giant axon. J. Physiol. (Lond.). 189:490.

Moore, L. E. 1969. Anion permeability of frog skeletal muscle. J. Gen. Physiol. 44:33.

Reuben, J. P., L. Girardier, and H. Grundfest. 1962. The chloride permeability of crayfish muscle fibers. Biol. Bull. 122:309.

Richards, C. D. 1969. Chloride fluxes in crab muscle fibers. J. Physiol. (Lond.). 202:211.

Selverstone, A. 1967. Structure and function of the transverse tubular system in crustacean muscle fibers. Am. Zool. 7:515.

evidence in favor of an exchange diffusion mechanism could be obtained by measuring the chloride transport number in dialyzed barnacle muscle fibres.
SHANES, A. M. 1958. Electrochemical aspects of physiological and pharmacological action in excitable cells. *Pharmacol. Rev.* 10:59.

SPURWAY, N. C. 1965. The site of anion interaction in frog skeletal muscle. *J. Physiol. (Lond.)* 178:51P.

TEORELL, T. 1953. Transport processes and electrical phenomena in ionic membranes. *Progr. Biophys. Mol. Biol.* 3:305.

VENOSA, R. A., A. C. RUARTE, and R. HOROWITZ. 1970. Stimulation of chloride efflux in frog striated muscle by aromatic acids. *Biophys. Soc. 14th Annu. Meet. Abstr.* 222a.

WEISER, H. S. 1949. Colloid Chemistry. John Wiley and Sons Inc., New York. 2nd edition. 296.

WRIGHT, E. M., and J. M. DIAMOND. 1968. Effects of pH and polyvalent cations on the selective permeability of gallbladder epithelium to monovalent ions. *Biochin. Biophys. Acta.* 163:57.