Intramembranous Charge Movement in Frog Cut Twitch Fibers Mounted in a Double Vaseline-Gap Chamber

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ABSTRACT Intramembranous charge movement was measured in cut twitch fibers mounted in a double Vaseline-gap chamber with either a tetraethylammonium chloride (TEA·Cl) or a TEA·SO₄ solution (13–14°C) in the central pool. Charge vs. voltage data were fitted by a single two-state Boltzmann distribution function. The average values of $\bar{V}$ (the voltage at which steady-state charge is equally distributed between the two Boltzmann states), $k$ (the voltage dependence factor), and $q_{\text{max}}/c_m$ (the maximum charge divided by the linear capacitance, both per unit length of fiber) were $V = -53.3 \text{ mV}$ (SEM, 1.1 mV), $k = 6.3 \text{ mV}$ (SEM, 0.3 mV), $q_{\text{max}}/c_m = 18.0 \text{ nC}/\mu\text{F}$ (SEM, 1.1 nC/μF) in the TEA·Cl solution; and $V = -35.1 \text{ mV}$ (SEM, 1.8 mV), $k = 10.5 \text{ mV}$ (SEM, 0.9 mV), $q_{\text{max}}/c_m = 36.3 \text{ nC}/\mu\text{F}$ (SEM, 3.2 nC/μF) in the TEA·SO₄ solution. These values of $k$ are smaller than those previously reported for cut twitch fibers and are as small as those reported for intact fibers. If a correction is made for the contributions of currents from under the Vaseline seals, $V = -51.2 \text{ mV}$ (SEM, 1.1 mV), $k = 7.2 \text{ mV}$ (SEM, 0.4 mV), $q_{\text{max}}/c_m = 22.9 \text{ nC}/\mu\text{F}$ (SEM, 1.4 nC/μF) in the TEA·Cl solution; and $V = -34.0 \text{ mV}$ (SEM, 1.9 mV), $k = 10.1 \text{ mV}$ (SEM, 1.1 mV), $q_{\text{max}}/c_m = 38.8 \text{ nC}/\mu\text{F}$ (SEM, 3.2 nC/μF) in the TEA·SO₄ solution. With this correction, however, the fit of the theoretical curve to the data is poor. A good fit with this correction can be obtained with a sum of two Boltzmann distribution functions. The first has average values $\bar{V} = -33.0 \text{ mV}$ (SEM, 2.8 mV), $k = 11.0 \text{ mV}$ (SEM, 0.5 mV), $q_{\text{max}}/c_m = 10.6 \text{ nC}/\mu\text{F}$ (SEM, 1.0 nC/μF) in the TEA·Cl solution; and $V = -20.0 \text{ mV}$ (SEM, 3.3 mV), $k = 17.0 \text{ mV}$ (SEM, 2.0 mV), $q_{\text{max}}/c_m = 36.4 \text{ nC}/\mu\text{F}$ (SEM, 2.3 nC/μF) in the TEA·SO₄ solution. The second has average values $\bar{V} = -56.5 \text{ mV}$ (SEM, 1.8 mV), $k = 2.9 \text{ mV}$ (SEM, 0.4 mV), $q_{\text{max}}/c_m = 13.2 \text{ nC}/\mu\text{F}$ (SEM, 1.0 nC/μF) in the TEA·Cl solution; and $V = -41.6 \text{ mV}$ (SEM, 1.4 mV), $k = 2.5 \text{ mV}$ (SEM, 0.8 mV), $q_{\text{max}}/c_m = 11.8 \text{ nC}/\mu\text{F}$ (SEM, 1.7 nC/μF) in the TEA·SO₄ solution. When a fiber is depolarized to near $\bar{V}$ of the second Boltzmann function, a slowly developing "hump" appears in the ON-segment of the current record.

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These results show that a steeply voltage-dependent component of charge, with properties similar to those of $Q_a$ charge in intact fibers (Adrian, R.H., and A. Peres. 1979. Journal of Physiology. 289:83–97), can be clearly resolved in cut fibers.

**INTRODUCTION**

The preceding article (Chandler and Hui, 1990) described measurements of linear capacitance in frog cut muscle fibers mounted in a double Vaseline-gap chamber. The end-pool segments of the fibers were treated briefly with saponin to render their plasma membranes (surface and transverse tubular membranes) permeable to small molecules and ions. According to one-dimensional cable theory, only about two-thirds of the transient "capacitive" current that is measured in such an experiment actually flows across the plasma membranes in the central-pool region. The remaining one-third flows either across the plasma membranes under the two Vaseline seals or directly from the current-passing end pool to the central pool, across the external longitudinal resistance under the Vaseline seal.

Similar pathways for current flow occur during intramembranous charge movement: some of the charge that is measured reflects a redistribution of charge within membranes in the central pool and the rest moves through other electrical pathways in the chamber. One of the initial aims of this investigation was to understand these pathways and to find out whether they might account for the previous findings that charge vs. voltage curves in cut fibers (work of others in Table VII B, column 6) are less steep than those reported in intact fibers (Table VII A, column 6). According to calculations in the first section of Results, however, the charge vs. voltage curve that is measured in a double Vaseline-gap experiment should have approximately the same voltage steepness as the charge vs. voltage curve of the fiber membrane. This suggested that the large values of $k$ that have been obtained in cut fibers in other laboratories might reflect a genuine property of charge movement in this preparation.

This article describes measurements of charge movement in cut fibers mounted in a double Vaseline-gap chamber, with either a tetraethylammonium chloride (TEA·Cl) or a TEA$_2$·SO$_4$ solution in the central pool. One of the interesting, and surprising, findings was that, when charge vs. voltage data were fitted with a single Boltzmann distribution function, the average values of $k$ were 6.3 mV (SEM, 0.3 mV) in the TEA·Cl solution, and 10.5 mV (SEM, 0.9 mV) in the TEA$_2$·SO$_4$ solution. These values are smaller than any of those previously reported for cut fibers and, in fact, are as small as those reported for intact fibers (Table VII, column 6). Moreover, a sum of two Boltzmann functions provided a significantly better fit to the charge vs. voltage data than did a single Boltzmann function. The two components of charge associated with the two Boltzmann functions appear to correspond to the $Q_a$ and $Q_v$ components that have been described in intact fibers (Adrian and Peres, 1979).

This article also shows that the charge movement that is measured at hyperpolarized potentials in a double Vaseline-gap experiment—for example, between −100 and −150 mV—contains a small contribution from intramembranous charge that moves in depolarized regions under the Vaseline seals. Consequently, the double Vaseline-gap technique is not reliable for measuring either intramembranous charge movement or capacitance at hyperpolarized potentials.
Although the theoretical analysis presented in this article is for a double Vaseline-gap technique, the general approach may be useful for analyzing results obtained with other Vaseline gap techniques, such as those that use one, three, or four seals.

A preliminary report of some of this work was presented to the Biophysical Society (Hui and Chandler, 1988).

METHODS

The experiments, which were performed at Yale University School of Medicine, were carried out on voltage-clamped frog cut twitch fibers that had been mounted in a double Vaseline-gap chamber (Fig. 1 A), as described in the preceding article (Chandler and Hui, 1990). $V_1(t)$ denotes the potential that is measured in the potential-measuring end pool EP₁, and $I_1(t)$ denotes the current that is injected into the current-passing end pool EP₂ and is collected in the central pool CP, which is maintained at earth potential. $V_2(t)$ denotes the potential in EP₂. Either a TEA-CI or a TEA₂SO₄ solution, maintained at 13-14°C, was used in the central pool. After a holding potential of $-90$ mV was maintained for at least 30 min, charge movement was measured by applying a sequence of depolarizing pulses of progressively increasing amplitude at a frequency of 1/min. Bracketing measurements at intermediate levels of depolarization were made at the end of a sequence. All traces shown in this article are single sweeps.

The next two subsections describe the procedures that were used to estimate charge movement currents from records of CONTROL and TEST $I_1(t)$ currents. The last subsection describes the contributions that are made to these charge movement currents by currents that cross the plasma membranes (surface and transverse tubular membranes), both in the central recording region and under the two Vaseline seals, and by currents that flow directly from the two end pools to the central pool, across the external longitudinal resistances under the Vaseline seals.

Determination of the CONTROL Capacitive Transient and CONTROL Conductance

The methods that were used to estimate the CONTROL capacitive transient and CONTROL conductance are similar to those employed in other laboratories. Electrical measurements were made while the voltage $V_1(t)$ was changed in a stepwise fashion between a holding potential of $-90$ mV and a hyperpolarizing potential of $-110$ mV. The pulse protocol for a single CONTROL measurement usually consisted of a prepulse from $-90$ mV to $-110$ mV followed by three voltage transitions $-110$ to $-90$ mV, $-90$ to $-110$ mV, and $-110$ to $-90$ mV (Fig. 2 A in Chandler and Hui, 1990). The interval of time between the two transitions from $-110$ to $-90$ mV was selected to coincide with an integral number of 60 Hz cycles plus half a cycle, so that the average of the two signals associated with the transitions from $-110$ to $-90$ mV was free of any noise synchronized to the line frequency. The CONTROL signals were usually signal averaged four times and the baseline that preceded the $-110$ to $-90$ mV transition was subtracted from the record.

The CONTROL $\Delta I_1(t)$ record was modified to increase the signal-to-noise ratio. The final part of each record, beginning 11 ms after the transition of $V_1(t)$ from $-110$ to $-90$ mV, was least-squares fitted by a sum of two exponential functions plus a constant. The theoretical curve was found to provide an excellent fit to the corresponding part of the $\Delta I_1(t)$ record so that, for further analysis, its values were substituted for those in the record. Thus, the first 10 ms of a CONTROL $\Delta I_1(t)$ record consisted of measured points and the rest consisted of points that were calculated from the fitted two exponential functions plus a constant. The fitted constant was used for the value of CONTROL $\Delta I_1(0)$. 
FIGURE 1. (A) Schematic diagram of the experimental chamber that was used to record electrical signals from a cut muscle fiber. CP denotes the central pool, of length $L_2$ (500 $\mu$m in our chamber), and EP$_1$ and EP$_2$ denote the two end pools. VS denotes the Vaseline seals, of length $L_1$ (300 $\mu$m in our chamber), that separate the two end pools from the central pool. EP$_1$ was used to measure the potential $V_1(t)$, and EP$_2$ was used to pass the current $I_s(t)$ and to measure the potential $V_2(t)$. (B) Spatial variation of the steady-state potentials $V(x, \infty)$ (internal) and $V_e(x, \infty)$ (external) along a cut fiber in which the plasma membrane resistance, $r_m$, is infinite. It has been assumed that there is no potential difference across the plasma membranes in the end pools because of the saponin treatment. (C) Spatial variation of the weighting function $w(x)$, Eq. A23, that is used to multiply the plasma membrane currents along the fiber to give their contributions to $I_s(t)$, Eqs. 4, 5, and A22. Additional information is given in the text.

The CONTROL $\Delta V(t)$ record was also modified. The baseline that preceded the transition from $-110$ to $-90$ mV was subtracted from the record. After the first 5 ms, the experimental points were least-squares fitted by a single exponential function plus a constant. The fit was excellent and the experimental points, after the first 5 ms, were replaced with the fitted points,
similar to the procedure used for the CONTROL $\Delta I_2(t)$ record. All points were then divided by the value of the fitted constant, which was used for the value of $\Delta V_I(\infty)$, to give a normalized voltage template that approached a value of unity at late times.

The CONTROL $\Delta I_2(t)$ record was assumed to consist of two components, ohmic (ionic) and capacitive. The ohmic component was estimated by multiplying the normalized voltage template by the value of CONTROL $\Delta I_2(\infty)$. The capacitive component was estimated by subtraction of the ohmic component from the CONTROL $\Delta I_2(t)$ record. The CONTROL conductance was obtained by dividing CONTROL $\Delta I_2(\infty)$ by CONTROL $\Delta V_I(\infty)$.

**Determination of Charge Movement Currents, $\Delta I_{cm}$**

TEST $\Delta I_2(t)$ signals were recorded during stepwise changes in $V_I(t)$, usually from and back to the holding potential of $-90$ mV. Each TEST pulse was preceded by its own sequence of CONTROL pulses. The TEST $\Delta I_2(t)$ record was corrected for capacitive and ohmic components by subtraction, respectively, of the CONTROL capacitive component, scaled by the ratio of TEST:CONTROL $\Delta V_I(\infty)$, and of the normalized voltage template, scaled by the product of CONTROL conductance times TEST $\Delta V_I(\infty)$. The noise that is introduced by these corrections is decreased by the substitution of calculated points for experimental points in the final parts of the CONTROL $\Delta I_2(t)$ and $\Delta V_I(t)$ records, as described in the preceding subsection. In this article, the term “TEST minus CONTROL current” will be used to refer to the corrected TEST current.

The records in Figs. 6 and 10 provide examples of TEST minus CONTROL currents. After each step depolarization, the current shows a transient outward component, which is thought to arise from intramembranous charge movement, that is followed by a nearly constant current, which is thought to arise from ionic movement across a nonlinear resistance in the plasma membranes (since the external resistances under the Vaseline seals are expected to be linear). On repolarization, the TEST minus CONTROL current shows a transient inward component that is followed by a current that is nearly constant and close to the level of the prestimulus baseline.

After either depolarization or repolarization, the time course of the late phase of the residual ionic current was assumed to follow a sloping straight line. This line was estimated by a least-squares fit of a single exponential function plus a sloping straight line to the final phase of the transient component (Chandler et al., 1976a). The sloping straight line from the fit (see Fig. 6), scaled by the normalized voltage template, was then used to estimate the ionic current at both late times and during the transient. The difference between the TEST minus CONTROL current and the ionic current is identified with charge movement and is denoted by $\Delta I_{cm}(t)$. The change in charge, $\Delta Q_{cm}$, that is produced by this current is given by

$$\Delta Q_{cm} = \int_0^\infty \Delta I_{cm}(t) \, dt.$$  \hspace{1cm} (1)

In practice, the upper limit of integration was the last point that was used for the least-squares fit that determined the sloping straight line for the residual ionic current.

In this article, the CONTROL capacitive transient was measured from $V_I = -110$ to $-90$ mV and $\Delta Q_{cm}$ was measured from a holding potential of $V_I = -90$ mV. Consequently, $\Delta Q_{cm} = 0$ at $V_I = -110$ and $-90$ mV. Throughout this article, each theoretical curve that has been used to fit a set of charge vs. voltage data (such as those in Figs. 3, 8, 9, 12, 13, and 14 A) has been corrected by subtraction of the appropriate sloping straight line to have a value of zero at $-110$ and $-90$ mV.
Contributions to Linear and Nonlinear Charge from Currents That Cross Plasma Membranes Either in the Central Recording Region or under the Two Vaseline Seals and from Currents That Flow from the Two End Pools to the Central Pool under the Vaseline Seals

Fig. 1 A shows a schematic diagram of the double Vaseline-gap chamber that was used in our experiments. Since saponin treatment was used to render the plasma membranes in the end pools permeable to small molecules and ions, we have assumed (in particular, in Fig. 1 B) that there is no potential difference across these treated membranes.

In the preceding article (Chandler and Hui, 1990), \( C_{\text{app}} \), the apparent linear capacitance of a cut twitch fiber mounted in a double Vaseline-gap chamber, was defined by

\[
C_{\text{app}} = \frac{\int_0^\infty \Delta I_{2,v}(t) \, dt}{\Delta V_1(\infty)}.
\]

\( C_{\text{app}} \) satisfies the relation

\[
C_{\text{app}} = l_{\text{app}} c_m,
\]

in which \( c_m \) is the linear capacitance per unit length of fiber and \( l_{\text{app}} \) is the apparent length of fiber that contributes to \( C_{\text{app}} \). The value of \( l_{\text{app}} \) depends on \( L_1 \) and \( L_2 \) (Fig. 1 A) and on the cable parameters \( r_m \) (membrane resistance), \( r_l \) (longitudinal internal resistance), and \( r_e \) (external longitudinal resistance under the Vaseline seals), all expressed in terms of a unit length of fiber. The value of \( l_{\text{app}} \) is independent of the value of \( c_m \).

In the eight experiments listed in Table IIA of Chandler and Hui (1990), the value of \( l_{\text{app}} \), 735–816 \( \mu \)m, was appreciably greater than that of \( L_1 \), 500 \( \mu \)m, mainly because the Vaseline seals do not provide perfect electrical insulation. Thus, the current that is collected in the central-pool region contains contributions from currents that cross the plasma membranes under the Vaseline seals and from currents that flow directly from the two end pools to the central pool across the external resistances under the Vaseline seals.

In the Appendix of this article, one-dimensional cable theory is used to derive an expression (Eq. A22) that relates the current that is collected in the central pool, \( I_2(t) \), to the various pathways of current flow within the chamber,

\[
I_2(t) = \int_0^{2L_1 + L_2} w(x) i_m(x, t) \, dx + \frac{V_1(t) + V_2(t)}{L_1 r_e}.
\]

\( i_m(x, t) \) represents the current density across the plasma membranes per unit length of fiber and \( L_1 r_e \) represents the external longitudinal resistance under each Vaseline seal. Fig. 1 C shows the weighting function \( w(x) \) plotted as a function of distance from the left-hand edge of the left-hand Vaseline seal to the right-hand edge of the right-hand Vaseline seal, from Eq. A23.

In the central-pool region (from \( L_1 \) to \( L_2 \)), the value of \( w(x) \) is unity; under each Vaseline seal (from 0 to \( L_1 \) and from \( L_1 + L_2 \) to \( 2L_1 + L_2 \)), it varies linearly from a value of unity at the central-pool edge to a value of zero at the end-pool edge.

As shown in the Appendix, Eq. 4 is simplified considerably if the value of \( r_m \) is sufficiently large that ohmic currents across the plasma membranes can be neglected. This approximation is expected to introduce little error into the estimate of charge movement since it introduces little error into the estimate of linear capacitance (Fig. 11 and associated text in Chandler and Hui, 1990). In this case, Eq. A31 shows that \( \Delta Q \), the change in both linear and nonlinear
charge that is measured in the preparation \( \Delta Q \) is given by

\[
\Delta Q = \int_0^{L_1 + L_2} w(x) \Delta q_m(x, \infty) \, dx + \frac{1}{L_1 \tau_e} \int_0^\infty \Delta V_{z \nu}(t) \, dt.
\]

(5)

\( \Delta q_m(x, \infty) \) is equal to the amount of charge per unit length of fiber, both linear and nonlinear, that is displaced across the plasma membranes in response to a change in potential \( \Delta V_\nu(\infty) \). \( \Delta V_{z \nu}(t) \) denotes the transient component of \( \Delta V_\nu(t) \), defined in Eq. A30. Thus, \( \Delta Q \) consists of two components. The first component, which is given by the first term in Eq. 5, is equal to \( \Delta q_m(x, \infty) \) weighted according to \( w(x) \). The value of \( w(x) \) in the central-pool region is unity and that under each Vaseline seal varies according to distance from the end pool (Fig. 1 C). The second component, which is given by the second term in Eq. 5, is the amount of charge carried by the current that is driven by the potential \( \Delta V_{z \nu}(t) \) across the external resistance under the Vaseline seal between the central pool and end pool 2.

The first term on the right-hand side of Eq. 5 can be calculated from \( w(x) \) (Fig. 1 C and Eq. A23) and an assumed relation between \( \Delta q_m \) and the steady-state membrane potential, \( V_m(x, \infty) \). \( V_m(x, \infty) \) is equal to the difference between \( V(x, \infty) \) and \( V(x, \infty) \), the steady-state potentials inside and outside a cut fiber, respectively. These are plotted in Fig. 1 B as a function of distance from the left-hand edge of the left-hand Vaseline seal. Since \( r_m \) is assumed to be infinite, no steady-state current is able to cross the plasma membranes and the relation between \( V(x, \infty) \) and \( x \) is linear for \( 0 \leq x \leq 2L_1 + L_2 \), with \( V(0, \infty) = V(\infty) \) and \( V(2L_1 + L_2, \infty) = V(\infty) \) (since the saponin treatment of the end-pool segments is assumed to render the plasma membranes very permeable to small ions). The value of \( V(x, \infty) \) is equal to zero in the central-pool region. Under the Vaseline seals, it varies linearly with distance, with \( V(0, \infty) = V(\infty) \), \( V(2L_1 + L_2, \infty) = V(\infty) \), and \( V(L_1, \infty) = V(L_1 + L_2, \infty) = 0 \).

There is no simple way to evaluate the second term on the right-hand side of Eq. 5. Although the value of the integral of \( \Delta V_{z \nu}(t) \) can be measured experimentally, \( \tau_e \) cannot be estimated reliably without the use of microelectrodes (Chandler and Hui, 1990). As considered next, however, this term can probably be neglected since both the experimental and (fitted) theoretical values of \( \Delta Q_m \) are normalized by \( C(\infty) \), the value of capacitance that is measured when \( V_i \) is changed from -110 to -90 mV. With \( r_m = 0.3 \text{M}/\text{cm}, r_i = 3 \text{M}/\text{cm}, r_\nu = 100 \text{M}/\text{cm}, c_m = 0.2 \mu \text{F}/\text{cm} \) and linear membrane properties, the theoretical value of \( \int_0^\infty \Delta V_{z \nu}(t) \, dt / L_1 \tau_e \) for measurements of capacitance is 0.05 times the total value (Table IV in Chandler and Hui, 1990). Hence, a 5% error is introduced into the theoretical value of \( C(\infty) \) by failure to include the \( \int_0^\infty \Delta V_{z \nu}(t) \, dt / L_1 \tau_e \) term. Since this error depends on \( r_m, r_i \), and \( r_\nu \), but not on \( c_m \), it is not expected to be very different for \( \Delta Q_m \). If it is the same, the theoretical estimates of \( \Delta Q_m \) and \( C(\infty) \) would be in error by the same relative amount and the theoretical value of \( \Delta Q_m / C(\infty) \) that is fitted to an experiment point would be without error. If the relative errors in \( \Delta Q_m \) and \( C(\infty) \) are different, an error would be introduced into the theoretical value of \( \Delta Q_m / C(\infty) \), but it seems likely that this error would be small.

**RESULTS**

**Calculations That Illustrate How the Measurement of a Charge vs. Voltage Curve Can Be Distorted by the Double Vaseline-Gap Recording Technique**

The calculations in this section were carried out on the assumption that intramembranous charge can exist in one of two states, which will be called resting and active; \( q \) denotes the amount of charge per unit length of fiber that is in the active state. The
steady-state value of $q$ was calculated from the equation for a two-state Boltzmann distribution,

$$q = \frac{q_{\text{max}}}{1 + \exp \left[ -(V - \overline{V})/k \right]} \quad (6)$$

in which $q_{\text{max}}$ represents the maximum amount of charge per unit length of fiber, $\overline{V}$ represents the voltage at which half the charge has moved into the active state, and $k$ is a voltage dependence factor.

The sigmoid curve in Fig. 2 A shows $q/q_{\text{max}}$ plotted as a function of membrane potential $V$, from Eq. 6 with $\overline{V} = -50$ mV and $k = 6$ mV. In a charge movement experiment, the current associated with a TEST pulse, from a voltage of $-90$ mV to $V$, is compared with that associated with a CONTROL pulse, from $-110$ to $-90$ mV, that has been scaled by the ratio TEST:CONTROL voltage change. If all the current came from membrane under uniform voltage control, $\Delta Q_{\text{em}}$ would be given by the difference between the sigmoid curve in Fig. 2 A and the straight line that intersects this curve at $V = -110$ and $-90$ mV (which is just visible in the right-hand part of Fig. 2 A).

In a double Vaseline-gap experiment, however, the plasma membranes from which current is collected are not under uniform voltage control. To estimate the charge movement that would be measured under these conditions, the charge vs. voltage curve in Fig. 2 A was used to represent charge movement in the plasma membranes and the spatial variations of $w(x)$ and steady-state membrane potential were taken from Fig. 1 C and B, respectively. The ratio $r_{e}/(r_{i}+r_{e})$ was set equal to 0.971, the value given by $r_{i} = 3 \text{ M\Omega/cm}$ and $r_{e} = 100 \text{ M\Omega/cm}$. The sigmoid curve in Fig. 2 B shows $Q$, the charge that would be measured if $V_{a}$ were taken from a very negative voltage to $V_{l}(\infty)$. The values of $Q$ have been normalized by $C(-\infty)$ and by $q_{\text{max}}/r_{i}$; $C(-\infty)$ represents the linear capacitance of the preparation, which can be evaluated with voltage steps at strongly negative potentials where there are no displacement currents from intramembranous charge movement. The curve in Fig. 2 B has a foot at negative potentials. This occurs because, as $V_{l}(\infty)$ is made progressively more negative, there is a decrease in the length of fiber that is strongly depolarized under each Vaseline seal adjacent to its end pool. Consequently, progressively more charge moves from the active to the resting state. At positive potentials, the curve approaches a value slightly greater than unity. This is because, for charge movement, the maximum value of the first term on the right-hand side of Eq. 5 is equal to $(L_{2} + L_{3})q_{\text{max}} = (0.08 \text{ cm})q_{\text{max}}$ whereas, for capacitance, the term is equal to $0.5[1 + \Delta V_{2}(\infty)/\Delta V_{1}(\infty)](L_{2} + 2L_{1}/3)c_{m} = [1 + (r_{i}/r_{e})(1 + 0.5L_{2}/L_{1})](L_{2} + 2L_{1}/3)c_{m} = (0.074 \text{ cm})c_{m}$ (Chandler and Hui, 1990). The amount of charge that would be measured in a charge movement experiment is given by the difference between the sigmoid curve in Fig. 2 B and the straight line that intersects it at $V = -110$ and $-90$ mV, similar to the situation described above for Fig. 2 A.

Fig. 2 C shows the contribution that is made by charge movement to membrane capacitance, plotted as a function of $V_{l}(\infty)$. The curve, which represents the derivative of the sigmoid curve in Fig. 2 B, has a foot at negative voltages, owing to intramembranous charge movement in the segments of fiber that are under the Vaseline seals adjacent to the end pools.
FIGURE 2. Theoretical estimate of the charge movement that would be measured in a double Vaseline-gap experiment on a cut fiber in which \( r_m \) is infinite and \( r_e/(r_i + r_e) = 0.971 \).

(A) \( q \), the intramembranous charge per unit length of fiber that is in the active state, is plotted against \( V \), the membrane potential. The curve was calculated for a two-state Boltzmann distribution, Eq. 6, with \( V = -50 \) mV and \( k = 6 \) mV and has been normalized by \( q_{\text{max}} \). (B) The sigmoid curve shows the theoretical contribution of the intramembranous charge in A to the charge that would be measured in a double Vaseline-gap experiment, for the initial condition that all the charge in the central-pool and Vaseline-seal regions is in the resting state. This contribution, denoted by \( Q \), has been normalized by \( C(-\infty) \) and by \( q_{\text{max}}/C_m \). It has been plotted against \( V_1(\infty) \), the steady-state potential measured in EP1 (Fig. 1 A). \( Q \) and \( C(-\infty) \) were calculated from the first factor on the right-hand side of Eq. 5. In A and B, sloping straight lines intersect the curves at \( V = -110 \) and -90 mV. (C) The curve shows the theoretical contribution to the measured capacitance that is made by the intramembranous charge in B, plotted against \( V_1(\infty) \). This contribution, denoted by \( C(V_1(\infty)) \) and normalized by \( C(-\infty) \cdot q_{\text{max}}/C_m \), is equal to the derivative of the sigmoid curve in B. See text for additional information.

The circles in Fig. 3 show the values of charge movement, \( \Delta Q_{\text{cap}} \), that would be measured in an experiment on the hypothetical fiber used for Fig. 2. The values of \( \Delta Q_{\text{cm}} \) are given by the differences between the values of the sigmoid curve and those of the straight line in Fig. 2 B. The sigmoid curve in Fig. 3 represents a best
least-squares fit of a two-state Boltzmann distribution function, Eq. 6, after subtraction of the straight line that intersected the Boltzmann curve at $-110$ and $-90$ mV. The fitted values of $\bar{V}$ and $k$ were $-50.6$ and $5.9$ mV, respectively, which are similar to the values that were used to generate the points, $-50$ and $6$ mV, respectively. This similarity indicates that, at least under some conditions, the double Vaseline-gap method can give reasonably reliable estimates of $\bar{V}$ and $k$. The maximal value of the curve in Fig. 3, however, is 0.80, indicating that an estimate of $q_{\text{max}}/C_m$ would be only 80% of the actual value.

![Figure 3](image3.png)

**Figure 3.** A Boltzmann distribution function fitted to charge vs. voltage points calculated for a hypothetical double Vaseline-gap experiment. The filled circles were obtained from the sigmoid curve in Fig. 2B after subtraction of the sloping straight line. The continuous curve represents the least-squares fitted Boltzmann distribution curve, also after subtraction of the sloping straight line that intersected that curve at $V = -110$ and $-90$ mV. The parameters of the fitted Boltzmann function are $\bar{V} = -50.6$ mV and $k = 5.9$ mV; its maximal value is 0.80.

![Figure 4](image4.png)

**Figure 4.** (A) A family of Boltzmann distribution functions of intramembraneous charge and (B) the corresponding charge that would be measured in a double Vaseline-gap experiment. The calculations and format are similar to those in Fig. 2, $A$ and $B$. $r_m$ was taken to be infinite, $r_e/(r_e + r_p) = 0.971$, and $\bar{V} = -50$ mV. The values of $k$ were $0, 2, 4, 6, 9, 12, 15,$ and $20$ mV.
Calculations similar to those in Fig. 2, A and B were carried out in which \( V \) was equal to \(-50\) mV (close to the average value that was found in our experiments with a TEA-CI solution in the central pool; see Table II) and \( k \) was varied from 0 to 20 mV. Fig. 4 A, which is similar to Fig. 2 A, shows the family of Boltzmann distribution functions that were calculated from Eq. 6. Fig. 4 B, which is similar to Fig. 2 B, shows

### Table I

| Assumed values | Fitted values | \( C(V,\infty)/[C(-\infty)q_{\text{mem}}/c_{\text{m}}] \) |
|----------------|---------------|------------------|
| \( V \) | \( k \) | Maximum | \( -150 \text{ mV} \) | \( -100 \text{ mV} \) |
| mV | mV | mV | mV | | |
| A  | | | | | |
| \(-50\) | 0 | \(-49.2\) | 0.6 | 0.82 | 0.00054 | 0.00185 |
| \(-50\) | 2 | \(-49.4\) | 2.6 | 0.82 | 0.00054 | 0.00184 |
| \(-50\) | 4 | \(-50.2\) | 4.3 | 0.81 | 0.00055 | 0.00187 |
| \(-50\) | 6 | \(-50.6\) | 5.9 | 0.80 | 0.00057 | 0.00193 |
| \(-50\) | 9 | \(-50.9\) | 8.4 | 0.79 | 0.00060 | 0.00217 |
| \(-50\) | 12 | \(-51.0\) | 11.1 | 0.78 | 0.00065 | 0.00265 |
| \(-50\) | 15 | \(-50.9\) | 14.0 | 0.80 | 0.00073 | 0.00322 |
| \(-50\) | 20 | \(-50.7\) | 19.3 | 0.84 | 0.00094 | 0.00401 |
| B  | | | | | |
| \(-70\) | 7 | \(-70.0\) | 10.0 | 1.01 | 0.00110 | 0.00428 |
| \(-60\) | 7 | \(-61.6\) | 10.4 | 0.93 | 0.00082 | 0.00289 |
| \(-50\) | 7 | \(-50.7\) | 6.7 | 0.80 | 0.00058 | 0.00198 |
| \(-40\) | 7 | \(-41.0\) | 7.4 | 0.89 | 0.00038 | 0.00130 |
| \(-30\) | 7 | \(-31.1\) | 7.8 | 0.97 | 0.00023 | 0.00078 |
| \(-20\) | 7 | \(-21.0\) | 8.1 | 1.03 | 0.00012 | 0.00041 |
| \(-10\) | 7 | \(-10.6\) | 8.0 | 1.06 | 0.00005 | 0.00017 |
| 0 | 7 | 0.0 | 7.8 | 1.06 | 0.00002 | 0.00006 |

Intramembranous charge movement was assumed to obey Eq. 6, the equation for a two-state Boltzmann distribution, with the values of \( V \) and \( k \) given in columns 1 and 2, respectively. The Boltzmann distribution functions for A and B are plotted in Figs. 4 A and 5 A, respectively. For each Boltzmann curve, the amount of charge movement that would be measured in a double Vaseline-gap experiment was calculated as described in the text and illustrated in Figs. 4 B (for part A of this table) and 5 B (for part B of this table). The difference between each calculated curve in Fig. 4 B or 5 B and the straight line that intersects it at \(-110\) and \(-90\) mV was determined at 5-mV intervals between \(-80\) and \(+20\) mV. The difference points were least-squares fitted by the difference between the Boltzmann curve and the straight line that intersects it at \(-110\) and \(-90\) mV, as illustrated in Fig. 3. The fitted values of \( V \) and \( k \) are given in columns 3 and 4, respectively, and the maximum values of the fitted curve are given in column 5. Columns 6 and 7 give the values of \( C(V,\infty)/[C(-\infty)q_{\text{mem}}/c_{\text{m}}] \) at \(-150\) and \(-100\) mV, respectively; these represent the slopes of the curves in Figs. 4 B (part A) and 5 B (part B) at the two voltages. See text for additional information.

The theoretical values of intramembranous charge movement were calculated from each of the curves in Fig. 4 B after subtraction of the straight line that intersected the corresponding family of charge vs. voltage curves for a fiber mounted in a double Vaseline-gap chamber. Each of the curves, including the one that was calculated with \( k = 0\) mV, has a significant foot at negative voltages.
the curve at -110 and -90 mV. Points between -80 and +20 mV, at 5-mV intervals, were fitted by a Boltzmann distribution function that also had been corrected by subtraction of the straight line that intersected the Boltzmann curve at -110 and -90 mV, similar to the procedure used in Fig. 3. Columns 1 and 2 of Table I A give the values of $V$ and $k$, respectively, that were used for each of the curves in Fig. 4. Columns 3 and 4 give the fitted values of $V$ and $k$, respectively, and column 5 gives the maximum values of the fitted curves.

Fig. 5 shows the results of another set of calculations, similar to those illustrated in Fig. 4, in which $k$ was equal to 7 mV (close to the average value that was found in our experiments with a TEA.Cl solution in the central pool; see Table II) and $V$ was varied from -70 to 0 mV. Columns 1-5 of Table I B give the parameters that are associated with each value of $V$.

The values of $V$ and $k$ in columns 3 and 4 in Table I, A and B are, in general, close to the corresponding values in columns 1 and 2. For $V \geq -50$ mV, the difference is $\leq 1.1$ mV, indicating that the double Vaseline-gap technique can provide reliable estimates of the position and steepness of a charge vs. voltage curve in this voltage range. On the other hand, the maximal values of the fitted curves (column 5) depend on the value of $V$. For $V = -50$ mV (close to the average value that was found in our experiments with a TEA.Cl solution in the central pool; see Table II), the double Vaseline-gap technique appears to underestimate $q_{\text{max}}/C_{\text{m}}$ by $\sim 20\%$.

**TEST minus CONTROL currents with a TEA.Cl Solution in the Central Pool**

The top trace in Fig. 6 A shows a record of $\Delta V_{g}(t)$ that was associated with a 200-ms depolarization from the holding potential of -90 mV to a TEST potential of -40
mV. The other traces in Fig. 6 show records of TEST minus CONTROL currents that were obtained from records of $\Delta I_d(t)$, with the procedure described in Methods. The vertical gain for the TEST minus CONTROL current traces in Fig. 6 B is one-third that in Fig. 6 A.

The first current record in Fig. 6 A, which is associated with a depolarization to $-70$ mV, shows a small outward current on depolarization, referred to as the "ON" current, which consists of an early transient component followed by a maintained component. After repolarization, there was a small transient inward current, referred to as the "OFF" current, that decayed to near the prestimulus baseline. The next current record, associated with a pulse to $-60$ mV, has an early transient outward component that is larger than that in the $-70$ mV trace. There is also a slight suggestion of a late, slowly decaying outward transient. This late component of ON current became progressively more prominent in the $-56$, $-52$, and $-48$ mV records and, at $-40$ mV, the amplitude of the late ON component exceeded that of the early ON component. Both the amplitude and the rates of development and decay of the late component increased as the TEST potential was made progressively more positive. The early and late components of ON charge currents are similar,
respectively, to the \( Q_d \) and \( Q_r \) components of charge that were first described by Adrian and Peres (1977, 1979) in intact fibers.

In contrast to the different time courses of the ON currents in Fig. 6, the OFF currents had approximately the same time course, but different amplitudes, after repolarization from all the TEST potentials that were studied.

In Fig. 6, the transient components of the ON and OFF currents, which are equal to the differences between the ON and OFF currents and their ionic components (both across the fiber membranes and across the external resistances under the Vaseline seals), are thought to arise from intramembranous charge movement. As described in Methods, the ionic currents were estimated by sloping straight lines that had been rounded according to the normalized voltage template. These lines were obtained from least-squares fits of a single exponential function plus a sloping straight line to the final decaying phase of each of the currents; the exponential function was included to allow for the final decaying phase of charge movement and was not used further in the analysis. For the OFF currents, the part of the trace that was used for the fit began when the transient component had decayed to 0.25 times its peak value and ended 600 ms after repolarization. The sloping straight lines are shown superimposed on the records in Fig. 6, A and B.

Since all the OFF currents in Fig. 6 had similar rapid time courses, it was possible to use the standard procedure described above to fit the single exponential function plus a sloping straight line to each trace. The time course of the ON currents were markedly different, however, and it was only for small depolarizations and large depolarizations that a reliable estimate of a sloping straight line for the ionic current could be obtained. In Fig. 6 B, the first and last points for each fit were selected by eye. The first point was on the final decaying phase of the charge movement and the last point, indicated by a vertical bar, was chosen just before a slowly developing inward current, possibly carried by Ca, became apparent (most prominent in the -10 and +10 mV traces).

Intramembranous Charge Movement with a TEA-Cl Solution in the Central Pool

\( \Delta I_{\text{cm}}(t) \), the current that is associated with intramembranous charge movement, is estimated from the difference between the TEST minus CONTROL current and the estimated ionic current (see preceding paragraph and Methods). \( \Delta Q_{\text{cm}} \), the charge that is carried by this current, is obtained by integration of \( \Delta I_{\text{cm}}(t) \), Eq. 1, from the beginning of the transient to the last point that was used for the least-squares fit to determine the sloping straight line that was used for the estimate of the ionic current. Fig. 7 shows values of ON (○) and negative OFF (●) \( \Delta Q_{\text{cm}} \) plotted as a function of TEST potential, \( \Delta V_1(\infty) \), from the experiment in Fig. 6. The data have been normalized by \( C(-100) \), the value of capacitance measured when \( V_1 \) was changed from -110 to -90 mV, which is used as an estimate of linear fiber capacitance.

In Fig. 7, the values of ON and negative OFF charge are similar for voltages in the ranges -80 ≤ \( V_1(\infty) \) ≤ -70 mV (not shown) and -50 ≤ \( V_1(\infty) \) ≤ -10 mV. Between -70 and -50 mV, however, the late component of the ON charge movement current prevented a reliable estimate of the ionic current (Fig. 6 A). Consequently, values of ON \( \Delta Q_{\text{cm}} \) were not estimated in this range. For \( V_1(\infty) = 10 \) and 30 mV, the
magnitude of the OFF charge is larger than that of the ON charge. This lack of charge equality was frequently observed with strong depolarizations. It was associated with the development of a slow inward current during the depolarizing pulse (Fig. 6 B, bottom trace) and, for that reason, is likely to be due to a contamination of the OFF charge by a tail of inward ionic current, possibly carried by Ca ions (Horowicz and Schneider, 1981). Consequently, the values of ON charge were used for the charge vs. voltage data at these two voltages.

**Use of a Single Boltzmann Distribution Function to Fit Charge vs. Voltage Data**

The filled circles in Fig. 8 A show $\Delta Q_{cm}/C(-100)$ data from Fig. 7 plotted as a function of $V_{1}(\infty)$. Negative OFF charge was used for $-80 \leq V_{1}(\infty) \leq -10$ mV and ON charge was used for $V_{1}(\infty) = 10$ and 30 mV. The theoretical curve represents a least-squares fit of the Boltzmann distribution function, Eq. 6. As is standard procedure throughout this article (see Methods), a sloping straight line has been subtracted so that the fitted curve has a value of zero at $-110$ mV (not shown) and at $-90$ mV. This correction is consistent with the $-90$ mV reference potential used for $\Delta Q_{cm}$ and with the change in potential from $-110$ to $-90$ mV used for the CONTROL pulse. This method of fitting $\Delta Q_{cm}$ data does not take into consideration the various pathways for current flow in a double Vaseline-gap chamber and, therefore, is referred to as a fit "without gap corrections." The best fit values in Fig. 8 A are $\bar{V} = -55.4$ mV, $k = 5.0$ mV, and $q_{\text{max}}/C_{m} = 16.2$ nC/µF.

Fig. 8 B shows the same $\Delta Q_{cm}/C(-100)$ data as Fig. 8 A. The theoretical curve, which represents a least-squares fit, was calculated to include currents from the different pathways in a double Vaseline-gap experiment, including the currents under the Vaseline seals (see Methods). This method of fitting $\Delta Q_{cm}$ data is referred to as a fit "with gap corrections." In Fig. 8 B, intramembranous charge movement in
the plasma membrane was described by the Boltzmann distribution function, Eq. 6, with \( V = -52.5 \) mV, \( k = 6.0 \) mV, and \( \frac{q_{\text{max}}}{C_m} = 21.4 \) nC/\( \mu F \). The fit is not as good as the one in Fig. 8 A, a point that is discussed below in connection with Table IV.

Table II gives parameters that were obtained by fitting data from ten fibers with the TEA-CI solution in the central pool. Columns 2, 3, and 4 give the values of \( V \), \( k \), and \( \frac{q_{\text{max}}}{C_m} \), respectively, for a single Boltzmann distribution function fitted without gap corrections. Columns 5, 6, and 7 give the values of the same parameters for a single Boltzmann distribution function fitted with gap corrections. Column 8 gives the values of \( \frac{r_e}{(r_i + r_e)} \) that were used for the gap correction fits. As expected from the information in Table I, the values of \( V \) and \( k \) obtained with gap corrections are similar to those obtained without gap corrections, whereas the values of \( \frac{q_{\text{max}}}{C_m} \) are larger by about 25%. The average values of \( k \), 6.3 mV in column 3 and 7.2 mV in
column 6, are smaller than any values previously reported for cut fibers (see Table VII B, column 6) and are similar to the smallest values reported for intact fibers (see Table VII A, column 6). This result shows that it is possible to obtain small values of $k$ in cut twitch fibers mounted in a double Vaseline-gap chamber. Consequently, the large values of $k$ reported by others, 13–22 mV, are probably not due to an artifact related to the various pathways of current flow in such a chamber.

### Table II

Parameters from Charge vs. Voltage Curves Fitted with a Single Boltzmann Function for Intramembranous Charge Movement, TEA·Cl Solution in the Central Pool

| Fiber reference | $V_0$ | $k$ | $q_{mem}/V_0$ | $V_0$ | $k$ | $q_{mem}/V_0$ | $r_s/(r_i + r_e)$ |
|-----------------|------|-----|---------------|------|-----|---------------|------------------|
| 120871          | -49.6 | 6.8 | 11.1          | -47.9 | 7.4 | 13.7          | 0.980            |
| 127872          | -50.5 | 6.4 | 20.9          | -48.5 | 6.7 | 25.5          | 0.985            |
| 331871          | -50.8 | 6.4 | 18.1          | -48.5 | 7.0 | 22.4          | 0.985            |
| 401872          | -55.4 | 5.0 | 16.2          | -52.5 | 6.0 | 21.4          | 0.991            |
| 406871          | -54.8 | 4.6 | 15.6          | -53.3 | 5.2 | 19.5          | 0.988            |
| 407871          | -54.2 | 7.7 | 17.0          | -52.4 | 9.5 | 22.6          | 0.984            |
| 407872          | -60.7 | 7.0 | 19.6          | -58.9 | 9.0 | 28.8          | 0.985            |
| 409871          | -49.4 | 7.9 | 22.3          | -47.1 | 8.4 | 26.7          | 0.991            |
| 409872          | -53.5 | 5.7 | 21.7          | -51.2 | 6.2 | 26.7          | 0.999            |
| 410871          | -53.6 | 5.8 | 17.1          | -51.8 | 6.4 | 21.2          | 0.988            |
| Mean            | -53.3 | 6.3 | 18.0          | -51.2 | 7.2 | 22.9          | 0.987            |
| SEM             | 1.1   | 0.3 | 1.1           | 1.1   | 0.4 | 1.4           | 0.001            |

$\Delta Q_{mem}/C(-100)$ vs. $V_l(\infty)$ data were obtained from cut muscle fibers that were mounted in a double Vaseline-gap chamber with the TEA·Cl solution in the central pool. Fiber 405872 is not included because it was first exposed to the TEA·SO$_4$ solution (see Table V). The data were least-squares fitted, without and with gap corrections, on the assumption that the steady-state distribution of membrane charge is described by Eq. 6, the equation for a two-state Boltzmann distribution. The fiber reference is given in column 1. The best fit values of $V_0$, $k$, and $q_{mem}/V_0$ for the fit without gap corrections (the procedure used for Fig. 8A) are given in columns 2, 3, and 4, respectively. The best fit values of $V_0$, $k$, and $q_{mem}/V_0$ for the fit with gap corrections (the procedure used for Fig. 8B) are given in columns 5, 6, and 7, respectively. Column 8 gives the values of $r_s/(r_i + r_e)$ that were obtained with the procedure described in Chandler and Hui (1990) and were used for the fits with gap corrections, columns 5–7. Fiber diameters, 88–117 μm. See text for additional information.

### Use of a Sum of Two Boltzmann Distribution Functions to Fit Charge vs. Voltage Data

In Fig. 8 A, the theoretical curve provides a good, but not excellent, fit to the experimental points. For $V_l(\infty) \leq -70$ mV, the data lie above the curve. Between -62 and -52 mV, the data show a voltage dependence that is steeper than that of the curve and, for voltages >-50 mV, the data show a voltage dependence that is less steep than that of the curve. Similar differences were observed in all the single Boltzmann fits without gap corrections that were used for Table II. In Fig. 8 B, the theoretical fit is worse than that in Fig. 8 A and this difference was also observed in
all the fibers used for Table II. It therefore seemed of interest to fit the charge vs. voltage data by a sum of two Boltzmann distribution functions.

Fig. 9, A and B show the fits obtained with a sum of two Boltzmann functions, without and with gap corrections, respectively. The fits in both panels are good, although the one in Fig. 9 A is somewhat better than the one in Fig. 9 B. The more steeply voltage dependent Boltzmann function is characterized by \( k = 1.2 \) mV (A) or \( 1.5 \) mV (B) and \( \bar{V} = -57.8 \) mV (A) or -58.2 mV (B). This steeply voltage-dependent component of OFF charge may be associated with the slowly developing component of ON charge, which we tentatively identify as \( Q_v \), that became apparent at voltages more positive than -60 mV (Fig. 6).

Table III gives the values of the parameters that were obtained by fitting a sum of two Boltzmann functions to the same charge vs. voltage data that were used in Table II. The format of Table III is similar to that of Table II except that two rows of
TABLE III
Parameters from Charge vs. Voltage Curves Fitted with a Sum of Two Boltzmann Functions for Intramembranous Charge Movement, TEA·Cl Solution in the Central Pool

| Fiber reference | Without gap corrections | With gap corrections |
|-----------------|-------------------------|----------------------|
|                 | (2) \( V \) \( mV \) | (3) \( k \) | (4) \( \frac{q_{\text{max}}}{C_m} \) | (5) \( V \) \( mV \) | (6) \( k \) | (7) \( \frac{q_{\text{max}}}{C_m} \) | (8) \( \frac{r_s}{(r_e + r_s)} \) |
| 120871          | -42.2                   | 15.1                 | 6.2 | -25.6 | 10.0 | 4.1 | 0.980 |
|                 | -51.6                   | 4.1                  | 6.5 | -52.5 | 4.1  | 9.8  |
| 127872          | -40.1                   | 11.0                 | 8.3 | -14.9 | 13.6 | 7.7  | 0.985 |
|                 | -53.1                   | 4.6                  | 13.6 | -51.9 | 4.7  | 20.1 |
| 331871          | -45.7                   | 12.5                 | 11.8 | -35.8 | 11.5 | 11.1 | 0.985 |
|                 | -53.7                   | 2.6                  | 7.9  | -54.3 | 2.4  | 11.8 |
| 401872          | -51.1                   | 9.4                  | 11.5 | -39.7 | 9.9  | 11.4 | 0.991 |
|                 | -57.8                   | 1.2                  | 6.2  | -58.2 | 1.5  | 10.8 |
| 406871          | -49.7                   | 9.9                  | 10.5 | -40.4 | 8.2  | 9.4  | 0.988 |
|                 | -56.8                   | 1.1                  | 6.7  | -57.7 | 1.4  | 11.0 |
| 407871          | -41.6                   | 13.6                 | 10.0 | -38.8 | 10.2 | 9.5  | 0.984 |
|                 | -58.2                   | 3.9                  | 8.9  | -58.8 | 3.8  | 12.4 |
| 407872          | -51.7                   | 8.7                  | 10.2 | -44.8 | 11.0 | 14.1 | 0.985 |
|                 | -65.3                   | 2.8                  | 9.4  | -65.3 | 3.0  | 13.3 |
| 409871          | -27.2                   | 19.5                 | 15.5 | -24.3 | 13.0 | 13.4 | 0.991 |
|                 | -53.1                   | 4.7                  | 13.4 | -52.9 | 4.2  | 16.1 |
| 409872          | -46.5                   | 11.9                 | 15.0 | -36.6 | 10.9 | 14.4 | 0.993 |
|                 | -56.4                   | 1.6                  | 9.8  | -56.5 | 1.6  | 14.5 |
| 410871          | -45.1                   | 15.4                 | 12.6 | -34.3 | 12.0 | 10.6 | 0.988 |
|                 | -55.9                   | 2.2                  | 8.8  | -56.5 | 2.3  | 12.6 |
| Mean            | -44.1                   | 11.2                 | 12.7 | -33.9 | 11.0 | 10.6 | 0.987 |
| SEM             | 2.3                     | 0.9                  | 1.0  | 2.8  | 0.5  | 1.0  | 0.001 |
| Mean            | -56.2                   | 2.9                  | 9.1  | -56.5 | 2.9  | 13.2 |
| SEM             | 1.2                     | 0.4                  | 0.8  | 1.3  | 0.4  | 1.0  |

The same \( \Delta Q / C(\sim 100) \) vs. \( V \) (m) data that were used in Table II were least-squares fitted, without and with gap corrections, on the assumption that the steady-state distribution of membrane charge is described by a sum of two Boltzmann functions. The fiber reference is given in column 1. The best fit values of \( V, k, \) and \( q_{\text{max}}/C_m \) for the fit without gap corrections (the procedure used for Fig. 9 A) are given in columns 2, 3, and 4, respectively. The best fit values of \( V, k, \) and \( q_{\text{max}}/C_m \) for the fit with gap corrections (the procedure used for Fig. 9 B) are given in columns 5, 6, and 7, respectively. For each fiber, the top and bottom rows give the values of the parameters for the Boltzmann functions with the larger and smaller values of \( k \), respectively. The values of \( r_s/(r_e + r_s) \) that were used for the fits with gap corrections, column 8, are the same as those in Table II.

The parameters follow each fiber reference, with the first row containing the parameters of the Boltzmann function with the larger value of \( k \). The parameters in columns 5–7, which were obtained with gap corrections, are expected to be more reliable than those in columns 2–4, which were obtained without gap corrections. The
Boltzmann distribution function with the larger value of \( k \) has, on average, \( \bar{V} = -33.0 \text{ mV} \) (column 5) and \( k = 11.0 \text{ mV} \) (column 6) whereas the one with the smaller value of \( k \) has \( \bar{V} = -56.5 \text{ mV} \) (column 5) and \( k = 2.9 \text{ mV} \) (column 6). Thus, on average, the steeply voltage-dependent Boltzmann function has a value of \( \bar{V} \) that is 23.5 mV more negative than that of the other Boltzmann function and has a value of \( k \) that is 0.26 times as large. The values of \( q_{\text{max}}/c_{\text{m}} \) for the two Boltzmann functions are similar (column 7) and are not significantly different from each other (\( P > 0.05 \) with the two-tailed \( t \) test).

**Residual Sums of Squares Associated with the Fits to Charge vs. Voltage Data**

Visual inspection shows that the fits in Fig. 9, A and B are better than the corresponding ones in Fig. 8, A and B. In fact, as shown by a statistical test (Table IV), a sum of two Boltzmann distribution functions gives a significantly better fit to the charge vs. voltage data than does a single Boltzmann function, either without or with gap corrections.

Column 1 of Table IV gives the fiber reference and column 2 gives the number of points, \( N \), that were used for each least-squares fit. Column 3 gives the values of \( \text{RSS}_{1\text{B}} \), the residual sum of squares that was calculated from the fits with one Boltzmann function, without gap corrections.

\[
\text{RSS}_{1\text{B}} = \sum_{i=1}^{N} (y_i - \hat{y}_{i,1\text{B}})^2. \tag{7}
\]

\( y_i \) represents the value of the \( i \)th experimental point and \( \hat{y}_{i,1\text{B}} \) represents the fitted value at the same voltage that was calculated with one Boltzmann function. Similarly, column 4 gives the values of \( \text{RSS}_{2\text{B}} \), the residual sum of squares that was calculated with a sum of two Boltzmann functions,

\[
\text{RSS}_{2\text{B}} = \sum_{i=1}^{N} (y_i - \hat{y}_{i,2\text{B}})^2, \tag{8}
\]

in which \( \hat{y}_{i,2\text{B}} \) represents the fitted value calculated with a sum of two Boltzmann functions. With each fiber, the residual sum of squares was decreased by a factor of two or more by increasing the number of Boltzmann functions from one to two.

Since a fit with one Boltzmann distribution function is a special case of a fit with a sum of two Boltzmann functions (in which one function makes no contribution), \( \text{RSS}_{2\text{B}} \leq \text{RSS}_{1\text{B}} \). It is necessary to use a statistical test to find out whether the difference between \( \text{RSS}_{2\text{B}} \) and \( \text{RSS}_{1\text{B}} \) is statistically significant or whether it can be attributed simply to the decrease in three degrees of freedom that is associated with the extra Boltzmann function used for \( \text{RSS}_{2\text{B}} \) compared with \( \text{RSS}_{1\text{B}} \). Since the value of a Boltzmann function, Eq. 6, depends on \( \bar{V} \) and \( k \) in a nonlinear manner, we have used a test based on the value of the likelihood ratio statistic \( \text{LRS} \),

\[
\text{LRS} = \frac{\text{RSS}_{1\text{B}} - \text{RSS}_{2\text{B}}}{\sigma^2}. \tag{9}
\]
(Bickel and Doksum, 1977), in which $\sigma^2$ is the true variance of the data set. The likelihood ratio statistic has a $\chi^2$ distribution with three degrees of freedom (equal to the difference in the number of parameters in two Boltzmann functions and in one Boltzmann function). If the $\chi^2$ statistic exceeds 7.8, the improvement in fit from one Boltzmann function to two Boltzmann functions is statistically significant at $P < 0.05$.

### Table IV

Residual Sums of Squares for Charge vs. Voltage Curves Fitted with a Single Boltzmann Function and with a Sum of Two Boltzmann Functions for Intramembranous Charge Movement, TEA·Cl Solution in the Central Pool

| Fiber reference | N  | One Boltzmann | Two Boltzmann | LRS | Residual sum of squares without gap corrections | Residual sum of squares with gap corrections |
|----------------|----|---------------|---------------|-----|-----------------------------------------------|--------------------------------------------|
|                |     | (nC/μF)^2     | (nC/μF)^2     |     |                                              |                                            |
| 120871         | 11  | 1.110         | 0.467         | 6.9 | 2.440                                        | 0.474                                       |
| 127872         | 15  | 4.718         | 2.512         | 7.9*| 11.914                                      | 3.877                                       |
| 381871         | 7   | 2.759         | 0.258         | 9.7*| 5.156                                        | 0.025                                       |
| 401872         | 20  | 10.088        | 0.652         | 202.4*| 26.193                                      | 2.196                                       |
| 406871         | 20  | 12.109        | 1.429         | 103.8*| 22.250                                      | 1.790                                       |
| 407871         | 21  | 10.429        | 2.920         | 38.6*| 16.533                                      | 3.076                                       |
| 407872         | 20  | 10.407        | 0.904         | 147.2*| 23.644                                      | 2.579                                       |
| 409871         | 20  | 14.042        | 3.475         | 42.6*| 21.877                                      | 4.325                                       |
| 409872         | 12  | 12.056        | 2.359         | 24.9*| 22.235                                      | 3.564                                       |
| 410871         | 12  | 5.804         | 0.489         | 65.2*| 11.770                                      | 0.825                                       |

Column 1 gives the fiber reference and column 2 gives $N$, the number of data points that were used for each fit. Columns 3 and 4 give the values of the residual sum of squares for fits with a single Boltzmann function (Eq. 7) and with a sum of two Boltzmann functions (Eq. 8), respectively, without gap corrections. Columns 6 and 7 give similar information for fits with gap corrections. Columns 5 and 8 give the estimated values of the likelihood ratio statistic (LRS, calculated from Eq. 10), for comparing the values in columns 3 and 4 and in columns 6 and 7, respectively; an asterisk denotes those values that are statistically significant at $P < 0.05$ with the likelihood ratio test (Bickel and Doksum, 1977). The TEA·Cl solution was used in the central pool. The Boltzmann parameters associated with the fits are given in Tables II and III. See text for additional information.

In general, the value of $\sigma^2$ is unknown but can be approximated by $\text{RSS}_{2B}/(N - 6)$. Accordingly, the values in column 5 of Table IV have been calculated from

$$LRS = \frac{\text{RSS}_{1B} - \text{RSS}_{2B}}{\text{RSS}_{2B}/(N - 6)}.$$  

In 9 out of 10 fibers (marked by an asterisk in column 5), the value of $LRS$ was >7.8, indicating that the improvement in fit from one Boltzmann function to a sum of two Boltzmann functions is statistically significant at $P < 0.05$.

Columns 6 and 7 in Table IV give the residual sums of squares for the fits, with gap corrections, with one Boltzmann function and with a sum of two Boltzmann functions, respectively. The values of the likelihood ratio statistic in column 8
(calculated from Eq. 10) are all >7.8, indicating that the improvement in the fit with a sum of two Boltzmann functions is statistically significant at $P < 0.05$ in all fibers.

In general, however, the fits with gap corrections are not as good as those without: in all fibers in Table IV, the values in column 6 are greater than those in column 3 and, in all but one fiber, the values in column 7 are greater than those in column 4. This is surprising since currents from the Vaseline seal regions are expected to make significant contributions to the total current that is measured, and the gap corrections are expected to deal with these contributions. Some possible reasons for the relatively poorer fits obtained with gap corrections are (a) ionic currents (for example, Ca currents after strong depolarizations) may make small contributions to the estimated charge movement current; (b) the distribution of membrane charge may not be described by simple two state Boltzmann distribution functions but may require somewhat different distribution functions; (c) TEST and CONTROL charge movement currents may contain contributions from charge 2 (Adrian and Almers, 1976; Schneider and Chandler, 1976) or from some other charge movement that was not included in the analysis; (d) the assumptions in the gap correction that $r_m$ is large and that $r_i$ and $r_e$ are constant along a fiber may be too idealized.

Whatever the reasons for the relatively poorer fits obtained with gap corrections, it is clear that a sum of two Boltzmann functions provides a significantly better fit to the charge vs. voltage data than does a single Boltzmann function. This conclusion applies to fits made either with or without gap corrections.

**Effect of a TEA$_2$\cdot SO$_4$ Central-Pool Solution on Charge Movement**

The charge vs. voltage data that we obtained from cut fibers in a TEA-CI solution have a voltage dependence that is steeper than that previously reported for cut fibers: when such data were fitted by a single Boltzmann distribution function, without gap corrections, the average value of $k$ was 6.3 mV in our experiments (Table II, column 3) and 13–22 mV in those of others (see Table VIIB, column 6). Since we used a TEA-CI solution in the central pool, whereas other workers used a TEA$_2$\cdot SO$_4$ solution, it seemed important to compare charge vs. voltage data in the same fiber with both kinds of solutions.

Fig. 10 shows TEST minus CONTROL current traces that were taken first with the TEA-CI solution in the central pool (panel A), then with the TEA$_2$\cdot SO$_4$ solution (panel B), and finally with the TEA-CI solution (panel C). The traces in the TEA-CI solution (panels A and C) are similar to those shown in Fig. 6. In particular, the ON currents show $Q_o$ humps for $V_1 \geq -54$ mV and possibly for $V_1 = -58$ mV. In Fig. 10, the traces in the TEA$_2$\cdot SO$_4$ solution (panel B) have been arranged to align with the TEA-CI traces (panels A and C) that were obtained at voltages 15–16 mV more negative. This was done to facilitate comparison of the traces in the two solutions, since the value of $V$ for a single Boltzmann fit in the TEA$_2$\cdot SO$_4$ solution was 15–18 mV more positive than that in the TEA-CI solution (see Fig. 12). In Fig. 10, the main difference between the traces in the TEA$_2$\cdot SO$_4$ solution (panel B) and those in the TEA-CI solution (panels A and C) at the same vertical position is the presence of larger early transient currents, both ON and OFF, in the TEA$_2$\cdot SO$_4$ solution. As a result, the more slowly developing $Q_o$ component appears to be less pronounced in
FIGURE 10. TEST minus CONTROL currents recorded from a cut muscle fiber bathed in either the TEA-Cl or the TEA₂-SO₄ solution. The values of the TEST potentials are indicated (in millivolts). (A) Currents measured in the TEA-Cl solution, 57–76 min after saponin treatment of the end-pool segments of the fiber. (B) Currents in the TEA₂-SO₄ solution, 110–129 min after saponin treatment. (C) Currents in the TEA-Cl solution, 167–189 min after saponin treatment. Fiber 406871; diameter, 98 μm.

the TEA₂-SO₄ solution than in the TEA-Cl solution. Another difference between the traces in the TEA₂-SO₄ solution (panel B) and those in the TEA-Cl solution (panels A and C) is that the traces in the TEA₂-SO₄ solution show less maintained ON current. This indicates that the relation between ionic current and membrane

FIGURE 11. Intramembranous charge movement \( \Delta Q_{\text{on}}/C(-100) \) plotted against \( V_i(\infty) \), from the experiment in Fig. 10. The open circles show the charge that was measured during the first exposure to the TEA-Cl solution, from the records in Fig. 10 A and others taken during the same sequence. The filled circles show the charge that was measured in the TEA₂-SO₄ solution, from the sequence in Fig. 10 B. The open squares show the charge that was measured during the second exposure to the TEA-Cl solution, from the sequence in Fig. 10 C. The values of \( \Delta Q_{\text{on}} \) were taken from measurements of negative OFF charge except in the TEA-Cl solution in which measurements of ON charge were used at \( V_i(\infty) = -10 \) and 0 mV.
potential is more linear in the TEA$_2$-SO$_4$ solution than in the TEA-Cl solution, consistent with a slight rectification of Cl current (Hutter and Noble, 1960).

Fig. 11 shows values of $\Delta Q_{cm}/C(-100)$ plotted as a function of $V_1(\infty)$, from the experiment illustrated in Fig. 10. The symbols $\bigcirc$ and $\square$ denote, respectively, data from the first (Fig. 10 A) and second (Fig. 10 C) exposure to the TEA-Cl solution, and $\bullet$ denotes data from the intermediate exposure to the TEA$_2$-SO$_4$ solution (Fig. 10 B). For $-55 \leq V_1 \leq -45$ mV, the points in the TEA$_2$-SO$_4$ solution are below those in the TEA-Cl solution whereas, at more positive potentials, they are above the TEA-Cl points. Thus, replacement of the TEA-Cl solution with the TEA$_2$-SO$_4$ solution appears to shift the charge vs. voltage relation to more positive potentials and to increase the maximum amount of charge that is available to move.

Fig. 12 shows the three sets of charge vs. voltage data in Fig. 11 with the curves calculated by least-squares fitting single Boltzmann functions without gap corrections. Panels A and C show, respectively, the data from the first and second exposures to the TEA-Cl solution and panel B shows the data from the intermediate exposure to the TEA$_2$-SO$_4$ solution. The values of the Boltzmann parameters for A, B, C are $V = -54.8, -39.4, -57.3$ mV, $k = 4.6, 9.2, 6.6$ mV, and $q_{\text{max}}/\epsilon_m = 15.6, 29.2, 14.4$ nC/$\mu$F, respectively. The TEA$_2$-SO$_4$ solution appears to shift $V$ by 15–18 mV, to multiply $k$ by 1.4–2, and to approximately double $q_{\text{max}}/\epsilon_m$. Similar changes were observed in two other experiments: fiber 401872, in which the second exposure to the TEA-Cl solution was omitted, and fiber 405872, in which the sequence of solution changes was reversed, from the TEA$_2$-SO$_4$ solution to the TEA-Cl solution to the TEA$_2$-SO$_4$ solution (Table V).

Table V gives values of $V$, $k$, and $q_{\text{max}}/\epsilon_m$ from six fibers that were studied with the TEA$_2$-SO$_4$ solution in the central pool. Columns 5–7 give the parameters obtained from fits without gap corrections and columns 8–10 gives those obtained from fits with gap corrections. Three of the fibers were also studied with the TEA-Cl solution and the values of the parameters obtained from fits without gap corrections are given in columns 2–4; these values are similar to those from the other fibers studied in that solution (Table II, columns 2–4). The numbers in parentheses in columns 4 and 7 give the order of the measurements. The average values of the parameters in the TEA$_2$-SO$_4$ solution, obtained from fits without gap corrections, are $V = -35.1$ mV, $k = 10.5$ mV, and $q_{\text{max}}/\epsilon_m = 36.3$ nC/$\mu$F. The average value of $k$, 10.5 mV, is smaller than any of those previously reported for cut fibers studied in a SO$_4$-containing solution (see Table VII B, column 6).

The average values of $V$, $k$, and $q_{\text{max}}/\epsilon_m$ in the TEA$_2$-SO$_4$ solution (Table V, columns 5–7) are significantly different ($P < 0.05$ with the two-tailed $t$ test) from those in the TEA-Cl solution (Table II, columns 2–4). The TEA$_2$-SO$_4$ solution appears to shift $V$ by 18 mV, to multiply $k$ by 1.7, and to double $q_{\text{max}}/\epsilon_m$.

The large increase in $q_{\text{max}}/\epsilon_m$ observed with the replacement of the TEA-Cl solution with the TEA$_2$-SO$_4$ solution is unexpected since charge movement currents are thought to represent movements of charges on integral membrane proteins and there is no reason to expect the amount of such charges, i.e., $q_{\text{max}}$, to be changed by the composition of the external solution. Since the approximate doubling of the value of $q_{\text{max}}/\epsilon_m$ could be produced by halving the value of $\epsilon_m$, it is of interest to
compare the values of $c_m$ [strictly speaking, $C(-100)$] in the three fibers in Table V that were exposed to both the TEA·Cl and the TEA$_2$·SO$_4$ solutions. In fiber 401872, $c_m$ was 0.205 $\mu$F/cm in the TEA·Cl solution and 0.196 $\mu$F/cm in the TEA$_2$·SO$_4$ solution; in fiber 405872, $c_m$ was 0.202 $\mu$F/cm in the TEA$_2$·SO$_4$ solution (initial exposure), 0.221 $\mu$F/cm in the TEA·Cl solution, and 0.198 $\mu$F/cm in the TEA$_2$·SO$_4$ solution (final exposure); in fiber 406871, $c_m$ was 0.159 $\mu$F/cm in the
TEA-CI solution (initial exposure), 0.158 μF/cm in the TEA₂SO₄ solution, and 0.167 μF/cm in the TEA-CI solution (final exposure). In these fibers, the value of $c_m$ in the TEA₂SO₄ solution was smaller by 3–9% than that in the TEA-CI solution. The average value of $c_m$ first measured in fibers initially exposed to the TEA₂SO₄ solution (0.172 μF/cm) was smaller by 10% than that obtained in fibers initially exposed to the TEA-CI solution (0.192 μF/cm), although the difference is not statistically significant (Chandler and Hui, 1990). Thus, $c_m$ appears to be 3–10% smaller in the TEA₂SO₄ solution than in the TEA-CI solution, although more experiments are required to determine whether this difference is genuine. In any event, the value of $c_m$ in the TEA₂SO₄ solution is clearly greater than half that observed in the TEA-CI solution, so that the doubling of $q_{\text{max}}/c_m$ from TEA-CI to TEA₂SO₄ reflects an almost twofold increase in $q_{\text{max}}$.

### Table V
Parameters from Charge vs. Voltage Curves in TEA-CI and TEA₂SO₄ Solutions
Fitted with a Single Boltzmann Function for Intramembranous Charge Movement

| Fiber reference | $V$ (mV) | $k$ (mV) | $q_{\text{max}}/c_m$ (nC/μF) | $V$ (mV) | $k$ (mV) | $q_{\text{max}}/c_m$ (nC/μF) | $V$ (mV) | $k$ (mV) | $q_{\text{max}}/c_m$ (nC/μF) | $r_o/(r_i + r_e)$ | Mean | SEM |
|-----------------|---------|--------|-----------------------------|---------|--------|-----------------------------|---------|--------|-----------------------------|-----------------|------|-----|
| 401872          | -55.4   | 5.0    | 16.2                        | -33.4   | 13.5   | 38.5                        | -32.2   | 14.2   | 43.2                        | 0.960           |      |     |
| 402871          | -41.0   | 7.3    | 25.8                        | -40.5   | 6.1    | 28.4                        | -38.5   | 11.5   | 40.4                        | 0.974           |      |     |
| 402872          | -29.8   | 10.9   | 48.0                        | -27.9   | 10.4   | 49.4                        | -27.9   | 10.4   | 49.4                        | 0.986           |      |     |
| 405871          | -34.7   | 11.5   | 37.0                        | -34.1   | 11.5   | 40.4                        | -34.1   | 11.5   | 40.4                        | 0.969           |      |     |
| 405872          | -32.2   | 10.7   | 39.4                        | -31.4   | 9.8    | 40.5                        | -31.4   | 9.8    | 40.5                        | 0.979           |      |     |
| 406871          | -55.7   | 4.5    | 16.2                        | -35.6   | 11.1   | 33.5                        | -34.3   | 10.6   | 35.6                        | 0.983           |      |     |
| 406872          | -54.8   | 4.6    | 15.6                        | -59.4   | 9.2    | 29.2                        | -38.2   | 8.5    | 30.7                        | 0.987           |      |     |
| Mean            | -55.3   | 4.7    | 16.0                        | -35.1   | 10.5   | 36.3                        | -34.0   | 10.1   | 38.8                        | 0.977           |      |     |
| SEM             | 0.3     | 0.2    | 0.2                         | 1.8     | 0.9    | 3.2                         | 1.9     | 1.1    | 5.2                         | 0.003           |      |     |

$\Delta Q_{\text{sub}}/C(-100)$ vs. $V_{(w)}$ data were obtained with the TEA-CI or the TEA₂SO₄ solution in the central pool. The data were least-squares fitted, without and with gap corrections, on the assumption that the steady-state distribution of membrane charge is described by Eq. 6. The fiber reference is given in column 1. The best fit values of $V$, $k$, and $q_{\text{max}}/c_m$ with the TEA-CI solution, without gap corrections, are given in columns 2, 3, and 4, respectively. The best fit values with the TEA₂SO₄ solution are given in columns 5, 6, and 7 (without gap corrections) and columns 8, 9, and 10 (with gap corrections). Column 11 gives the values of $r_o/(r_i + r_e)$ that were estimated from CONTROI measurements and used for the fits with gap corrections, columns 8–10. The numbers in parentheses in columns 3 and 4 indicate the chronological order of the measurements.
A possible explanation for the larger value of $q_{\text{max}}$ in the TEA₂·SO₄ solution is that charge 2 makes a contribution to the CONTROL $\Delta I_g(t)$ record and that this contribution is greater in the TEA·Cl solution than in the TEA₂·SO₄ solution. According to this explanation, a contribution of charge 2 to the CONTROL $\Delta I_g(t)$ record would result in a reduction in the estimate of charge movement in the TEST minus CONTROL record and, consequently, a reduction in the estimate of $q_{\text{max}}$; this reduction would be greater in the TEA·Cl solution because the contribution from charge 2 to the CONTROL $\Delta I_g(t)$ is assumed to be greater. Since the value of $c_m$ at $-100$ mV is directly proportional to the amount of charge in the CONTROL $\Delta I_g(t)$ record, this explanation would also be consistent with $c_m$ being smaller in the TEA₂·SO₄ solution than in the TEA·Cl solution, as discussed in the preceding paragraph. The following observations, however, make this explanation unlikely.

First, in intact fibers bathed by an isotonic TEA·Cl solution, the increase in capacitance between $-185$ and $-100$ mV is $\approx 4\%$ (Huang, 1981b) or less (Schneider and Chandler, 1976), suggesting that the contribution of charge 2 to the amount of charge measured in the CONTROL $\Delta I_g(t)$ record in an isotonic TEA·Cl solution is small, probably no more than 4%. Second, in intact fibers bathed by hypertonic solutions, this increase in capacitance is the same with either a TEA·Cl solution or a TEA₂·SO₄ solution (Huang, 1981b). Although these experiments were carried out with hypertonic solutions, rather than with isotonic solutions as were used in our experiments, they suggest that the contribution of charge 2 to the amount of charge in the CONTROL $\Delta I_g(t)$ record is the same with either TEA·Cl or TEA₂·SO₄ in the external solution.

Fig. 13 shows the same charge vs. voltage data as Fig. 12, fitted, with gap corrections, by a sum of two Boltzmann distribution functions. The values of the Boltzmann parameters are given in the legend of the figure.

Charge vs. voltage data, similar to those in Figs. 12 B and 13 B, were obtained from five fibers in the TEA₂·SO₄ solution. Similar to the results with the TEA·Cl solution (Table IV), the data were better fitted by a sum of two Boltzmann functions than by a single Boltzmann function. In all fibers, the improvement in fit was statistically significant ($P < 0.05$ with the likelihood ratio test with three degrees of freedom).

Table VI gives the values of $\bar{V}$, $k$, and $q_{\text{max}}/c_m$ from the five fibers studied in the TEA₂·SO₄ solution. Columns 2–4 give the values associated with the Boltzmann distribution function with the larger value of $k$ and columns 5–7 give the corresponding values associated with the Boltzmann function with the smaller value of $k$. Column 8 gives the values of $r_e/(r_e + r_i)$ that were used for the fits. The last two rows in the table give average values and SEM from the experiments in the TEA·Cl solution (Table III, columns 5–7).

Table VI shows that the average values of $\bar{V}$ in the TEA₂·SO₄ solution are more positive than those in the TEA·Cl solution by 13.0 mV (column 2) and 14.9 mV (column 5). The average values of $k$ in column 3 and of $q_{\text{max}}/c_m$ in column 4 are significantly different in the TEA₂·SO₄ and the TEA·Cl solutions ($P < 0.05$ with the two-tailed $t$ test); the TEA₂·SO₄ solution appears to increase $q_{\text{max}}/c_m$ by a factor of slightly more than 3 and to increase $k$ by about 50%. On the other hand, the average
FIGURE 13. Least-squares fits of a sum of two Boltzmann functions, with gap corrections, to the charge vs. voltage data in Fig. 11. Same format as Fig. 12.

(A) TEA-Cl solution, first exposure. One of the Boltzmann functions is determined by $V = -40.4$ mV, $k = 8.2$ mV, and $q_{\text{max}}/C_m = 9.4$ nC/µF. The other Boltzmann function is determined by $V = -57.7$ mV, $k = 1.4$ mV, and $q_{\text{max}}/C_m = 11.0$ nC/µF. $r/(r_1 + r_2) = 0.988$. (B) TEA$_2$SO$_4$ solution. One of the Boltzmann functions is determined by $V = -30.1$ mV, $k = 12.6$ mV, and $q_{\text{max}}/C_m = 28.6$ nC/µF. The other Boltzmann function is determined by $V = -44.2$ mV, $k = 1.8$ mV, and $q_{\text{max}}/C_m = 7.7$ nC/µF. $r/(r_1 + r_2) = 0.987$. (C) TEA-Cl solution, second exposure. One of the Boltzmann functions is determined by $V = -38.8$ mV, $k = 6.0$ mV, and $q_{\text{max}}/C_m = 7.4$ nC/µF. The other Boltzmann function is determined by $V = -61.6$ mV, $k = 2.6$ mV, and $q_{\text{max}}/C_m = 11.1$ nC/µF. $r/(r_1 + r_2) = 0.988$. The curves were fitted with the same procedure used for Fig. 9B.

Values of $k$ in column 6 and of $q_{\text{max}}/C_m$ in column 7 are not significantly different in the TEA$_2$SO$_4$ and the TEA-Cl solutions ($P > 0.05$ with the two-tailed $t$ test). Thus, the TEA$_2$SO$_4$ solution appears to change the component of charge movement that is associated with the Boltzmann function with the larger value of $k$ but not to change the component associated with the other Boltzmann function.
Contribution of Intramembranous Charge Movement to the Capacitance Measured in a Cut Fiber Mounted in a Double Vaseline-Gap Chamber

Fig. 2 B shows the charge vs. voltage curve that was calculated for a cut fiber, mounted in a double Vaseline-gap chamber, with intramembranous charge that is distributed according to the single Boltzmann distribution illustrated in Fig. 2 A. The curve in Fig. 2 C shows the capacitance that the intramembranous charge movement in Fig. 2 B is expected to contribute to the total capacitance of the preparation; it is the derivative of the charge vs. voltage curve. The curve is bell-shaped.

| Fiber reference | $V_1$ | $k_1$ | $q_1$ | $V_2$ | $k_2$ | $q_2$ | $r_c/(r_i + r_c)$ |
|-----------------|-------|-------|-------|-------|-------|-------|------------------|
| 401872          | -10.4 | 23.0  | 34.3  | -44.2 | 5.3   | 16.7  | 0.969            |
| 402872          | -16.3 | 16.5  | 41.7  | -36.8 | 1.2   | 14.4  | 0.986            |
| 405871          | -20.7 | 19.6  | 39.2  | -41.9 | 3.0   | 11.9  | 0.969            |
| 405872          | -22.6 | 13.3  | 38.3  | -40.8 | 1.0   | 8.5   | 0.979            |
| 406871          | -30.1 | 12.6  | 28.6  | -44.2 | 1.8   | 7.7   | 0.987            |
| Mean            | -20.0 | 17.0  | 36.4  | -41.6 | 2.5   | 11.8  | 0.978            |
| SEM             | 5.3   | 2.0   | 2.3   | 1.4   | 0.8   | 1.7   | 0.004            |

From TEA-Cl experiments

| Fiber reference | $V_1$ | $k_1$ | $q_1$ | $V_2$ | $k_2$ | $q_2$ | $r_c/(r_i + r_c)$ |
|-----------------|-------|-------|-------|-------|-------|-------|------------------|
| Mean            | -33.0 | 11.0  | 10.6  | -56.5 | 2.9   | 13.2  | 0.987            |
| SEM             | 2.8   | 0.5   | 1.0   | 1.3   | 0.4   | 1.0   | 0.001            |

$\Delta Q_{\text{m}}/C(-100)$ vs. $V_{1(\infty)}$ data were obtained with the TEA$_2$SO$_4$ solution in the central pool. The data were least-squares fitted, with gap corrections, on the assumption that the steady-state distribution of membrane charge is described by a sum of two Boltzmann functions. The fiber reference is given in column 1. Columns 2, 3, and 4 give the best fit values of $V_1$, $k_1$, and $q_1$ respectively, for the Boltzmann function with the larger value of $k$; columns 5, 6, and 7 give the values associated with the smaller value of $k$. Column 8 gives the values of $r_c/(r_i + r_c)$ that were used for the fits, from column 11 in Table V. No parameters are given for fiber 402871, one of the fibers used in Table V, since the least-squares fitting program for a sum of two Boltzmann functions did not converge with the charge vs. voltage data from that fiber. The bottom two rows give the average values and SEM of the measurements made in the TEA-Cl solution, from columns 5-7 in Table III. Fiber diameters, 95-129 $\mu$m.

with a foot on the left-hand side. Thus, although almost all the intramembranous charge is in the resting state at membrane potentials more negative than $-80$ mV (Fig. 2 A), charge movement makes a contribution to capacitance at potentials as negative as $V_1 = -150$ mV. This contribution arises from membranes under the Vaseline seals, in regions near the end pools where the fiber is less hyperpolarized than in the central-pool region.

Fig. 14 shows results from an experiment in which charge vs. voltage data (panel A) were obtained with bracketing measurements of capacitance vs. voltage data.
The smooth curve in Fig. 14A shows the single Boltzmann distribution function that was fitted, with gap corrections, to the charge vs. voltage data. The fitted parameters ($V = -45.9$ mV, $k = 6.7$ mV, and $q_{\text{max}}/c_m = 23.9$ nC/$\mu$F) are similar to the average values given in Table II, columns 5–7. (These parameters are not included in Tables II and III because there were not enough data points between $-60$ and $-20$ mV to reliably fit the charge vs. voltage data with a sum of two Boltzmann functions).

Fig. 14B shows capacitance vs. voltage data taken before (○) and after (□) the charge movement data in Fig. 14A were obtained. The smooth curve in Fig. 14B shows the capacitance vs. voltage relation that is expected for a constant linear
capacitance plus a contribution from charge movement. This was obtained by (a) taking the derivative of the original fitted curve in Fig. 14 A, before subtraction of the sloping straight line that intersects it at -110 and -90 mV, (b) adding unity to the derivative, and (c) scaling the resulting curve to give a least-squares fit. The scaling constant, 8.73 nF, is equal to $C(-\infty)$, the capacitance that, in theory, would be measured at extremely negative values of $V_1$ where there should be no displacement currents from charge movement. According to the calculated curve in Fig. 14 B, the capacitance at -150 and -100 mV that was contributed by charge movement was 1.1% and 4.0%, respectively, of that contributed by the linear capacitance.

The numbers 1.1% and 4.0% are in good agreement with the results of the theoretical calculations given in Table I. Columns 6 and 7 give the values of $C(V_1(\infty))/[C(-\infty)q_{\text{max}}/\epsilon_m]$ that were calculated for a single Boltzmann distribution function for charge, with gap corrections, for the values of $V$ and $k$ given in columns 1 and 2, respectively. These values, when multiplied by the value of $q_{\text{max}}/\epsilon_m$, give the contribution that intramembranous charge movement makes to capacitance, expressed as a fraction of the value of linear capacitance. For $V = -50$ mV and $k = 7$ mV (which are close to the average values obtained in our experiments with the TEA-CI solution, columns 5 and 6 of Table II), $C(V_1(\infty))/[C(-\infty)q_{\text{max}}/\epsilon_m] = 0.00058$ (nC/μF)$^{-1}$ at -150 mV and 0.00198 (nC/μF)$^{-1}$ at -100 mV. Multiplication of these values by the average value of $q_{\text{max}}/\epsilon_m$, 22.9 nC/μF (Table II, column 7), gives 0.013 and 0.045, respectively. Thus, the capacitance at -150 and -100 mV that is contributed by charge movement is 1.3% and 4.5%, respectively, of that contributed by the linear capacitance.

The general conclusion of this section is that, in a double Vaseline-gap experiment on a cut muscle fiber, the capacitance measured at a voltage more negative than -100 mV can contain a small contribution from intramembranous charge movement that occurs in membranes that are depolarized under the Vaseline seals near the end pools. In a typical cut fiber studied under our experimental conditions with the TEA-CI solution in the central pool, charge movement is expected to make a contribution to capacitance that is ~1% of the linear capacitance at -150 mV and ~4% of the linear capacitance at -100 mV.

**DISCUSSION**

The experiments reported in this article show, for the first time, that charge vs. voltage curves recorded from cut twitch fibers can be as steeply voltage dependent as those from intact fibers. Table VII compares results on intact (A) and cut (B) fibers from several laboratories. Column 1 gives the reference source and column 2 gives the temperature at which the measurements were made. Column 3 gives the predominant anion that was present in the external solution; only experiments that were carried out with a Cl-containing or a SO$_4$-containing solution have been included in this table. Column 4 gives the number of fibers that were used for each study. Columns 5, 6, and 7 give, respectively, the average values of $V$, $k$, and $q_{\text{max}}/\epsilon_m$ that were obtained by fitting a single Boltzmann distribution function, Eq. 6, to the charge vs. voltage data. This table includes information only from those articles in which the average values of these parameters are given.
### Table VII

Parameters from Charge vs. Voltage Curves in Frog Intact and Cut Fibers Fitted with a Single Boltzmann Function for Intramembranous Charge Movement

| Reference                          | Temperature | Anion  | n   | k  | \(q_{max}/c_0\) |
|------------------------------------|-------------|--------|-----|----|-----------------|
| A. Intact fibers                   |             |        |     |    |                 |
| Schneider and Chandler (1973)      | 2           | Cl     | 5   | -49| 11 N.G.         |
| Adrian and Amers (1976)            | 2-6         | SO\(_4\) | 7   | -48.6| 12.6 32.0       |
| Chandler et al. (1976a)            | 0-1         | Cl     | 6   | -44.1| 7.8 24.5        |
| Shlevin (1979)                     | 2-5         | Cl     | 2   | -37.8| 8.0 31.6        |
| Shlevin (1979)                     | 2-5         | SO\(_4\) | 1   | -38.2| 8.6 21.6        |
| Hollingworth and Marshall (1981)   | 2.5         | Cl     | 6   | -41.9| 7.3 23.9        |
| Rakowski (1981)                    | 5           | Cl     | 24  | -32.1| 11.1 23.3       |
| Hui (1983a)                        | 5-7         | Cl     | 8   | -36.5| 7.0 23.7        |

| B. Cut fibers                      |             |        |     |    |                 |
| Horowicz and Schneider (1981)      | 1-4         | SO\(_4\) | 18  | -34.9| 16.0 26.3       |
| Luttgau et al. (1983)              | 2-4         | SO\(_4\) | 5   | -39.5| 15.0 28.2       |
| Mezler et al. (1986)               | 5-10        | SO\(_4\) | 6   | -24.2| 21.5 27.8       |
| Csornoch et al. (1987)             | 3-5         | SO\(_4\) | 2   | -35.1| 12.7 21.8       |
| Csornoch et al. (1988)             | 4-6         | SO\(_4\) | 3   | -46.9| 13.6 23.3       |
| Feldmeyer et al. (1988)            | 4           | SO\(_4\) |    | NG  | -40.5 15.2 29.9 |
| This article (without gap corrections) | 13-14    | SO\(_4\) | 6   | -35.1| 10.5 36.3       |
| This article (without gap corrections) | 13-14    | Cl     | 10  | -55.3| 6.3 18.0        |
| This article (with gap corrections) | 13-14     | SO\(_4\) | 6   | -34.0| 10.1 38.8       |
| This article (with gap corrections) | 13-14     | Cl     | 10  | -51.2| 7.2 22.9        |

Sections A and B give results from intact and cut fibers, respectively. Column 1 gives the article reference and column 2 gives the temperature. Column 3 gives the predominant anion that was present in the external solutions. TEA was the predominant cation in all external solutions except that used by Feldmeyer et al. (1988), which contained Na; the concentration of free Ca was normal except for the measurements of Hui (1983a), who used 11.8 mM CaCl\(_2\). In the cut fiber experiments, Cs and glutamate were the predominant cation and anion, respectively, in the internal solutions. Column 4 gives the number of fibers that were studied. Columns 5, 6, and 7 give the average values of \(V\), \(k\), and \(q_{max}/c_0\), respectively, that were obtained by fitting a single Boltzmann function to the charge vs. voltage data. Most of the results with intact fibers were obtained with the three-microelectrode method applied to the end of a fiber (Adrian et al., 1970). The only exception is the work of Hollingworth and Marshall (1981), who used the three-microelectrode method applied to the middle of a fiber (Adrian and Marshall, 1977). Movement in intact fibers was reduced by making the external solutions hyperosmotic with sucrose. The results with cut fibers were obtained with a single Vaseline-gap technique (Kovacs and Schneider, 1978) or a double Vaseline-gap technique (Kovacs et al., 1983), with internal and external solutions of normal osmolarity; movement was reduced by stretch. The last four rows in B give results from the experiments in this article. In columns 5-7, the values obtained without gap corrections were taken from Table V, columns 5-7 (SO\(_4\)) and from Table II, columns 2-4 (Cl); the values with gap corrections were taken from Table V, columns 8-10 (SO\(_4\)) and from Table II, columns 5-7 (Cl). NG, not given.

The comparison of our cut fiber results with the cut fiber results of others should be made with the fits of a single Boltzmann function without gap corrections, since this was the fitting procedure used by others. Our average value of \(k\) in the TEA\(_2\)SO\(_4\) solution (without gap corrections), 10.5 mV, is smaller than those reported by others, 12.7-21.5 mV (Table VII B, column 6). (A similar comparison cannot be made with the TEA\(_2\)Cl solution since this solution has not been used...
previously with cut fibers.) The reason for the smaller value of $k$ in our experiments is not known.

On the other hand, the comparison of our results on cut fibers with those of others on intact fibers should be made with the fits of a single Boltzmann distribution function with gap corrections. With gap corrections, our average values of $k$, 10.1 mV with the TEA$_2$·SO$_4$ solution and 7.2 mV with the TEA·Cl solution (Table VII B, column 6), are similar to the corresponding values obtained in intact fibers (Table VII A, column 6). Our average values of $V$, -34.0 mV with the TEA$_2$·SO$_4$ solution and -51.2 mV with the TEA·Cl solution (Table VII B, column 5), and of $q_{\text{max}}/c_m$, 38.8 nC/µF with the TEA$_2$·SO$_4$ solution and 22.9 nC/µF with the TEA·Cl solution (Table VII B, column 7) are also similar to the values from intact fibers (Table VII A, columns 5 and 7). The conclusion from these comparisons is that, under the conditions of our experiments, the charge vs. voltage curves in cut fibers appear to be similar to those in intact fibers.

\textit{Separation of Charge into $Q_\alpha$ and $Q_\gamma$ Components}

One of the main conclusions from our experiments is that charge vs. voltage data are fitted significantly better by a sum of two Boltzmann distribution functions than by a single Boltzmann function (Table IV). The curve for intramembranous charge vs. voltage appears to be asymmetrical, with a steep curvature at potentials negative to the midpoint and a shallow curvature at more positive potentials. A similar asymmetry has been observed in intact fibers (Adrian and Almers, 1976; Hui, 1983a). Another similarity between the charge movement currents observed by us in cut fibers and those observed by others in intact fibers is the presence of a late component ("hump" component) that becomes apparent when the fiber is depolarized to potentials where the charge vs. voltage curve is steeply voltage dependent (Adrian and Peres, 1977, 1979; Huang, 1981a; Hui, 1983a, b). Adrian and Peres (1979) have given the name $Q_\gamma$ to this component of charge movement and $Q_\alpha$ to the other, earlier component of charge movement that is less steeply voltage dependent. Subsequent workers (Huang, 1982; Hui, 1983a, b; Vergara and Caputo, 1983) have suggested that $Q_\gamma$ is more closely related to sarcoplasmic reticulum (SR) Ca release and contractile activation than $Q_\alpha$.

The last four rows in Table VI give the average values of the Boltzmann parameters that are associated with $Q_\alpha$ (columns 2–4) and $Q_\gamma$ (columns 5–7) in our cut fiber experiments. A change from the TEA·Cl solution to the TEA$_2$·SO$_4$ solution resulted in a 13–15-mV positive shift in $V$ for both $Q_\alpha$ and $Q_\gamma$ (columns 2 and 5, respectively). This shift is similar to that observed for the threshold of contraction for long duration pulses, 13 mV (Adrian et al., 1969). Changing from the TEA·Cl solution to the TEA$_2$·SO$_4$ solution also resulted in a 50% increase in the value of $k$ for $Q_\alpha$ (column 3), without any significant change in that for $Q_\gamma$ (column 6), and a three-fold increase in the value of $q_{\text{max}}/c_m$ for $Q_\alpha$ (column 4), without any significant change in that for $Q_\gamma$. Thus, the species of anion that is used in the central-pool solution appears to change the amount, and perhaps the apparent valence, of the $Q_\alpha$ charge but not of the $Q_\gamma$ charge. The reason for this interesting effect is not known.
Table VIII gives the average values of estimates of the Boltzmann parameters for $Q_a$ and $Q_v$ from experiments on intact fibers (A), from a single previous experiment on a cut fiber (B, first row), and from our experiments on cut fibers (B, last four rows). The format of columns 1–4 is the same as that in Table VII. Columns 5, 6, and 7 give the average values of $\bar{V}$, $k$, and $q_{\text{max}/C_m}$, respectively, that have been estimated for $Q_a$ and columns 8–10 give the corresponding values for $Q_v$. Our values of $\bar{V}$ and $k$ are in reasonable agreement with those of others whereas the values of $q_{\text{max}/C_m}$ are somewhat different. With a TEA$_2$SO$_4$ solution, Adrian and Peres (1979) and Huang (1982) found that $Q_v$ accounted for more than half the total charge whereas we found that it accounted for only one-fourth of the total. In a TEA·CI solution, Hui (1983b) found that $Q_v$ accounted for about one-fourth of the total charge whereas we found that it accounted for about half.

In the separation of charge movement into $Q_a$ and $Q_v$ components, Adrian and Peres (1979), Huang (1982), Hui (1983b), and we have treated the components as independent processes that act in parallel (Table VIII). Melzer et al. (1986) used a somewhat different approach and separated charge movement into two components, $Q_1$ and $Q_2$, that are linked according to a three-state two-transition model,

$$
A \overset{Q_1}{\Rightarrow} B \overset{Q_2}{\Rightarrow} C.
$$

Their average values of $k$ were 12.3 mV for the first transition and 12.9 mV for the

| Table VIII |
|---|
| Separation of Charge into $Q_a$ and $Q_v$ Components |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) |
| Reference | Temperature | Anion | $n$ | $\bar{V}$ | $k$ | $q_{\text{max}/C_m}$ | $\bar{V}$ | $k$ | $q_{\text{max}/C_m}$ |
| °C | mV | mV | nC/µF | mV | mV | nC/µF |
| A. Intact fibers |
| Adrian and Peres (1979) | 2.5–4.6 | SO$_4$ | 7 | −26 | 18 | 18 | −46 | NG | 23 |
| Huang (1982) | 3–6 | SO$_4$ | NG | −21* | 15* | 10* | −37* | 6* | 15 |
| Hui (1983b) | 5–7 | CI | 8 | −56.2 | 7.6 | 18.5 | −87.4 | 3.8 | 5.2 |
| B. Cut fibers |
| Vergara and Caputo (1983) | 9 | SO$_4$ | 1 | $\parallel$ | $\parallel$ | $\parallel$ | −34.2 | 3.6 | 5.7 |
| This article | 13–14 | SO$_4$ | 6 | −20.0 | 17.0 | 36.4 | −41.6 | 2.5 | 11.8 |
| This article | 13–14 | CI | 10 | −33.0 | 11.0 | 10.6 | −56.5 | 2.9 | 13.2 |

*Values estimated from Fig. 9 in Huang (1982).
†Value of tetracaine resistant charge.
§The relation between $Q_v$ charge and voltage was linear between −55 and 0 mV so that a Boltzmann distribution function could not be reliably fitted.
N.G., not given.
second transition. Our results are also consistent with this sequential model except that the value of \( k \) for the \( Q_1 \) transition is significantly smaller than that for the \( Q_2 \) transition (Hui and Chandler, manuscript submitted for publication).

**Role of Charge Movement in the Regulation of Ca Release from the SR**

Near the voltage threshold for Ca release from the SR, both in intact fibers (Baylor et al., 1979, 1983; Miledi et al., 1981) and in cut fibers (Maylie et al., 1987a, b), the amplitudes of both the Ca transient and the estimated rate of Ca release are steeply voltage dependent; they increase e-fold with each additional 2–4 mV of depolarization. These results, which were obtained in TEA·Cl and NaCl external solutions, can be compared with our charge movement results in the TEA·Cl solution. The estimates of \( k \) (Table III, column 6) were, on average, 11.0 mV for \( Q_a \) (range, 8.2–13.6 mV) and 2.9 mV for \( Q_v \) (range, 1.4–4.7 mV). The 2–4 mV range for an e-fold increase in the amplitude of the Ca transient and of the peak rate of SR Ca release is essentially the same as the range 1.4–4.7 mV for the \( k \) of \( Q_v \) and clearly different from the range 8.2–13.6 mV for the \( k \) of \( Q_a \), consistent with the idea that \( Q_v \) (Adrain and Peres, 1979) plays a role in the regulation of SR Ca release (Huang, 1982; Hui, 1983a, b; Vergara and Caputo, 1983).

Unfortunately, our experiments do not indicate whether the \( Q_0 \) component of charge movement is related to the \( Q_v \) component or whether it plays any role in the regulation of SR Ca release. If \( Q_a \) and \( Q_v \) were related, the finding that the values of \( k \) for the two components are different by a factor of 4–7 (average values in Table VI) is of interest. It indicates that the apparent valence (which is inversely proportional to \( k \)) associated with \( Q_v \) is four to seven times that associated with \( Q_a \). If charge movement is due to an intramembranous rearrangement of the dihydropyridine binding protein, as suggested by Rios and Brum (1987), the \( Q_a \) component might reflect the rearrangement of a single protein molecule and the \( Q_v \) component might reflect the rearrangement of a group of four protein molecules, as might occur since dihydropyridine binding proteins are able to cluster in groups of four within the transverse tubular membrane (Block et al., 1988).

**Advantages and Limitations of the Double Vaseline-Gap Technique**

Cut fibers, studied under the conditions of our experiments in which a steeply voltage-dependent \( Q_v \) can be characterized, may be useful for the investigation of possible relationships between \( Q_a \) charge, \( Q_v \) charge, and SR Ca release. For this purpose, cut fibers have at least two advantages over intact fibers: the signal-to-noise ratio of the current signal is larger (Vaseline-gap recording vs. microelectrode recording) and it is not necessary to block movement with hyperosmotic solutions that may have deleterious effects on the fibers (Sanchez and Stefani, 1978).

There are also some limitations associated with the double Vaseline-gap method for recording current. Membrane currents from the central-pool region and from under both Vaseline seals contribute to the total current. Although the weighting function \( w(x) \) can be used to estimate this contribution (Fig. 1 C), the derivation of the functional form of \( w(x) \) relies on the values of \( r_i \) and \( r_e \) being constant along the fiber and it is not known whether this is true. The derivation does not take into
account any inactivation of charge movement, of the type described by Chandler et al. (1976b) and by Adrian and Peres (1979), that might occur at depolarized membrane potentials near the end pools in the Vaseline-seal region; however, contributions from these regions are expected to be small since \( w(x) \) is small near the end pools.

Another limitation of the double Vaseline-gap technique is that the charge movement or capacitance that is measured at hyperpolarized potentials, for example, between \(-150\) and \(-100\) mV, is expected to contain small contributions from charge movement that occurs at depolarized potentials under the Vaseline seals next to the end pools (Figs. 2, 4, 5, and 14). For this reason, this method cannot be used to reliably measure charge movement in cut fibers at hyperpolarized potentials (cf., Brum and Rios, 1987), and the four Vaseline-gap method, recently introduced by Rios et al. (1989), may be advantageous.

**APPENDIX**

This section is concerned with how to relate the properties of charge movement that are measured in a double Vaseline-gap experiment (Fig. 1 A) to those of charge movement within the plasma membranes. The analysis represents an extension of that presented by Chandler and Hui (1990), which was concerned with the measurement of linear capacitance.

According to one-dimensional cable theory,

\[
\frac{\partial V_i(x, t)}{\partial x} = r_i i_i(x, t) \tag{A1}
\]

and

\[
\frac{\partial V_e(x, t)}{\partial x} = r_e i_e(x, t). \tag{A2}
\]

\( V_i(x, t) \) and \( V_e(x, t) \) represent the internal and external potentials, respectively; \( r_i \) and \( r_e \) represent the internal and external longitudinal resistances per unit length of fiber; \( i_i(x, t) \) and \( i_e(x, t) \) represent the internal and external longitudinal currents with the convention that current flow from right to left is positive (Fig. 1 A).

Under the left-hand Vaseline seal,

\[
i_i(x, t) + i_e(x, t) = 0 \tag{A3}
\]

and, under the right-hand Vaseline seal,

\[
i_i(x, t) + i_e(x, t) = I_e(t). \tag{A4}
\]

\( I_e(t) \) represents the current that is injected into the end pool labeled EP.

The membrane current per unit length of fiber, \( i_m(x, t) \), satisfies the usual cable relations

\[
i_m(x, t) = \frac{\partial i_i(x, t)}{\partial x} \tag{A5}
\]

\[
= -\frac{\partial i_e(x, t)}{\partial x}. \tag{A6}
\]

Under the left-hand Vaseline seal, where \( 0 \leq x \leq L_i \) (Fig. 1 A), Eq. A5 can be integrated from \( x \) to \( L_i \) and then combined with Eqs. A3 and A2 to give

\[
\int_x^{L_i} i_m(\xi, t) \, d\xi = i_i(L_i, t) - i_i(x, t) \tag{A7}
\]
\[ i(t) = i(L_1, t) + i_x(x, t) \quad (A8) \]
\[ = i(L_1, t) + \frac{1}{r_e} \frac{\partial V_e(x, t)}{\partial x} \quad (A9) \]

Eq. A9 can be integrated from \( x = 0 \) to \( L_1 \) to give
\[ \int_0^{L_1} dx \int_x^{L_1} i_m(\xi, t) \, d\xi = L_1 i(L_1, t) - \frac{1}{r_e} V_1(t). \quad (A10) \]

\( V_1(t) \) represents the potential in the end pool labeled EP_1 (Fig. 1 A). The left-hand side of Eq. A10 can be integrated by parts,
\[ \int_0^{L_1} dx \int_x^{L_1} i_m(\xi, t) \, d\xi = x \int_x^{L_1} i_m(\xi, t) \, d\xi \bigg|_0^{L_1} + \int_0^{L_1} x i_m(x, t) \, dx \quad (A11) \]
\[ = \int_0^{L_1} x i_m(x, t) \, dx. \quad (A12) \]

Eqs. A10 and A12 can be combined to give
\[ \int_0^{L_1} \left( \frac{x}{L_1} \right) i_m(x, t) \, dx = i(L_1, t) - \frac{V_1(t)}{L_1 r_e}. \quad (A13) \]

In the central-pool region, where \( L_1 \leq x \leq L_1 + L_2 \), Eq. A5 can be integrated to give
\[ \int_{L_1}^{L_1 + L_2} i_m(x, t) \, dx = i(L_1 + L_2, t) - i(L_1, t). \quad (A14) \]

The region under the right-hand Vaseline seal, where \( L_1 + L_2 \leq x \leq 2L_1 + L_2 \), can be analyzed in the same manner that was used for the region under the left-hand Vaseline seal (Eqs. A7–A9). Eq. A5 is integrated from \( L_1 + L_2 \) to \( x \) and then combined with Eqs. A4 and A2,
\[ \int_{L_1 + L_2}^x i_m(\xi, t) \, d\xi = i(L_1 + L_2, t) - i(L_1 + L_2, t) \quad (A15) \]
\[ = i(L_1 + L_2, t) - \frac{1}{r_e} \frac{\partial V_e(x, t)}{\partial x} - i(L_1 + L_2, t). \quad (A16) \]

On integration from \( L_1 + L_2 \) to \( 2L_1 + L_2 \), Eq. A17 becomes
\[ \int_{L_1 + L_2}^{2L_1 + L_2} dx \int_{L_1 + L_2}^x i_m(\xi, t) \, d\xi = L_1 I_2(t) - \frac{1}{r_e} V_2(t) - L_1 i(L_1 + L_2, t). \quad (A18) \]

\( V_2(t) \) represents the potential in the end pool labeled EP_2 (Fig. 1 A). The left-hand side of Eq. A18 can be integrated by parts,
\[ \int_{L_1 + L_2}^{2L_1 + L_2} dx \int_{L_1 + L_2}^x i_m(\xi, t) \, d\xi = x \int_{L_1 + L_2}^x i_m(\xi, t) \, d\xi \bigg|_{L_1 + L_2}^{2L_1 + L_2} - \int_{L_1 + L_2}^{2L_1 + L_2} x i_m(x, t) \, dx \quad (A19) \]
\[ = \int_{L_1 + L_2}^{2L_1 + L_2} (\frac{2L_1 + L_2 - x}{2L_1 + L_2}) i_m(x, t) \, dx. \quad (A20) \]
Eqs. A18 and A20 can be combined to give

\[
\int_{L_1 + L_2}^{2L_1 + L_2} \left( \frac{2L_1 + L_2 - x}{L_1} \right) i_m(x, t) \, dx = I_2(t) - \frac{1}{L_1 r_e} V_2(t) - i_i(L_1 + L_2, t). \tag{A21}
\]

The left-hand sides of Eqs. A13, A14, and A21 give the integrals of \( i_m(x, t) \) across the left-hand Vaseline-seal region, the central-pool region, and the right-hand Vaseline-seal region, respectively. These equations can be added to give

\[
\int_0^{2L_1 + L_2} w(x) i_m(x, t) \, dx = I_2(t) - \frac{V_i(t) + V_2(t)}{L_1 r_e}, \tag{A22}
\]

in which \( w(x) \), a weighting function for \( i_m(x, t) \), is given by

\[
w(x) = \begin{cases} 
\frac{x}{L_1}, & 0 \leq x \leq L_1 \\
1, & L_1 \leq x \leq L_1 + L_2 \\
\frac{(2L_1 + L_2 - x)/L_1}{L_1 + L_2 \leq x \leq 2L_1 + L_2}. 
\end{cases} \tag{A23}
\]

Fig. 1 C shows \( w(x) \) plotted as a function of \( x \).

Eqs. A22 and A23 do not depend on the properties of the plasma membranes and, in particular, on whether they behave as a linear electrical circuit. The first term on the right-hand side of Eq. A22 represents the current that is injected into the current-passing end pool. The second term represents the current that flows into the central pool across the external resistances (with values equal to \( L_1 r_e \)) under the two Vaseline seals that separate the central pool from the two end pools. Thus, \( L_2(t) \) is equal to the currents that cross \( L_1 r_e \) plus the product of \( w(x) \) times the currents that cross the plasma membranes.

If \( r_m \) is sufficiently large that ohmic plasma membrane currents can be neglected (see Methods),

\[
i_m(x, t) = \partial q_m(x, t)/\partial t, \tag{A24}
\]

in which \( q_m(x, t) \) is the charge per unit length of fiber, both linear and nonlinear, that is displaced across the plasma membranes. Eq. A22 can then be written

\[
\int_0^{2L_1 + L_2} w(x) \frac{\partial q_m(x, t)}{\partial t} \, dx = I_2(t) - \frac{V_i(t) + V_2(t)}{L_1 r_e}. \tag{A25}
\]

If \( V_i(t) \) is changed by an amount \( \Delta V_i(t) \) from a reference steady-state level, the changes in \( V_i(t) \), \( I_2(t) \), and \( q_m(x, t) \)—denoted by \( \Delta V_i(t) \), \( \Delta I_2(t) \), and \( \Delta q_m(x, t) \), respectively—can be incorporated into Eq. A25 to give

\[
\int_0^{2L_1 + L_2} w(x) \frac{\partial q_m(x, t)}{\partial t} \, dx = \Delta I_2(t) - \frac{\Delta V_i(t) + \Delta V_2(t)}{L_1 r_e}. \tag{A26}
\]

Since the assumption of large \( r_m \) gives

\[
\Delta I_2(\infty) = \frac{\Delta V_i(\infty) + \Delta V_2(\infty)}{L_1 r_e}, \tag{A27}
\]
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Eq. A26 can be written

\[ \int_0^{L_1+L_2} w(x) \frac{\partial q_m(x, t)}{\partial t} \, dx = \Delta I_{2,\nu}(t) - \frac{\Delta V_{2,\nu}(t)}{L_1 r_c}, \]  

(A28)

in which \( \Delta I_{2,\nu}(t) \) and \( \Delta V_{2,\nu}(t) \) are defined by

\[ \Delta I_{2,\nu}(t) = \Delta I_2(t) - \Delta V_1(t) \frac{\Delta I_4(\infty)}{\Delta V_1(\infty)}, \]  

(A29)

and

\[ \Delta V_{2,\nu}(t) = \Delta V_2(t) - \Delta V_1(t) \frac{\Delta V_4(\infty)}{\Delta V_1(\infty)}. \]  

(A30)

Eq. A28 can be integrated from \( t = 0 \) to \( \infty \) to give

\[ \int_0^{L_1+L_2} w(x) \Delta q_m(x, \infty) \, dx = \int_0^\infty \Delta I_{2,\nu}(t) \, dt - \frac{1}{L_1 r_c} \int_0^\infty \Delta V_{2,\nu}(t) \, dt, \]  

(A31)

in which \( \Delta q_m(x, \infty) \) represents the change in steady-state distribution of charge across the plasma membranes that accompanies \( \Delta V_1(\infty) \). Eq. A31 gives the desired relation between the results of experimental measurements (right-hand side) and the properties of the charge rearrangements that occur in the plasma membranes (left-hand side).

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