Membrane Capacitance in Frog Cut Twitch Fibers Mounted in a Double Vaseline-Gap Chamber

W. KNOX CHANDLER and CHIU SHUEN HUI

From the Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut 06510; and the Departments of Physiology/Biophysics and Medicine, Indiana University School of Medicine, Indianapolis, Indiana 46223

ABSTRACT In experiments on cut muscle fibers mounted in a double Vaseline-gap chamber, electrical measurements are usually made by measuring the voltage $V_1(t)$ in one end pool and by passing current $I_2(t)$ from the other end pool to the central pool, which is usually clamped to earth potential. The voltage in the current-passing end pool is denoted by $V_2(t)$. This article describes how the value of the holding current, $I_h$, and the values of $\Delta V_2(\infty)/\Delta V_1(\infty)$ and $\Delta I_2(\infty)/\Delta V_1(\infty)$ that are associated with a small change in $V_1(t)$ can be used to estimate the linear cable parameters $r_m$, $r_i$, and $r_e$ in a cut fiber that has been equilibrated with a Cs-containing internal solution. $r_m$, $r_i$, and $r_e$ represent, respectively, the resistance of the plasma membranes, the internal longitudinal resistance, and the external longitudinal resistance under the Vaseline seals, all for a unit length of fiber. The apparent capacitance, $C_{app}$, of the preparation is defined to equal $\int_0^\infty \Delta J_{2\infty}(t)\,dt/\Delta V_1(\infty)$, in which $\Delta J_{2\infty}(t)$ represents the transient component of current that is associated with a change in $V_1(t)$ of amplitude $\Delta V_1(\infty)$. A method is described to estimate $c_m$, the capacitance of the plasma membranes per unit length of fiber, from $C_{app}$ and the values of $r_m$, $r_i$, and $r_e$. In experiments carried out with a tetraethylammonium chloride (TEA·Cl) solution at 13–14°C in the central pool, $c_m$ remained stable for as long as 3–4 h. The values of $c_m$, 0.19 μF/cm on average, and their variation with fiber diameter are similar to published results from intact fibers. This article also describes the different pathways that are taken by the current that flows from the current-passing end pool to the central pool. Approximately two-thirds of $\Delta J_{2\infty}(t)$ flows across the capacitance of the plasma membranes in the central-pool region. The rest flows either across plasma membranes that are 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. [There is also a current that flows directly from the voltage-measuring end pool to the central pool but this does not contribute to $\Delta J_{2\infty}(t)$.]
When the voltage inside a cell is changed from one level to another, some electrical charge is displaced across the capacitance of the plasma membranes (in muscle, taken to include both surface and transverse tubular membranes). If the capacitance is constant, the amount of charge should be directly proportional to the change in membrane potential. In many excitable cells, however, there is an extra component of charge that arises from a nonlinear intramembranous charge movement (Armstrong and Bezanilla, 1973; Schneider and Chandler, 1973; see also Almers, 1978; and Huang, 1988).

In a frog twitch muscle fiber, the currents from such charge movement become prominent during depolarizations to a level near or beyond the threshold potential for the activation of contraction, an observation that led to the suggestion that such currents might play a role in excitation--contraction coupling (Schneider and Chandler, 1973). When the relation between nonlinear charge movement and membrane potential is fitted by the function for a two-state Boltzmann distribution, the voltage dependence factor (or voltage steepness factor) is 7-13 mV in intact fibers (Schneider and Chandler, 1973; Adrian and Almers, 1976; Chandler et al., 1976; Shleivin, 1979; Hollingworth and Marshall, 1981; Rakowski, 1981; Hui, 1983) and 13-22 mV in cut fibers (Horowicz and Schneider, 1981; Luttgau et al., 1983; Melzer et al., 1986; Csernoch et al., 1987, 1988; Feldmeyer et al., 1988). The finding of different voltage dependence factors in intact and cut fibers is surprising since many properties of the myoplasmic Ca signals recorded with Ca indicators, including the voltage steepness near threshold, are similar in the two preparations (Baylor et al., 1979, 1983; Miledi et al., 1981; Maylie et al., 1987a, b).

A possible explanation for the different values of the voltage dependence factor is that different methods are used to record charge movement in intact and cut fibers. In cut fibers, a single or double Vaseline-gap method is frequently employed in which Vaseline seals are used to electrically isolate the end pool(s) from the central pool where the current is collected (Kovacs and Schneider, 1978; Kovacs et al., 1983). If a seal provided perfect insulation, all the current collected in the central pool will have crossed the plasma membranes in that region. If a seal does not provide perfect insulation, however, some of the current that is collected will have crossed plasma membranes under the seal where the membrane potential may be different from that in the central pool. Because of such currents, a possible uncertainty is introduced into the estimate of the voltage dependence of nonlinear charge movement. In experiments on intact fibers, the three-microelectrode technique is usually used in which current is estimated near the end of a fiber where the variation of potential can be relatively small (Adrian et al., 1970). Consequently, the problem of voltage nonuniformity is greatly reduced.

The work described in this article was undertaken to understand the pathways for current flow in a double Vaseline-gap experiment. For simplicity, the measurements and analysis have been restricted to linear cable properties; the following article (Hui and Chandler, 1990) gives an application to nonlinear charge movement. Cut muscle fibers were mounted in a double Vaseline-gap chamber. The myoplasm was equilibrated with a Cs-containing internal solution in the end pools, after the plasma
membranes there had been rendered permeable by a brief saponin treatment. A small change in voltage in one end pool, $\Delta V_1(t)$, was imposed by passing a current, $\Delta I_g(t)$, from the other end pool to the central pool, which was clamped at earth potential. The change in voltage in the current-passing end pool is denoted by $\Delta V_2(t)$. The value of the holding current, $I_h$, and the steady-state values of $\Delta V_2(\infty)/\Delta V_1(\infty)$ and $\Delta I_g(\infty)/\Delta V_1(\infty)$ are used to estimate the linear cable parameters $r_m$, $r_i$, and $r_e$, which represent, respectively, the resistance of the plasma membranes, the internal longitudinal resistance, and the external longitudinal resistance under the Vaseline seals, all for a unit length of fiber.

Once $r_m$, $r_i$, and $r_e$ are known, $c_m$ (the capacitance of the plasma membranes per unit length of fiber) can be estimated from the charge that is carried by the transient "capacitive" current following a step change in $V_1(t)$. According to theoretical calculations for the chamber used in our experiments, with a 500-μm central-pool region and two 300-μm Vaseline-seal regions, only about two-thirds of the transient current that is collected in the central pool actually flows across the plasma membranes in that region. The rest flows either across the plasma membranes under the two Vaseline seals or directly across the external seal resistance that separates the central pool from the current-passing end pool. If the contributions from these seal currents are ignored, the estimate of fiber capacitance per unit length would be too large by a factor of roughly 1.5.

Although the cable analysis presented in this article applies to a double Vaseline-gap method, it should be possible to use a similar approach to analyze measurements made with a single Vaseline-gap method (Kovacs and Schneider, 1978) or with the potentiometric method of Hille and Campbell (1976).

METHODS

The experiments were carried out at Yale University on cut twitch fibers (Hille and Campbell, 1976) from semitendinosus muscles of cold-adapted Rana temporaria. The experimental methods were adapted from those of Kovacs et al. (1983) and are described in Irving et al. (1987). A fiber was dissected and cut in a 120 mM K glutamate relaxing solution, then transferred and mounted in a double Vaseline-gap chamber (Fig. 1). The end-pool segments of the fiber were exposed for 2 min to a relaxing solution that contained 0.01% saponin (Endo and Iino, 1980), to render the plasma membranes permeable to the internal solution. The permeabilized segments of the fiber are denoted by dashed lines in Fig. 1. After a thorough rinsing with saponin-free relaxing solution, internal solution was introduced into the two end pools where it remained during the rest of the experiment. At least 45 min was allowed for equilibration of the myoplasm with the end-pool solutions before electrical measurements were started. Table I A gives the composition of the relaxing and the internal solutions. Table I B gives the composition of the solutions used in the central pool; the experiments in all the figures, except some of those in Fig. 9, were carried out with the tetraethylammonium chloride (TEA-Cl) solution in the central pool.

Fig. 1 shows a diagram of the experimental chamber and electrical connections to the apparatus. Two end pools, EP$_1$ and EP$_2$, were used for measuring potential and for passing current, respectively. These were separated by two Vaseline seals VS, of length $L_{VS}$, from the central-pool region CP, of length $L_{CP}$. Throughout this article, the potential difference across the saponin-treated plasma membranes in the end pools is assumed to be zero; consequently, the potentials in EP$_1$ and EP$_2$ are the same as those at points 1 and 2, respectively. The
FIGURE 1. Schematic diagram of a cut muscle fiber, with saponin-treated end-pool segments, mounted in a double Vaseline-gap chamber. \( V_1, V_a, V_b, \) and \( V_2 \) denote the potential at points 1, a, b, and 2, respectively. Throughout this article, the assumption is made that, because of the saponin treatment, the potentials in EP1 and EP2 are the same as \( V_1 \) and \( V_2 \), respectively. See text for additional information.

A. Solutions

|                        | Glutamate | PIPES | EGTA | MgSO_4 | Glucose | Cs_2-ATP | Cs_2-creatine phosphate |
|------------------------|-----------|-------|------|--------|---------|----------|------------------------|
| Relaxing solution      | 120 (K)   | 5     | 0.1 (K) | 1.0 | 0 | 0 | 0 |
| Internal solution      | 45.5 (Cs) | 5     | 20.0 (Cs) | 6.8 | 5 | 5.5 | 20 |

B. Central-pool solutions

| TET-A-CI | TET-A-SO_4 | RbCl | Na_2HPO_4 | NaH_2PO_4 | CaCl_2 | CaSO_4 | TTX |
|----------|------------|------|-----------|-----------|--------|--------|-----|
| mM       | mM        | mM   | mM       | mM        | mM     | mM     | mM  |
| TET-A-CI | 120        | 0    | 2.5      | 2.15      | 0.85   | 1.8    | 0   | 1  |
| TET-A-SO_4 | 0        | 80   | 2.5      | 2.15      | 0.85   | 0      | 7   | 1  |

A. The major cation associated with glutamate and EGTA is given in parentheses. The internal solution contained a total concentration of 0.4 mM Ca and had a free Mg concentration that was calculated to be 1.0 mM. The pH was adjusted to 7.0 by adding KOH to the relaxing solution or CsOH to the internal solution. B. The pH of the central-pool solutions was 7.1.
apparatus. In CP and EPz, separate side pools were used for measuring potential and for passing current.

The electrical measurements were made with the voltage-clamp technique. The amplifier at the top of Fig. 1 was used to clamp \( V_1 \) to a value nearly equal to that of the command potential; command pulses were rounded by a 0.5-ms time constant. The potential in the central pool, \( V_c \), was maintained at earth by a feedback amplifier circuit (Fig. 1). \( I_z \) was determined from the voltage across the resistance \( R \). Electrical signals corresponding to \( V_1 \), \( V_2 \), and \( I_z \) were connected to the inputs of Tektronix, Inc. (Beaverton, OR) AM 502 amplifiers, with the bandwidth set from DC to 3 kHz. An 11/73 LSI computer system (Digital Equipment Corp., Marlboro, MA) generated the command potential and sampled the outputs of the AM 502 amplifiers in the sequence \( V_2: I_z: V_1: I_z: V_2: I_z: V_1: I_z \) at a frequency of 5,000 or 12,500 sequences per second; values from five successive sequences were averaged and stored on a disk for subsequent analysis. At the end of some of the experiments, a conventional 3 M KCl-filled microelectrode was inserted near point \( a \) (Fig. 1) to measure \( V_a \). The resistance of the microelectrodes was 13–30 MΩ in Ringer’s solution at room temperature.

The experiments were carried out with a striation spacing of 3.5–3.6 μm, a temperature of 13–14°C, and a holding potential \( V_1 = -90 \) mV. The statistical significance of a difference between two sets of results was sometimes determined with the two-tailed \( t \) test; if \( P < 0.05 \), the difference was considered to be significant.

**RESULTS**

Electrical Signals Associated with ±20-mV Steps Applied to a Voltage-clamped Cut Muscle Fiber Mounted in a Double Vaseline-Gap Chamber

Fig. 2 A shows changes in \( V_1 \) [\( \Delta V_1(t) \)] and in \( I_z \) [\( \Delta I_z(t) \)] associated with +20- and -20-mV step changes in the command potential in a cut fiber with the TEA·Cl solution in the central pool. 200 ms before data sampling began, the potential at \( V_1 \) was switched from the normal holding potential of -90 mV to -110 mV. The records in Fig. 2 A show a 40-ms baseline at \( V_1 = -110 \) mV followed by the response to a +20 mV step (interval \( a \)), then to a -20 mV step (interval \( b \)), and finally to a +20 mV step (interval \( c \)); this is the protocol that we usually used to obtain control currents and voltages in experiments on intramembranous charge movement (Hui and Chandler, 1990). The \( \Delta V_1(t) \) signal is rounded, owing to the 0.5-ms delay that was applied to the step command potential and to the 3-KHz bandwidth of the amplifier that was used to process the signal. Accompanying each step change in \( V_1 \), there was a large, transient component on the \( \Delta I_z(t) \) record, in the same direction as the \( \Delta V_1(t) \) signal, that was followed by a maintained steady level.

The top two pairs of traces in Fig. 2 B show the segments of the \( \Delta V_1(t) \) and \( \Delta I_z(t) \) records in Fig. 2 A that accompanied the depolarizing and hyperpolarizing transitions. The upper trace in each pair, labeled \((a + c)/2\), shows the average of the segments from intervals \( a \) and \( c \) in panel A. The lower trace in each pair, labeled \( b \), shows the segment from interval \( b \). The trace below the \( \Delta I_z \) pair shows the sum of the depolarizing and hyperpolarizing \( \Delta I_z(t) \) segments, plotted at high gain. There is a transient inward current, of peak amplitude \( \sim 1 \) nA, immediately after the change in potential. This is not due to a difference in the shape of the depolarizing and hyperpolarizing \( \Delta V_1(t) \) waveforms since the sum of the two \( \Delta V_1 \) records in Fig. 2 B was flat (not shown). The area associated with the nonlinear component of \( \Delta I_z(t) \) corresponds to 3.6 pC of charge, which is equal to 1.4% of the charge contributed by
the transient component of $\Delta I_2(t)$ (Fig. 3 B). A similar current was observed in two other experiments of this type, in which the amounts of charge were 1.3% and 0.6% of those contributed by the transient component of $\Delta I_2(t)$. Although the origin of the extra current has not been determined, one possibility is a rapidly decaying inward ionic current that crossed the plasma membranes when they were hyperpolarized from $-90$ to $-110$ mV.

The lowermost record in Fig. 2 B shows the difference between the $\Delta I_2(t)$ segments in intervals $a$ and $c$ in Fig. 2 A. The difference trace is flat, indicating that the two $\Delta I_2(t)$ segments were essentially identical.

Fig. 3 A shows the positive going $\Delta V_1(t)$ and $\Delta I_2(t)$ traces from Fig. 2 B and the $\Delta V_2(t)$ trace recorded at the same time. Irving et al. (1987) used the variables $a_1$ and $a_2$ to denote the steady-state ratios $\Delta V_2(\infty)/\Delta V_1(\infty)$ and $\Delta I_2(\infty)/\Delta V_1(\infty)$, respectively. Fig. 3 B repeats the traces in Fig. 3 A and shows $\Delta V_1(t)$ traces, scaled by $\Delta V_2(\infty)/\Delta V_1(\infty)$ and $\Delta I_2(\infty)/\Delta V_1(\infty)$, superimposed on the $\Delta V_2(t)$ and $\Delta I_2(t)$ traces, respectively. The differences between the original $\Delta V_2(t)$ and $\Delta I_2(t)$ traces and the scaled $\Delta V_1(t)$ traces represent the transient components of these signals, which are denoted by $\Delta V_{2,\text{tr}}(t)$ and $\Delta I_{2,\text{tr}}(t)$, respectively. $\Delta I_{2,\text{tr}}(t)$, then, represents the current collected in end pool 2 minus a component of current that is ohmic and simply proportional to $\Delta V_1(t)$. 

![Figure 2](image-url)
In this article, $G_{\text{app}}$, the apparent conductance of a fiber mounted in a double Vaseline-gap chamber, will be defined by

$$G_{\text{app}} = \frac{\Delta I_2(\infty)}{\Delta V_1(\infty)},$$

and $C_{\text{app}}$, the apparent capacitance of the preparation, will be defined by

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

$L_{\text{app}}$, the apparent length of a fiber that contributes to $C_{\text{app}}$, is defined by

$$C_{\text{app}} = L_{\text{app}} c_m.$$
Eqs. A18 and A19 give two relations (for $a_1$ and $a_2$) between three parameters ($r_m$, $r_i$, and $r_e$). With these equations, the values of $r_m$, $r_i$, and $r_e$ can be estimated from the values of $a_1$ and $a_2$ if one other value is known: that of $r_m$, $r_i$, $r_e$, or a relation between them.

One of the methods used by Irving et al. (1987) was to estimate the value of $r_e/(r_i + r_e)$ from a measurement made with a microelectrode inserted into a fiber near location $a$ in Fig. 1. According to Eq. A4 in their article, $r_e/(r_i + r_e) = \Delta V_1(0)/\Delta V_2(0)$.

Table II A gives information about cable parameters in eight fibers in which microelectrodes were used. Columns 2–4 give the values of $a_1$, $a_2$, and $I_h$ that were measured just before a microelectrode was inserted into the fiber near location $a$ in Fig. 1, at the end of an experiment. Once the microelectrode was in place, a $-50$-mV command pulse was applied to the input of the voltage-clamp amplifier. The value of $\Delta V_1(\infty)/\Delta V_2(\infty)$ was measured and used for the estimate of $r_e/(r_i + r_e)$ in column 5. Columns 6–8 give the values of $r_m$, $r_i$, and $r_e$ that were calculated from Eqs. A18 and A19 by an iterative computer program. For the calculations, the values of $r_i$ and $r_e$ were constrained so that $r_e/(r_i + r_e)$ was equal to the value in column 5. Column 9 gives the values of $l_{app}$ that should be used in Eq. 3, and column 10 gives the values of $c_m$. If the Vaseline seals provided perfect electrical insulation so that $r_e$ was infinite, $l_{app}$ should be approximately equal to the length of the central-pool region, $L_2$, which in our experiments was 500 μm (Fig. 1). In the experiments in Table II A, however, the average value of $l_{app}$ was 772.8 μm (column 9). The reasons why $l_{app}$ is so much larger than 500 μm are given in the Discussion.

**Estimates of $r_m$, $r_i$, and $r_e$ Estimated from $I_h$**

Each value of $r_e/(r_i + r_e)$ in column 5 of Table II A was determined with a microelectrode, which, because of concern of possible damage to the fiber, was inserted only at the end of an experiment. Since this estimate of $r_e/(r_i + r_e)$ may not apply to the initial condition of a fiber, it seemed desirable to find another way to estimate either $r_m$, $r_i$, or $r_e$, or a relation between them, that did not require the use of a microelectrode. For this purpose, Irving et al. (1987) used the value of $I_h$ and a relation between $I_h$ and $r_m$, $r_i$, and $r_e$ (their Eq. A30 or A31). Their relation requires a value for $V_{RP}$, the normal resting potential that would be measured across the plasma membranes in the absence of current flow. In their experiments, the cut fibers were exposed to end-pool solutions containing 124 mM K and to a central-pool solution containing either 2.5 mM K (Ringer's solution) or 2.5 mM Rb (TEA·Cl solution). After equilibration with the end-pool solutions, the myoplasm was expected to contain approximately 124 mM K, a concentration similar to that found in intact fibers (Godt and Maughan, 1988). Consequently, Irving et al. (1987) assumed that $V_{RP}$ in their cut fibers was $-90$ mV, similar to the resting potential of a normal intact fiber bathed with either Ringer's solution or the TEA·Cl solution.

The cut fibers studied in this article were equilibrated with end-pool solutions that contained Cs instead of K. Since the resting potential has not been measured in intact fibers in which almost all the K has been replaced with Cs, the value of $V_{RP}$ is unknown. Hence, the equation used by Irving et al. (1987) to relate $I_h$ to $r_m$, $r_i$, and $r_e$ must be modified to apply to the experiments reported in this article.
TABLE II

Estimates of Linear Cable Parameters Obtained from Fibers in Which a Microelectrode Was Used to Determine \( r_0/(r_0 + r_c) \)

| Fiber reference | \( a_1 \) | \( a_2 \) | \( \Delta l \) | \( r_0/(r_0 + r_c) \) | \( r_c \) | \( r_0 \) | \( l_{app} \) | \( \xi_a \) |
|-----------------|--------|--------|---------|----------------|------|------|-------|------|
|                 | \( \mu S \) | nA     | M\( \Omega \)/cm | M\( \Omega \)/cm | M\( \Omega \)/cm | \( \mu m \) | \( \mu F/cm \) |
| A. \( r_0/(r_0 + r_c) \) estimated with microelectrodes | | | | | | | |
| D16862          | 1.188  | 1.637  | -77.2   | 0.964  | 0.179 | 2.236 | 59.9  | 803.4 | 0.2863 |
| D17862          | 1.190  | 0.723  | -31.7   | 0.975  | 0.209 | 5.026 | 196.0 | 769.6 | 0.1070 |
| D18862          | 1.149  | 0.703  | -31.0   | 0.975  | 0.294 | 4.046 | 157.8 | 768.2 | 0.1338 |
| D19861          | 1.169  | 0.873  | -40.2   | 0.979  | 0.161 | 3.674 | 171.3 | 754.6 | 0.1502 |
| D22861          | 1.137  | 1.448  | -64.2   | 0.984  | 0.090 | 1.778 | 109.3 | 734.9 | 0.2191 |
| D23861          | 1.161  | 0.860  | -40.4   | 0.971  | 0.282 | 3.599 | 120.5 | 781.1 | 0.1517 |
| D23862          | 1.204  | 0.987  | -49.9   | 0.960  | 0.325 | 4.055 | 97.3  | 816.1 | 0.1631 |
| 107871          | 1.155  | 1.194  | -52.5   | 0.979  | 0.127 | 2.462 | 114.8 | 754.5 | 0.2548 |
| Mean            | 1.169  | 1.503  | -48.4   | 0.973  | 0.208 | 3.360 | 128.4 | 772.8 | 0.1833 |
| SEM             | 0.008  | 0.121  | 5.7     | 0.003  | 0.030 | 0.389 | 15.6  | 9.5   | 0.0223 |
| B. \( r_c \) estimated from \( l_{app} \) | | | | | | | |
| D16862          | 1.188  | 1.637  | -77.2   | 0.974  | 0.099 | 2.201 | 82.5  | 773.9 | 0.2972 |
| D17862          | 1.190  | 0.723  | -31.7   | 0.975  | 0.207 | 5.024 | 195.9 | 768.7 | 0.1071 |
| D18862          | 1.149  | 0.703  | -31.0   | 0.980  | 0.209 | 4.010 | 196.5 | 749.3 | 0.1372 |
| D19861          | 1.169  | 0.873  | -40.2   | 0.977  | 0.178 | 3.687 | 166.6 | 762.4 | 0.1487 |
| D22861          | 1.137  | 1.448  | -64.2   | 0.982  | 0.101 | 1.785 | 97.4  | 743.9 | 0.2164 |
| D23861          | 1.161  | 0.860  | -40.4   | 0.978  | 0.184 | 3.561 | 158.3 | 760.0 | 0.1559 |
| D23862          | 1.204  | 0.987  | -49.9   | 0.970  | 0.181 | 3.991 | 129.0 | 788.3 | 0.1689 |
| 107871          | 1.155  | 1.194  | -52.5   | 0.980  | 0.123 | 2.459 | 120.5 | 752.0 | 0.2556 |
| Mean            | 1.169  | 1.503  | -48.4   | 0.977  | 0.160 | 3.340 | 142.1 | 762.3 | 0.1859 |
| SEM             | 0.008  | 0.121  | 5.7     | 0.010  | 0.016 | 0.387 | 14.9  | 5.1   | 0.0229 |
| C. Ratio of values in part B to those in part A | | | | | | | |
| D16862          | 1.010  | 0.554  | 0.984  | 1.377  | 0.965 | 1.038 |
| D17862          | 1.000  | 0.989  | 1.000  | 0.999  | 0.990 | 1.001 |
| D18862          | 1.005  | 0.711  | 0.991  | 1.245  | 0.975 | 1.025 |
| D19861          | 0.998  | 1.109  | 1.004  | 0.914  | 1.010 | 0.990 |
| D22861          | 0.998  | 1.128  | 1.004  | 0.891  | 1.012 | 0.988 |
| D23861          | 1.007  | 0.656  | 0.989  | 1.314  | 0.975 | 1.028 |
| D23862          | 1.010  | 0.557  | 0.984  | 1.326  | 0.966 | 1.036 |
| 107871          | 1.001  | 0.964  | 0.999  | 1.050  | 0.997 | 1.003 |
| Mean            | 1.004  | 0.854  | 0.994  | 1.140  | 0.987 | 1.014 |
| SEM             | 0.002  | 0.085  | 0.003  | 0.070  | 0.007 | 0.007 |

Column 1 gives the fiber references. Columns 2-4 give the values of \( a_1, a_2, \) and \( \Delta l \) that were measured just before the fiber was impaled with a microelectrode near location \( a \) indicated in Fig. 1. Column 5 gives the values of \( r_c/(r_c + r_c) \); in part A, this is equal to the value of \( \Delta V_c(0)/\Delta V_c(\infty) \) determined with a microelectrode and, in part B, it was calculated from the values in columns 7 and 8. Columns 6–8 give the values of \( r_c, r_0, \) and \( r_c \). In part A, these were calculated from the values of \( a_1, a_2, \) and \( \Delta l \) with an iterative computer program based on Eqs. A18 and A19; the values of \( r_c \) and \( r_c \) were constrained so that \( r_c/(r_c + r_c) \) was equal to the values determined from the microelectrode measurements. Column 5. In part B, the values of \( r_c \) in column 8 were calculated from the values of \( r_c \) in column 4, with Eqs. 4 and 5; the values in columns 6 and 7 were calculated with an iterative computer program, similar to the one used for part A, in which the value of \( r_c \) was constrained to equal that given in column 8. Column 9 gives the values of \( l_{app} \) that were calculated with Eq. A50 from the values of the cable parameters in columns 6–8. Column 10 gives the values of \( c_a \), that were obtained from the values of \( C_{app} \) and \( l_{app} \) (column 9), with Eq. 3. Columns 5–10 in part C give the ratios of the corresponding values in part B to those in part A. Fiber diameters, 84–116 \( \mu m \).
Several modifications were tested, and the one that was finally adopted is a relation between $I_h$ and $r_e$. This relation was suggested by calculations made with the one-dimensional cable equations. With typical values of $r_m$, $r_i$, and $r_e$, most of the $I_h$ that is calculated to enter the central pool does not cross the plasma membranes but flows directly from the two end pools across the external resistances under the two Vaseline seals. For example, with $r_m = 0.3 \, \text{M} \Omega \cdot \text{cm}$, $r_i = 3 \, \text{M} \Omega / \text{cm}$, and $r_e = 100 \, \text{M} \Omega / \text{cm}$, with $V_1 = V_{RP} = -90 \, \text{mV}$, and with linear variation of $V_r$ (the reversal potential of the plasma membranes under the Vaseline seals, Irving et al., 1987), $I_h$ was calculated to be $-64.51 \, \text{nA}$ (Eq. A2; $b_2$ was calculated with Eq. A30 in Irving et al., 1987), of which $-63.36 \, \text{nA}$, given by

$$
\frac{V_1 + V_2}{L_1 I_h},
$$

behaved as though it flowed directly across the external resistances under the two Vaseline seals.

It therefore seemed reasonable to estimate $r_e$ from the equation

$$
r_e = \frac{V_1 + V_2}{L_1 I_h}.
$$

Eq. 4 holds exactly if the value of $r_m$ is infinite so that all $I_h$ crosses the external resistances under the Vaseline seals. Since $r_m$ is not infinite, Eq. 4 introduces a small error, which, in the example given in the preceding paragraph, was 1.8%.

The resting value of $V_2$ was not monitored in our experiments. It was estimated from $V_1 (= -90 \, \text{mV})$ and the equation

$$
V_2 = V_1 + \frac{a_1 - 1}{a_2} \cdot I_h,
$$

which is easily derived from Eqs. A1 and A2 and the relation $b_1 = (a_1 - 1)b_2/a_2$ (Eq. 17 in Irving et al., 1987).

Once the estimate of $r_e$ has been made, $r_m$ and $r_i$ can be calculated from the values of $a_1$ and $a_2$ with Eqs. A18 and A19. Columns 5-10 of Table II B give the values of the cable parameters that were obtained with this method. The estimates of $r_m/(r_i + r_e)$, $r_i$, $l_{app}$, and $c_m$ in Table II B are extremely close to those obtained with microelectrodes, Table II A, but the estimates of $r_m$ and $r_e$ are not as good. The ratios of the values in Table II B to those in Table II A are given in Table II C and are, on average, 1.004 for $r_m/(r_i + r_e)$, 0.834 for $r_m$, 0.994 for $r_i$, 1.140 for $r_e$, 0.987 for $l_{app}$, and 1.014 for $c_m$.

The conclusion of this section is that Eqs. 4 and 5, combined with Eqs. A18 and A19, can be used to give extremely reliable estimates of $r_m/(r_i + r_e)$, $r_i$, $l_{app}$, and $c_m$ and less reliable estimates of $r_m$ and $r_e$. This method for estimating cable parameters will be used in the rest of this article.

Changes in the Linear Cable Parameters during the Course of an Experiment

Figs. 4-8 show values of cable parameters, plotted as a function of time after saponin treatment, from an experiment in which the TEA-Cl solution was in the central pool. Fig. 4 shows values of $a_1$ (panel A) and $a_2$ (panel B). $a_1$ remained relatively constant during the four hour experiment whereas $a_2$ progressively increased. Fig. 5 A shows the values of $-I_h$ and Fig. 5 B shows the values of $r_e$ that were calculated from $a_1$, $a_2$, and $I_h$ with Eqs. 4 and 5. $I_h$ increased in amplitude during the experiment and $r_e$ decreased.
Figure 4. Values of \( a_1 \) and \( a_2 \) measured during the time course of a cut fiber experiment. (A) \( a_1 \) \( = \frac{\Delta V_{t}(s)}{\Delta V_{t}(\infty)} \); and (B) \( a_2 \) \( = \frac{\Delta L_{t}(s)}{\Delta V_{t}(\infty)} = G_{\text{app}} \) plotted against time following saponin treatment of the end-pool segments. Same fiber as used for Figs. 2 and 3.

The values of \( a_1 \), \( a_2 \), and \( r_e \) in Figs. 4 A, 4 B, and 5 B were used to estimate the other cable parameters from Eqs. A18, A19, and A50. \( r_m \) (Fig. 6 A) decreased somewhat during the first 2 h of the experiment and then remained reasonably constant. \( r_i \) (Fig. 6 B) was relatively stable throughout the experiment. \( r_d/(r_i + r_e) \) (Fig. 7 A) decreased slightly, from a value of 0.983 at the beginning of the experiment to a value of 0.977 at the end. \( I_{\text{app}} \) (Fig. 7 B) increased slightly throughout the experiment.

Figure 5. Values of \( I_h \) (panel A) and \( r_e \) (panel B) during the time course of a cut fiber experiment. \( r_e \) was estimated from the values of \( I_h \) (panel A) and of \( a_1 \) and \( a_2 \) (Fig. 4), with Eqs. 4 and 5. Same experiment as in Fig. 4.
Most of the changes that were observed in Figs 4–7 can be explained by a progressive decrease in \( r_c \) (Fig. 5 B) and an early decrease in \( r_m \) (Fig. 6 A). These produced progressive increases in \( a_2 \) (Fig. 4 A) and in the absolute value of \( l_h \) (Fig. 5 A), a slight progressive decrease in \( r_c/(r_i + r_e) \) (Fig. 7 A), and a slight progressive increase in \( l_{\text{app}} \) (Fig. 7 B).

---

**Figure 6.** Values of \( r_m \) (panel A) and \( r_i \) (panel B) during the time course of a cut fiber experiment. These parameters were estimated from the values of \( a_i \) and \( a_2 \) in Fig. 4 with Eqs. A18 and A19; \( r_c \) was constrained to equal the values in Fig. 5 B. Same experiment as in Figs. 4 and 5.

**Figure 7.** Values of \( r_c/(r_i + r_e) \) (panel A) and \( l_{\text{app}} \) (panel B) during the time course of a cut fiber experiment. \( r_c/(r_i + r_e) \) was calculated from the values in Figs. 5 B and 6 B; the origin of the ordinate is 0.90 rather than 0. \( l_{\text{app}} \) was calculated with Eq. A50. Same experiment as in Figs. 4–6.
Fig. 8 shows $C_{app}$ (panel A) and $c_m$ (panel B) from the experiment illustrated in Figs. 4–7. After the first 1–1.5 h, the value of $c_m$ progressively decreased ~3% per h. In 24 experiments, the average value of the rate of change of $c_m$ from the beginning of an experiment to the end, was 0.48%/h (SEM, 0.76%/h), which is not significantly different from zero. Thus, under our experimental conditions, $c_m$ in cut fibers appears to remain stable for periods of time as long as 3–4 h.

**Effect of Fiber Diameter on the Estimated Values of $r_i$ and $c_m$**

$r_i$ was estimated in 28 fibers in which the TEA·Cl solution was in the central pool. The average value of the first measurements on each fiber was 2.978 MΩ/cm (SEM, 0.134 MΩ/cm). With the TEA$_2$SO$_4$ solution in the central pool, the average value was 3.756 MΩ/cm (five fibers; SEM, 0.369 MΩ/cm), which is not significantly different from that in the TEA·Cl solution.

![Figure 8](image)

**FIGURE 8.** Values of $C_{app}$ (panel A) and $c_m$ (panel B) during the time course of a cut fiber experiment. $C_{app}$ was determined from $\int_0^t \Delta I_{ref}(t) \, dt/AV$ (Eq. 2); $c_m$ was calculated from $C_{app}$ with Eq. 3 and the values of $l_{app}$ in Fig. 7 B. Same experiment as in Figs. 4–7.

If $R_i$, the specific resistance of myoplasm, is independent of fiber diameter, the value of $r_i$ should vary inversely with the cross sectional area of a fiber. In the highly stretched fibers that were used in our experiments, the cross section is approximately circular (Blinks, 1965) so that its area should be approximately equal to $\pi d^2/4$, in which $d$ represents the fiber diameter. Fig. 9 A shows the values of $r_i$ plotted against $(100 \mu m/d)^2$. The filled and open circles are from the TEA·Cl and TEA$_2$SO$_4$ experiments, respectively. The straight line represents a least-squares fit, constrained to intersect the origin, to the TEA·Cl data. Its slope corresponds to a value of 225 Ω·cm for $R_i$. This value, obtained at 13–14°C, is similar to those obtained from K-containing cut fibers at ~18°C, 242 Ω·cm with Ringer's solution in the central pool and 214 Ω·cm with a TEA·Cl solution in the central pool (Irving et al., 1987).
This similarity is expected since K and Cs have similar values of mobility in aqueous solutions (Robinson and Stokes, 1959); the slightly greater mobility of Cs, compared with that of K at the same temperature, is partially compensated by the slightly lower temperature that was used for the Cs experiments.

The value of \( c_m \) was also estimated in the two solutions. The average value was 0.1916 \( \mu F/cm \) (28 fibers; SEM, 0.0098 \( \mu F/cm \)) in the TEA·Cl solution and 0.1721 \( \mu F/cm \) (5 fibers; SEM, 0.0196 \( \mu F/cm \)) in the TEA·SO\(_4\) solution. These two values are not significantly different from each other.

\( c_m \) contains contributions from both surface and transverse tubular membranes. If capacitance is expressed in terms of \( c_m \), the fiber capacitance per square centimeter of surface area, the contribution from the surface membranes is expected to be constant whereas that from the tubular membranes is expected to vary linearly with fiber diameter (Hodgkin and Nakajima, 1972). Fig. 9 B shows \( c_m \) plotted against \( d \); the filled and open circles denote data obtained with the TEA·Cl and TEA·SO\(_4\) solutions, respectively. The straight line shows a least-squares fit to the TEA·Cl data, constrained to intersect the ordinate at 0.9 \( \mu F/cm^2 \), the value of the specific capacitance assumed for the surface membrane (Hodgkin and Nakajima, 1972). If the specific capacitance of the transverse tubular membranes is also 0.9 \( \mu F/cm^2 \) (Hodgkin and Nakajima, 1972), the slope of the straight line corresponds to a value of 2,265/cm for the ratio of tubular surface area to fiber volume. This value lies

![Figure 9](image-url)
within the range of values estimated from electron micrographs: 3,000/cm for frog sartorius fibers stretched to 120–150% of slack length (Peachey, 1965; striation spacing not given), 2,200/cm for frog sartorius fibers at rest length (Mobley and Eisenberg, 1975; striation spacing, 2.6 μm), and 1,700/cm for frog semitendinosus fibers (Mobley and Eisenberg, 1975; striation spacing, 3.4 μm).

The average value of \( r_m \) was 0.186 MΩ·cm (28 fibers; SEM, 0.009 MΩ·cm) in the TEA·Cl solution and 0.569 MΩ·cm (5 fibers; SEM 0.032 MΩ·cm) in the TEA₂·SO₄ solution. The threefold difference between these values, which is statistically significant, is consistent with the conductance of the plasma membrane for Cl being approximately twice that for K (Hodgkin and Horowicz, 1959; Hutter and Noble, 1960). Since the estimates of \( r_m \) are considered to be somewhat unreliable (see p. 234), no attempt has been made to determine the relation between \( r_m \) and fiber diameter.

Voltage-Clamp Transients Recorded from a Linear Electrical Model of a Muscle Fiber Mounted in a Double Vaseline-Gap Chamber

To test our method for estimating linear cable parameters, we constructed a model electrical circuit to represent a cut muscle fiber mounted in a double Vaseline-gap chamber. One reason was to find out whether all the capacitive transient in a double Vaseline-gap experiment is brief, and easy to resolve, or whether there is a slow component, such as might originate from plasma membranes under the Vaseline seals, that cannot be measured reliably.

Fig. 10 A shows a diagram of the circuit that was used to represent the region of fiber under the Vaseline seals (from 1 to a and from 2 to b in Fig. 1) and Fig. 10 B shows the circuit that was used to represent the region of fiber in the central pool (from a to b in Fig. 1). For simplicity, the electrical properties of the plasma membranes were represented by a resistive and a capacitive element in parallel. The capacitive element, which is located electrically on the surface of the fiber, represents the combined capacitance of the surface and transverse tubular membranes. The electrical properties of the longitudinal pathways for current flow, both inside the fiber and outside the fiber under the Vaseline seals, were represented by purely resistive elements. The values of the resistors and capacitors in the model circuit are given in the legend of Fig. 10. They correspond to \( r_m = 0.3 \) MΩ·cm, \( r_i = 3 \) MΩ/cm, \( r_e = 100 \) MΩ/cm, and \( c_m = 0.2 \) μF/cm, values that are similar to those determined in our cut fiber experiments (Table II).

\( \Delta V_1(t) \), \( \Delta V_2(t) \), and \( \Delta I_2(t) \) (not shown) were recorded from the voltage-clamped model circuit following a +20-mV step applied to the command input of the clamp amplifier (Fig. 1). The records were similar to those obtained from muscle fibers (Figs. 2 and 3) except that the \( \Delta I_{2tr}(t) \) and \( \Delta V_{2tr}(t) \) signals had larger amplitudes and shorter durations than those in Fig. 3. This is expected for \( \Delta I_{2tr}(t) \) since all the capacitance in the model circuit was placed as though it were in the surface membranes, whereas most of the capacitance in a muscle fiber is in the transverse tubular membranes where the charging time is delayed by the series resistance of the luminal solution. It is also expected for \( \Delta V_{2tr}(t) \) since \( \Delta V_{2tr}(t) \) provides the driving voltage for \( \Delta I_{2tr}(t) \). The time course of \( \Delta I_{2tr}(t) \) after a step change in \( \Delta V_1(t) \) was rapid and the transient component appeared to be completed within 10 ms.
double Vaseline-gap method introduces no obvious delay in the current transient that is longer than a few milliseconds.

The measured values of $a_1$ (1.1499), $a_2$ (0.9723 μS), and $C_{app}$ (0.01565 μF) were within 1% of the theoretical values of $a_1$ (1.1504), $a_2$ (0.9633 μS), and $C_{app}$ (0.01562 μF) that were calculated from Eqs. A18, A19, A49, and A50. This agreement shows that the lumped circuit in Fig. 10 is a good approximation to the actual distributed circuit for a cut muscle fiber mounted in a double Vaseline-gap chamber. The agreement between the measured and theoretical values of $C_{app}$ also shows that the
DISCUSSION

This article shows how measurements of \( a_1 \) \( = \Delta V_2(\infty)/\Delta V_1(\infty) \), \( a_2 \)
\( = G_{app} = \Delta I_2(\infty)/\Delta V_1(\infty) \), and \( I_b \) can be used to estimate the linear cable parameters
\( r_m, r_i, \) and \( r_e \) in a cut muscle fiber that is mounted in a double Vaseline-gap chamber.
Once these parameters are known, a reliable estimate of \( l_{app} \) can be obtained from
Eq. A50 and Eq. 3 can then be used to estimate \( c_m \) from the measured value of \( C_{app} \).
With this procedure, the average value of \( l_{app} \) in our experiments was estimated to be 772.8 \( \mu \)m (Table II A). The following paragraphs explain why this value is so much
larger than 500 \( \mu \)m, the width of the central-pool region in our experimental
chamber (\( L_2 \) in Fig. 1).

The current that is injected into the current-passing end pool takes several
different pathways to reach the central pool where it is collected. These pathways are
analyzed exactly in Appendix B, with equivalent electrical circuits (see Fig. 12) to
represent the segments of a fiber in the central-pool region and under the two
Vaseline seals. The main results of this analysis can be expressed, approximately, by
the circuit in Fig. 11 A. \( G_j \) represents a conductive element and \( \Gamma_j \) represents a
parallel network of resistors and capacitors arranged as shown in Fig. 11 B, with \( j = \)
1, 2, or 3. \( C_j \) will be used to represent the sum of the values of the individual
capacitors in the \( \Gamma_j \) circuit in Fig. 11 B. \( V_1 \) and \( V_2 \) \( (V_c = 0 \text{ mV}) \) are experimentally
measurable voltages as defined in Fig. 1. \( V_m \) represents the average potential inside a
fiber in the central-pool region, with steady-state changes, \( \Delta V_m(\infty) \), estimated by
\[
\Delta V_m(\infty) = \frac{[\Delta V_1(\infty) + \Delta V_2(\infty)]}{2}
\]
\[
= \frac{a_1 + 1}{2} \cdot \Delta V_1(\infty).
\]

The circuit in Fig. 11 A shows five pathways for current flow into the central pool,
the point labeled \( V_c = 0 \). These are listed below in order from left to right:
1. Across \( G_3 \) from voltage \( V_1 \): \( G_3 = (L_1r_e)^{-1} \)
2. Across \( G_2, \Gamma_2 \) from voltage \( V_m \): \( G_2 = L_1/(3\cdot r_m), C_2 = L_1\cdot c_m/3 \)
3. Across \( G_1, \Gamma_1 \) from voltage \( V_m \): \( G_1 = L_2/r_m, C_1 = L_2\cdot c_m \)
4. Across \( G_2, \Gamma_2 \) from voltage \( V_m \): \( G_2 = L_1/(3\cdot r_m), C_2 = L_1\cdot c_m/3 \)
5. Across \( G_3 \) from voltage \( V_2 \): \( G_3 = (L_1r_e)^{-1} \)

Pathways 1 and 5 represent, respectively, circuits for current flow from EP and
EP2 to CP across the external resistances under the Vaseline seals. Pathways 2 and 4
represent, respectively, circuits for current flow across the plasma membranes under
the Vaseline seals that separate EP and EP2 from CP. Pathway 3 represents a circuit
for current flow across the plasma membranes in the central-pool region.

Table III gives the values of the conductance and capacitance associated with each
of the five pathways in Fig. 11 A. These were calculated with \( r_m = 0.3 \text{ M}\Omega\cdot\text{cm}, r_i = \)
3 \text{ M}\Omega/cm, \( r_e = 100 \text{ M}\Omega/cm, \) and \( c_m = 0.2 \text{ \mu F/cm}, \) values that are close to those
obtained in our experiments (Table II). The information is arranged in three sections
according to whether current is driven by \( V_1 \), \( V_m \), or \( V_2 \). Column 1 gives the values of \( \Delta V_j(\infty)/\Delta V_1(\infty) \), in which \( j = 1, m, \) or 2. \( \Delta V_1(\infty)/\Delta V_1(\infty) \) is obviously unity,
\[ \frac{\Delta V_m(\infty)}{\Delta V_1(\infty)} \] was calculated from Eq. 7, and \[ \frac{\Delta V_2(\infty)}{\Delta V_1(\infty)} (=a_1) \] was calculated from Eq. A18.

Column 2 in Table III gives the formulas for the conductance and total capacitance of each of the admittance elements in Fig. 11A. Column 3 gives the value of each conductive element. Each of these elements makes a contribution to \( G_{\text{app}} \) according to the value of its conductance (column 3) times the ratio \( \frac{\Delta V_j(\infty)}{\Delta V_1(\infty)} \) (column 1). The value of this product is given in column 4. The first five

![Diagram](https://example.com/diagram.png)

**Figure 11.** (A) A circuit that describes, approximately, the pathways for current flow in a double Vaseline-gap chamber in which a muscle fiber is mounted (Fig. 1). \( V_1 \) and \( V_2 \) represent the potentials in end pools \( \text{EP}_1 \) and \( \text{EP}_2 \), respectively. \( V_c \), which is clamped to 0 mV, represents the potential in the central pool. \( V_m \) represents an estimate of the average potential inside a fiber in the central-pool region; its value is calculated from Eq. 7. \( G_1 \), \( G_2 \), and \( G_3 \) represent purely conductive elements. \( \Gamma_1 \) and \( \Gamma_2 \) represent networks of resistive and capacitive elements as shown in B. The \( G_i \), \( \Gamma_1 \), and \( \Gamma_2 \) elements represent the admittance of the surface and transverse tubular membranes in a segment of fiber of length \( L_i \) and \( L_i/3 \), respectively (Fig. 1). \( G_i \) represents the external longitudinal conductance under a Vaseline seal, \( (L_i r_i)^{-1} \). (B) An admittance element of the kind used in A. The total capacitance of the \( \Gamma_1 \) element is denoted by \( C_i \) and is equal to the sum of the individual capacitors. See text, Table III, and Appendix B for additional information.

Entries in this column give the contributions made by the five conductances in Fig. 11A (pathways 1–5). The next entry, row \( i \), gives the sum of these five conductances, 0.9676 \( \mu \)S. This value is closely similar to 0.9633 \( \mu \)S (row \( ii \)), the value of \( a_2 \) calculated from Eq. A19 with \( r_m = 0.3 \) M\( \Omega \)-cm, \( r_1 = 3 \) M\( \Omega \)-cm, and \( r_e = 100 \) M\( \Omega \)-cm. This similarity shows that the circuit in Fig. 11A provides a reliable estimate of \( G_{\text{app}} \) for these values of cable parameters.
Column 5 in Table III gives the values of the total capacitance of the three $\Gamma_j$ elements in Fig. 11 A. The first three values in column 6 give the contributions to $C_{\text{app}}$ from currents that flow across these elements. These values are given by the product of the values in columns 1 and 5.

It is also possible that current flow across the $G_s$ elements in Fig. 11 A (pathways 1 and 5) might contribute to $\Delta I_{z,v}(t)$ and therefore to $C_{\text{app}}$. Any current that flows from $V_1$ across $G_s$ (pathway 1) does not contribute to $\Delta I_{z,v}(t)$ since it is included in the scaled $\Delta V_i(t)$ waveform that is subtracted from $\Delta I_{z,v}(t)$ to give $\Delta I_{z,v}(t)$ (Fig. 3 B). The current that flows from $V_2$ across $G_s$ (pathway 5), however, does contribute to $\Delta I_{z,v}(t)$. The $\Delta V_{z,v}(t)$ waveform (Fig. 3 B) produces a transient current across $G_s$ that makes a contribution to $C_{\text{app}}$ of 0.00081 $\mu$F. This value was calculated from

Table III

| Values of the Resistance and Total Capacitance of the Elements in the Circuit in Fig. 11 A |
|---------------------------------------------------------------|
| $\Delta V_i(\infty)/\Delta V_i(\infty)$ | Formula | Value | $G$ | Value | $C$ |
|-------------------------------------------|---------|-------|-----|-------|-----|
|                                           |         |       |     |       |     |
|                                           | $(L, r_j)^{-1}$ | 0.3333 | 0.3333 |       |     |
| $V_1$: | $G_s$ | 1.0000 |       |     |     |
| $V_2$: | $G_s$ | 1.0752 | $L_{s,v}/3$ | 0.0533 | 0.0533 |
|        | $G_s$ | 1.0752 | $L_{s,v}/3$ | 0.1667 | 0.1792 |
|        | $C_s$ | 1.0752 | $L_{s,v}$ | 0.0100 | 0.01075 |
|        | $G_s$ | 1.0752 | $L_{s,v}/3$ | 0.0333 | 0.0333 |
|        | $C_s$ | 1.0752 | $L_{s,v}$ | 0.0200 | 0.0215 |
| $V_2$: | $G_s$ | 1.1504 | $(L, r_j)^{-1}$ | 0.3333 | 0.3835 |
| $V_1$: | $G_s$ | 1.0000 |       | 0.9676 | 0.9676 |
| $V_2$: | $G_s$ | 1.0752 | $L_{s,v}/3$ | 0.0533 | 0.0533 |
| $V_1$: | $G_s$ | 1.0752 | $L_{s,v}/3$ | 0.1667 | 0.1792 |
| $V_2$: | $C_s$ | 1.0752 | $L_{s,v}$ | 0.0100 | 0.01075 |
| $V_1$: | $G_s$ | 1.0752 | $L_{s,v}/3$ | 0.0333 | 0.0333 |
| $V_2$: | $C_s$ | 1.0752 | $L_{s,v}$ | 0.0200 | 0.0215 |

This table is arranged in three sections according to whether the current is driven by $V_1$, $V_2$, or $V_3$. Column 1 gives the values of $\Delta V_i(\infty)/\Delta V_i(\infty)$, in which $j = 1, m, or 2$; Column 2 gives the formulas for the elements $G_s$ and $C_s$, in which $j = 1, 2, or 3$. Column 3 gives the values of $G_s$ calculated from the formulas in column 2. Column 4 gives the products of the corresponding values in columns 1 and 5; these represent the contributions of $G_s$ to $C_{\text{app}}$ (Eq. B22). Column 5 gives the values of $C_s$ calculated from the formulas in column 2. The first three values in column 6 give the contributions of $C_s$ to $C_{\text{app}}$ given by the products of the corresponding values in columns 1 and 5. The fourth value in the row labeled $V_2$: $G_s$ gives the contribution that is made by $\Delta V_{z,v}(t)$ to $C_{\text{app}}$. Column 7 gives the values in column 6 divided by $c_v$. The last two rows of columns 4, 6, and 7 give the values of $G_{\text{app}}$, $C_{\text{app}}$, and $l_{\text{app}}$, respectively. Row (i) gives the values determined with the circuit in Fig. 11 A, which are equal to the sum of the values in the table. Row (ii) gives the exact values calculated from Eqs. A19, A49, and A50. All values in the table were calculated with $r_v = 0.3 \Omega \cdot cm$, $r_s = 3 \Omega/cm$, $r_s = 100 \Omega/cm$, and $c_v = 0.2 \mu F/cm$. See Discussion and Appendix B for additional information.

The method for estimation of this value is given in the text.

$V_1$ across $G_s$ (pathway 1) does not contribute to $\Delta I_{z,v}(t)$ since it is included in the scaled $\Delta V_i(t)$ waveform that is subtracted from $\Delta I_{z,v}(t)$ to give $\Delta I_{z,v}(t)$ (Fig. 3 B). The current that flows from $V_2$ across $G_s$ (pathway 5), however, contributes to $\Delta I_{z,v}(t)$. The $\Delta V_{z,v}(t)$ waveform (Fig. 3 B) produces a transient current across $G_s$ that makes a contribution to $C_{\text{app}}$ of 0.00081 $\mu$F. This value was calculated from
\[ G_3 \int_0^\infty \Delta V_{2,p} \cdot t \, dt / \Delta V_1(\infty), \]

with Eq. A40, and is listed in column 6 of Table III on the line labeled \( V_2: G_3 \).

The next value in column 6 of Table III, row i, gives the sum of the four contributions, 0.01586 \( \mu \text{F} \). This value is closely similar to 0.01562 \( \mu \text{F} \) (row ii), the value of \( C_{\text{app}} \) calculated from Eqs. A49 and A50 with \( r_m = 0.3 \text{ M}\Omega \cdot \text{cm}, r_i = 3 \text{ M}\Omega / \text{cm}, r_e = 100 \text{ M}\Omega / \text{cm}, \) and \( c_m = 0.2 \mu \text{F/cm} \). This similarity shows that the circuit in Fig. 11 A provides a reliable estimate of \( C_{\text{app}} \) for these values of cable parameters.

Column 7 in Table III gives the values of the capacitances from column 6 divided by \( c_m \). Each entry corresponds to the length of fiber that appears to contribute to the value of capacitance in column 6. These values depend only on \( r_m, r_i, r_e, L_1, \) and \( L_2 \), and not on \( c_m \).

The main conclusion of this analysis and that in Appendix B is that the measured current flow in a double Vaseline-gap chamber, in which a cut fiber has been mounted, can be accurately interpreted in terms of the circuit shown in Fig. 11 A. With \( r_m = 0.3 \text{ M}\Omega \cdot \text{cm}, r_i = 3 \text{ M}\Omega / \text{cm}, r_e = 100 \text{ M}\Omega / \text{cm}, \) and \( c_m = 0.2 \mu \text{F/cm} \), the following conclusions hold (Table III):

1. The errors introduced into the estimates of \( G_{\text{app}}, C_{\text{app}}, \) and \( I_{\text{app}} \) are < 2%.
2. \( G_1 \), the conductance that is attributed to the plasma membranes in the central-pool region (pathway 3), accounts for only ~20% of \( G_{\text{app}} \). Most of \( G_{\text{app}} \) is contributed by the two \( G_3 \) elements (pathways 1 and 5) that exist because \( r_e \) is not infinite.
3. \( C_1 \), the total capacitance that is contributed by the plasma membranes in the central-pool region (pathway 3), accounts for about two-thirds of \( C_{\text{app}} \). The remaining one-third is due to the two \( C_2 \) elements (pathways 2 and 4) and the right-hand \( G_3 \) element (pathway 5).

The analysis in this section, which has been developed for linear circuit elements, will be extended in the following article (Hui and Chandler, 1990) to include nonlinear membrane capacitive elements, for use in the interpretation of measurements of intramembranous charge movement in cut fibers.

**APPENDIX A**

The purpose of this section is to analyze some of the electrical properties of a cut muscle fiber mounted in a double Vaseline-gap chamber (Fig. 1) and to determine the relation between \( c_m \) and \( C_{\text{pp}} \) (Eqs. 2 and 3). In the analysis, the saponin treatment of the end-pool segments of the fiber is assumed to render the plasma membranes sufficiently permeable to ions that the potentials in EP_1 and EP_2 are the same as those inside the fiber at points 1 and 2, respectively. The theoretical procedure is similar to that employed by Irving et al. (1987), who were interested in estimating \( r_m, r_i, \) and \( r_e \) under conditions that were similar to ours.

If the steady-state behavior of the system is linear, then, in the notation of Eqs. 15 and 16 in Irving et al. (1987), measurements of steady-state current and voltage should obey

\[ V_2 = a_1 V_1 + b_1, \quad (A1) \]

\[ I_2 = a_2 V_1 + b_2, \quad (A2) \]

and changes in steady-state current and voltage should obey

\[ \Delta V_2 = a_1 \Delta V_1, \quad (A3) \]

\[ \Delta I_2 = a_2 \Delta V_1. \quad (A4) \]
The constants $a_1$ and $a_2$ can be evaluated from
\[
\begin{pmatrix}
  a_1 \\
  a_2
\end{pmatrix}
= S^{-1}
\begin{pmatrix}
  1 & 0 \\
  0 & -1
\end{pmatrix}
F S
\begin{pmatrix}
  1 \\
  0
\end{pmatrix},
\] (A5)
which is the differential form of Eq. A30 or A31 in Irving et al. (1987). $S$ and $F$ are 2 x 2 matrices of the form,
\[
S = \begin{pmatrix}
  s_{11} & s_{12} \\
  s_{21} & s_{22}
\end{pmatrix},
\] (A6)
and
\[
F = \begin{pmatrix}
  f_{11} & f_{12} \\
  f_{21} & f_{22}
\end{pmatrix}.
\] (A7)
The elements of $S$ are given by the equations
\[
s_{11} = \frac{(r_e + r_i)}{r_e},
\] (A8)
\[
s_{12} = -L_1 r_i,
\] (A9)
\[
s_{21} = \frac{f(L_1/\Lambda_1)}{L_1 r_e},
\] (A10)
\[
s_{22} = \frac{r_e + r_i f(L_1/\Lambda_1)}{r_e + r_i},
\] (A11)
in which
\[
f(L_1/\Lambda_1) = \frac{L_1/\Lambda_1}{\tanh (L_1/\Lambda_1)}.
\] (A12)
$\Lambda_1$, which is the length constant of the fiber under the Vaseline seals, is equal to $[r_m/(r_e + r_i)]^{1/2}$.

The elements of $F$ are given by the equations
\[
f_{11} = \cosh (L_2/\Lambda_2),
\] (A13)
\[
f_{12} = -\Lambda_2 r_i \sinh (L_2/\Lambda_2),
\] (A14)
\[
f_{21} = -\sinh (L_2/\Lambda_2)/(\Lambda_2 r_i),
\] (A15)
\[
f_{22} = \cosh (L_2/\Lambda_2).
\] (A16)
$\Lambda_2$, which is the length constant of the fiber in the central-pool region, is equal to $[r_m/r_e]^{1/2}$.

Since the determinant of $S$ is unity, the elements of the inverse matrix $S^{-1}$ are given by
\[
S^{-1} = \begin{pmatrix}
  s_{22} & -s_{12} \\
  -s_{21} & s_{11}
\end{pmatrix}.
\] (A17)
Eqs. A6–A17 can be incorporated into Eq. A5 to give
\[
a_1 = (f_{11}s_{11} + f_{12}s_{21})s_{22} + (f_{21}s_{11} + f_{22}s_{21})s_{12},
\] (A18)
\[
a_2 = -(f_{11}s_{11} + f_{12}s_{21})s_{21} - (f_{21}s_{11} + f_{22}s_{21})s_{11}.
\] (A19)
Eqs. A5–A19, which apply to steady-state measurements, can be modified with Laplace transforms to deal with transients, following the approach given in the Appendix of Adrian and Almers (1974) and in Appendix B of Schneider and Chandler (1976). The Laplace transform of a function \( y(t) \) is denoted by \( \tilde{y}(p) \) and is defined by

\[
\tilde{y}(p) = \int_0^\infty y(t) \exp (-pt) \, dt,
\]

in which \( p \) is a complex variable. With Laplace transforms, the equations analogous to Eqs. A3 and A4 are

\[
\Delta \tilde{V}_S(p) = a_1(p) \Delta \tilde{V}_i(p)
\]

and

\[
\Delta \tilde{I}_c(p) = a_2(p) \Delta \tilde{V}_i(p),
\]

respectively. \( a_1(p) \) and \( a_2(p) \) are evaluated from Eqs. A5–A19, after the terms in the \( S, F, \) and \( S^{-1} \) matrices have been modified by substitution of \( \bar{y}_m(p) \), the admittance of the plasma membranes (surface and transverse tubular membranes) per unit length of fiber, for \( g_m (=1/r_m) \), the conductance of the same membranes. In the distributed model of the transverse tubular system used by Adrian et al. (1969) and Schneider and Chandler (1976),

\[
y_m(p) = g_m + p c'_m + \sum_n \frac{p c_n}{1 + p r_n c_n}.
\]

\( g_m \), the first term on the right-hand side of Eq. A23, represents the ohmic membrane conductance. The other terms on the right-hand side represent the admittance of the \( \Gamma \) element illustrated in Fig. 11B; \( c'_m \) represents the component of membrane capacitance that does not have a series resistance and \( r_n, c_n \) represents the \( n \)th resistive, capacitive series element. All elements are for a unit length of fiber.

The conductance of the plasma membranes is given by

\[
g_m = y_m(0)
\]

and the capacitance, \( c_m \), is given by

\[
c_m = c'_m + \sum_n c_n
\]

\[
= \lim_{p \to 0} \frac{dy_m}{dp},
\]

which is usually written

\[
c_m = y'_m(0).
\]

(Note that \( c'_m \) in Eqs. A23 and A25 is not a derivative.)

With voltage-clamp records such as those in Fig. 3, the apparent capacitance of the segment of fiber under study, \( C_{app} \), is usually taken to equal \( \int_0^\infty \Delta I_{2,n}(t) \, dt/\Delta V_i(\infty) \), Eq. 2, in which \( \Delta I_{2,n}(t) \) is given by

\[
\Delta I_{2,n}(t) = \Delta I_2(t) - \Delta V_i(t) \frac{\Delta I_2(\infty)}{\Delta V_i(\infty)}.
\]
The integral of $\Delta I_{z,n}(t)$ can be evaluated by taking the limit, as $p \to 0$, of the Laplace transform of $\Delta I_{z,n}(t)$,

$$\int_0^\infty \Delta I_{z,n}(t) \, dt = \lim_{p \to 0} \overline{\Delta I_{z,n}}(p). \quad (A29)$$

The Laplace transform of Eq. A28 is given by

$$\overline{\Delta I_{z,n}}(p) = \overline{\Delta I_4}(p) - \overline{\Delta V_1}(p) \frac{\Delta I_4(\infty)}{\Delta V_1(\infty)}$$

$$= \left[ a_2(p) - \frac{\Delta I_4(\infty)}{\Delta V_1(\infty)} \right] \overline{\Delta V_1}(p)$$

$$= [a_2(p) - a_2(0)] \overline{\Delta V_1}(p), \quad (A30)$$

since

$$\frac{\Delta I_4(\infty)}{\Delta V_1(\infty)} = \lim_{p \to 0} \frac{\overline{\Delta I_4}(p)}{\overline{\Delta V_1}(p)} = a_2(0). \quad (A31)$$

which is denoted by

$$\frac{\Delta I_4(\infty)}{\Delta V_1(\infty)} = a_2(0). \quad (A32)$$

If $a_2(p)$ is expanded in a Maclaurin’s series, Eq. A32 can be written

$$\overline{\Delta I_{z,n}}(p) = [a_2(0) + pa_2'(0) + p^2a_2''(0)/2! + \cdots - a_2(0)] \overline{\Delta V_1}(p). \quad (A33)$$

In the limit, as $p \to 0$,

$$\lim_{p \to 0} \overline{\Delta I_{z,n}}(p) = a_2(0) \lim_{p \to 0} p \overline{\Delta V_1}(p)$$

$$= a_2(0) \Delta V_1(\infty). \quad (A34)$$

Consequently,

$$\int_0^\infty \Delta I_{z,n}(t) \, dt \overline{\Delta V_1}(\infty) = a_2(0), \quad (A35)$$

and similarly,

$$\int_0^\infty \Delta V_{z,n}(t) \, dt \overline{\Delta V_1}(\infty) = a_1(0). \quad (A36)$$

Thus, $G_{\text{pp}} = \Delta I_4(\infty)/\Delta V_1(\infty) = a_2(0)$ (Eq. A35) and $C_{\text{pp}} = a_2'(0)$ (Eq. A39).

The Laplace transform equations that are analogous to Eqs. A8–A11, A13–A16, and A18–A19 reduce to these equations in the limit as $p \to 0$. The first derivatives of $a_i(p)$ and
248 THE JOURNAL OF GENERAL PHYSIOLOGY

VOLUME 96 1990

a_2(p), taken in the limit as p \rightarrow 0, can be evaluated from Eqs. A8–A11, A13–A16, and A18–A19 and the equations

\[ f'_{12}(0) = \left[ \frac{\sinh \left( \frac{L_2}{A_2} \right)}{L_2/A_2} \right] \cdot (L_2 \xi_m/2), \]  

(A41)

\[ f'_{12}(0) = -\cosh \left( \frac{L_2}{A_2} \right) \left( \frac{\sinh \left( \frac{L_2}{A_2} \right)}{L_2/A_2} \right) \cdot (L_2 \xi_m/2), \]  

(A42)

\[ f'_{21}(0) = -\left[ \frac{(L_2/A_2) \cosh \left( \frac{L_2}{A_2} \right) + \sinh \left( \frac{L_2}{A_2} \right)}{2L_2/A_2} \right] \cdot (L_2 \xi_m), \]  

(A43)

\[ f'_{22}(0) = f'_{11}(0), \]  

(A44)

\[ s'_{11}(0) = 0, \]  

(A45)

\[ s'_{12}(0) = 0, \]  

(A46)

\[ \frac{\sinh \left( \frac{L_1}{A_1} \right) \cosh \left( \frac{L_1}{A_1} \right) - \frac{L_1}{A_1}}{(2L_1/3A_1) \sinh^2 \left( \frac{L_1}{A_1} \right)} \frac{r_e + r_i}{r_e} \cdot (L_1 \xi_m/3). \]  

(A47)

\[ s'_{22}(0) = \left[ \frac{\sinh \left( \frac{L_1}{A_1} \right) \cosh \left( \frac{L_1}{A_1} \right) - \frac{L_1}{A_1}}{(2L_1/3A_1) \sinh^2 \left( \frac{L_1}{A_1} \right)} \right] \cdot (L_1 r_i)(L_1 \xi_m/3). \]  

(A48)

In Eqs. A8–A11 and A13–A16, the right-hand side is a function of r_m, r_i, r_c, L_1, and L_2. In Eqs. A41–A48, the right-hand side is equal to \xi_m times a function of r_m, r_i, r_c, L_1, and L_2 (this function is zero in Eqs. A45 and A46). Consequently, a_1'(0) and a_2'(0) are each equal to a function of r_m, r_i, r_c, L_1, and L_2 that is multiplied by \xi_m.

Eq. 3 was used to define \( l_{app} \) according to

\[ l_{app} = \frac{C_{app}/\xi_m}{c_m}. \]  

(A49)

Eqs. 2, A39, and A49 can be combined to give

\[ l_{app} = \left[ \frac{a_2'(0)}{c_m} \right]. \]  

(A50)

Hence, \( l_{app} \) is equal to a function of r_m, r_i, r_c, L_1, and L_2.

For the numerical evaluation of Eq. A50, the Laplace transform equation that is analogous to Eq. A19 is differentiated with respect to p and the limit taken as p \rightarrow 0,

\[ l_{app} = -[f_{11} s_{11} + 2f_{12} s_{12} + f_{22} s_{22} + s_{11} f'_{11} + s_{22} f'_{22} + s_{12} f'_{12} + s_{21} f'_{21} + s_{11} f'_{22} + s_{22} f'_{22}]/c_m. \]  

(A51)

\( s_{11}, s_{12}, s_{21}, s_{22}, f_{11}, f_{12}, f_{21}, f_{22}, \) and \( f_{22} \) are calculated from Eqs. A8–A11 and A13–A16, \( f'_{11}(0), f'_{12}(0), f'_{21}(0), f'_{22}(0), s'_{11}(0), s'_{12}(0), s'_{21}(0), \) and \( s'_{22}(0) \) are calculated from Eqs. A41–A48.

In the analysis of the experimental records described in the text and in Table II, r_m, r_i, and r_c were estimated from a_i, a_n, and either a value of r_c/(r_i + r_c) estimated with a microelectrode or a value of r_c estimated from \( I_{nc} \).

APPENDIX B

The purpose of this section is to analyze the different pathways for current flow in a double Vaseline-gap experiment on a cut muscle fiber with saponin-treated end-pool segments (Fig. 1). Fig. 12 shows three equivalent circuits that represent the segments of the fiber under the Vaseline seals and in the central-pool region. In these circuits, the admittance element A(p)
between two points is represented by

\[ A_j(p) = G_j + \Gamma_j(p), \]  

(B1)

in which \( p \) is the Laplace transform variable (cf. Appendix A) and \( j = I, II, III, IV, \) or \( V \). \( G_j \) is a conductive element and \( \Gamma_j(p) \) is a network of resistors and capacitors as illustrated in Fig. 11 B. \( C_j \) is used to denote the sum of the individual capacitors.

The functional form of each \( A_j(p) \) element is derived from the one-dimensional cable equations on the assumption that the values of \( r_m (=1/g_m) \), \( r_i \), \( r_o \) and \( c_m \) are constant and independent of position along the fiber. Once \( A_j(p) \) has been determined, \( G_j \) and \( C_j \) can be obtained from the relations

\[ G_j = A_j(0) \]  

and

\[ C_j = A'_j(0) - \Gamma'_j(0) \]  

(B3)

Eqs. B2 and B3 are similar to Eqs. A24 and A27, respectively.

---

**Figure 12.** Three equivalent electrical circuits that are used to analyze the electrical properties of a cut fiber mounted in a double Vaseline-gap chamber (Fig. 1). (A and C) Triangle equivalent circuits are used to represent the electrical properties of the regions under the Vaseline seals between EP1 and CP (A) and between CP and EP2 (C). (B) A star equivalent circuit is used to represent the electrical properties of the region in the central pool. Points 1, a, b, c, and 2 correspond to the locations shown in Fig. 1; point ab does not correspond to a particular location in Fig. 1. See Appendix B and Table IV for additional information.

Fig. 12 A shows a triangle equivalent circuit that represents the electrical properties of the fiber segment that is under the left-hand Vaseline seal (Fig. 1). There are three points where, in theory, voltage can be measured and current can be injected: 1, a, and c. The point pairs 1:a, 1:c, and a:c are connected by three admittance elements \( A_1(p) \), \( A_2(p) \), and \( A_3(p) \), respectively.

It is well known from cable theory (for example, see the Appendix of Irving et al., 1987) that, if current does not enter the circuit at point 1, \( V_1 = [(r_i/(r_i + r_o))V_o \) (with \( V_o = 0 \) mV). From this it follows that \( A_1(p)/A_2(p) \) must be constant and equal to \( r_i/r_o \). If points a and c are connected together, the admittance between this point and point 1 is equal to \( A_1(p) + A_2(p) \).
The cable equations show that $A_{\text{m}}(\rho) + A_{\text{n}}(\rho)$ is independent of membrane properties and is equal to $(L_r r_c)^{-1} + (L_r r_c)^{-1}$. This relation can be combined with $A_{\text{n}}(\rho)/A_{\text{m}}(\rho) = \tau_i/\tau_c$ to show that $A_{\text{m}}(\rho)$ and $A_{\text{n}}(\rho)$ are purely conductive with

$$G_{\text{m}} = (L_c \tau_c)^{-1}, \quad (B4)$$

$$G_{\text{n}} = (L_r \tau_r)^{-1}. \quad (B5)$$

The functional form of $A_{\text{m}}(\rho)$ can be determined by calculating the admittance between points $a$ and $c$ with point 1 floating. The variable $x$ is used to denote longitudinal distance along the Vaseline seal with $x = 0$ corresponding to the left-hand edge of the seal (Fig. 1). The potential at point $c$ is assumed to be zero. According to one-dimensional cable theory, $\Delta V(x, \rho)$, the Laplace transform of the voltage change across the fiber membrane at $x$, is given by

$$\Delta V(x, \rho) = \Delta V(L_{\text{m}}, \rho) \frac{\sinh [x/\Theta_i(\rho)]}{\sinh [L_i/\Theta_i(\rho)]}, \quad (B6)$$

$$\Theta_i(\rho) = [\gamma_m(\rho) \cdot (\tau_i + \tau_c)]^{-1/2}, \text{in which } \gamma_m(\rho) \text{ is given by Eq. A23.}$$

From one-dimensional cable theory, the current $I(a)\Delta V(x, t)/\partial x$ at point $a$ is given by

$$I(a) = \frac{1}{\Theta_i(\rho)(\tau_i + \tau_c)} \tanh [L_i/\Theta_i(\rho)], \quad (B7)$$

and the admittance that would be measured between points $a$ and $c$ is given by

$$\frac{\Delta I(a)}{\Delta V(L_{\text{m}}, \rho)} = \frac{1}{\Theta_i(\rho)(\tau_i + \tau_c)} \tanh [L_i/\Theta_i(\rho)], \quad (B8)$$

Eq. B9 gives the admittance that is contributed by $A_{\text{m}}(\rho)$ connected in parallel with $G_{\text{m}}$ and $G_{\text{n}}$ arranged in series. $G_{\text{m}}$ and $G_{\text{n}}$ can be obtained from the network equation for this parallel/series circuit plus Eqs. B2–B5 and B9,

$$G_{\text{m}} = 3[L_i/\Lambda_2 - \tanh (L_i/\Lambda_1)] / (L_i/\Lambda_2)^2 \tanh (L_i/\Lambda_1) \cdot (L_i g_m/3), \quad (B10)$$

and

$$G_{\text{n}} = \left[ \frac{(L_i/\Lambda_1) \sinh (L_i/\Lambda_1) \cosh (L_i/\Lambda_1)}{(\tau_c)^2 \sinh^2 (L_i/\Lambda_1)} - 1 \right] \cdot (L_i g_m/3). \quad (B11)$$

$\Lambda_1$ represents the length constant of the fiber under the Vaseline seals, equal to $[\tau_m/(\tau_i + \tau_c)]^{1/2} = \Theta_i(0)$. Eqs. B10 and B11 have been arranged so that the factors in brackets on the right-hand sides approach unity as $L_i/\Lambda_1$ approaches zero.

Fig. 12 B shows a star equivalent circuit that represents the electrical properties of a fiber segment that is in the central-pool region (Fig. 1). Current can be injected and voltage can be measured at three points: $a$, $b$, and $c$. Admittance elements $A_{\text{m}}(\rho)$ connect points $a$ and $ab$ (the central node) and points $ab$ and $b$; $A_{\text{n}}(\rho)$ connects points $ab$ and $c$. 

The potential at point $c$ is assumed to be zero. According to one-dimensional cable theory, $\Delta V(x, \rho)$, the Laplace transform of the voltage change across the fiber membrane at $x$, is given by
If point \( c \) is maintained at earth potential and point \( b \) is floating, a voltage \( \Delta V(L_1, p) \) at point \( a \) (where \( x = L_1 \)) would produce a current \( \Delta I(p) \) from point \( a \) to point \( c \) that satisfies,

\[
\frac{\Delta I(p)}{\Delta V(L_1, p)} = \frac{\tanh \left[ \frac{L_2}{\Theta_1(p)} \right]}{\Theta_2(p) r_1},
\]  

in which \( \Theta_1(p) = \left[ \gamma_1(p) r_1 \right]^{-1/2} \).

If point \( b \) is connected to point \( c \), and both points are maintained at earth potential,

\[
\frac{\Delta I(p)}{\Delta V(L_1, p)} = \Theta_1(p) r_1 \tanh \left[ \frac{L_2}{\Theta_2(p)} \right].
\]  

Eq. B12 gives the admittance for \( A_1(p) \) and \( A_2(p) \) connected in series, and Eq. B13 gives the admittance for \( A_3(p) \) connected in series with \( A_1(p) \) and \( A_2(p) \) connected in parallel. The network equations for these parallel/series circuits can be combined with Eqs. B2, B3, B12, and B13 to give

\[
G_{IV} = \left[ \frac{L_2/2A_2}{\tanh \left( L_2/2A_2 \right)} \right] \cdot (2/L_2 r_1),
\]  

\[
G_V = \left[ \sinh \left( \frac{L_2}{A_2} \right) \right] \cdot (L_2 r_m),
\]  

\[
C_{IV}(0) = \left[ \frac{\sinh \left( \frac{L_2}{A_2} \right) \cosh \left( \frac{L_2}{2A_2} \right) - L_2/2A_2}{(L_2/3A_2) \sinh \left( \frac{L_2}{2A_2} \right)} \right] \cdot (L_2 r_m/6),
\]  

and

\[
C_V(0) = \left[ \frac{(L_2/2A_2) \cosh \left( \frac{L_2}{A_2} \right) - \sinh \left( \frac{L_2}{A_2} \right)}{2L_2/A_2} \right] \cdot (L_2 r_m),
\]

in which \( A_2 = \left[ \gamma_2/p_1 \right]^{1/2} = \Theta_2(0) \). Eqs. B14–B17 have been arranged so that the factors in brackets on the right-hand sides approach unity as \( L_2/A_2 \) approaches zero.

Fig. 12C shows a triangle equivalent circuit that describes the electrical properties of the fiber segment that is under the right-hand Vaseline seal in Fig. 1. This circuit is identical to that in Fig. 12A so that its elements are also given by Eqs. B4, B5, B10, and B11.

In Fig. 12, there are five pathways for current flow into point \( c \). In \( A \), current can flow from \( \Delta V_1(p) \) across \( G_{II} \) and from \( \Delta V_2(p) \) across \( G_m \) and \( r_V(p) \). In \( B \), it can flow from \( \Delta V_{ab}(p) \) across \( G_v \) and \( r_V(p) \). In \( C \), it can flow from \( \Delta V_v(p) \) across \( G_{III} \) and \( \Delta V_3(p) \) and from \( \Delta V_v(p) \) across \( G_{III} \). \( \Delta I_3(p) \) is given by the sum of these five components,

\[
\Delta I_3(p) = G_{II} \left[ \Delta V_1(p) + \Delta V_2(p) \right] + \left[ G_{III} + \Gamma_{II}(p) \right] \frac{\Delta V_3(p) + \Delta V_2(p)}{\Delta V_1(p)} + \left[ G_v + \Gamma_v(p) \right] \frac{\Delta V_{ab}(p)}{\Delta V_1(p)}. \]  

If the apparent admittance of a cut fiber mounted in a double Vaseline-gap chamber is defined by \( \Delta I_3(p)/\Delta V_1(p) \) and is represented by \( G_{app} + \Gamma_{app}(p) \), the right- and left-hand sides of Eq. B18 can be divided by \( \Delta V_1(p) \) to give

\[
G_{app} + \Gamma_{app}(p) = G_{II} \left[ 1 + \frac{\Delta V_2(p)}{\Delta V_1(p)} \right] + \left[ G_{III} + \Gamma_{II}(p) \right] \frac{\Delta V_3(p) + \Delta V_2(p)}{\Delta V_1(p)} + \left[ G_v + \Gamma_v(p) \right] \frac{\Delta V_{ab}(p)}{\Delta V_1(p)}. \]
$G_{\text{app}}$ is given by

$$G_{\text{app}} = G_{\text{II}} \left[ 1 + \frac{\Delta V_a(\infty)}{\Delta V_i(\infty)} \right] + G_{\text{III}} \frac{\Delta V_a(\infty) + \Delta V_b(\infty)}{\Delta V_i(\infty)} + G_v \frac{\Delta V_m(\infty)}{\Delta V_i(\infty)}$$  \hspace{1cm} (B20)

and $C_{\text{app}}$, which is equal to $C_{\text{app}}(0)$, is given by

$$C_{\text{app}} = G_{\text{II}} \alpha I(0) + G_{\text{III}} \frac{\Delta V_a(\infty) + \Delta V_b(\infty)}{\Delta V_i(\infty)} + C_m \left. \frac{d}{dp} \left( \frac{\Delta V_b(p)}{\Delta V_i(p)} \right) \right|_{p=0} + C_v \left. \frac{d}{dp} \left( \frac{\Delta V_m(p)}{\Delta V_i(p)} \right) \right|_{p=0}.$$  \hspace{1cm} (B21)

Table IV gives the contributions that the various terms in Eqs. B20 and B21 make to $G_{\text{app}}$ and $C_{\text{app}}$. These were calculated with the cable parameters $r_m = 0.3 \, \text{M} \Omega \cdot \text{cm}$, $r_a = 3 \, \text{M} \Omega / \text{cm}$, $r_v = 100 \, \text{M} \Omega / \text{cm}$, and $c_m = 0.2 \, \text{F} / \text{cm}$. See Appendix B for additional information.

**Table IV**

Values of the Resistance and Total Capacitance of the Elements in the Circuit

in Fig. 12

| $\Delta V_i(\infty)/\Delta V_v(\infty)$ | Formula | Factor | (4) | (6) | (7) | (8) |
|---|---|---|---|---|---|---|
| | | | $G$ | $C$ | $\mu F$ | $\mu F$ | $\mu F$ | $\mu m$ |
| $V_i$: | $G_{\text{II}}$ | $1.0060$ | $(L_{r_a}/r_a)^{-1}$ | $1.0000$ | $0.3333$ | $0.3333$ |
| $G_{\text{III}}$ | $1.0350$ | $L_{r_m}/3$ | $0.9800$ | $0.0327$ | $0.0356$ |
| $C_{\text{III}}$ | $1.0350$ | $L_{c_m}/3$ | $0.9605$ | $0.00192$ | $0.00198$ | $98.9$ |
| $V_a$: | $G_a$ | $1.0575$ | $L_{r_m}/3$ | $1.0042$ | $0.1674$ | $0.1770$ | $0.00006$ | $1.2$ |
| $C_a$ | $1.0575$ | $L_{c_m}/3$ | $1.0083$ | $0.01098$ | $0.01066$ | $533.2$ |
| $V_b$: | $G_{\text{II}}$ | $1.0982$ | $L_{r_m}/3$ | $0.9800$ | $0.0327$ | $0.0359$ | $0.00006$ | $1.8$ |
| $G_{\text{III}}$ | $1.0982$ | $L_{r_m}/3$ | $0.9605$ | $0.00192$ | $0.00210$ | $105.5$ |
| $V_c$: | $G_n$ | $1.1504$ | $(L_{r_a}/r_a)^{-1}$ | $1.0000$ | $0.5333$ | $0.5855$ | $0.00081$ | $40.3$ |
| Total | | | | $0.9633$ | $0.01562$ | $780.9$ |

This table is arranged in five sections according to the voltage that drives the current. Column 1 gives the values of $\Delta V_i(\infty)/\Delta V_v(\infty)$, in which $j = 1, a, b, c, b, o, or 2$. Column 2 gives the formulas for the values of elements $G_j$ and $C_j$ as either $L_i/A_i$ or $L_v/A_v$ approaches zero, with $j =$ II, III, or V. To obtain the exact values of these elements, the values calculated from the formulas in column 2 must be multiplied by the factors given in column 3, which were calculated from Eqs. B10, B11, B15, and B17. These values are given in column 4. Column 5 gives the contributions of $G_j$ to $G_{\text{app}}$ (right-hand side of Eq. B20), given by the product of the corresponding values in columns 1 and 4. Each entry in column 6 gives the product of the value calculated from the formula in column 2 and the factor in column 3. Column 7 lists the six contributions to $C_{\text{app}}$ that appear on the right-hand side of Eq. B21. The three values on the lines labeled $C_{\text{III}}$ and $C_{\text{V}}$ represent the products of corresponding values in columns 1 and 6. The value in the bottom row represents the contribution that $\Delta V_v(a)$ makes to the total capacitance (first term on the right-hand side of Eq. B21). The other two values, in rows $V_vG_a$ and $V_vG_{\text{III}}$, arise from the last two terms on the right-hand side of Eq. B21. The values in column 8 were obtained by dividing the values in column 7 by $c_m$. The values in the bottom row were calculated with $r_m = 0.3 \, \text{M} \Omega \cdot \text{cm}$, $r_a = 3 \, \text{M} \Omega / \text{cm}$, and $c_m = 0.2 \, \mu \text{F} / \text{cm}$. See Appendix B for additional information.
CHANDLER AND HUI  Membrane Capacitance in Cut Muscle Fibers  253

\[ r_c = 100 \, \text{MΩ/cm}, \quad \varepsilon_m = 0.2 \, \mu\text{F/cm}, \] values that are similar to those obtained in our experiments (Table II). The information is arranged in five sections according to whether current is driven by \( V_1, V_a, V_{ab}, V_b, \) or \( V_2 \). Column 1 gives the values of \( \Delta V_1(\omega)/\Delta V_1(\omega) \), with \( j = 1, a, ab, b, \) or 2. Column 2 gives the mathematical formulas for each conductive and capacitive element in the limit as \( L_i/A_i \) or \( L_j/A_j \) approaches zero. Since \( L_i/A_i \) and \( L_j/A_j \) are not, in general, zero, each formula must be multiplied by a factor that may be different from unity. Column 3 gives the values of these factors. The factor for \( C_n \) is unity (Eq. B5) and the others, which were calculated from the factors in brackets in Eqs. B10, B11, B15, and B17, are close to unity.

Column 4 in Table IV gives the values of the conductive elements in the right-hand side of Eq. B20; these are equal to the product of the values in column 3 and those calculated from the formulas in column 2. Each element makes a contribution to \( G_{\text{app}} \) given in column 5, that is equal to the product of the corresponding values in columns 1 and 4. The sum of the five values in column 5, 0.9633 \( \mu \text{S} \) (last row), is exactly equal to the value of \( a_4(0) \) that was calculated from Eq. A35.

Column 6 in Table IV gives the values of the capacitive elements in the right-hand side of Eq. B21; these are equal to the values in column 3 times those calculated from the formulae in column 2. Each element makes a contribution to \( C_{\text{app}} \) given in column 7, that is equal to the product of the values in columns 1 and 6. There are three other contributions to \( C_{\text{app}} \) that do not involve \( C_{\text{III}} \) or \( C_v \) directly. The largest of these arises from the current that is produced by \( \Delta V_{v1}(\omega) \) across \( C_v \). This contribution, which is given by the first term on the right-hand side of Eq. B21, is listed in Table IV, column 7 in the row \( V_2:G_v, V_b:G_v \), and the other two contributions are small and are given by the last two terms on the right-hand side of Eq. B21. They are given in rows \( V_v:G_{\text{III}} \) and \( V_v:G_{\text{III}} \) of Table IV.

Column 8 in Table IV gives the values in column 7 divided by \( \varepsilon_m \). This ratio corresponds to the length of fiber that appears to contribute to the value of capacitance in column 7. It depends on \( r_m, r_a, L_{ab}, \) and \( L_{ab} \), but not on \( \varepsilon_m \).

The sums of the six values in column 7, 0.01562 \( \mu \text{F} \), and of those in column 8, 780.9 \( \mu \text{m} \), are exactly equal to the values of \( a_1(0) \) and \( a_4(0)/\varepsilon_m \), respectively, that were calculated from Eqs. A39 and A50.

The equivalent circuit in Fig. 12 can be simplified by making certain reasonable approximations that are suggested by inspection of the values in Table IV. First, the contributions to \( C_{\text{app}} \) made by \( G_v \) connected to \( V_v \), and by \( G_{\text{III}} \) connected to \( V_2 \), are small and can be neglected. Second, the values of the factors in column 3 are all close to unity so that the formulas in column 2 can be used without correction. Third, since \( \Delta V_{a_1}(\omega)/\Delta V_1(\omega) \approx \Delta V_{a_1}(\omega)/\Delta V_2(\omega) + \Delta V_{v1}(\omega)/\Delta V_2(\omega) \)/2 (column 1), all the elements \( G_{\text{III}}, G_{v1}(\omega), G_1(\omega), \) and \( \Gamma_1(\omega) \) can be connected in parallel to point \( ab \). Fourth, \( \Delta V_{a_1}(\omega) \approx \Delta V_{a_1}(\omega) + \Delta V_{v1}(\omega) \)/2.

These approximations correspond to the situation in which the conductance of the plasma membranes approaches zero and Eqs. B20 and B21 become

\[ G_{\text{app}} = G_{\text{III}} \left[ 1 + \frac{\Delta V_2(\omega)}{\Delta V_1(\omega)} \right] + \frac{\Delta V_1(\omega) + \Delta V_2(\omega)}{2 \Delta V_1(\omega)} \quad (\text{B22}) \]

and

\[ C_{\text{app}} = C_{\text{III}} a_1(0) + \frac{\Delta V_1(\omega) + \Delta V_2(\omega)}{2 \Delta V_1(\omega)} \cdot \quad (\text{B23}) \]

respectively. \( G_{\text{III}} = (L_{2v} \varepsilon_m)^{-1}, G_{v1} = L_3 \varepsilon_m/3, C_{\text{III}} = L_i \varepsilon_m/3, G_v = L_2 \varepsilon_m, \) and \( C_v = L_1 \varepsilon_m. \)

The circuit in Fig. 11A is based on the approximations listed in the preceding two paragraphs. Although the circuit is not intended to be used as an equivalent circuit, it can be
used to calculate current flow into the point labeled \( V_i \) by applying the voltages \( V_1, V_2, \) and \( V_3 \) to the circuit elements, as indicated. The points labeled \( V_1, V_2, \) and \( V_3 \) in Fig. 11A correspond, respectively, to the points labeled 1, 2, and 3 in Fig. 12. The point labeled \( V_m \) in Fig. 11A corresponds to the point labeled ab in Fig. 12; \( \Delta V_m(0) = [\Delta V_1(0) + \Delta V_2(0)]/2 \). The admittance elements \( G_i, \Gamma_i(p) \) and \( G_m, \Gamma_m(p) \) in Fig. 11A correspond to \( G_v, \Gamma_v(p) \) and \( G_m, \Gamma_m(p) \), respectively, in Fig. 12. \( G_i \) in Fig. 11A corresponds to \( G_i \) in Fig. 12.

Table III gives the values of the different elements of the circuit in Fig. 11A that were calculated with \( r_m = 0.3 \) M\( \Omega \cdot \text{cm}, r_i = 3 \) M\( \Omega \cdot \text{cm}, r_c = 100 \) M\( \Omega \cdot \text{cm}, \) and \( c_m = 0.2 \) \( \mu \)F/cm. The values in Table III are close to the corresponding values in Table IV, indicating that the circuit in Fig. 11A can be used to provide an accurate estimate of the properties of the equivalent circuit in Fig. 12. The values of \( G_{app}, C_{app}, \) and \( I_{app} \) are 0.9676 \( \mu \)S, 0.01586 \( \mu \)F, and 792.8 \( \mu \)m, respectively, with the approximate circuit in Fig. 11A (row i in Table III) and 0.9633 \( \mu \)S, 0.01562 \( \mu \)F, and 780.9 \( \mu \)m, respectively, with the exact equivalent circuit in Fig. 12 (row ii in Table III and last row in Table IV).

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