Linear Electrical Properties of the Transverse Tubules and Surface Membrane of Skeletal Muscle Fibers

MARTIN F. SCHNEIDER

From the Department of Physiology and Pharmacology, Duke University, Durham, North Carolina 27706. Dr. Schneider's present address is Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06510.

ABSTRACT With the use of two intracellular microelectrodes and a circuit designed to compensate for the effects of stray capacitances around the electrodes, transfer impedance measurements were made at frequencies from 0.5 to 1000 c/s on frog sartorius muscle fibers bathed in 7.5 mM K Ringer solution. Complete AC cable analyses performed at 46, 100, 215, 464, and 1000 c/s showed that the fibers behaved as ideal one-dimensional cables having purely resistive internal impedances ($R_i = 102 \pm 11 \, \Omega \text{ cm}$). Two circuits were considered for fiber inside-outside impedance, a four lumped parameter circuit and a parallel resistance and capacitance shunted by the input impedance of a lattice model for the T-system. Least squares fits to fiber input impedance phase angles were better with the latter circuit than with the former. With the use of the lattice model the specific capacitance of both the surface and transverse tubule membranes was found to be 1 $\mu$F/cm$^2$ and the internal resistivity of the tubules to be about 300 $\Omega$ cm.

The local activation experiments of Huxley and Taylor (1958) and of Huxley and Straub (1958) indicated that the transverse tubules of skeletal muscle fibers form the system which conducts the influence of surface membrane depolarization into the fiber for initiating contraction. Knowledge of the electrical properties of the structures in this system is consequently important for understanding excitation-contraction coupling. The experiments to be described were carried out to determine the passive linear electrical properties of both the T-system and surface membrane of frog muscle fibers.

Impedance measurements made over a range of frequencies may provide a means of separating the T-system impedance from that of the surface membrane. Falk and Fatt (1964) demonstrated that the input impedance of frog
muscle fibers closely approximates that of a cable having inside-outside1 impedance elements consisting of separate resistance and capacitance paths in parallel with a resistance and capacitance in series. However, a variety of different circuits can have impedance properties similar to or identical with those of the circuit proposed by Falk and Fatt.

With either the Falk and Fatt circuit or any circuit electrically equivalent to it as the inside-outside impedance element of a fiber, radial potential gradients would be impossible since such circuits are composed exclusively of lumped resistances and capacitances. Radial gradients are possible only with circuits in which some resistance or capacitance must be spatially distributed through the fiber cross-section. Consequently, assuming the contractile system to be uniform across the fiber, only circuits of the latter type can account for the findings that deep myofibrils are activated later (Gonzalez-Serratos, 1966) and require a larger surface membrane depolarization for activation (Adrian, Costantin, and Peachey, 1969) than those near the fiber surface. The results of the present paper indicate that a closer approximation to fiber input impedance can be obtained using a circuit having some spatially distributed elements than can be obtained using circuits having only lumped impedance elements.

**THEORY**

If it is assumed that muscle fibers have the properties of ideal one-dimensional cables, that they extend indefinitely in both directions, and that both their internal and inside-outside impedances are linear and independent of the axial coordinate then the fiber transfer impedance $Z$, the ratio of $V$ to $I_0$ where $I_0$ is the AC current applied at a given point in the fiber and $V$ the resulting AC potential at distance $x$ from that point, is given by the cable equation

$$Z = Z_0 e^{-\gamma x}$$

(King, 1955; Falk and Fatt, 1964). The fiber input impedance $Z_0$ and propagation constant $\gamma$ are given by

$$Z_0 = (z_i/4\gamma)^{1/2}$$

and

$$\gamma = (z_i\gamma)^{1/2},$$

where $z_i$ is the fiber internal impedance and $\gamma$ its inside-outside admittance, both per unit length of fiber. $Z_0$, $Z$, $z_i$, $\gamma$, and $\gamma$ may all be functions of fre-

1 Inside-outside impedance will be used to denote the impedance, distributed along the length of a fiber, separating the myoplasm from the extracellular fluid adjacent to the surface membrane.
quency \( f \). Expressing \( z_i \) and \( y \) in terms of their magnitudes \(| z_i |\) and \(| y |\) and phase angles \( \phi_z \) and \( \phi_y \), equation (1) can be rewritten as

\[
Z = \left| \frac{z_i}{4y} \right|^{1/2} \exp \left[ -\frac{x}{\lambda_f} \right] \exp \left[ j \left( \frac{\phi_z - \phi_y}{2} + j x \frac{d\phi}{dx} \right) \right],
\]

where

\[
\lambda_f = \left[ \left| \frac{z_i}{4y} \right|^{1/2} \cos \left( \frac{\phi_z + \phi_y}{2} \right) \right]^{-1},
\]

\[
\frac{d\phi}{dx} = -\frac{360}{2\pi} \left| \frac{z_i}{4y} \right|^{1/2} \sin \left( \frac{\phi_z + \phi_y}{2} \right)
\]

and \( j = (-1)^{1/2} \). The transfer impedance magnitude \(| Z |\) is thus an exponential function of \( x \) having a space constant \( \lambda_f \) and a value \(| Z_0 | = | \frac{z_i}{4y} |^{1/2} \) at \( x = 0 \), whereas the transfer impedance phase angle \( \phi \) is a linear function of \( x \) having a slope \( d\phi/dx \), given here in degrees per unit fiber length, and a value \( \phi_0 = (\phi_z - \phi_y)/2 \) at \( x = 0 \). Equations (4) through (6) are algebraically simpler than the equivalent equations in which the variables are expressed in terms of their real and imaginary components (cf. Tasaki and Hagiwara, 1957; Falk and Fatt, 1964). If \(| Z_0 |\), \( \phi_0 \), \( \lambda_f \), and \( d\phi/dx \) are known, the phase angles and magnitudes of \( z_i \) and \( y \) for the frequency under consideration can be calculated using

\[
\phi_z = \phi_0 + \arctan \left( \frac{-2\pi}{360 \lambda_f} \frac{d\phi}{dx} \right),
\]

\[
\phi_y = -2\phi_0 + \phi_z,
\]

\[
| z_i | = \frac{2 | Z_0 |}{\lambda_f \cos \left( \frac{\phi_z + \phi_y}{2} \right)}
\]

and

\[
| y | = \frac{| z_i |}{4 | Z_0 |^{1/2}}.
\]

For the special case of \( \phi_z = 0 \) over the frequency range of interest, \(| Z_0 |\), \( \phi_0 \) and either \( \lambda_f \) or \( d\phi/dx \) can be used to calculate \(| z_i |\) and \(| y |\). In this case \( \phi_y \) is equal to \(-2\phi_0\), \(| z_i |\) is given by either

\[
| z_i | = \frac{4\pi}{360 \sin \phi_0} | Z_0 | \frac{d\phi}{dx}
\]
or by

\[ |z_i| = \frac{2 |Z_0|}{\lambda_y \cos \phi_0} \]  

and \( |y| \) can be calculated using equation (10).

The above equations hold for any \( y \). In order to evaluate a particular circuit for \( y \) the elements in that circuit were related to fiber input impedance, an experimentally determined parameter. Two circuits were considered. The first (Fig. 1 A) was the four lumped parameter circuit proposed by Falk and Fatt (1964) in which \( r \) and \( c \) represent properties of the surface membrane and \( r_s \) and \( c_s \) properties of the current path via the T-system. Using this circuit as the element of fiber inside-outside admittance,

\[ y = \frac{1}{r_m} + \frac{r_e (2\pi f c_e)^2}{1 + \left(2\pi f c_e r_e\right)^2} + j \left(2\pi f c_e + \frac{2\pi f c_e}{1 + \left(2\pi f c_e r_e\right)^2}\right), \]  

where \( r_m, r_e, c_e, \) and \( c_m \) are referred to a unit length of fiber. If the myoplasm is purely resistive (\( \phi_{st} = 0 \)),

\[ -\phi_0 = \frac{1}{2} \arctan \left(\frac{Df + Af}{1 + Bf^2}\right), \]  

where the variables \( A, B, \) and \( D \) are defined as \( 8\pi^2 c_m r_m^2 r_e, 4\pi^2 c_m r_m (r_e + r_m), \) and \( 2\pi (c_e + c_m) r_m \), respectively. The term in parentheses in equation (14) corresponds to \( b/g \) as used by Freygang, Rapoport, and Peachey (1967).

The second circuit (Fig. 1 B) consisted of a parallel lumped resistance \( r_s \) and capacitance \( c_s \), representing the surface membrane, in parallel with the input admittance \( y_{in} \) of a lattice model for the T-system (Appendix 1) divided by the sarcomere length \( \eta \). Using this distributed parameter circuit \( y \) would
be given by

\[ y = \frac{1}{r_{s}} + j2\pi f c_{s} + \frac{y_{in}}{\eta}, \tag{15} \]

where \( r_{s}, c_{s}, \) and \( y_{in}/\eta \) refer to a unit length of fiber. For \( y_{in} \) given by equation (31) and for \( \phi_{s} = 0, \)

\[-\phi_{0} = \frac{1}{2} \arctan \left\{ \left[ \text{Im} \{ (K_{3} + j2\pi f)J_{0}[j(K_{3} + j2\pi fK_{1})^{1/2}] \} \right. \right. \]

\[ + \left. \left. \text{Im} \{ K_{2}j(K_{3} + j2\pi fK_{1})^{1/2}J_{0}[j(K_{3} + j2\pi fK_{1})^{1/2}] \} \right] \right\} - \arctan \left\{ \left[ \text{Im} \{ J_{0}[j(K_{3} + j2\pi fK_{1})^{1/2}] \} \right. \right. \]

\[ + \left. \left. \text{Re} \{ K_{2}j(K_{3} + j2\pi fK_{1})^{1/2}J_{0}[j(K_{3} + j2\pi fK_{1})^{1/2}] \} \right] \right\} \right\} \tag{16} \]

where \( \text{Re} \) and \( \text{Im} \) accompanied by bracketed material denote respectively the real and imaginary terms of the expression within brackets, the variables \( K_{1}, K_{2}, \) and \( K_{4} \) and the functions \( J_{0} \) and \( J'_{0} \) are as defined in Appendix 1, and the variable \( K_{3} \) is equal to \( 1/r_{s} c_{s}. \)

**Methods**

**Material and Solution**

Experiments were carried out on sartorius muscles of *Rana pipiens* at 24–26°C in a solution (7.5 mM K Ringer) containing 115 mM NaCl, 7.5 mM KCl, 1.8 mM CaCl\(_{2}\), and 1 mM tris (hydroxymethyl) aminomethane maleate buffer (Gomory, 1955), pH 7.1. The high K concentration relative to 2.5 mM K of normal Ringer served to decrease the shunting effect of fiber damage at the site of electrode penetration.

**Optics**

The optical system consisted of a compound binocular microscope equipped with water immersion objective and with a total magnification of 400×. In order to facilitate microscopic observation, only fibers near the edge of a muscle were studied. A piece of plastic film, inserted between objective and microscope body, eliminated any resistive path from bath to ground via the objective. The microscope was mounted so that it could be moved a few millimeters in any direction parallel to the bath surface without disturbing muscle or electrodes, thus providing a method of measuring
electrode separations greater than the field diameter. Distance measurements were made using a calibrated eyepiece scale. The sarcomere length of each fiber was calculated using the mean of three determinations of the number, to the nearest quarter band pair, of dark-bright band pairs in a 25 μ length of fiber. The relative depth of each fiber edge was read from a calibrated fine focus adjustment when the condenser was stopped down and the microscope focused at the level at which the region just inside the edge changed from bright to dark. The relative depth of the fiber surface approximately midway between its edge projections was determined by focusing on connective tissue strands, having diameters of 1–2 μ, lying in contact with the fiber surface. All relative depths were taken as the mean of seven determinations. The fiber width at the same axial coordinate that was used for the depth measurements was also determined.

**Microelectrodes**

Two capillary microelectrodes were used to make impedance measurements on individual fibers in the muscle. Voltage-monitoring electrodes were filled with 3 M KCl, had resistances of from 9 to 16 MΩ, and were not controlled for tip potentials. Current-passing electrodes were filled with 2 M sodium citrate acidified to pH 5.5 with citric acid, had resistances of from 15 to 30 MΩ, and passed currents of at least 30 namp peak to peak at 10 c/s without showing evidence of nonlinearities. Electrodes satisfying the last condition were generally found to be linear over the range of frequencies and currents used in the experiments.

**Circuit**

A circuit (Figs. 2 and 3) similar to that employed by Freygang et al. (1967) was used in order to compensate for the effects of stray capacitances around the electrodes. The current and voltage electrodes were assumed to be electrically equivalent to the lumped resistances $R_i$ and $R_v$, respectively, in parallel with the lumped capacitances to bath $C_i$ and $C_v$ and the capacitance between cell interior and an electrode in the "in" position was assumed negligible (Fig. 3). With the negative input terminals of amplifiers $VA$ and $IA$ held respectively at negligible potential difference from ground and $E_1 + E_2$, all current through $R_v$ passed through the feedback resistance $R_{vf}$ around $VA$ and the only current through $R_{vf}$ which did not pass through $R_v$ was the current through the interelectrode coupling capacitance $C$. The latter was compensated for by setting the gain of the differentiator (Fig. 2) so that $V_{SR} = 0$ when the voltage electrode was in the cell, the current electrode was outside the cell but within a few microns of its site of penetration, and the amplitude and frequency of the driving voltage $V_d$ were greater than those used when both electrodes were in the cell. An analysis of the circuit, including the balancing procedure, is outlined in Appendix 2. The experimentally determined phase shift in the balanced circuit was at most 0.2° at frequencies up to $1.5 \times 10^4$ c/s.

Resting potentials were measured under the condition of zero current through $R_v$ by holding $E_2$ equal to zero and adjusting $E_1$ to give $V_{SR} = 0$ both before and after

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645

The author is indebted to Dr. Robert S. Eisenberg for much of the circuit design and assembly.
the voltage electrode had penetrated the fiber. The difference in $E_i$ settings was taken as the resting potential.

*Calibration and Impedance Magnitude Determination*

In order to measure impedance magnitudes and to correct observed impedances for small deviations from ideal cable behavior due to fiber input impedance not being negligible compared to electrode resistances, it was necessary to determine the values of $R_v$ and $R_i$ while both electrodes were in the fiber. Three calibration procedures were used: (1) the ratio $(V_b/E)_{cal}$ of the step change in $V_b$ due to ±10 mv steps $E$ applied to the bath was determined; (2) with $V_b$ at 0.5 c/s the slope $(V_b/V_i)_{cal}$ of the line produced by displaying $V_b$ vertically and $V_i$ horizontally was determined; (3) with $V_b$ left at 0.5 c/s the slope $(V_b/V_i)_{cal}$ of the line produced by displaying $V_b$ vs. $V_i$ was determined. Simultaneous solution of the equations (Appendix 3) corresponding to each of the three calibration procedures gave the equations for
calculating \( R_v \), \( R_t \), and either fiber input resistance \( R_0 \) or transfer resistance \( R \). Straight lines in procedures (2) and (3) indicated that the phase angle of \( Z_0 \) or \( Z \) at 0.5 c/s was zero, i.e., \( Z_0 = R_0 \) or \( Z = R \), and that the system was linear over the voltage displacement used. The validity of the calibration procedure was established by correctly measuring the values of three resistors used to simulate \( R_v \), \( R_t \), and \( R_0 \).

With \( R_v \) and \( R_t \) known and the differentiator balanced, \( Z_{z=0} \), the transfer impedance for electrode separations sufficiently small that the AC potential decrement between electrodes could be neglected when making magnitude calculations and correcting for noninfinite electrode resistances, was determined using

\[
Z_{z=0} = \left( \frac{GR_v}{R_t R_v} \right) \left| \frac{V_s}{V_f} \right| \cos \theta_{z=0} - \frac{1}{R_v} - \frac{1}{R_t} - j \left( \frac{GR_v}{R_t R_v} \left| \frac{V_s}{V_f} \right| \sin \theta_{z=0} \right)^{-1}
\]

(Appendix 2), where \( \theta_{z=0} \) is the phase angle of \( V_s/V_s \) at this short interelectrode distance and \( -G \) the gain of the second stage amplifier in the voltage-monitoring circuit. For the case of significant potential decrement between electrodes \( Z \) was calculated as

\[
Z = \left| \frac{V_s}{V_f} \right| \left[ R_v R_t \cos \theta + |Z_{z=0}| (R_v + R_t) \cos (\theta + \theta_{z=0}) \right]
\]

\[
+ j \left| \frac{V_s}{V_f} \right| \left[ R_v R_t \sin \theta + |Z_{z=0}| (R_v + R_t) \sin (\theta + \theta_{z=0}) \right]
\]

(Appendix 3), where \( \theta \) is the phase angle of \( V_s/V_s \) at electrode separation \( x \).

**Experimental Procedure**

Measurements were made at three or four different interelectrode distances ranging in each fiber from between 8 and 30 \( \mu \) to between 500 and 700 \( \mu \). The angular component of electrode separation was between 45 and 90°. Oscilloscopic Lissajous figures of \( V_s \) vs. \( V_s \) were photographed for subsequent determination of the phase angle and magnitude of \( V_s/V_s \) from projected film images. Since phase angles close to 90° cannot be accurately determined from Lissajous figures, any phase angles which appeared to be greater than about 60° were determined during the course of the experiment using a phase-shifting circuit (Dytronics Standard Phase Generator, Model 410, Dytronics, Inc., Columbus, Ohio). With \( V_s \) as its input signal, the phase generator was set so that its output signal \( V_p \) was perfectly in phase with \( V_s \), as indicated by closure of the Lissajous figure of \( V_p \) vs. \( V_p \), and the phase angle of \( V_s/V_s \) read directly from the setting; only \( |V_s/V_s| \) was subsequently determined from a photograph of \( V_s \) vs. \( V_s \). At the shortest interelectrode distance successive frequencies from 1–1000 c/s differing by a factor of 1.47 were used and the three calibration procedures carried out both before and after scanning the frequency spectrum. All calculations were made using average values of \( R_v \), \( R_t \), and \( R_0 \). At the longer electrode separations successive frequencies from 46.4 to 1000 c/s differing by a factor...
of (1.47)^2 were generally used and, if magnitude measurements were also to be made, the calibration procedures carried out once at each separation. \( V_s \) was adjusted so that the membrane potential always remained within 5 mV of its resting value, thus minimizing membrane nonlinearities. Records showing evidence of nonlinearities were not analyzed.

![Diagram](image)

**Figure 3.** Equivalent circuit for electrodes, fiber, and stray capacitances around the experimental apparatus. Switches \( S_i \) and \( S_v \) represent the locations, "in" or "out" of the fiber, of current and voltage electrodes. \( R_{bi} \) and \( R_{b2} \), each representing the resistance of a silver chloride electrode and agar bridge (Fig. 2), were negligible compared to the 1 MΩ feedback resistance around IA.

**Data Analysis**

Using rearrangements of equations (17) or (18), respectively, \( |Z_{z=0}| \) and \( \phi_{z=0} \) or \( |Z| \) and \( \phi \) values were calculated from magnitudes and phase angles of \( V_s/V_s \). When both \( \phi \) and \( |Z| \) were determined at several interelectrode distances at a given frequency, \( \phi_0 \) and \( |Z_0| \) were found as the \( x = 0 \) intercepts of phase-distance and magnitude-distance plots, respectively. Otherwise \( \phi_0 \) was calculated using

\[
\phi_0 = \phi - x \frac{d\phi}{dx}
\]

and, assuming \( \phi_{z_i} = 0 \), \( |Z_0| \) was calculated using

\[
|Z_0| = |Z| \exp \left[ \frac{2\pi x (d\phi/dx)}{360 \tan \phi_0} \right].
\]
where the values of $\phi$ and $|Z|$ were those determined at the shortest interelectrode distance. If $d\phi/dx$ was not experimentally determined at a given frequency it was approximated as described in Appendix 4. Assuming $z_i$ to be independent of frequency, values of $r_m$, the fiber inside-outside resistance times unit length, were calculated as

$$r_m = \frac{4R_0^2}{|\bar{z}_i|}, \tag{21}$$

where $|\bar{z}_i|$ denotes the mean of the $|z_i|$ values calculated at 46.4, 100, 215, 464, and 1000 c/s by means of equation (11). Values of $|Z_i|$ the specific internal impedance magnitude, were calculated as the product of $|z_i|$ and fiber cross-section area, the latter determined assuming either elliptical (Schneider, 1969) or circular fiber cross-sections.

**Curve Fitting**

The best values, in the least squares sense, of the variables $K_1$, $K_2$, and $K_4$ or $A$, $B$, and $D$ were determined for each fiber by digital computer using equation (16) with $K_2$ constant or equation (14), respectively. Since these equations are nonlinear in the variables to be determined the iterative routine described by Wolberg (1967, Chap. 3) was used. It was assumed that the absolute errors in $\phi_0$ were independent of $f$ and that errors in $f$ were negligible. Since the number of independent variables in the equation for $\phi_0$ is one less than the number necessary to specify both $\phi_0$ and $|Z_0|$ the variables determined by the curve-fitting procedure were functions of circuit parameter ratios or products.

The products $r_{ce_0}$, $r_{cm_0}$, and $r_{ce_0}$ of parameters in the four lumped parameter circuit were calculated using

$$r_{ce_0}^2 + (D/2\pi)(r_{ce_0})^2 - (B/4\pi^2)r_{ce_0} - A/8\pi^3 = 0, \tag{22}$$

$$r_{cm_0} = A/8\pi^3(r_{ce_0})^2 \tag{23}$$

and

$$r_{ce_0} = (D/2\pi) - r_{cm_0}. \tag{24}$$

Equation (22) was solved for $r_{ce_0}$ by computer. Values of $r_m$ calculated by means of equation (21) were used to extract values of $r_e$, $c_m$, and $c_e$ from parameter products. An equivalent fiber radius, the radius of a circle of area equal to the average of the fiber cross-section areas determined on the basis of elliptical and circular cross-sections, was used to refer $r_m$, $r_e$, $c_m$, and $c_e$ to a unit area of fiber surface giving $R_m$, $R_e$, $C_m$, and $C_e$, respectively.

The products and ratio $r_{ce_0}$, $r_1/r_w$, and $r_{ce_0}$ of parameters (Appendix 1) in the distributed parameter circuit were determined from $K_1$, $K_2$, and $K_4$, respectively, using rearrangements of equations (32) through (34). $r_{ce_0}$ was calculated using 3.75 $\mu^{-1}$ (Peachey, 1965) for the length of T-tubule per unit cross-section area in the
plane of the lattice while $r_c$ and $r_l/r_o$ were calculated using equivalent fiber radii. $r_c$ was given by $1/K_3$. Since

$$\frac{1}{r_m} = \frac{1}{r_s} + \frac{g_{in}}{\eta},$$

(25)

where $g_{in}$ is the input admittance of a lattice when $f = 0$, $r_s$ was calculated as

$$r_s = r_m\left[K_3 + K_4F\left\{(K_2)^{1/2}\right\}\right].$$

(26)

The function $F(x)$ was defined as $jxJ'_x/jxJ_x$ and could be obtained from tables compiled by Onoe (1958) or calculated using a series expansion given by the same author. $r_s$ values were used to extract parameter values from ratios or products. Equivalent fiber radii were used to refer $r_s$ and $c_s$ to a unit area of fiber surface membrane giving $R_s$ and $C_s$. A T-tubule perimeter of $0.2\mu$ (Peachey, 1965) was used to refer $r_o$ and $c_o$ to a unit area of tubule membrane giving $R_o$ and $C_o$ and a cross-section area of $2 \times 10^{-3}\mu^2$ (Peachey, 1965) used to convert $r_l$ to the specific resistivity $R_1$ of the tubule contents.

**RESULTS**

The mean ($\pm$SE) resting potential of the eight fibers studied was $66 \pm 2$ mV and their sarcomere lengths ranged from 2.53 to 2.75 $\mu$. The transfer impedance phase angles at short interelectrode distances ($8-29\mu$) of seven fibers are plotted as a function of frequency in Fig. 4 A. In agreement with the results of Falk and Fatt (1964) and of Freygang et al. (1967) the observed phase-frequency plots differed markedly from the sigmoid input impedance phase-frequency plots calculated for a cable having inside-outside impedance elements consisting of a resistance in parallel with a capacitance. The normalized transfer impedance magnitudes corresponding to the phase angles of Fig. 4 A are plotted in Fig. 4 B. As expected of a cable having an inside-outside impedance made up of any combination of resistances and capacitances, the transfer impedance magnitudes decreased monotonically with frequency. The maximum change in electrode resistance during the time that both electrodes were in a fiber at the shortest interelectrode distance was 8%, with the mean absolute change in electrode resistance being 2.8%. Consequently, the condition, necessary for accurate magnitude measurements, that electrode resistance remain constant over the time that an electrode was in a fiber was fulfilled. The average ratio of $R_0$ values determined after to before scanning the frequency spectrum at the shortest interelectrode distance was 0.98.

In order to obtain information about fiber inside-outside impedance from transfer impedance data two types of analyses were required. First, input
Impedances had to be determined from transfer impedances by compensating for fiber one-dimensional cable properties (equation 1) and for any effects of the three-dimensional spread of current from the microelectrode tip (Falk and Fatt, 1964; Eisenberg and Johnson, 1970). Second, the relative contributions of the internal and inside-outside impedances to fiber input impedance (equation 2) had to be evaluated. Experimental data for the

![Graph A](image1.png)

**Figure 4.** Fiber transfer impedances measured at various frequencies using short interelectrode distances. Fiber indices and interelectrode distances corresponding to each symbol are as follows: $\triangledown$ 31, 2 (24 $\mu$m), $\circ$ 32, 1 (29 $\mu$m), $\circ$ 32, 2 (11 $\mu$m), $\circ$ 32, 3 (19 $\mu$m), $\circ$ 36, 1 (8 $\mu$m), $\triangle$ 38, 2 (20 $\mu$m), and $\circ$ 39, 1 (14 $\mu$m). A, transfer impedance phase angles. B, normalized transfer impedance magnitudes. Observed magnitudes were divided by the following factors for normalization: $\triangle$ 0.425, $\circ$ 0.320, $\circ$ 0.270, $\circ$ 0.202, $\circ$ 0.320, $\circ$ 0.250, and $\circ$ 0.255.
analyses consisted of transfer impedance phase angles and magnitudes determined at several different interelectrode distances and frequencies. Four fibers were studied and the results from one are presented in Figs. 5 through 7. As predicted for an ideal one-dimensional cable, the phase angles measured at a given frequency increased linearly (Fig. 5) and the magnitudes decreased exponentially (Fig. 6) with electrode separation. Since $-d\phi/dx$ increased with frequency (Fig. 5) the differences between $\phi_{x=0}$ and the phase-distance plot intercept at $x = 0$ also increased with frequency. This is seen in Fig. 7 A which presents the transfer impedance phase-frequency plot for a 15 $\mu$ interelectrode distance (squares), the zero distance intercepts of phase-distance plots at several frequencies (triangles), and the phase angles of

![Figure 5](image1.png)

**Figure 5.** Transfer impedance phase-distance plots. The frequencies, in c/s, corresponding to each symbol were $\circ$ 46.4, $\square$ 100, $\triangle$ 215, $\triangledown$ 464, and $\bigcirc$ 1000. Lines drawn by eye. Fiber 31,3.

![Figure 6](image2.png)

**Figure 6.** Transfer impedance magnitude-distance plots. Numbers above each line give frequency, in c/s, at which measurements were made and vertical bars mark position of $\lambda_f$. Lines drawn by eye. Fiber 31,3.
$V_{A}/V_{B}$ for the 15 $\mu$ electrode separation (circles). The difference between the square and circle at a given frequency represents the phase-angle error which would have been introduced by assuming fiber input impedance to be negligible compared to the electrode resistances. Fig. 7 B completes the data for the fiber by giving its transfer impedance magnitude-frequency plot as observed at an interelectrode distance of 15 $\mu$.

Tentatively assuming that no significant deviations from ideal one-dimensional cable behavior were present the $x = 0$ intercepts of phase-distance and

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**Figure 7.** Input and transfer impedances of a fiber. A, transfer impedance phase angles recorded at an interelectrode distance of 15 $\mu$ (○), phase angles of $V_{A}/V_{B}$ for the 15 $\mu$ interelectrode distance (□), and input impedance phase angles determined as $x = 0$ intercepts of phase-distance plots at five frequencies (▵). B, transfer impedance magnitude-frequency plot for a 15 $\mu$ interelectrode distance. Fiber 31,3.
magnitude-distance plots could be taken as $\phi_0$ and $|Z_0|$, respectively, and equation (7) used to calculate $\phi_{xi}$. The values thus obtained for $-\phi_{xi}/2$, the quantity which must be subtracted from $-\phi_0$ to give $\phi_{vi}/2$, are presented in Fig. 8 and were considered to be insignificantly different from zero. $|z_i|$ could be calculated by means of equation (9) after having calculated the apparent value of $\phi_{xi}$ or, if $\phi_{xi}$ was assumed equal to zero, by either equation (11) or (12). Values obtained by each of the methods of calculation at a series of frequencies in one fiber are presented in Fig. 9. Since $|z_i|$ values calculated using equation (11) are directly proportional to $d\phi/dx$ they are poorly determined at low frequencies where $d\phi/dx$ is subject to large relative errors. With the use of data from four fibers mean values of the normalized internal impedance magnitude $|z_i|/|z_i|$ were determined and, as seen in Fig. 10, were found to be insignificantly different from unity at frequencies from 46.4 to 1000 c/s. $|z_i|$ was the mean of the $|z_i|$ values determined at 46.4, 100, 215, 464, and 1000 c/s in a fiber using a given method of calculation. The mean

![Figure 8. Internal impedance phase angles determined at various frequencies in four fibers. Fiber indices corresponding to each symbol are 31,3 (○), 32,2 (○), 36,1 (△), and 38,2 (v).](image)

![Figure 9. Fiber internal impedance magnitudes at various frequencies. Values calculated using the complete ac cable analysis are represented by ○, those calculated assuming $\phi_{xi} = 0$ and using $\phi_0$, $|Z_0|$, and $\lambda_f$ by △, and those calculated assuming $\phi_{xi} = 0$ and using $\phi_0$, $|Z_0|$, and $d\phi/dx$ by ◼. Fiber 38,2.](image)
(± se) |Zi| values determined using equations (9), (11), and (12) were 4.20 ± 0.57, 3.99 ± 0.58, and 4.24 ± 0.56 MΩ/cm, respectively.

The facts that the magnitude-distance and phase-distance plots exhibited no consistent deviation from the behavior predicted for an ideal one-dimensional cable, that \( \phi_a \) was not found to be significantly different from zero, and that |zi| did not vary significantly with frequency were all consistent with the conclusion that the fibers behaved as one-dimensional cables having purely resistive internal impedances. Consequently, all further calculations were made using \(-2\phi_0\) for \( \phi_a \) and equations (10) and (11) to calculate |y| and

\[
\frac{|z_i|}{|z_j|} = \frac{-\phi_0}{\omega} - \frac{i\phi_0}{\omega^2} - \frac{i^2\phi_0}{\omega^3} - \frac{i^3\phi_0}{\omega^4} - \frac{i^4\phi_0}{\omega^5}
\]

Figure 10. Normalized internal impedance magnitudes. Symbols used to represent the three methods of calculation are the same as in Fig. 9. Each point represents mean ±se of values from fibers 31, 3, 32, 2, 36, 1, and 38, 2.

\(|z_i|\), respectively. \( \phi_0 \) was calculated using equation (19) and \(|Z_0|\) using equation (20). Since the difference between \( \phi \) and \( \theta \) at a given frequency was found to be independent of distance in each fiber completely analyzed, \( d\phi/dx \) measurements in the other fibers were made using graphs of \( \theta \) as a function of \( x \).

Taking the 0.5 c/s values of \(|Z_0|\) as \( R_0 \), \( r_m \) values were calculated for each fiber and are listed in Table I. Table I also lists the values of the specific internal impedance magnitude calculated for each fiber assuming either elliptical or circular cross-section. Although the two assumed cross-section shapes gave different |Zi| values for the same fiber, the mean value of |Zi| for either shape was the same.

Fig. 11 A presents the values of \(-\phi_0\) determined in one fiber and the best theoretical phase-frequency plot calculated for the fiber using the four lumped parameter circuit. Although the theoretical curve follows the points quite closely, it has a more pronounced crest and trough than are present in the experimental results. That this deviation was systematic and not random is
demonstrated in Fig. 12 in which theoretical minus experimental values of $-\phi_{a}$ are presented for each of the eight fibers studied. In almost all cases the differences exhibit a positive peak at a frequency slightly under 100 c/s and a negative peak in the neighborhood of 300 c/s. This systematic deviation of theoretical from experimental points indicates either that there was some systematic error in the method or that a fiber cannot be perfectly represented using the four lumped parameter circuit. It should be noted that the misfit cannot be corrected by including a shunt resistance across $c$.c.

| Fiber index | Fiber area | $Z_{1}$ | Fiber area | $Z_{1}$ |
|-------------|------------|--------|------------|--------|
| Fiber index | Fiber area | $Z_{1}$ | Fiber area | $Z_{1}$ |
| 31,1        | 147        | 5.31   | 2580       | 137    | 2060   | 110    |
| 31,2        | 160        | 5.51   | 2270       | 125    | 3070   | 169    |
| 32,1        | 39        | 3.80   | 3330       | 126    | 3190   | 121    |
| 32,2        | 110        | 2.86   | 3010       | 86     | 2710   | 78     |
| 32,3        | 79         | 2.48   | 4050       | 101    | 4570   | 114    |
| 36,1        | 104        | 4.21   | 1760       | 74     | 2160   | 91     |
| 38,2        | 84         | 3.39   | 2250       | 76     | 1680   | 57     |
| 39,1        | 112        | 2.37   | 2710       | 64     | 3990   | 94     |

Mean 108 3.52 99 104 ±12 ±0.42 ±10 ±12

Table I
Inside-outside resistance, internal resistance, and internal resistivity of fibers in 7.5 mM K Ringer

Assumed cross-section shape

| Fiber index | $\ell_{m}$ | $\ell_{i}$ | Fiber area | $Z_{1}$ | Fiber area | $Z_{1}$ |
|-------------|------------|------------|------------|--------|------------|--------|
| Fiber index | Fiber area | $Z_{1}$ | Fiber area | $Z_{1}$ |
| 31,2        | 147        | 5.31       | 2580       | 137    | 2060       | 110    |
| 31,3        | 160        | 5.51       | 2270       | 125    | 3070       | 169    |
| 32,1        | 79         | 3.80       | 3330       | 126    | 3190       | 121    |
| 32,2        | 110        | 2.86       | 3010       | 86     | 2710       | 78     |
| 32,3        | 70         | 2.49       | 4050       | 101    | 4570       | 114    |
| 36,1        | 104        | 4.21       | 1760       | 74     | 2160       | 91     |
| 38,2        | 84         | 3.39       | 2250       | 76     | 1680       | 57     |
| 39,1        | 112        | 2.37       | 2710       | 64     | 3990       | 94     |

Mean 108 3.52 99 104 ±12 ±0.42 ±10 ±12

Fig. 11 B presents $-\phi_{a}$ data from the same fiber as illustrated in Fig. 11 A and the best theoretical phase-frequency plot calculated using form 1 of the lattice model. The fit is better than that obtained using the four lumped parameter circuit. Fig. 13 presents the differences between theoretical and experimental values of $-\phi_{a}$ for each fiber. There is no evidence of a systematic deviation of theoretical from experimental points when using the lattice model. The mean ratio of the sum of least squares using the lattice model to the sum of least squares for the same fiber using the lumped parameter circuit was 0.62, with only two of the eight ratios, 1.14 and 1.02, being greater than unity. The values of $R_{e}$, $C_{r}$, $C_{e}$, and $R_{1}$ determined for each fiber are presented in Table III. Interpretation of the data in terms of form 1 of the lattice model gave lower values of surface membrane capacitance than were obtained
with the four lumped parameter circuit. With the use of the structural data presented by Peachey (1965), both tubule and surface membranes were found to have specific capacitances close to 1 \( \mu F/cm^2 \). The \( R_1 \) values presented in Table III are for the out of register case and would be 25% higher assuming in register branching. They are approximately three times the 90 \( \Omega \) cm resistivity (Fatt, 1964) of Ringer solution.

The values in Table III were determined assuming the tubule membrane conductance \( g_o \) to be zero. When \( g_o \) and, therefore, \( K_2 \) were allowed to be nonzero, convergence was not obtained in attempts to determine values of \( K_1, K_2, K_3, \) and \( K_4 \) which gave the best fit to observed input impedance phase angles. However, using various nonzero values of \( K_2 \) as constants and determining the values of \( K_1, K_3, \) and \( K_4 \) which then gave the best fit to \( -\phi_0 \) data, fits as good, in the sum squares sense, as those for \( K_2 = 0 \) were obtained.
Figure 12. Differences between theoretical and experimental input impedance phase angles. Theoretical values calculated using the four lumped parameter circuit. Fiber indices are indicated at left.

**Table II**

**Best Values of the Parameters in the Four Lumped Parameter Circuit for the Inside-Outside Impedance of a Muscle Fiber**

| Fiber Index | $r_e/r_m$ | $r_s/r_m$ | $r_p/r_m$ | $r_m/r_m$ | $a^*$ | $R_m$ | $R_e$ | $C_m$ | $C_e$ |
|-------------|-----------|-----------|-----------|-----------|-------|-------|-------|-------|-------|
| 31,2        | 0.092     | 1.38      | 0.387     | 3.08      | 27.2  | 2.52  | 230   | 1.22  | 1.68  |
| 31,3        | 0.092     | 1.34      | 0.428     | 3.47      | 29.1  | 2.94  | 271   | 1.18  | 1.58  |
| 32,1        | 0.122     | 1.87      | 0.642     | 2.81      | 32.2  | 1.60  | 195   | 1.76  | 3.28  |
| 32,2        | 0.160     | 1.51      | 0.690     | 2.84      | 30.2  | 2.09  | 336   | 1.36  | 2.06  |
| 32,3        | 0.173     | 2.82      | 0.870     | 1.78      | 37.0  | 1.62  | 279   | 1.10  | 3.12  |
| 36,1        | 0.202     | 1.48      | 1.046     | 3.49      | 25.0  | 1.63  | 329   | 2.15  | 3.18  |
| 38,2        | 0.122     | 1.68      | 0.678     | 3.30      | 25.0  | 1.31  | 160   | 2.52  | 4.23  |
| 39,1        | 0.128     | 1.73      | 0.793     | 3.59      | 32.6  | 2.29  | 292   | 1.57  | 2.72  |

| Mean        | 0.136     | 1.73      | 0.692     | 3.04      | 29.1  | 2.00  | 262   | 1.61  | 2.73  |

| ±SE         | ±0.014    | ±0.17     | ±0.077    | ±0.21     | ±1.4  | ±0.20 | ±2.2  | ±0.18 | ±0.32 |

*Equivalent fiber radius.

Forms 1 and 2 of the lattice model thus appear to be electrically equivalent. Since the latter has an additional variable, one of its variables could be set arbitrarily and the remaining ones adjusted so as to make the form 2 impedance properties the same as those of form 1.
Figure 13. Differences between theoretical and experimental input impedance phase angles. Theoretical values calculated using form 1 of the lattice model. Fiber indices are indicated at left.

**Table III**

| Fiber index | $R_s$ (kΩ cm$^2$) | $G_s$ (µF/cm$^2$) | $G_m$ (µF/cm$^2$) | $R_i^*$ (Ω cm) |
|-------------|-------------------|-------------------|-------------------|----------------|
| 31,2        | 2.52              | 0.74              | 0.54              | 249            |
| 31,3        | 2.94              | 0.74              | 0.51              | 263            |
| 32,1        | 1.60              | 0.98              | 0.95              | 191            |
| 32,2        | 2.09              | 0.89              | 0.64              | 365            |
| 32,3        | 1.62              | 0.46              | 0.79              | 273            |
| 36,1        | 1.63              | 1.43              | 1.19              | 428            |
| 38,1        | 1.31              | 1.56              | 1.49              | 293            |
| 39,1        | 2.29              | 0.97              | 0.73              | 309            |
| Mean        | 2.00              | 0.97              | 0.85              | 289            |
| ±SE         | ±0.20             | ±0.13             | ±0.12             | ±27            |

*Tabulated values are for out of register case; values for in register case would be 1.25 times higher.

Fig. 14 gives the locus of values of $C_s$, $C_m$, $G_s$ ($= 1/R_s$), and $G_m$ ($= 1/R_m$) which fit the data from one fiber. The values are plotted as a function of the fraction $G_s/G_m$ of total inside-outside conductance $G_m$ ($= 1/R_m$) attributed to the surface membrane. The form 1 parameters correspond to $G_s/G_m = 1$. As
the relative conductance attributed to the fiber surface was decreased and that attributed to the tubules increased, \( C_s \) increased and \( C_w \) decreased. The tubule internal resistivity, not plotted in Fig. 14, varied by less than 1% over the entire range of fractional surface conductances; the \( R_1 \) values in Table III are thus valid for form 2 as well as for form 1. Table IV gives the values of \( R_s, R_w, C_s, \) and \( C_w \) calculated for \( G_s/G_m = 0.5 \), a lower bound on that quantity if all chloride conductance of the fiber is due to the surface membrane.

![Figure 14](image-url)

**Figure 14.** Parameter value combinations giving equally good fits to the input impedance data from a fiber. Values calculated using form 2 of the lattice model. Fiber 39, 1.

**Table IV**

| Fiber index | \( R_s \) | \( R_w \) | \( C_s \) | \( C_w \) |
|-------------|---------|---------|--------|--------|
|             | k\( \Omega \) cm\(^2\) | k\( \Omega \) cm\(^2\) | \( \mu F/cm^2 \) | \( \mu F/cm^2 \) |
| 31,2        | 5.03    | 19.7    | 0.71   | 0.58   |
| 31,3        | 5.87    | 22.6    | 0.72   | 0.54   |
| 32,1        | 3.20    | 28.5    | 0.93   | 1.05   |
| 32,2        | 4.19    | 16.2    | 0.85   | 0.71   |
| 32,3        | 3.23    | 15.2    | 0.40   | 0.93   |
| 36,1        | 3.25    | 10.2    | 1.36   | 1.41   |
| 38,2        | 2.62    | 8.2     | 1.49   | 1.66   |
| 39,1        | 4.57    | 20.2    | 0.94   | 0.82   |
| Mean        | 4.00    | 17.6    | 0.92   | 0.96   |
| \( \pm SE \) | \( \pm 0.39 \) | \( \pm 2.3 \) | \( \pm 0.12 \) | \( \pm 0.14 \) |

**Table IV**

**Best Circuit Parameter Values Using Form 2 of the Lattice Model**

\( G_s/G_m = 0.5 \) in the Circuit for Fiber Inside-Outside Impedance
DISCUSSION

The mean $\epsilon_a/\epsilon_m$ value of 1.73 found in the present experiments agrees well with the values of 1.74 reported by Falk and Fatt (1964) and of 1.80 reported by Freygang et al. (1967), both for fibers in normal (2.5 mM K) Ringer. When the low frequency time constant $r_m (\epsilon_a + \epsilon_m)$ was multiplied by 2.2, the ratio of $r_m$ values in normal to 7.5 mM K Ringer (unpublished observations), a value of 17.8 msec was obtained which is in fair agreement with the values of 21.0 msec obtained by Falk and Fatt (1964) and of 14.3 msec obtained using the data in Table II of Freygang et al. (1967). The agreement of $\epsilon_a/\epsilon_m$ and of $r_m (\epsilon_a + \epsilon_m)$ values is consistent with fiber capacitances being unaffected by the addition of 5 mM KCl to normal Ringer solution and indicates that the fiber population was comparable to those studied by the authors cited. However, the mean values of $C_m$ and $C_s$ were about 35% lower than the respective values of 2.6 and 4.1 $\mu$F/cm$^2$ determined by Falk and Fatt (1964). The difference may be due to the fact that in the present experiments fiber cross-section areas were measured, giving a mean equivalent radius of 30 $\mu$m, and $|Z_i|$ values experimentally determined, giving a mean $|Z_i|$ of 102 $\Omega$ cm, whereas Falk and Fatt (1964) assumed a mean fiber radius of 50 $\mu$m and an internal resistivity of 200 $\Omega$ cm.

That fiber input impedance data exhibit a smoother transition in the intermediate frequency range than is predicted using the four lumped parameter circuit was reported by both Falk and Fatt (1964) and Freygang et al. (1967) and this was also true of the present data. Falk and Fatt concluded, however, that the fit obtained using a distributed model, electrically equivalent to the lattice model proposed here, was worse than that obtained using the four lumped parameter circuit, a conclusion contrary to that of the present paper. The discrepancy is due to the fact that Falk and Fatt fit their model assuming low frequency equivalence of the distributed path and $r_s$ and $\epsilon_s$ in series, with $r_s$ and $\epsilon_s$ assumed equal to $r_m$ and $\epsilon_m$, respectively, whereas in the present case each model was fit independently. If fiber inside-outside impedance were that of a parallel resistance-capacitance surface membrane shunted by a path composed of distributed series resistance and capacitance, approximating it using the four lumped parameter circuit would result in the distributed capacitance located near the fiber surface and thus having little resistance in series with it being attributed to the surface membrane. The surface membrane capacitance calculated for a fiber must consequently be lower when its input impedance data are interpreted using the distributed model than when using the lumped parameter circuit. The different distribution of capacitance in the two cases accounts for the fact that $\epsilon_a/\epsilon_m$ was found to be 2.4 times lower than the ratio of tubule to surface membrane areas predicted for a 30 $\mu$m radius fiber (Peachey, 1965) whereas $C_s$ and $C_m$ were approximately equal.

A second reason for Falk and Fatt's rejection of the distributed model de-
pended on the interpretation of transverse impedance measurements made on whole sartorius muscles and analyzed in terms of a circuit for fiber transverse impedance having two parallel paths, each consisting of a resistance and capacitance in series. The resistivity $R_z$ and capacitance $C_z$ of one path were interpreted as arising in the tubule lumen and at the mouths of the tubules, respectively, whereas the resistivity $R_i$ and capacitance $C_m$ of the second path were assigned respectively to the myoplasm and surface membrane (Fatt, 1964). Since the value determined for $R_z$ was an order of magnitude too low to account for the $R_z$ value obtained using microelectrodes, it was concluded that $R_z$ must be due to a structure other than the tubule lumen (Falk and Fatt, 1964). However, it is equally likely that $R_z$ is a measure not of the tubule resistivity but of some other property of the muscle. An additional result of the transverse impedance experiments which might be taken as evidence against the distributed model is that they gave a $C_m$ value of 2.6 $\mu$F/cm$^2$ (Fatt, 1964), in good agreement with the $C_m$ value obtained by Falk and Fatt from analysis of input impedance data using the lumped parameter circuit. However, as in the case of the intracellular measurements, the surface capacitance value obtained from transverse impedance data may be high because of some tubule capacitance being attributed to the surface membrane. In fact, the presence of a distributed series resistance and capacitance might explain the 70° phase angle seen in the transverse impedance loci (Bozler and Cole, 1935; Fatt, 1964).

In the present experiments the tubule internal resistivity was found to be over three times that of Ringer solution. Since $R_i$ was calculated as being proportional to the T-system volume per unit fiber cross-section area in the lattice plane, to make $R_i$ equal to the resistivity of Ringer the T-system volume would have to be over three times lower than the value used. Although Peachey and Schild (1968) found that the fraction of the T-system volume accessible to ferritin might be as low as one-third, their estimate of the ferritin volume was a lower limit and they concluded that the entire T-system is probably accessible to ferritin and, therefore, presumably also to extracellular fluid. A high tubule internal resistivity would be consistent with the finding that the effective space constant of the T-system is shorter than can be explained on the basis of the tubule contents having the resistivity of Ringer solution (Adrian, Costantin, and Peachey, 1969).

Assuming the T-system contribution to fiber chloride conductance to be minimal (Hodgkin and Horowicz, 1960; Eisenberg and Gage, 1969), 0.5 would be a safe lower bound on $G_i/G_m$. In normal Ringer the ratio of chloride to potassium conductance is 2 to 1 for small displacements from the resting potential (Hodgkin and Horowicz, 1959; Hutter and Noble, 1960). Addition of 5 mM KCl causes a twofold increase in fiber inside-outside conductance (unpublished observations; Jenerick, 1953); since chloride conductance follows the constant-field equation (Hodgkin and Horowicz, 1959; Hutter and
Noble, 1960) and chloride is distributed passively across the fiber membrane (Adrian, 1961), addition of 5 mM KCl caused an increase in chloride conductance by a factor calculated to be 2.0 when -90 and -66 mV were taken as the membrane potentials in normal and 7.5 mM K Ringer. Consequently, the ratio of chloride to potassium conductance in 7.5 mM K Ringer should also be 2 to 1, $G_c/G_m$ should be greater than 0.5, and the values of surface and tubule resistance and capacitance should be between those presented in Tables III and IV. Using an alternative assumption, consistent with the findings of Adrian, Costantin, and Peachey (1969) and the $R_1$ values determined from the present data, that the specific conductances of surface and tubule membranes are equal, mean ($\pm$ se) values of 0.90 $\pm$ 0.12 and 1.04 $\pm$ 0.15 $\mu$F/cm² were calculated for $C_s$ and $C_e$, respectively, and of 9.73 $\pm$ 1.05 kΩ cm² for the membrane resistance. However, if impedances not considered in the model, such as an impedance between tubule membrane and myoplasm due to some component of the sarcoplasmic reticulum, are present in the fiber the resistance and capacitance of the surface and tubule membranes might be quite different from values calculated using the present model.

The internal resistivity value of 102 $\Omega$ cm found in the present experiments on sartorius muscle fibers in 7.5 mM K Ringer at 24–26°C is considerably lower than the $R_1$ values determined by several previous authors. From transverse impedance measurements on whole sartorius muscles in 2.5 mM K Ringer containing sucrose as a partial substitute for NaCl, Bozler and Cole (1935) found $R_1$ to be 264 and 253 $\Omega$ cm (temperature unspecified) in two muscles and Fatt (1964) found the mean $R_1$ of nine muscles to be 202 $\Omega$ cm (18°C). Using longitudinal currents and external electrodes, Katz (1948) found the mean $R_1$ of nine bundles of one to four adductor magnus or adductor longus fibers in normal Ringer to be 188 $\Omega$ cm (22.5°C). However, using longitudinal currents and single semitendinosus or bicep fibers hung vertically through a moist air gap, Tamasige (1950) found $R_1$ to be 94 $\Omega$ cm at 25°C and to have a $Q_{10}$ of 2. Since $|z_i|$ is only 10% higher in normal than in 7.5 mM K Ringer (unpublished observations), the present results agree with those of Tamasige. The source of the discrepancy between these results and those in the three other reports is not known. The finding that $|z_i|$ is independent of frequency is in agreement with the results of longitudinal impedance measurements made at frequencies from 10 to 10,000 c/s on whole sartorius muscles (Sperelakis and Hoshiko, 1961).

**APPENDIX 1**

*A Lattice Model for the T-System in Frog Muscle Fibers*³

Fiber cross-sections were assumed circular and the T-system lattice assumed to be composed of a series of concentric rings of T-tubule with adjacent rings joined by a

³A similar model for the T-system has been analyzed in a recent paper by Adrian, Chandler, and Hodgkin (1969).
set of radial T-tubule elements of length \( \delta \). Adjacent radial elements of a given annulus were separated by a circumferential distance \( \delta \) at their points of intersection with the inner of the two rings they joined giving a Vernier arrangement along the ring which neighboring annuli had in common. The analysis was carried out for each of two extreme cases: (1) assuming all radial elements of adjacent annuli to be perfectly in register (Fig. 15 A) and (2) assuming them all to be perfectly out of register (Fig. 15 B). The principal paths of internal current for these two cases are illustrated in Fig. 15 C and D.

Assuming the fractional drop in tubule membrane potential between the starting points of adjacent radial elements in neighboring annuli to be negligible, the membrane impedances and internal resistances of the elements depicted in Fig. 15 C or D were treated as lumped parameters and combined to give a single electrically equivalent radial element of length \( \delta \) having an internal resistance \( r'_i \) per unit length and membrane impedance \( z'_w \) times unit length. For equal input impedances at the start of each radial element in a given annulus the total current through all equivalent elements of an annulus could be considered as passing along a single element having an internal resistance per unit length equal to \( r'_i / 2\pi \Delta r \) and a membrane impedance times unit length equal to \( z'_w / 2\pi \Delta r \), where \( 2\pi \Delta r \) gives the number of radial elements in the annulus having the midpoints of its radial elements at distance \( r \) from the fiber axis and \( 2\Delta \) is the length of tubule per unit area in the plane of the lattice.

Applying current to the lattice,

\[
\frac{dE}{dr} = -\frac{Ir'_i}{2\pi \Delta r} \tag{27}
\]

and

\[
\frac{dI}{dr} = -\frac{E2\pi \Delta r}{z'_w} \tag{28}
\]

giving

\[
\frac{d^2E}{dr^2} + \frac{1}{r} \frac{dE}{dr} - \frac{r'_i}{z'_w} E = 0, \tag{29}
\]
where \( E \) is the change in tubule internal potential referred to the isopotential myoplasm in the lattice plane and \( I \) is the internal current, positive in the direction of increasing \( r \), in the lattice. Solving equation (29) using the boundary conditions \( I = 0 \) at \( r = 0 \) and \(-E = E_s \) at \( r = a \), where \( E_s \) is the change in fiber surface membrane potential at the axial coordinate of the lattice and \( a \) is the fiber radius, the lattice input admittance \( y_{in} \) was found to be given by

\[
y_{in} = \frac{2\pi\Delta j(\frac{r_1}{z_w})^{1/2} J_0(j(\frac{r_1}{z_w})^{1/2} a)}{r_1 J_0(j(\frac{r_1}{z_w})^{1/2} a)}
\]

(30)

where \( J_0 \) accompanied by bracketed material denotes the Bessel function of zero order of the argument in brackets and \( J'_0 \) with bracketed material denotes differentiation of \( J_0 \) with respect to the entire argument in brackets.

If \( r_1 \) and \( z_w \) are the tubule internal resistance per unit length and membrane impedance times unit length, \( z_w = z_{cw}/2 \) and \( r_1 = 5r_{1/4} / 4 \) for the out of register case whereas for the in register case, assuming \( 2z_w/\delta \gg r_1 \delta/2 \), \( z_w = z_{cw}/2 \), and \( r_1' = r_1 \). Assuming the tubules to have a simple RC membrane of resistance \( r_w \) times unit length and capacitance \( c_w \) per unit length, the lattice input admittance would be given by

\[
y_{in} = \eta c K_d j(K_2 + j2\pi f K_1)^{1/2} J'_0[j(K_2 + j2\pi f K_1)^{1/2}]
\]

(31)

where

\[
K_1 = \frac{r_w a^2}{\sigma},
\]

(32)

\[
K_2 = \frac{r_1 a^2}{\delta c_w},
\]

(33)

\[
K_4 = \frac{\sigma^4 \pi^4}{r_w c}
\]

(34)

and \( \sigma \) equals \( \frac{3}{2} \) for the in register case and \( \frac{3}{2} \) for the out of register case. The surface membrane capacitance \( c_s \) per unit fiber length and sarcomere length \( \eta \) were introduced to simplify the expression used to fit the lattice model to experimental values of \(-\phi_0\). In form 1 of the model \( K_2 \) was set equal to zero whereas in form 2 \( K_4 \) could take on nonzero values.

**Appendix 2**

**Electronic Compensation for the Effects of Stray Capacitances**

Applying Ohm's law to the circuit around the electrodes (Fig. 3) and using the equations \( \epsilon_t = I_{R_t} / (A_x + 1) \), \( \epsilon_r = -V_r / A_y \), and \( I_{R_r} = -V_r (A_y + 1) / (R_{r_f} A_y) \),

\[
\epsilon_t = I_{R_t} / (A_x + 1), \quad \epsilon_r = -V_r / A_y, \quad \text{and} \quad I_{R_r} = -V_r (A_y + 1) / (R_{r_f} A_y)
\]
where $\epsilon_1$, $A_1$ and $I_{Rf}$ represent the potential between input terminals, the open loop gain, and the current through the feedback resistance of amplifier $IA$ and $\phi$, $A_V$, and $I_{Rf}$ represent the same respective parameters of amplifier $VA$, a set of equations was obtained and solved for $V_V$ as a function of $V_\phi$, $A_V$, $A_1$, and the various impedances in the recording circuit. For the case of both electrodes being in the fiber at a sufficiently small separation that the potential decrement between them was negligible,

$$V_V = -A_V \left[ \frac{V_\phi Z_0}{R_V R_I + Z_0 (R_V + R_I)} + V_\phi (j2\pi fC_c) \right]$$

$$V_\phi \left[ \frac{R_V R_I}{R_V R_I + Z_0 (R_V + R_I)} + j2\pi f R_V C_V \right] \left[ \frac{R_V R_I}{R_V R_I + Z_0 (R_V + R_I)} + j2\pi f R_V C_V \right]$$

$$+ \frac{A_I + 1}{R_f} + j2\pi f (C_I + C_V + C_{t0}) + \frac{R_V + R_I}{R_V R_I + Z_0 (R_V + R_I)}$$

For the case of the voltage electrode being in the fiber and the current electrode being just outside the fiber

$$V'_V = -A_V \left[ V_\phi (j2\pi fC_c) \right]$$

$$+ \frac{V_\phi}{R_V R_I} \left( \frac{R_V}{R_V + Z_0} + j2\pi f R_V C_V \right) \left( 1 + j2\pi f R_V C_V \right)$$

$$+ \frac{A_I + 1}{R_f} + j2\pi f (C_V + C_I + C_{t0}) + \frac{R_V + R_I}{R_V R_I + Z_0 (R_V + R_I)}$$

$$\left[ \frac{A_V + 1}{R_f} + \frac{1}{Z_{0} + R_V} + j2\pi f (C_C + C_V + C_{t0}) \right]$$

$$- \frac{1}{R_V R_I} \left( \frac{R_V}{R_V + Z_0} + j2\pi f R_V C_V \right)^2$$

$$- \frac{A_I + 1}{R_f} + j2\pi f (C_V + C_I + C_{t0}) + \frac{R_V + R_I}{R_V R_I + Z_0 (R_V + R_I)}$$
where the primed symbols denote variables which may in this case take on values different from their values in the case of both electrodes being in the fiber. It was assumed that the displacement of the current electrode during its insertion into the fiber was sufficiently small that all stray capacitances were unchanged by that procedure. The open loop gain magnitudes \(|A_F|\) and \(|A_I|\) were assumed to be greater than \(10^3\) at frequencies up to \(1.15 \times 10^6\) c/s. \(C_V\) and \(C_I\) were both assumed to be less than 20 pF since the electrodes were less than 2 cm immersed in the bathing solution \(\) (Nastuk and Hodgkin, 1950), \(R_V\) was greater than 5 M\(\Omega\), and \(R_I\) greater than 10 M\(\Omega\). With these limits and with \(C_C + C_{Ig}\) less than 80 pF, less than 0.5% error was introduced by setting the denominators of the expressions for both \(V_V\) and \(V_I\) equal to \(-A_V/R_{VF}\). Using the same limits, both \(|(R_V + R_I)/(R_V R_I + Z_0(R_V + R_I))|\) and \(|(R_V + R_I + Z_0)/(R_V R_I + Z_0)\) were less than 0.03% of \(|A_I/R_{IF}|\) and were neglected.

With the voltage electrode in the fiber and the current electrode just outside the fiber the feedback resistance around amplifier CA was adjusted so that \(V_h\) was equal to zero giving

\[
0 = -GV_V + G_p V_c',
\]

(37)

where \(G\) and \(G_p\) were the gains of the negative and positive channels of the differential amplifier (DA) and \(V_c\) was the output voltage of amplifier CA. After the current electrode was inserted, \(V_y = V_c(V_s/\rho_s)\) so that

\[
V_y = -GV + V_p V_V'
\]

(38)

and

\[
\frac{V_y}{V_b} = \frac{G R_{VF}Z_0}{R_V R_I + Z_0(R_V + R_I)} + \frac{G R_{IF}}{R_V R_I (1 - \frac{R_I}{R_I}) (1 + j2\pi f R_V C_V)} \left( \frac{1 + R_I}{R_{IF}} + j2\pi f (C_I + C_V + C_{Ig}) \right)
\]

(39)

\[
- R_V R_I \left\{ \left( \frac{R_V}{R_V + Z_0} - R_I \right) (1 + j2\pi f R_V C_V) \right\} + \left( \frac{R_V^2}{R_V + Z_0} + R_I - R_I \right) (1 + j2\pi f R_V C_V) \right\}
\]

For \(R_V\) less than 20 M\(\Omega\) and \(R_I\) less than 30 M\(\Omega\) together with the above mentioned parameter value limits, neglecting the last term in equation (39) gave rise to an error of less than 0.3%. Assuming a change of less than 10% in \(R_I\) on penetrating...
the fiber and for $|Z_0|$ values greater than 0.05 MΩ neglecting the second term in equation (39) introduced an error of less than 1%.

**APPENDIX 3**

**Electrode Resistance Calibration and Impedance Magnitude Calculation**

For the case of nonnegligible potential decrement between electrodes the circuit (Fig. 16 A) for the first calibration procedure was analyzed using the general steady-state

![Circuit diagram](Image)

**Figure 16.** Circuit used to determine electrode resistances and fiber transfer impedance magnitudes when electrode separation was not negligible. Stray capacitances have been omitted. A, representation of circuit when recording the change in $V$ due to voltage step $E$ applied to the bath. B, representation of circuit for recording $V_I$ and $V_S$, $V_B$ and $I_I$, or $V_B$ and $V_S$.

solution

$$V - E = Ae^{-x/l} + Be^{x/l}$$  

(40)

of the differential equation for a one-dimensional cable (see Taylor, 1963) where $V$ is the internal potential of the cable with respect to ground at distance $x$, $E$ is the potential step applied to the bath, and $A$ and $B$ are constants evaluated by means of boundary conditions on the fiber internal currents at the $x$ coordinates of the voltage and current electrodes. Solving for $A$ and $B$, the equation

$$\frac{V_B}{E}_{\text{Cal.}} = \frac{GR_T R_0 (1 - e^{-\pi l}) + GR_T R_T}{R_0 (1 - e^{-\pi l}) + R_0 (R_T + R_T) + R_T R_T}$$  

(41)

was obtained. In the second and third calibration procedures the resistance between current electrode and virtual ground was $2R_0$ in parallel with the input resistance of a length $x$ of cable terminated by the two parallel resistances $R_T$ and $2R_0$ (Fig. 16 B).
It was assumed that at 0.5 c/s the current through \( C_r \) was negligible compared to that through \( R_1 \). Using equations for the input resistance of a cable terminated by an arbitrary resistance (Johnson and Sommer, 1967) and for potential as a function of distance in a terminated cable (Sommer and Johnson, 1968), the equation for the second calibration procedure was given by

\[
\frac{V_{g}}{V_{t}}_{\text{Cal} \ 1} = \frac{(R_0 + R_f)(R_0 + R_T)e^{ik} - R_0^2 e^{-ik}}{((R_0 + R_T)e^{ik} - R_0)R_{1f}} \tag{42}
\]

and the equation for the third calibration procedure by

\[
\frac{V_{g}}{V_{t}}_{\text{Cal} \ 3} = -GR_{V_f}R_0 \frac{R_f}{R_{1f}(R_{V_f}e^{ik} - R_0(1 - e^{ik}))} \tag{43}
\]

Since \( R_0 \) was previously determined from measurements at negligible electrode separation, equations (41) through (43) constitute a set of three equations in the three variables \( R_V, R_1, \) and \( R = R_0 e^{ik} \). Rearranging and neglecting terms which contributed less than 0.25% to \( R_V \) or \( R_1 \), the equations used in calculating \( R_V, R_1, \) and \( R \) were obtained. For the case of negligible potential decrement between electrodes equations (41) through (43) with \( x = 0 \) were solved simultaneously to give the equations used in calculating \( R_V, R_1, \) and \( R_0 \).

Under the condition of electronic compensation for the effects of current through the stray capacitances, \( V_{g}/V_{t} \) for nonnegligible potential decrement between electrodes was given by

\[
\frac{V_{g}}{V_{t}} = \frac{GR_{V_f}Z_0 e^{-\gamma x} - Z_0 e^{-\gamma x}}{(Z_0 + R_t)(Z_0 + R_V) - Z_0 e^{-\gamma x}} \tag{44}
\]

Rearranging equation (44), neglecting a term which contributed less than 0.25% to \( |Z_0 e^{-\gamma x}| \), and approximating \( \phi_0 \) by \( \theta_{z_{n0}} \) and \( |Z_0| \) by \( |Z_{z_{n0}}| \) gave equation (18) or calculating \( Z \). The use of \( \theta_{z_{n0}} \) to approximate \( \phi_0 \) is preferable to the use of \( \theta_{z_{n0}} \) since at the higher frequencies \( \phi_0 \) was closer to the former than to the latter (Fig. 7 A).

**APPENDIX 4**

**Method of Approximating \( d\phi/dx \) Values for Frequencies at which Phase-Distance Data Were Not Obtained**

Assuming one-dimensional cable behavior with \( \phi_i = 0 \) and \( |z_i| \) independent of frequency,

\[
\left( \frac{d\phi}{dx} \right)_{f_1} = \left( \frac{Z_0}{Z_0} \right)_{f_1} \frac{\sin (-\phi_0)_{f_1} \left( \frac{d\phi}{dx} \right)_{f_5}}{\sin (-\phi_0)_{f_5}} \left( \frac{d\phi}{dx} \right)_{f_5}, \tag{45}
\]

where the subscript on each variable denotes the frequency at which the variable takes its value. Using impedance magnitudes and phase angles recorded at a short interelectrode distance \( x \) to approximate their values at \( x = 0 \), \( d\phi/dx \) could be ap-
proximated as

\[
\left( \frac{d\phi}{dx} \right)_{f_1} \approx \frac{|Z|}{|Z|} \frac{\sin (-\phi)_{f_1}}{\sin (-\phi)_{f_2}} \left( \frac{d\phi}{dx} \right)_{f_2}.
\]

(46)

As \( x \) approaches zero or as \( f_1 \) approaches \( f_2 \), the error involved in the approximation approaches zero. In order to minimize any systematic error which might be introduced by using data obtained at either the next highest frequency \( f_{i+} \) (\( = 1.47f_i \)) or next lowest frequency \( f_{i-} \) (\( = f_i/1.47 \)) to approximate \( d\phi/dx \) at a frequency \( f_i \) at which it was not determined, the approximation employed was

\[
\left( \frac{d\phi}{dx} \right)_{f_i} \approx \frac{\sin (-\phi)_{f_i}}{2Z_{f_i}} \left[ \frac{|Z|_{f_{i+}}}{\sin (-\phi)_{f_{i+}}} \left( \frac{d\phi}{dx} \right)_{f_{i+}} + \frac{|Z|_{f_{i-}}}{\sin (-\phi)_{f_{i-}}} \left( \frac{d\phi}{dx} \right)_{f_{i-}} \right],
\]

(47)

where \( x \) was a short interelectrode distance. If \( d\phi/dx \) was not determined at a given frequency \( f_1 \) and also not determined at either \( f_1/1.47 \) or \( 1.47f_1 \), the approximation employed was that of equation (46) where \( x \) was a short interelectrode distance and \( f_2 \) was the frequency closest to \( f_1 \) at which \( d\phi/dx \) was determined. Since the factor \( x(d\phi/dx) \) which was subtracted from \( \phi \) to give \( \phi_0 \) was always less than 3 degrees, an error of as much as 10% in the approximated value of \( d\phi/dx \) would give rise to an error of less than 0.3 degree in the calculated value of \( \phi_0 \). At frequencies lower than the highest used in these experiments, \( d\phi/dx \) was smaller, thus giving rise to a smaller maximum error in \( \phi_0 \).

It is a pleasure to thank Dr. Paul Horowicz for his suggestions, support, and critical comments throughout the course of this work, and Dr. Robert Eisenberg for advice and assistance during its early phases.

This work was supported by research grants from the U. S. Public Health Service (GM-08803) and the National Science Foundation (GB-2537) and was completed in partial fulfillment of the requirements for the Ph.D. degree in the Department of Physiology and Pharmacology, Duke University, Durham, North Carolina.

The author was supported by Training Grant NIH 5 T01 GM 00929.

An abstract of this paper was presented at the Third International Biophysics Congress, Cambridge, Massachusetts, September, 1969.

Received for publication 11 March 1970.

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