Longitudinal Impedance of Single Frog Muscle Fibers

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ABSTRACT The longitudinal impedance of single skeletal muscle fibers has been measured from 1 to 10,000 Hz in an oil gap apparatus which forces current to flow longitudinally down the fiber. The impedance observed is purely resistive in some fibers from the semitendinosus muscle and in two fibers from the sartorius muscle. In other fibers from the semitendinosus muscle a small phase shift is observed. The mean value of the maximum phase shift observed from all fibers is −1.07°. The artifacts associated with the apparatus and method are examined theoretically and it is shown that one of the likely artifacts could account for the small phase observed. It is concluded that the longitudinal impedance of skeletal muscle fibers is essentially resistive and that little, if any, longitudinal current crosses the membranes of the sarcoplasmic reticulum.

INTRODUCTION

The impedance of skeletal muscle fibers to the longitudinal flow of current is of some physiological interest: It might be expected to reflect the linear electrical properties of the sarcoplasmic reticulum, it surely influences the shape and conduction velocity of the action potential (Adrian and Peachey, 1973), and so, on both counts, it should influence excitation contraction coupling. The nature of the longitudinal impedance is also important in the interpretation of a number of experiments on the electrical properties of muscle fibers, including experiments on the mechanism of the action potential (Adrian et al., 1970 *a, b*), on the linear equivalent circuit of muscle fibers (Valdiosera et al., 1974 *a, b*), and on the analysis of the linear (Hodgkin and Nakajima, 1972 *a, b*; Adrian and Almers, 1974) and nonlinear (Schneider and Chandler, 1973) capacitance of the tubular system.

Some time ago Schneider (1970) measured the transfer impedance of muscle fibers with microelectrodes and sought to determine the longitudinal impedance by a procedure involving the subtraction of quantities measured at different separations of the microelectrodes. This procedure is necessarily fraught with difficulty and subject to systematic errors since it involves re-

peated impalements of a fiber and also the subtraction of two numbers of comparable size, but the analysis does show that the longitudinal impedance is not dominated by a capacitive reactance. Other exepriments (Mobley et al., 1973) suggest, however, that the longitudinal impedance includes a small capacitive reactance.

We have investigated the longitudinal impedance of two preparations of muscle fibers, skinned muscle fibers (Mobley et al., 1974) and isolated intact fibers. The former preparation has no surface membrane and so has negligible transverse impedance which might mask the longitudinal impedance we wish to measure; on the other hand, it is not clear to what extent the skinned preparation is an adequate model of the interior of an intact muscle fiber. The experiments reported here were performed on intact fibers in normal physiological condition, but the experiments are sensitive to errors produced by the impedance of the surface and tubular membranes, and we had to investigate the significance of these errors. Our measurements are made with an oil gap apparatus (Cole and Hodgkin, 1939) that forces current to flow longitudinally, down the impedance we seek to measure.

The results presented in this paper show that the longitudinal impedance of intact muscle fibers is essentially a resistance. Small deviations from purely resistive behavior are observed, but they probably result from experimental difficulties.

METHODS

Apparatus and Procedure

Fig. 1 shows the presumed pattern of current flow in a muscle fiber and the experimental apparatus. Currents were kept sufficiently small (from 2 to 7 nA root mean square) that the preparation behaved as a linear circuit element. Checks showed that in this range of current the impedance observed was independent of the amount of current passed through the muscle fiber. Fig. 1 of Mobley et al. (1974) shows the apparatus explicitly; that paper also describes the experimental details and the correction for stray capacitance.

The Appendix to this paper presents an analysis of the equivalent circuit of the preparation. Briefly, the analysis shows that we measure $Z(L)$ the total impedance of the ends of the fiber (in the conducting pools of Ringer) in series with the impedance of a length L (centimeters) of fiber in the oil gap:

$$Z(L) \simeq zL + Z_1 + Z_2 \qquad (1)$$

where Z_1, Z_2 are the input impedances of the ends of the fiber (ohms) and z is the longitudinal impedance of a unit length of the fiber (ohms per centimeter). The equation is a crude approximation since shunting is ignored and the ends of the fiber are assumed to be infinitely long. The shunting caused by the longitudinal flow of current in the thin layer of conducting solution between the fiber and the oil is certainly im-
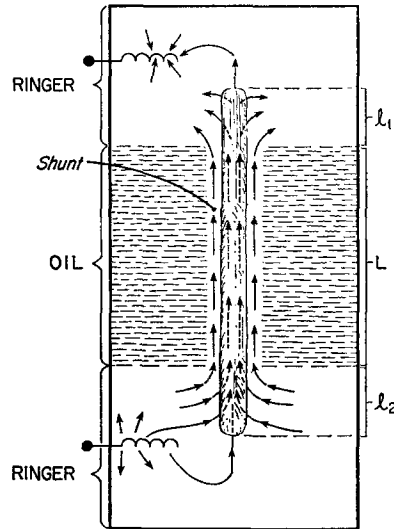
FIGURE 1. A representation of the experimental apparatus. The fiber is suspended vertically with each end bathed in Ringer and the middle bathed in an oil gap. Current flows from one bath of Ringer solution to the other by way of the fiber and a small layer of Ringer solution between the fiber and the oil; this layer of solution shunts the impedance of the fiber. The current flowing by way of the fiber first must cross the membrane at the end of the fiber, then flow longitudinally along the fiber in the oil gap, and then cross the membrane at the other end of the fiber. The impedance to current flowing across the ends of the fiber is similar to the input impedance of the fiber and is often called an end effect. The impedance to current flowing along the length of the fiber is essentially the longitudinal impedance of the fiber. Since the end effect is not expected to depend on the length of the oil gap (L), the difference between measurements at two different lengths is, in principle, the longitudinal impedance.

portant and the theoretical treatment of this shunt must be quite precise if the experiments and results are to be fully understood. Since there is little information concerning the shunt, the Appendix treats it in a simple way, as a uniformly distributed resistance, and shows that changes in the shunt could be important in our results.

The Appendix shows that, to a first approximation, the longitudinal impedance we wish to measure is given by the difference between the impedance measured at two gap lengths:

$$\frac{Z_L(\Delta L)}{\Delta L} \cong \frac{Z(L_2) - Z(L_1)}{L_2 - L_1} \cong z. \tag{2}$$

Preparation

Single muscle fibers were isolated from the semitendinosus muscle or sartorius muscle of the frog *Rana pipiens*. Very large frogs were used and the diameter of the muscle fibers was also large, up to 170 μm. The isolation of the sartorius fibers was somewhat troublesome since it is necessary to clean the entire length of the fibers and to pare

down the tendon so it can fit in the apparatus. Fibers were bathed in a Ringer solution of 115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, and 2.6 mM Tris HCl, 0.4 mM Tris base (pH 7.2) unless otherwise noted. Experiments were performed at room temperature of some 20°C. All fibers gave vigorous twitches at the end of the experiment.

RESULTS

Fig. 2 shows the phase angle of the impedance $Z_L(\Delta L)$ of a sartorius muscle fiber. The phase angle is negligible over the entire range of frequencies. The relation of the phase angle presented in the figure to the raw experimental data is explained in the Methods section and the Appendix of this paper and is illustrated in Fig. 5 of Mobley et al., (1974). Similar results were obtained from one other sartorius fiber; we were unable to perform more experiments on sartorius fibers because of the difficulty in completely isolating these very long fibers.

Fig. 3 shows the phase angle of the impedance $Z_L(\Delta L)$ of a semitendinosus fiber which had negligible phase shift. While this was a common result, we also found significant phase shift in many fibers. Fig. 4 shows the results from
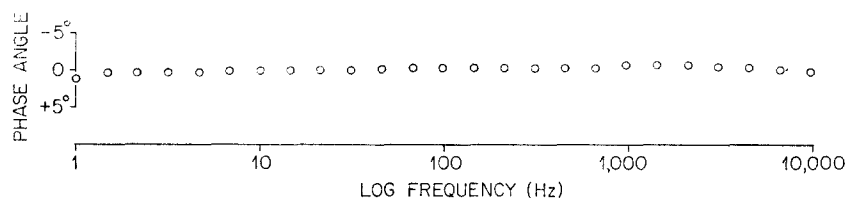

FIGURE 2.   The phase angle of the longitudinal impedance $Z_L(\Delta L)$. The results are from a sartorius fiber with an apparent resistivity of 92 ohm-cm.
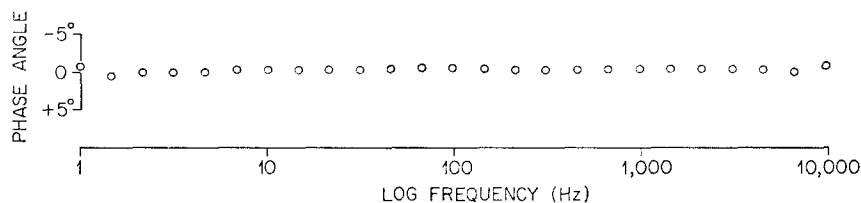

FIGURE 3.   The phase angle of the longitudinal impedance $Z_L(\Delta L)$. The results are from a semitendinosus fiber with apparent resistivity of 81 ohm-ch.
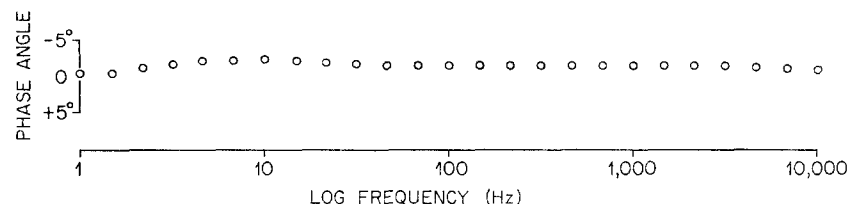

FIGURE 4.   Phase angle of the longitudinal impedance $Z_L(\Delta L)$. The results are from a semitendinosus fiber with an apparent resistivity of 97 ohm-cm. This fiber had almost the largest phase shift we observed.

a fiber that showed significant phase shift.[1] There was a definite pattern of the phase shift relative to frequency in this and the other fibers that showed significant phase shift. The phase angle reached a maximum at a relatively low frequency and although the phase angle then declined with frequency, the decline with frequency was very slight making the phase angle appear almost constant in the range of higher frequencies. The phase angle approached zero near the maximum frequency, 10,000 Hz.

We thought it possible that the presence or absence of phase shift might be correlated with the resting potential of the fibers and so two experiments were performed in solutions which presumably depolarized the fibers. Fig. 5 shows the phase angle observed from a fiber bathed in a Ringer solution of 115 mM K^+ methanesulfonate, 2.5 mM KCl, 1.8 mM $CaSO_4$, and buffered with Tris. The maximum phase shift was some $-1.5°$ even though the resting potential would be expected to be about -10 mV. Another experiment was performed in a Ringer solution to which 12.5 mM KCl had been added; the maximum
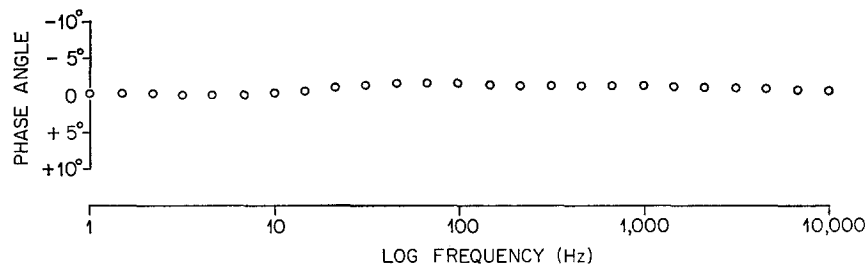

FIGURE 5.   The phase angle of the longitudinal impedance $Z_L(\Delta L)$ of a fiber bathed in a solution with high potassium concentration (see text).

phase observed in this case was some $-1.2°$, even though the fiber would be expected to be quite depolarized. It seems unlikely then that the presence or absence of phase shift is correlated with the resting potential. Table I shows the results from 14 muscle fibers. The first two fibers are from the sartorius, the rest are from the semitendinosus. The first column gives the largest phase angle observed, whether that of the maximum of a phase distribution (10 Hz, Fig. 4) or that due to a random error (1 Hz, Fig. 2). The next column gives the major and minor axes of the fiber; numbers marked with an asterisk were not measured but rather estimated. The estimate was made by taking the mean value of the ratio of the major to minor axis from fibers where both axes were measured and applying it to fibers where only the major axis was measured. The third column gives the mean value (and standard deviation) of the magnitude of the impedance recorded at all 25 frequencies from 1–

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[1] We do not present averaged results from our 14 successful experiments because the variance of the mean is comparable to the mean itself and so averaged results are badly biased (Valdiosera et al., 1974 b, Appendix).

TABLE I

RESULTS FROM 14 MUSCLE FIBERS

	ϕ_{max}	Major, minor axis	$|z_i|$	R_i	$|z|/r$
	degrees	*μm*	*MΩ/cm*	*ohm-cm*	
	−1.48	120,74	1.168 (0.056)	74	1.28
	1.33	139,111	1.050 (0.032)	92	0.84
	0.74	83,49*	2.898 (0.079)	81	1.09
	−2.45	102,60*	1.838 (0.046)	90	0.88
	−2.42	92,54*	2.783 (0.063)	103	0.64
	−2.22	127,75*	1.776 (0.074)	141	0.20
	−1.58	91,54*	3.787 (0.098)	152	0.11
	−1.60	117,69*	0.967 (0.038)	65	1.60
	0.59	108,64*	1.821 (0.045)	72	1.35
	0.68	122,72*	2.466 (0.068)	124	0.36
	−1.79	117,69*	2.895 (0.123)	199	−0.15
	−0.62	163,90	1.411 (0.025)	117	0.44
	−1.86	135,90	1.614 (0.052)	145	0.17
	−2.25	167,72	0.959 (0.041)	97	0.74
Mean	−1.07	120,72	1.959	111	0.68
SD	1.34	25,17	0.875	38	0.52

* Numbers not measured but estimated.

10,000 Hz. The column labeled R_i gives the *apparent resistivity* of the sarcoplasm determined from the real part of the observed impedance and the major and minor axes of the fiber. Note that it includes the important shunting effect of current flow in the thin layer of solution outside the muscle fiber. The figures are much less than measured by more reliable techniques (for example, 169 ohm-cm reported by Hodgkin and Nakajima 1972 *a*, *b*) because of the shunting. Indeed, the amount of shunting (the ratio of the magnitude of the longitudinal impedance z to the longitudinal resistance r of the shunt in a unit length of fiber; see the last column in Table I) can be calculated from the ratio 169 ohm-cm to the apparent resistivity. A similar calculation can be used to estimate that the thickness of the layer of solution between the oil and the fiber is some 10 μm. This figure should not be taken too seriously, however, since it is sensitive to assumptions about the shape of the fiber.

Table II gives data concerning the magnitude of the various components of the impedance at different gap lengths so one can estimate the sensitivity of the results to end effects. One column gives the impedance of the length of fiber in the oil gap ("gap impedance" $= R_iL$) and another column gives the magnitude of the impedance of the ends of the fiber in the conducting pools of Ringer ("end impedance" $= |Z_T| = |Z(L) - R_iL|$). The fibers shown in Table II include two sartorius fibers (the first two), one semitendinosus fiber (the last) which showed almost no phase shift, and one semitendinosus fiber with a substantial phase shift. Note that as expected the end effect is relatively

TABLE II

MAGNITUDE OF THE COMPONENTS OF THE OBSERVED IMPEDANCE

Fiber	Gap length L	End length l_2	End length l_1	Gap impedance*	End impedance*
	cm	*cm*	*cm*	*MΩ*	*MΩ*
Sartorius	0.70	0.90	3.40	0.723	0.222
	1.70	0.90	2.40	1.76	0.222
Sartorius	0.80	1.50	2.20	0.905	0.839
	1.30	1.50	1.70	1.47	0.839
Semitendinosus	0.50	0.30	1.50	0.492	0.638
	1.00	0.30	1.00	0.983	0.638
Semitendinosus	0.50	0.70	2.00	0.695	0.892
	1.00	0.70	1.50	1.39	0.892

* See text for precise definition.
Each fiber is measured at two gap lengths and the results of both measurements are shown.

more important in the short semitendinosus fibers. Also note that there is no particular difference between the semitendinosus fibers with and without phase shift.

Artifacts

We now analyze the artifacts likely to be produced by our apparatus and preparation to see if they might account for the small phase angle observed in many semitendinosus fibers (Table I). Numerical calculations were performed using the expression for the observed impedance $Z_L(\Delta L)$ derived in the Appendix (Eq. 2 *a* and, where appropriate, 3 *a*). We thank Mr. C. Clausen for help in programming. The shunt admittance y was represented by the lumped model of Falk and Fatt, 1964 (see also Valdiosera et al., 1974 *a*) using the circuit parameters reported by Valdiosera et al., 1974 *b* (Table VII). The resistivity of the layer of Ringer solution between the fiber and the oil was taken to be 80 ohm-cm; the DC length constant of the fiber in the oil was then computed from the thickness of the layer of solution, the resistivity, and the internal resistance of a unit length of fiber.

A likely source of artifact is drift in the properties of the preparation produced by a general deterioration with time. This artifact has been ruled out by making our measurements in different sequence, sometimes working at short gap length first, sometimes working at long gap length first. Our results did not depend on the order of measurement.

Another possible source of artifact is the finite length of the ends of the fiber in the pools of Ringer. We assumed that the impedance of the fiber in the conducting solution does not change as the length of the gap is changed, which assumption implies that the length of fiber in a pool of conducting solution

must either be "infinite" (many length constants) or must not change when the length of the gap is changed. In practice the length of the fiber in the upper pool was held constant and the length of the fiber in the lower pool was kept as long as possible (see Table II). In experiments with sartorius fibers the length of fiber in both end pools was essentially infinite (greater than five length constants), but in experiments with semitendinosus fibers this was not the case. The length in the upper pool was adjusted so that the shortest length in the lower pool would be at least three length constants. The length of the upper pool was always greater than 3 mm. Since significant phase angles were only observed in semitendinosus fibers, we were suspicious that the finite length of the fiber in the conducting pools might account for the observation. Fig. 6 shows the predicted phase angle of $Z_L(\Delta L)$ when the interior of the fiber is purely resistive and the fiber length is 18 mm. In the lower curve (B) the length of fiber in one conducting pool was 2 mm and that in the other pool was 6 and 11 mm (the corresponding gap lengths are 10 and 5 mm). The pre-
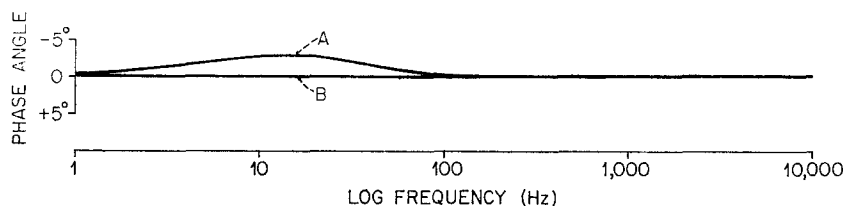

FIGURE 6. Theoretically predicted plots of the phase angle of the impedance $Z_L(\Delta L)$. Curve B was computed for a set of parameters which models reasonably well the experimental situation. Curve A was computed assuming a large change in the length of fiber in both pools of Ringer (as specified in the text). Note that even in this case the phase predicted at high frequencies is different from that observed.

dicted phase angle is indistinguishable from zero as might be expected since both 6 and 11 mm are many length constants. The other curve (A) was computed assuming a change in the length of the fiber in both pools of Ringer. The length of the longer end of the fiber was again 6 and 11 mm, but in this case the shorter end of the fiber was assumed to change from a length of 1–2 mm. Since these lengths are comparable to a length constant, one would expect some phase shift and some is in fact seen in Fig. 6 (A). The phase shift is quite small if we use a realistic value of the thickness of the shunt layer (some 5–10 μm); the calculation shown was done with thickness of 1 μm to maximize the effect. Since the predicted phase shift is small and occurs only at low frequencies, whereas we observe phase shift over a wide range of frequencies, we conclude that changes in the length of fiber in the conducting pool do not account for the phase shift sometimes observed experimentally.

It seemed possible that a change in electrical properties of the preparation

correlated with gap length might account for the experimentally observed phase shift. Calculations were made assuming, for instance, that the membrane resistance was higher at the long gap length than at the short gap length. Of course, if the membrane resistance is assumed to vary in the opposite manner, the sign of the phase shift is reversed. The phase predicted occurs again only at low frequencies and so cannot account for the phase shift observed.

Another source of artifact is a change in the shunt resistance with a change in the length of the gap, for instance caused by a change in the mean thickness of the thin layer of Ringer solution between the oil and the fiber. The theoretical analysis (Eqs. 3 *a* and 5 *a* illustrate this most clearly) shows that there are two effects of the shunt: one, on the longitudinal term (the first term) and two, on the "end" effect (the second term). The end effect is particularly sensitive to changes in the shunt (see Eq. 5 *a*) and so in cases where the end effect is itself significant (when the length of the fiber is not too large), it should be particularly important. Fig. 7 shows the phase predicted for a
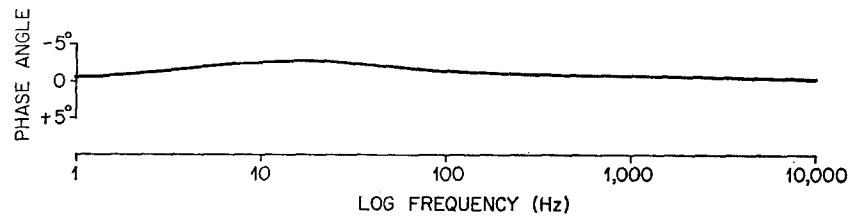

FIGURE 7.   Theoretically predicted plot of the phase angle of the impedance $Z_L(\Delta L)$. The curve was computed allowing the size of the shunt resistance *r* to vary with gap length. Note that even at high frequencies there is a definite phase angle. This curve has a shape similar to that observed experimentally.

fiber 18 mm long with the length of fiber in one end pool 2 mm. The length of the other end of the fiber was 6 mm and 11 mm at the two gap lengths of 10 and 5 mm. The shunt was assumed to be 2.5-μm thick at the longer gap length and 5-μm thick at the shorter gap length. The phase predicted is reasonably similar to the experimental results which show significant phase shift; the curves reach a maximum phase angle in the midfrequency range but show significant phase at the high frequencies as well. Furthermore, a similar calculation for a long fiber like a sartorius shows a phase shift reduced by a factor of three or so, as expected from consideration of the relative size of the terms in Eq. 4 *a*. We conclude then that changes in the thickness of shunting layer of solution *might* account for the phase angle often observed. We cannot be sure that this artifact does in fact cause the observed phase because our methods do not directly measure the shunt resistance.

A further way to test the idea that the transverse impedance of the ends of the fiber are responsible for the apparent phase shift is to shunt out this impedance by skinning just the ends of the fiber. In two experiments on fibers with skinned ends the phase shift was less than 1° over the entire frequency range and the fibers did not show the pattern of phase shift with respect to frequency as shown in Fig. 4. This result suggests that the impedance of the ends of the fiber is responsible in some way for the phase shift observed, but it does not distinguish between the various possible types of artifact.

DISCUSSION

There have been two methods used to measure the impedance of muscle fibers to longitudinal current flow. One method uses microelectrodes to measure the transfer impedance of the fiber at different longitudinal separations of the microelectrodes and the other method uses an oil gap apparatus to measure the composite of the end effects and the longitudinal impedance.

In the measurements with microelectrodes the longitudinal impedance is determined indirectly, since it is the transfer impedance which is measured. Unfortunately, the equations relating the phase angle of the longitudinal impedance to the measured transfer impedance require repeated impalement of the muscle fiber and the subtraction of numbers of comparable size (Schneider, 1970; see also the more powerful method of Mathias, 1973, pp. 71–78). Thus, estimates of the longitudinal impedance by this method are subject to serious random and systematic error. For instance, it is usually assumed that the properties of the fiber are uniform down the length of the fiber. This assumption is reasonable if one is interested only in the transfer impedance itself but is less reasonable if one is interested in the small difference between the phase angle of transfer impedances. In that case the localized shunts near each of the microelectrodes, and possible nonuniform membrane resistance (produced by the interaction of the DC current through the shunts and the anomalous resistance of the fiber membrane) might produce important longitudinal nonuniformity in the impedance of the surface membrane. Perhaps such phenomena account for the small longitudinal phase shift often apparent in microelectrode experiments (Mobley et al., 1973; Valdiosera and Mathias, personal communication) although we cannot rule out the possibility that other errors contribute, or indeed that the phenomenon might be real.

The other method that has been used to measure the longitudinal impedance has been the oil gap method used here. The oil gap method requires the dissection of a clean long single muscle fiber and the mounting of the fiber in the experimental apparatus; the experiments can be difficult. In addition,

the results are subject to electrical artifacts which are called end effects and are analyzed in the Appendix. Previous experiments (Mobley et al., 1973) using this apparatus on intact muscle fibers showed a small phase shift, but the number of experiments in which the end effects were correctly treated was rather small, and it may be that the mean phase observed in a long run of experiments would have been close to ours. Experiments using this apparatus have also been performed on skinned muscle fibers (Mobley et al. 1974) and no phase shift was observed. The physiological state of the skinned preparation is not well known, however, and one must be particularly concerned since fibers may swell upon skinning (Matsubara and Elliott, 1972). We have recently skinned muscle fibers in a solution which prevents swelling (120 mM K methane sulfonate, 4 mM $MgCl_2$, 4 mM Na_2ATP, 4 mM K_2EGTA, 20 mM Tris maleate buffer adjusted to pH 5) and these fibers show no phase shift (less than 1° in two fibers; mean $R_i = 227$ Ωcm).

We conclude that the longitudinal impedance of single muscle fibers, like the longitudinal impedance of skinned muscle fibers, is essentially resistive, and that the interior of a muscle fiber can be represented in many electrical models as a resistor. Our conclusions are reached for several reasons: (a) The phase angle of a number of fibers is zero over the entire frequency range; (b) the appearance of phase shift in other fibers is inconsistent, suggesting the presence of an uncontrolled experimental variable, probably an artifact; (c) theoretical calculations show that a number of artifacts are likely to be of the same size as the phase angle sometimes observed. Indeed, the theory shows that one type of artifact also reproduces the shape of the phase plot; (d) long muscle fibers, which should give results much less sensitive to any of the artifacts considered, show no phase shift; (e) muscle fibers skinned at the ends, which also should give results much less sensitive to many artifacts, show no phase shift.

It remains possible that the phase shift often observed is a real property of muscle fibers. However, the phase shift is near the limit of resolution of our apparatus and procedure and since its appearance is inconsistent, we believe that further investigation and/or discussion of the possible significance of the phase shift would require substantial improvements in the apparatus and procedures.

We originally started the investigation of longitudinal impedance in order to learn more about the electrical function of the sarcoplasmic reticulum. Since our investigation revealed no properties other than those expected of sarcoplasm itself, we can infer little about the electrical properties of the sarcoplasmic reticulum. Perhaps we are justified in suggesting that under the conditions of our analysis little if any longitudinal current flows across the membranes of the sarcoplasmic reticulum.

An Analysis of the Oil Gap Apparatus

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APPENDIX

Introduction

The impedance of single muscle fibers was determined by measuring the current produced by a sinusoidal voltage applied between the ends of the muscle fiber, separated by an oil gap (see Fig. 8). This appendix analyzes the experimental situation using certain plausible assumptions concerning the electrical properties of the fiber and the
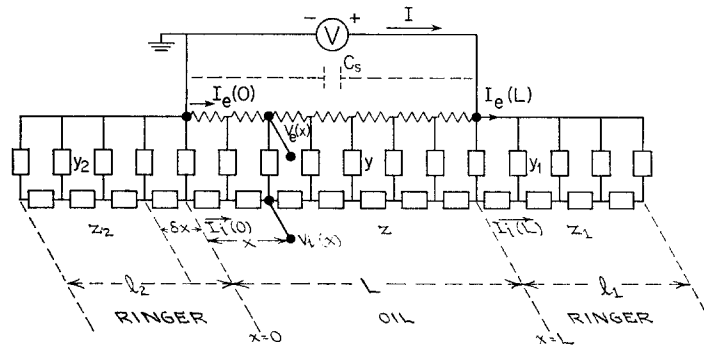

FIGURE 8. The variables and sign conventions used in the analysis. The symbols are defined in the text. Note that the impedances, admittances, currents, and voltages are in general given by complex numbers, while the distances are, of course, described by real numbers.

geometry of the current flow in the extracellular medium. The assumed electrical properties of the fiber determine differential equations which describe the current flow in the fiber; the assumptions about current flow in the extracellular medium determine the boundary conditions and thus the particular solutions. A less general or explicit analysis has been presented by Cole (1968) and Cole and Hodgkin (1939).

The fiber is assumed to have the following properties: (a) It can be described by a one-dimensional cable structure, Fig. 8. The fiber is assumed to be uniform in its properties within the oil gap and in each pool of conducting solution. The properties of the fiber need not be the same in these three locations, however. Three-dimensional effects are ignored since there is no point source of current and since in the frequency range of interest the spread of current occurs over distances larger than two fiber diameters (Eisenberg and Johnson, 1970; Peskoff and Eisenberg, 1973).

(b) The fiber is analyzed assuming that a sarcomere is very much smaller than the distance over which current spreads; that is, the fiber is assumed to behave like a distributed electrical circuit. The situation where this is not the case has been ana-

lyzed (Mathias, 1973) and our assumption has been shown to be sufficiently accurate under our conditions.

(*c*) Each repeating structure in the cable (the combination of y, *r*, and z) represents the linear electrical properties of a sarcomere: y is the admittance between the inside and the outside of a unit length of the fiber; z is the longitudinal impedance of a unit length of the interior; and *r* is the longitudinal resistance of a unit length of the thin layer of solution outside the fiber in the oil gap.

The external medium is assumed to have the following properties: (*a*) The resistivity of the Ringer bathing the ends of the fiber is considered sufficiently small so that the external solution is isopotential.

(*b*) The boundary between the oil and the Ringer is abrupt.

(*c*) The layer of solution outside the fiber in the oil gap is considered sufficiently thin that there are only longitudinal potential gradients, no radial or circumferential gradients being considered significant.

(*d*) The oil is a perfect insulator.

We use the standard conventions of elementary circuit theory (Desoer and Kuh, 1969) and use complex numbers shown in boldface type to represent currents and voltages. The complex numbers are, in fact, the Laplace transforms of the physical currents and voltages; in the case that the current and voltages are sinusoids, the complex numbers have simple significance. The amplitude of the complex number is the amplitude of the sinusoid, the phase of the complex number is the phase of the sinusoid.

Nomenclature and Symbols

$V_i(x)$; $V_e(x)$	Internal and external potential in the oil gap (volts)
$V_m(x)$	$V_i(x) - V_e(x)$ membrane potential (volts)
V	Potential difference between the two Ringer pools (volts)
I	Current delivered to the pool (amperes)
$I_i(x)$; $I_e(x)$	Internal and external current in the oil gap (amperes)
r	Resistance of a unit length of external solution ($\Omega\text{-cm}^{-1}$)
z; z_1, z_2	Longitudinal impedance in the oil gap and the two Ringer pools ($\Omega\text{-cm}^{-1}$)
$Z(L)$	$Z(L) = V/I$
$Z_L(\Delta L)$	$Z_L(\Delta L) = Z(L_2) - Z(L_1)$, the impedance ($\Omega$) of a length $\Delta L = L_2 - L_1$
Z_1; Z_2	Characteristic impedance (sometimes called input impedance) of the section of the fiber in the Ringer pool assuming no longitudinal current flowing at the ends $(Z_1 = (z_1/y_1)^{1/2} \coth \Gamma_1 l_1$; $Z_2 = (z_2/y_2)^{1/2} \coth \Gamma_2 l_2$; King, 1965)
Γ; Γ_1, Γ_2	Propagation constants in the different regions; at DC these are the reciprocal of the length "constant" (cm^{-1}) $\Gamma = [y(r + z)]^{1/2}$; $\Gamma_1 = [z_1 y_1]^{1/2}$; $\Gamma_2 = (z_2 y_2)^{1/2}$
L	Length of the oil gap (centimeters)
l_1; l_2	Length of the end sections in the Ringer pools (centimeters)
ΔL	Difference between two oil gap lengths (centimeters)
x	Distance into the oil gap from the oil-Ringer interface (centimeters)

Calculations

The impedance $\mathbf{Z}(L)$ is given by $\mathbf{V}/\mathbf{I}$.
An expression for $\mathbf{Z}(L)$ can be obtained by Ohm's law as follows:

$$\frac{d\mathbf{V}_e(x)}{dx} = -r\mathbf{I}_e,$$

$$\frac{d\mathbf{V}_i(x)}{dx} = -\mathbf{z}\mathbf{I}_i,$$

$$\frac{d\mathbf{I}_e(x)}{dx} = \mathbf{y}(\mathbf{V}_i - \mathbf{V}_e) = \mathbf{y}\mathbf{V}_m(x),$$

and the continuity of currents

$$-\frac{d\mathbf{I}_e(x)}{dx} = \frac{d\mathbf{I}_i(x)}{dx},$$

whence

$$\frac{d^2\mathbf{V}_m}{dx^2} = \mathbf{\Gamma}^2\mathbf{V}_m,$$

which has the general solution

$$\mathbf{V}_m = \mathbf{A} \sinh \mathbf{\Gamma}x + \mathbf{B} \cosh \mathbf{\Gamma}x, \tag{1 a}$$

where $\mathbf{A}$ and $\mathbf{B}$ are arbitrary constants.
With Eq. 1 a

$$\mathbf{I}_i(x) = -\frac{\mathbf{yA}}{\mathbf{\Gamma}} \cosh \mathbf{\Gamma}x - \frac{\mathbf{yB}}{\mathbf{\Gamma}} \sinh \mathbf{\Gamma}x + c_1,$$

$$\mathbf{I}_e(x) = \frac{\mathbf{yA}}{\mathbf{\Gamma}} \cosh \mathbf{\Gamma}x + \frac{\mathbf{yB}}{\mathbf{\Gamma}} \sinh \mathbf{\Gamma}x + c_2,$$

$$\mathbf{V}_i(x) = \frac{\mathbf{zyA}}{\mathbf{\Gamma}^2} \sinh \mathbf{\Gamma}x + \frac{\mathbf{zyB}}{\mathbf{\Gamma}^2} \cosh \mathbf{\Gamma}x - c_1 \mathbf{z}x + c_3,$$

$$\mathbf{V}_e(x) = -\frac{r\mathbf{yA}}{\mathbf{\Gamma}^2} \sinh \mathbf{\Gamma}x - \frac{r\mathbf{yB}}{\mathbf{\Gamma}^2} \cosh \mathbf{\Gamma}x - c_2 rx + c_4,$$

where c_1, c_2, c_3, and c_4 are constants of integration.
At the oil-Ringer interfaces (choosing $\mathbf{V}_e[0] = 0$):

$$\mathbf{I}_i(0) + \mathbf{I}_e(0) = -\mathbf{I},$$

$$\mathbf{V}_i(0) = -\mathbf{I}_i(0)\mathbf{Z}_2,$$

at $x = 0$, and

$$\mathbf{V}_i(L) - \mathbf{V}_e(L) = \mathbf{V}_m(L) = \mathbf{I}_i(L)\mathbf{Z}_1,$$

$$\mathbf{V}_e(L) = \mathbf{V},$$

at $x = L$.

For this set of boundary conditions, the constants are:

$$c_1 = -\frac{r\mathbf{I}}{r + z},$$

$$c_2 = -\frac{z\mathbf{I}}{r + z},$$

$$c_3 = c_4 = \frac{ry\mathbf{B}}{\mathbf{\Gamma}^2},$$

$$\mathbf{B} = \mathbf{Z}_2\left(\frac{y\mathbf{A}}{\mathbf{\Gamma}} + \frac{r\mathbf{I}}{r + z}\right),$$

$$\mathbf{A} = -\frac{ry\mathbf{I}}{\mathbf{\Gamma}} \frac{\mathbf{Z}_2\mathbf{\Gamma}\cosh\mathbf{\Gamma}L + y\mathbf{Z}_1\mathbf{Z}_2\sinh\mathbf{\Gamma}L + \mathbf{Z}_1\mathbf{\Gamma}}{(\mathbf{\Gamma}^2 + \mathbf{Z}_1\mathbf{Z}_2 y^2)\sinh\mathbf{\Gamma}L + \mathbf{\Gamma}y(\mathbf{Z}_1 + \mathbf{Z}_2)\cosh\mathbf{\Gamma}L}.$$

Hence the impedance

$$\mathbf{Z}(L) = \frac{\mathbf{V}_e(L)}{\mathbf{I}} = \frac{rz}{r + z}L$$

$$+ \left(\frac{r}{r + z}\right)^2 \frac{\mathbf{\Gamma}^2(\mathbf{Z}_1 + \mathbf{Z}_2)\sinh\mathbf{\Gamma}L + 2\mathbf{\Gamma}\mathbf{Z}_1\mathbf{Z}_2 y\cosh\mathbf{\Gamma}L - 2\mathbf{\Gamma}\mathbf{Z}_1\mathbf{Z}_2 y}{(\mathbf{\Gamma}^2 + \mathbf{Z}_1\mathbf{Z}_2 y^2)\sinh\mathbf{\Gamma}L + \mathbf{\Gamma}y(\mathbf{Z}_2 + \mathbf{Z}_2)\cosh\mathbf{\Gamma}L}. \qquad (2\,a)$$

Simplifications

If the length of the oil gap L is sufficiently large so that $|\mathbf{\Gamma}L| \geq 5$ in the frequency range of interest, the hyperbolic sine and cosine can be replaced approximately by

$$\sinh\mathbf{\Gamma}L \approx \cosh\mathbf{\Gamma}L \approx \frac{\exp(\mathbf{\Gamma}L)}{2}.$$

Similarly, if the lengths of the fiber in the Ringer are also large so that the hyperbolic cotangent (see p. 109) can be replaced by one, then $\mathbf{Z}_1$ and $\mathbf{Z}_2$ no longer depend on L. Under these conditions $\mathbf{Z}(L)$ is

$$\mathbf{Z}(L) = \frac{r}{r + z}\left\{zL + \frac{r}{\mathbf{\Gamma}}\left[\frac{1}{1 + \mathbf{\Gamma}/\mathbf{Z}_1 y} + \frac{1}{1 + \mathbf{\Gamma}/\mathbf{Z}_2 y}\right]\right\} + 0\left(\exp(-\mathbf{\Gamma}L)\right). \quad (3\,a)$$

Since the second term is independent of L, the difference between the impedances

measured at two large oil gap lengths is

$$\mathbf{Z}_L(\Delta L) = \frac{r\mathbf{z}}{r + \mathbf{z}} \Delta L = \mathbf{Z}(L_2) - \mathbf{Z}(L_1). \qquad (4\,a)$$

If the shunting is very small ($r \gg |\,\mathbf{z}\,|$), then the phase angle of $\mathbf{Z}_L$ is just the phase angle of the longitudinal impedance, the phase angle we wish to measure (see text Eq. 1). On the other hand, if there is significant shunting, the whole Eq. (4 a) must be used, and the relation between the phase angle observed and the phase angle of the longitudinal impedance depends on the longitudinal impedance and shunt resistances themselves.

If the electrical properties of the fiber are the same in oil and in Ringer, i.e., $\mathbf{y} = \mathbf{y}_1 = \mathbf{y}_2$, and $\mathbf{z} = \mathbf{z}_1 = \mathbf{z}_2$, Eq. 3 a can be written as

$$\mathbf{Z}(L) = \frac{\mathbf{z}L}{1 + \dfrac{\mathbf{z}}{r}} + \frac{2\mathbf{Z}_1}{\left(1 + \dfrac{\mathbf{z}}{r}\right)^{3/2}} \frac{1}{\left(\dfrac{\mathbf{z}}{r}\right)^{1/2} + \left(1 + \dfrac{\mathbf{z}}{r}\right)^{1/2}} + 0\,(\exp\,(-\Gamma L)), \qquad (5\,a)$$

where $\mathbf{z}/r$ is the ratio of the longitudinal impedance of the bulk of the fiber to the resistivity of the external medium in the oil gap. Eq. 5 a shows that the end effect (the second term) depends on the amount of shunting (the ratio $\mathbf{z}/r$) and so changes in the shunt would be expected to change the end effect and cause errors if Eq. 4 a (or equivalently Eq. 1 of the text) is used to analyze the experimental results. Detailed analysis shows that the end effect is particularly sensitive to shunting when the shunting is small, that is when $\mathbf{z}/r \ll 1$. This sensitivity accounts for the results illustrated in Fig. 7 of the paper.

Eq. 5 a also suggests further applications of the oil gap technique. Note that the slope and intercept of the function (regarding $\mathbf{Z}(L)$ as a function of L) can be determined experimentally. If the longitudinal impedance is purely resistive, then the phase angle of the intercept is the phase angle of the characteristic (that is, input) impedance $\mathbf{Z}_1$, even in the presence of shunting. If there is no shunting, then the magnitude of the intercept is the magnitude of the characteristic impedance. In this way, at least in principle, the oil gap method can be used to measure the characteristic impedance of a muscle fiber. Since three-dimensional effects are not expected to be present in such experiments, measurements of this sort could serve as a check on the results of experiments with microelectrodes.

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