

Impedance of Frog Skeletal Muscle Fibers in Various Solutions

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ABSTRACT The linear circuit parameters of 140 muscle fibers in nine solutions are determined from phase measurements fitted with three circuit models: the disk model, in which the resistance to radial current flow is in the lumen of the tubules; the lumped model, in which the resistance is at the mouth of the tubules; and the hybrid model, in which it is in both places. The lumped model fails to fit the data. The disk and hybrid model fit the data, but the optimal circuit values of the hybrid model seem more reasonable. The circuit values depend on sarcomere length. The conductivity of the lumen of the tubules is less than, and varies in a nonlinear manner with, the conductivity of the bathing solution, suggesting that the tubules are partially occluded by some material like basement membrane which restricts the mobility of ions and has fixed charge. The $\times 2.5$ hypertonic sucrose solution used in many voltage clamp experiments produces a large increase in the radial resistance, suggesting that control of the potential across the tubular membranes would be difficult to achieve. Glycerol-treated fibers have 90% of their tubular system insulated from the extracellular solution and 10% connected to the extracellular solution through a high resistance. We discuss the implications of our results for calculations of the nonlinear properties of muscle fibers, including the action potential and the radial spread of contraction.

The transverse tubular system of skeletal muscle fibers is intimately involved in the mechanism by which an action potential on the surface membrane initiates contraction in the depths of a muscle fiber. The electrical properties of the tubular system determine the radial spread of potential and so these properties have considerable functional significance. They also help to determine the conduction velocity and shape of the action potential itself. It is possible to measure the linear passive electrical properties of the tubules by measuring the impedance of an entire muscle fiber (Valdiosera et al. 1974 *a*) and then interpreting the impedance with an electrical model (Valdiosera et al., 1974 *b*), a branch of which represents the properties of the tubular system. This approach was developed by Falk and Fatt (1964),

followed by Freygang et al. (1967) and Schneider (1970). Some electrical properties can also be estimated from measurements of the voltage response to a step function of current and from the shape and conduction velocity of the foot of the action potential (Hodgkin and Nakajima, 1972 *a, b*). Several conclusions of the analysis can be checked by measurements (Gage and Eisenberg, 1969; Hodgkin and Nakajima, 1972 *a, b*; Nakajima et al., 1973) on glycerol-treated muscle fibers (Howell, 1969; Krolenko, 1969) which have little intact tubular system (Eisenberg and Eisenberg, 1968).

Recently, Peachey and Adrian (1973) have shown that the shape and conduction velocity of the action potential across the surface membrane should be quite sensitive to the passive electrical properties of the tubular system (Peachey, 1973). Several circuit models of the passive electrical properties, which seem to differ only in detail, predict quite different shapes and longitudinal conduction velocities for the surface action potential and predict radically different conduction of the action potential radially down the tubules into the muscle fiber. The circuit models proposed for the tubular system differ primarily in the structural attribution of the resistance to radial current flow. The lumped model puts all the resistance to radial current flow in one place, most likely the mouth of the tubules; the disk model puts the resistance to radial current flow in the lumen of the tubules; and the hybrid model puts the resistance in both locations.

The experiments reported in this paper are designed to determine the values of the circuit parameters of muscle fibers in a variety of solutions. The absolute value of the circuit parameters depends, of course, on the equivalent circuit chosen to represent the fiber and so some attention is paid to the choice of the "best" equivalent circuit. The relative value of the circuit parameters in different conditions is reasonably independent of the choice of circuit model and so most of our qualitative conclusions are not dependent on the choice of the best equivalent circuit. Four types of experiments are reported: experiments on muscle fibers bathed in Ringer solution but held at different sarcomere lengths; experiments on muscle fibers bathed in hypertonic solutions, including the solution in which many voltage clamp experiments have been performed (Adrian et al., 1970 *a, b*); experiments on muscle fibers bathed in solutions of varying conductivity; and experiments performed on glycerol-treated muscle fibers.

The circuit values show a dependence of the capacitance of the surface membrane on sarcomere length, a nonlinear dependence of the conductivity of the lumen of the tubules on the conductivity of the bathing solution, and suggest that the conductivity of lumen of the tubules is much lower than the conductivity of the bathing solution. Furthermore, the luminal resistivity in the hypertonic solution used in voltage clamp experiments is very high. Glycerol-treated fibers are shown to have little tubular capacitance, as pre-

dicted by Eisenberg and Eisenberg, 1968, and confirmed by Gage and Eisenberg, 1969. This result is in qualitative disagreement with the suggestion of Nakajima et al. (1973) that most of the tubular system is intact in glycerol-treated fibers, and is in quantitative disagreement with the data of Hodgkin and Nakajima (1972 *b*). We conclude that 90% of the tubular system in glycerol-treated fibers is "perfectly" insulated from the extracellular space and makes no contribution to the electrical properties we observe. The residual 10% of the tubular system is in an abnormal state: the radial resistance in series with the membrane of the residual tubules is 50–60 times higher than in normal fibers (Nakajima et al., 1973) and so these residual tubules should have little effect on many of the electrical properties of glycerol-treated fibers.

METHODS

Preparation, Solution, and Procedure

The preparation used in these experiments was the sartorius muscle of the frog *Rana pipiens*. Large frogs were chosen in which the muscle was some 5 cm long. After dissection the muscle was placed in the bath containing one of the solutions described in Table I. The muscle was soaked in the solution for 1 h before experimental measurements were made at room temperature, about 22°C. The muscle was observed with a polarizing compound microscope, arranged to focus and move independently of the preparation. The column of the microscope was split and hinged so the objective and column could be moved out of the way while manipulations of the preparation were made. We are indebted to Mr. N. Ricchiuti for the design of the

TABLE I
TABLE OF SOLUTIONS

Solution	Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	Cl ⁻	Methane sulfonate	Sucrose	Conductivity
	<i>mM</i>	<i>mM</i>	<i>mM</i>	<i>mM</i>	<i>mM</i>	<i>mM</i>	<i>mM</i>	<i>mmho/cm</i>
A Ringer	115	2.5	1.8	—	124	—	—	12.2
B Hypertonic NaCl	230	2.5	1.8	—	239	—	—	22.3
C Hypertonic sucrose (× 2)	115	2.5	1.8	—	124	—	232	10.2
D Hypertonic sucrose (× 2.5)	115	2.5	1.8	—	124	—	350	9.5
E 1 CCCP	120	2.5	1.8	—	14.7	114	—	9.5
F ½ CCCP	54.7	2.5	1.8	—	14.5	48.9	131	4.7
G ¼ CCCP	22.0	2.5	1.8	—	14.4	16.3	196	2.4
H ⅛ CCCP	5.82	2.5	1.8	—	14.5	—	228	1.3
I High Ca ⁺⁺	115	2.5	5	5	140	—	—	—
J Super Ca ⁺⁺	—	2.5	60	30	245	—	—	—

Solutions also contained 0.38 mM Tris and 2.61 mM Tris⁺, adjusted to pH 7.2 except High Ca⁺⁺ and Super Ca⁺⁺ which were adjusted to pH 6.5. The conductivity was measured at 1,000 Hz with a Pt/Pt black conductivity cell standardized with 0.1 M and 0.01 M KCl solutions.

apparatus. Sarcomere length was measured with a $\times 40$ (numerical aperture 0.75) water immersion lens and filar eyepiece (total magnification $\times 773$) and adjusted, usually to $2.5 \mu\text{m}$. This procedure was repeated each time a fiber was selected for impalement, since there was considerable variation in sarcomere length from fiber to fiber, particularly across the width of the muscle. Microelectrodes, of 15–25 $\text{M}\Omega$ resistance, were placed in position under microscopic control using an air objective (total magnification $\times 393$) and inserted into the fiber with measured sarcomere length. It is necessary to use an air objective instead of a water immersion objective, even though the microscopic image is degraded, in order to avoid the formation of a meniscus and the accompanying large increase in the stray capacitances C_V and C_I (Valdiosera et al., 1974 *a*, Fig. 1). The current and voltage waveforms were displayed on an oscilloscope after the microelectrodes were inserted into the fiber and carefully checked for asymmetry and other signs of nonlinearity at amplitudes considerably in excess of those used experimentally. The current microelectrode was filled with a mixture of 1.7 M K^+ Citrate (pH 6.5) and 0.7 M KCl. Resting potential and the resistance of the voltage microelectrode¹ were measured and then phase measurements were taken at some six frequencies per decade, with the 10 Hz measurement repeated every decade. The DC potential of the voltage electrode was continuously monitored and at the end of a set of phase measurements the electrode resistance and resting potential were again measured. The voltage electrode was then withdrawn just outside the fiber, keeping the current electrode in the fiber, in order to measure the extracellular potentials. Measurements of electrode resistance¹ were again made. A map of the fiber was drawn showing the location of the microelectrodes and the projected width (“diameter”) of the fiber. A typical location is shown in Fig. 2 of Valdiosera et al., 1974 *b*: “position 1.” The voltage recording electrode was then removed along the fiber and inserted 500, 1,500, and 2,500 μm from the current electrode. Measurements were made of resting potential, the resistance of the current and voltage electrodes,¹ and the magnitude of the current and potential at 1 Hz in order to determine the DC length constant of the fiber (Hodgkin and Nakajima, 1972 *a, b*).

Biological Variation

These experiments were designed to measure the impedance of fibers in a number of conditions. Because of the duration of such experiments, it is not often possible to measure all the circuit parameters from one muscle fiber, and it is almost impossible to make measurements on the same muscle fiber in two different conditions. Thus, we are faced with two unfortunate consequences: First, the value of the DC length constant and the input resistance are often measured in different fibers from those in which the phase plot is measured. Second, different solutions are studied on different populations of muscle fibers.

We feel that the problems in the measurements of the length constant and input resistance are not serious. Measurements of \underline{R}^* , which depend on both the length constant and the input resistance, are quite reproducible. For instance, in a set of measurements from 10 fibers made about a year before the measurements reported

¹ Actually, the sum of the fiber resistance and the resistance of the voltage microelectrode is measured, but this is close enough to the resistance of the microelectrode for our purposes.

here R^* was within 5% of the value reported. Values of R^* from fibers of different sarcomere length are within 10% of each other. Furthermore, Table II shows that estimates made from those fibers with phase measurements and those fibers without phase measurements are quite similar.

The comparison of different populations of muscle fibers in different solutions is a more serious source of error. First, such comparisons can cause confusion. It might

TABLE II
DC PROPERTIES OF A UNIT LENGTH OF FIBER

Condition	Fibers with phase measurements					Fibers without phase measurements				
	$-V_m$	No. fibers	\bar{r}	r_i	a	$-V_m$	No. fibers	\bar{r}	r_i	a
	mV		$\Omega\text{-cm} \times 10^3$	$\Omega/\text{cm} \times 10^3$	μm	mV		$\Omega\text{-cm} \times 10^3$	$\Omega/\text{cm} \times 10^3$	μm
Ringer $\sigma = 2.5 \mu\text{m}$	91.4 (1.2)	8	120 (25)	3.37 (0.31)	41 (2)	91.6 (0.7)	9	101 (5)	3.09 (0.29)	44 (3)
Ringer $\sigma = 2.0 \mu\text{m}$	90.6 (1.2)	11	120 (15)	4.61 (0.65)	37 (2)	—	—	—	—	—
Hypertonic NaCl: $\sigma = ?$	91.0 (4.4)	3	248 (55)	7.76 (0.38)	29 (1)	—	—	—	—	—
Hypertonic sucrose, $\times 2$ $\sigma = ?$	76.8 (1.5)	9	168 (23)	10.3 (2.3)	29 (3)	—	—	—	—	—
Hypertonic sucrose, $\times 2.5$ $\sigma = 2.5 \mu\text{m}$	65.4 (3.0)	6	130 (7)	6.45 (1.10)	33 (3)	60.7 (1.7)	9	161 (20)	5.63 (0.49)	34 (1)
1 CCP $\sigma = 2.5 \mu\text{m}$	87.8 (0.6)	6	456 (78)	5.47 (1.33)	36 (5)	85.1 (0.9)	5	524 (88)	5.48 (1.67)	35 (4)
$\frac{1}{2}$ CCCP $\sigma = 2.5 \mu\text{m}$	87.5 (1.6)	8	244 (44)	4.43 (0.96)	40 (5)	84.1 (3.4)	3	319 (74)	3.11 (0.91)	44 (5)
$\frac{1}{4}$ CCCP $\sigma = 2.5 \mu\text{m}$	—	—	—	—	—	80.4 (1.0)	12	285 (16)	3.34 (0.38)	42 (2)
$\frac{1}{8}$ CCP $\sigma = 2.5 \mu\text{m}$	76.5 (2.9)	2	226 (49)	4.66 (0.74)	34 (3)	76.3 (1.2)	7	250 (45)	4.75 (0.63)	35 (2)
Glycerol treated $\sigma = 2.5 \mu\text{m}$	—	—	—	—	—	73.5 (1.8)	23	133 (14)	6.57 (1.30)	33 (2)

V_m is the initial resting potential.

a is the radius calculated assuming a value for R_i as described in the text. The numbers in parentheses are the standard error of the measurement.

appear puzzling at first that the population of fibers at a shorter sarcomere length have a smaller diameter than fibers at a long sarcomere length. This does not violate the principle that a muscle fiber shortens at constant volume, but is most likely caused by the different size of the fibers in the two populations.

Second, the difference in size of the fibers in different populations makes it difficult to compare the values of the parameters of the T system, unless one uses morphometric or theoretical considerations (Peachey, 1965; Hodgkin and Nakajima, 1972 *a, b*) to scale the parameters of the T system with respect to fiber size.

Third, the different states of the frogs in the different populations might be a

serious source of uncertainty. We doubt that this is the case, at least for the parameters involved in the calculation of the capacitance of the surface membrane (namely, the length constant, the input resistance, the internal resistivity and the phase plot itself) since the value of C_m^* in fibers at constant volume and sarcomere length is remarkably constant. Consideration of Table V shows that the *range* of values of C_m^* is about 4% in the hybrid model. These measurements were taken from many muscles at different seasons over the course of 18 mo and there thus appears to be very little variability in the state of the fibers. Furthermore, the close agreement between our results and those of Hodgkin and Nakajima (see Fig. 9) also suggests that biological variation is less than might be supposed.

We conclude then that there is some uncertainty introduced into our conclusions by biological variation, particularly into estimates of the properties of the tubular system, but that these uncertainties are not likely to change the qualitative conclusions concerning the actions of various solutions. These uncertainties do prevent us from quoting meaningful standard errors of the derived circuit parameters (except in Table X).

Effects of Shunting

There are three sources of shunting present in our experiments: the shunting caused by the voltage and current microelectrodes (each of which is a pathway to ground in our setup, see Fig. 1 of Valdiosera et al., 1974 *a*) and the shunting caused by local damage around the microelectrode. The attenuation caused by the shunting of the microelectrodes is taken into account by the loading correction discussed in Valdiosera et al., 1974 *a*, and since the correction is small (never more than 1.1°), the residual uncertainty should be small. The DC shunting of the resting potential could, and probably should be removed in future experiments by modifying the experimental apparatus to isolate the current microelectrode from ground (by placing a large capacitor in series with the output of the oscillator) and by adjusting the DC potential at the top of voltage microelectrode to the resting potential (by connecting the positive input of the input operational amplifier to an adjustable DC voltage source). We can estimate the amount of shunting by the change in resting potential observed when the current microelectrode is inserted into the fiber, although, of course, the presence of anomalous rectification in our preparation makes this estimate precarious (Hodgkin and Nakajima, 1972 *a*). In all solutions except $\times 2.5$ hypertonic sucrose the change in potential is about 3 mV, a value which would be expected from the shunting effect of the microelectrode itself, without the effect of local damage. In $\times 2.5$ hypertonic sucrose the change in potential is greater, about 7 mV. Because our microelectrodes have high resistance (15–25 M Ω) and because of our stringent stability requirements, we suspect that the shunting caused by local damage is negligible, except in $\times 2.5$ hypertonic sucrose. In that solution the local damage is significant and would be expected to shift the phase plot downwards some 2°, according to a calculation similar to that shown in Fig. 5 of Falk and Fatt, 1964.

Electrical Apparatus

The circuitry used to measure impedance is discussed in detail in Valdiosera et al., 1974 *a*. Important changes in the apparatus and methods from those of Schneider

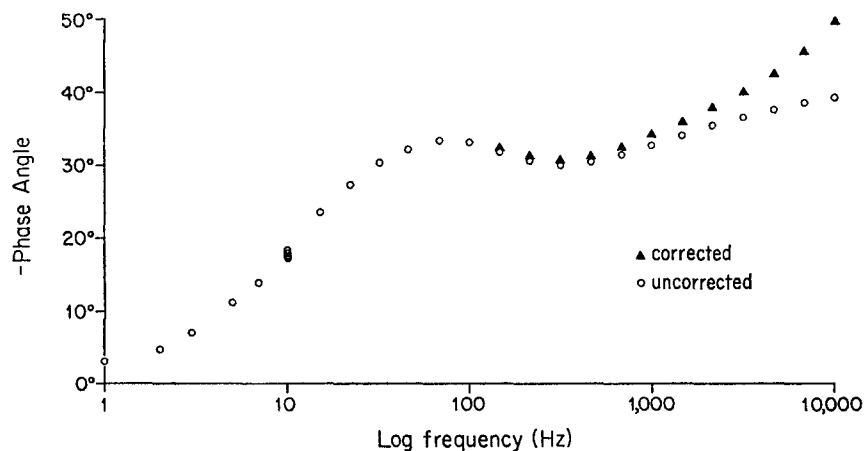
(1970) include:

(a) Use of a different input amplifier circuit which allows a phase error of less than 2° at a frequency of 10 kHz. (b) Shielding of the current microelectrode with conductive paint to minimize a previously unrecognized artifact, the extracellular potential produced by the flow of (capacitive) current across the wall of the microelectrode and then through the resistance of the bathing solution. (c) Theoretical and experimental analysis of the residual extracellular potential allowing a subtraction procedure to remove the effects of the residual artifact. (d) Measurement of the phase shift at the tip of the microelectrodes. (e) The use of phase-sensitive detectors to measure impedance. (f) The calibration of the apparatus in the presence of noise.

RESULTS

Measurements of Fibers in Normal Ringer

The phase shift between sinusoidal current and potential, applied and recorded with microelectrodes, was measured in a number of muscle fibers bathed in normal Ringer solution. Fig. 1 shows the results from a fiber with scatter, size of the extracellular potential, and drift close to the mean observed values. The open circles are the raw experimental data, corrected only for phase shift in the recording apparatus (Valdiosera et al., 1974 *a*, Fig. 2). The filled triangles are data corrected for the measured extracellular potential and loading effect (Valdiosera et al., 1974 *a*, Eq. 9 and Fig. 3). After every five measurements, readings were taken at a frequency of 10 Hz in order to determine the drift of the properties of the preparation. Fibers which showed drift greater than some 2° were rejected.



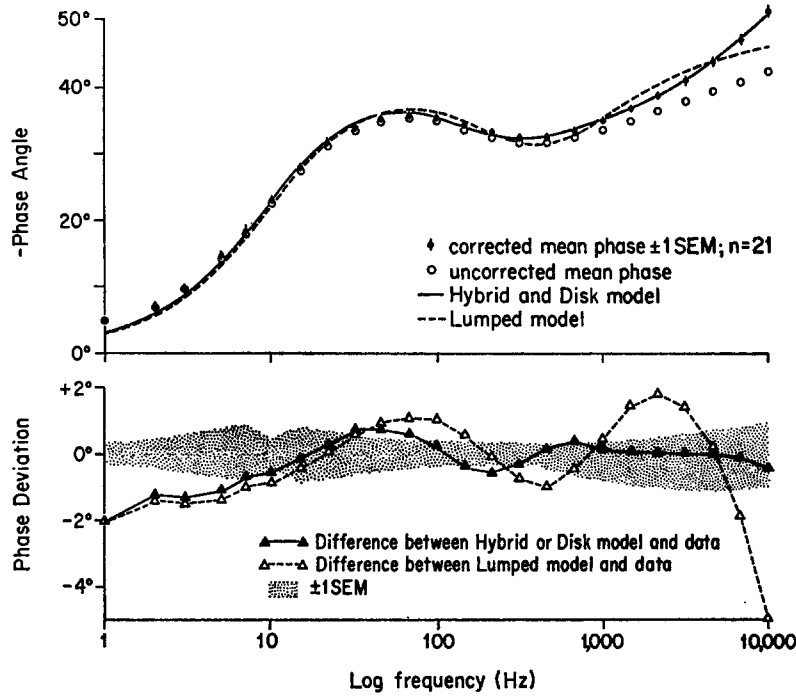


FIGURE 2. Phase and phase deviation plots of fibers in normal Ringer at a sarcomere length of $\sigma = 2.5 \mu\text{m}$. Each point in the upper plot is the mean (shown ± 1 SEM) of the phase data measured at that frequency from 21 fibers, corrected for loading and extracellular potential. The uncorrected data are also given. The lower plot shows the deviation between the theoretical value of phase, computed with the optimal set of parameter values for each model, and the experimentally observed phase at each frequency. The straight lines connecting the points are drawn for the sake of visual clarity and have no special significance. The stipled area represents the domain of phase which lies within 1 SEM of the corrected phase angle shown in the upper plot. The optimal values of the circuit parameters, their standard deviations, and the goodness of fit parameters are given in the various tables. The data taken at a sarcomere length of $2 \mu\text{m}$ are qualitatively similar, as are the fits of the different circuit models, but the optimal values of the parameters differ quantitatively.

Fig. 2 shows the results from 21 muscle fibers in normal Ringer held at a sarcomere length of $2.5 \mu\text{m}$. It would be best to analyze the results from each fiber individually, fitting the phase data from each fiber with the three circuit models under consideration (Valdiosera et al., 1974 *b*) and determining the best values of the circuit parameters and their standard errors in each case. Such a procedure would permit the variance of the data and circuit values to be split into two classes, one associated with the biological variation from fiber to fiber and the other caused by instrumental errors, drift, and systematic errors in the curve-fitting procedure. The computer time necessary to fit one set of data with three circuit models is considerable and costly (about \$30).

Since we report the results from about 140 fibers, it is not practical to follow the optimal procedure and fit the data from each fiber individually. We have followed the alternative procedure of averaging our data frequency by frequency (see Appendix). Thus, the phase point at each frequency in Fig. 2 (open circles in the upper part of the figure) is the mean value of the phase measured from 21 fibers at that frequency. The standard error of the mean is also displayed. The data, corrected for extracellular potential and loading effects of the microelectrodes (filled circles), were fit with three circuit models (Valdiosera et al., 1974 *b*) and the optimal values of the circuit elements and their standard deviations were determined (see Tables III, IV discussed below.) The points at low frequencies were routinely assigned a lower weight ($w_i = 0.1, 0.2, 0.3, 0.5, 0.7$ were the weights for the frequencies $f_i = 1, 2, 3, 5, 7$) since there is evidence of a systematic misfit at these frequencies.² Although this procedure is recommended by Hamilton (1964), it has little if any, effect on the results of the curve-fitting analysis. The various lines in the upper part of Fig. 2 show the best fit of each circuit model. Here, as in several other cases, the hybrid and disk models produce fits indistinguishable by eye. The lower part of the figure shows the deviation between the theoretical curves and the experimental data, each point representing the difference between the phase measured and the phase predicted at that frequency by a circuit model. The lines connecting the points in the lower part of the figure were drawn by hand to improve visual appearance; the stipled area represents the domain of phase which lies within 1 SEM of the observed phase angle.

Tables III and IV show the properties of the fibers and the optimal values of the circuit elements. The mean resting potential on both insertion and removal of the microelectrode is shown, as is the drift in phase during the course of the measurements. The parameter R measures the goodness of fit and is defined in Valdiosera et al., 1974 *b* and Hamilton (1964 and 1965). *The R-test takes into account the number of adjustable variables.* Since we typically make measurements at 24 frequencies and use four or five adjustable parameters (depending on the circuit model) we have 19 or 20 statistical degrees of freedom. This means that values of R which differ by a factor greater than 1.17 are significantly different at a probability level of 0.05, and values of R which differ by a factor greater than 1.32 are significantly different at a probability of 0.005. The circuit variables used in Tables III and IV are defined in Table I of Valdiosera et al., 1974 *b*. Briefly, τ_c is the overall time constant of the fiber, that which would be measured by analysis of the voltage response to a step function of current; c_{ec} is the normalized variable which expresses the fraction of the total capacitance which is in the tubular system; r_{are} is the

² The cause of this misfit is not known. Possible causes include: nonlinearities and time dependence in the membrane or microelectrodes, including the residual effects of "creep" (Almers, 1972 *a, b*), a longitudinal phase shift (see Discussion), or an effect of the sarcoplasmic reticulum.

TABLE III
MEASUREMENTS OF CIRCUIT PARAMETERS OF THE HYBRID MODEL

Condition	$-V_m$	No. fibers	Drift	R	\underline{rg}	ccc	$rare$	rer	P
	<i>mV</i>		<i>Degree</i>	<i>%</i>	<i>ms</i>	$\times 10^{-1}$	$\times 10^{-1}$	$\times 10^{-2}$	$\times 10^{-2}$
Ringer $\sigma = 2.5 \mu\text{m}$	89.5, 82.7 (0.7, 0.9)	21	0.80 (0.06)	1.15	16.3 (0.3)	7.95 (0.03)	1.41 (0.11)	5.28 (0.12)	-1.55 (0.04)
Ringer $\sigma = 2.0 \mu\text{m}$	90.6, 86.6 (1.2, 1.7)	12	1.02 (0.12)	1.75	19.4 (0.3)	7.46 (0.06)	1.82 (0.32)	3.94 (0.18)	-0.87 (0.06)
Hypertonic NaCl; $\sigma = ?$	86.8, 79.0 (2.2, 2.6)	11	1.22 (0.17)	1.34	24.2 (0.4)	7.72 (0.04)	1.67 (0.21)	3.81 (0.11)	-1.12 (0.04)
Hypertonic sucrose $\times 2; \sigma = ?$	77.4, 73.1 (1.2, 1.3)	16	1.15 (0.15)	1.62	22.3 (0.5)	8.17 (0.03)	1.80 (0.15)	7.28 (0.25)	-1.29 (0.05)
Hypertonic sucrose $\times 2.5; \sigma = 2.5 \mu\text{m}$	67.0, 63.0 (1.6, 1.8)	16	0.98 (0.12)	0.83	23.5 (0.3)	8.05 (0.02)	1.65 (0.08)	24.6 (0.4)	-0.98 (0.04)
1 CCCP $\sigma = 2.5 \mu\text{m}$	85.9, 80.7 (0.9, 1.2)	12	0.67 (0.12)	1.05	52.9 (1.0)	7.91 (0.04)	1.06 (0.16)	2.27 (0.07)	-0.58 (0.02)
$\frac{1}{2}$ CCCP $\sigma = 2.5 \mu\text{m}$	86.9, 85.4 (1.6, 1.4)	14	0.84 (0.11)	1.24	33.4 (0.6)	7.85 (0.02)	1.72 (0.12)	7.52 (0.18)	-0.82 (0.02)
$\frac{1}{4}$ CCCP $\sigma = 2.5 \mu\text{m}$	83.2, 76.1 (1.0, 1.4)	10	0.82 (0.09)	0.94	45.9 (0.9)	7.94 (0.02)	1.99 (0.08)	13.5 (0.32)	-0.60 (0.02)
$\frac{1}{8}$ CCCP $\sigma = 2.5 \mu\text{m}$	78.3, 75.5 (1.7, 1.8)	10	0.54 (0.09)	1.23	36.4 (0.8)	8.18 (0.03)	0.098 (0.073)	19.2 (0.6)	-0.91 (0.03)

The first number in the column labelled V_m is the resting potential after impalement by *both* microelectrodes; the second number is the resting potential measured upon withdrawal of the voltage microelectrode.

See Table IV for values of *gwg*.

Numbers in parentheses are the standard error of the measurement.

normalized variable which determines the location of the resistance to radial current flow in the lumen of the tubules. If $rare = 1$, all the radial resistance is in the "access" resistance at the mouth of the tubules, and the circuit model is said to be "lumped;" if $rare = 0$, all the radial resistance is in the lumen of the tubules and the circuit model is that of a "disk;" if $rare$ is between 0 and 1, the radial resistance is in both places and the circuit model is a "hybrid." The variable rer is the ratio of the total radial resistance to the DC resistance of the fiber; P is the proximity parameter which describes the three-dimensional spread of potential; and *gwg* is the ratio of the conductance of the walls of the tubules to the DC conductance of the fiber. Impedance measurements cannot determine *gwg* since this parameter has hardly any effect on the phase plot and so it has been estimated from the data of Eisenberg and Gage, 1969.

Similar measurements were taken from 12 muscle fibers in normal Ringer held at a sarcomere length of $2.1 \mu\text{m}$. The results are shown in Tables III and IV where it can be seen that the circuit parameters vary with sarcomere length.

Derived Circuit Parameters

In the results presented so far the circuit parameters are determined by the phase measurements alone and we would not be surprised if the uncertainty

TABLE IV
MEASUREMENTS OF THE CIRCUIT PARAMETERS OF THE DISK MODEL

Condition	R	\underline{rc}	cec	gwg^*	rer	P
	%	ms	$\times 10^{-1}$	$\times 10^{-1}$	$\times 10^{-2}$	$\times 10^{-1}$
Ringer $\sigma = 2.5 \mu\text{m}$	1.22	16.1 (0.3)	8.72 (0.02)	1.82	4.81 (0.01)	-2.10 (0.04)
Ringer $\sigma = 2.0 \mu\text{m}$	1.82	19.3 (0.3)	8.31 (0.01)	1.82	3.50 (0.02)	-1.27 (0.06)
Hypertonic NaCl $\sigma = ?$	1.43	23.9 (0.4)	8.54 (0.03)	1.1	3.43 (0.10)	-1.57 (0.04)
Hypertonic sucrose $\times 2$ $\sigma = ?$	1.87	22.1 (0.5)	9.12 (0.02)	1.82	6.84 (0.27)	-2.32 (0.06)
Hypertonic sucrose $\times 2.5$ $\sigma = 2.5 \mu\text{m}$	1.33	23.9 (0.5)	8.71 (0.02)	1.82	23.0 (0.6)	-1.81 (0.06)
1 CCCP $\sigma = 2.5 \mu\text{m}$	1.18	52.5 (1.0)	8.43 (0.04)	5.0	2.10 (0.06)	-0.72 (0.02)
$\frac{1}{2}$ CCCP $\sigma = 2.5 \mu\text{m}$	1.24	33.3 (0.7)	8.62 (0.02)	5.0	6.83 (0.20)	-1.24 (0.03)
$\frac{1}{4}$ CCCP $\sigma = 2.5 \mu\text{m}$	1.58	46.7 (1.6)	8.75 (0.03)	5.0	12.4 (0.6)	-1.16 (0.04)
$\frac{1}{8}$ CCCP $\sigma = 2.5 \mu\text{m}$	1.21	36.1 (0.8)	8.20 (0.03)	5.0	19.1 (0.6)	-0.90 (0.03)

Numbers in parentheses are the standard error of the parameter. See Table III for V_m , number of fibers, and drift data.

* Assumed value, not measured (see text and Valdiosera et al., 1974 b).

introduced by experimental error is smaller than the uncertainty introduced by imperfections in the theoretical circuit models (see Valdiosera et al., 1974 a, b). In order to determine the electrical properties of the structures which correspond to the various circuit elements, additional information is needed beyond the results already given. It is customary to refer the electrical properties of various structures to the outer surface area of a hypothetical fiber, of circular cross section, without folding or invagination of the surface membrane. We denote such parameters by an asterisk to indicate clearly that they depend quantitatively on an unlikely structural assumption (see Table I of Valdiosera et al., 1974 b). The determination of this set of parameters from the measured values already presented requires information concerning the DC resistance of the fiber and the cross-sectional area and perimeter of the fiber. We have measured the DC resistance directly and have determined the structural parameters by measuring the DC length constant of the fiber. Assuming a value for the internal resistivity from Hodgkin and Nakajima (1972 a, b), we have calculated the radius of an "equivalent" fiber with a circular cross section. This procedure is evaluated and discussed in Hodgkin and Nakajima (1972 a, b).

Determination of the properties of a square centimeter of the membrane of the tubular system, or of the conductivity of the lumen of the tubules, requires additional structural information, concerning the morphometric parameters of the tubular system. We have used the parameters measured by Peachey (1965) in his pioneering study, in the form presented by Adrian et al., 1969, but it should be clearly understood that there is much uncertainty concerning these figures. First, the parameters were not measured by a standard technique of structural analysis (for example, stereology) and so it is difficult to evaluate their sensitivity to systematic or statistical error. Second, the effect of fixation, dehydration, embedding, and sectioning on the morphometric parameters is not clear. Third, the shape of the cross section of the tubules is known to depend drastically on the chemicals and procedure used to fix the muscle (Birks and Davey, 1972; Davey, 1973) and so the true volume fraction ρ and the volume to surface ratio ζ of the tubules is not known. Fourth, the structure of the mouth of the tubules is not known, the few images which have been seen are a very small sample of the observations which have been attempted. The difficulty in observing the opening of the tubules could have several causes: the structure might be destroyed by fixation except in a few cases; the structure might be so convoluted, either naturally or after fixation, that two-dimensional images cannot be interpreted (B. Eisenberg, 1971). In the latter case the few images reported would be the result of an unusual combination of section orientation and tubular structure. In any case the conclusion seems inescapable that there is no reliable information concerning the structure of a *typical* tubular opening, or of typical tubules in the immediate vicinity of the surface membrane.

Derived Circuit Parameters for Fibers in Normal Ringer

Tables V and VI present the derived parameters for the hybrid and disk models. The numbers were computed from the measured parameters using the definitions and morphometric parameters of Valdiosera et al., 1974 *b*, Adrian et al., (1969: Eqs. 1–3) and Peachey (1965).

We now consider the apparent dependence of the various circuit parameters on sarcomere length. It is difficult to reach conclusions concerning the dependence of the parameters of the T system on sarcomere length since the two populations of frogs had quite different diameters.³ Such conclusions await further experiments on the same fiber at two different sarcomere lengths.

The variation of the properties of the surface membrane is more clear. It can be seen in the parameters of any of the circuit models and considerably exceeds the variation expected if the total area of the surface membrane and

³ However, analysis of the data from only those fibers for which we have measurements of both length constant and phase angle (see Table II) yields circuit parameters very close to those shown in Tables V, VI, and VII.

TABLE V
DERIVED CIRCUIT PARAMETERS OF THE HYBRID MODEL

Condition	R^*	a	R_i	C_m^*	C_w^*	C_w	R_s^*	R_L^*	G_L
	$\Omega\text{-cm}^2$	μm	$\Omega\text{-cm}$	$\mu\text{F/cm}^2$	$\mu\text{F/cm}^2$	$\mu\text{F/cm}^2$	$\Omega\text{-cm}^2$	$\Omega\text{-cm}^2$	mmho/cm
Ringer $\sigma = 2.5 \mu\text{m}$	2,847	42.2	169	1.18	4.55	0.72	21	129	5.45
Ringer $\sigma = 2.0 \mu\text{m}$	2,587	36.5	169	1.90	5.59	1.02	19	83	7.33
Hypertonic NaCl $\sigma = ?$	4,532	28.8	202	1.21	4.21	0.97	29	144	3.33
Hypertonic sucrose $\times 2$ $\sigma = ?$	3,009	28.7	202	1.36	6.05	1.41	39	180	2.66
Hypertonic sucrose $\times 2.5$ $\sigma = 2.5 \mu\text{m}$	3,151	34.0	202	1.46	6.01	1.18	128	647	0.876
1 CCCP $\sigma = 2.5 \mu\text{m}$	9,809	35.3	169	1.13	4.26	0.80	24	198	2.97
$\frac{1}{2}$ CCCP $\sigma = 2.5 \mu\text{m}$	6,350	41.3	169	1.13	4.13	0.67	82	396	1.74
$\frac{1}{4}$ CCCP $\sigma = 2.5 \mu\text{m}$	7,359	41.7	169	1.29	4.96	0.79	196	792	0.878
$\frac{1}{8}$ CCCP $\sigma = 2.5 \mu\text{m}$	5,412	34.7	169	1.22	5.50	1.06	10	1,031	0.561

TABLE VI
DERIVED CIRCUIT PARAMETERS OF THE DISK MODEL

Condition	C_m^*	C_w^*	C_w	R_L^*	G_L
	$\mu\text{F/cm}^2$	$\mu\text{F/cm}^2$	$\mu\text{F/cm}^2$	$\Omega\text{-cm}^2$	mmho/cm
Ringer $\sigma = 2.5 \mu\text{m}$	0.73	4.94	0.78	137	5.13
Ringer $\sigma = 2.0 \mu\text{m}$	1.26	6.18	1.13	91	6.68
Hypertonic NaCl $\sigma = ?$	0.77	4.51	1.04	155	3.10
Hypertonic Sucrose $\times 2$ $\sigma = ?$	0.64	6.70	1.56	206	2.32
Hypertonic Sucrose $\times 2.5$ $\sigma = 2.5 \mu\text{m}$	0.98	6.60	1.53	725	0.660
1 CCCP $\sigma = 2.5 \mu\text{m}$	0.84	4.51	0.85	206	2.86
$\frac{1}{2}$ CCCP $\sigma = 2.5 \mu\text{m}$	0.73	4.51	0.73	434	1.59
$\frac{1}{4}$ CCCP $\sigma = 2.5 \mu\text{m}$	0.79	5.55	0.89	913	0.761
$\frac{1}{8}$ CCCP $\sigma = 2.5 \mu\text{m}$	1.20	5.47	1.05	1,034	0.561

See Table V for R^* , R_i and a .

total volume of the fiber were independent of sarcomere length, the surface membrane simply unfolding as the fiber stretches (Hodgkin, 1954, Martin, 1954) like a stopped up concertina filled with incompressible fluid (Elliot et al., 1963). It has been suggested that the apparent variation of C_m^* is really produced by a variation in the internal resistivity with sarcomere length, and since we have not measured the resistivity directly, we cannot rule out this possibility. The large variation of the capacitance of the surface membrane

TABLE VII
DERIVED CIRCUIT PARAMETERS OF THE LUMPED MODEL

Condition	R	C_m^*	C_w^*	C_w	R_A^*
	%	$\mu F/cm^2$	$\mu F/cm^2$	$\mu F/cm^2$	$\Omega\text{-cm}^2$
Ringer $\sigma = 2.5 \mu m$	2.98	2.16	3.43	0.54	176
Ringer $\sigma = 2.0 \mu m$	2.65	2.95	4.49	0.82	113
Hypertonic NaCl $\sigma = ?$	2.77	1.96	3.32	0.77	191
Hypertonic sucrose $\times 2$ $\sigma = ?$	4.41	2.26	4.68	1.09	212
Hypertonic sucrose $\times 2.5$ $\sigma = 2.5 \mu m$	4.97	1.94	3.93	0.77	706
1 CCCP $\sigma = 2.5 \mu m$	2.49	1.13	4.26	0.80	222
$\frac{1}{2}$ CCCP $\sigma = 2.5 \mu m$	3.92	1.62	2.98	0.48	493
$\frac{1}{4}$ CCCP $\sigma = 2.5 \mu m$	4.17	1.61	2.88	0.46	1,052
$\frac{1}{8}$ CCCP $\sigma = 2.5 \mu m$	4.21	1.90	2.89	0.56	1,101

R^* , a , R_i are given in Table V.

Values of R greater than 3% indicate a serious misfit of the experimental data.

in a unit length of fiber (c_m) can be explained this way, but the small variation of the resistance r cannot. Another explanation of the variation of C_m^* with sarcomere length is that there is a complex rearrangement of the surface membrane as the fiber is stretched.

Measurements in Hypertonic Solutions

Measurements of impedance were made from fibers in three hypertonic solutions (solutions B, C, D, in Table I) but unfortunately measurements in the first two solutions were performed before we were aware of the importance of sarcomere length. The muscles were stretched to some 130% of the resting length but the sarcomere length was not measured. The striking difference between the phase characteristics of fibers in solutions C and D (Figs. 3 and

4) results in a large change in the estimates of the circuit parameters. Since the main difference in the two sets of data is in the height of the low frequency maximum, one would expect that the parameter r_{er} would be responsible for most of the difference between the curves (see Fig. 3 of Valdiosera et al., 1974 *b*) and this is indeed the case. It is also possible that the low resting potentials of fibers in the $\times 2.5$ hypertonic solution could produce this change in

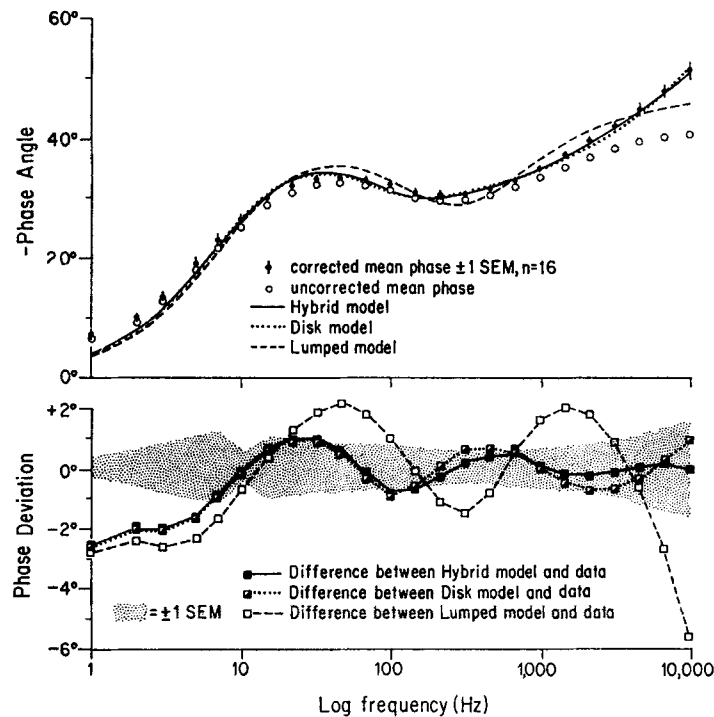


FIGURE 3. Phase and phase deviation plots of fibers in $\times 2$ hypertonic sucrose at an unknown sarcomere length. The plots are described in the legend of Fig. 2. The data from fibers in hypertonic NaCl, and the fits of the theory, are qualitatively similar, although quantitatively quite different (see Tables).

the phase plot through some unknown mechanism. Measurements were also taken from fibers in a $\times 2$ hypertonic NaCl solution and the extracellular potential in this case was small as would be expected. The results were fitted using a value of gwg determined from Eisenberg and Gage (1969) assuming the chloride conductance to be proportional to the external chloride concentration although in view of the increase in membrane resistance in the hypertonic NaCl solution a different value of gwg should have been used. This is of little significance since gwg has almost no effect on the phase characteristic. The value of R_i was that reported by Hodgkin and Nakajima (1972 *a*) for a somewhat different solution.

It is surprising that C_w^* appears to increase in these sucrose solutions, even though it is unlikely that the tubular system swells in these solutions (Birks and Davey, 1972). The apparent increase in the luminal resistance in these hypertonic solutions is also hard to explain. The increased value of R^* could

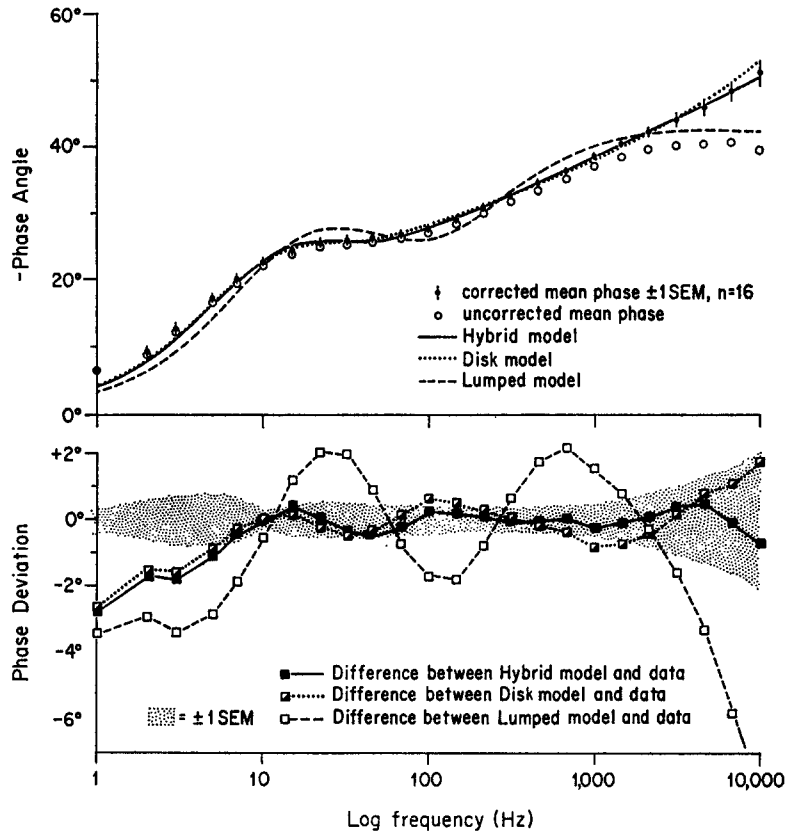


FIGURE 4. Phase and phase deviation plots of fibers in $\times 2.5$ hypertonic sucrose, a solution used in many voltage clamp experiments to inhibit movement, at a sarcomere length of $\sigma = 2.5 \mu\text{m}$. The plots are described in the legend to Fig. 2. The striking difference between these data and those recorded from fibers at the same sarcomere length in normal Ringer (Fig. 2), or at unknown sarcomere length in $\times 2$ hypertonic sucrose, (Fig. 3) can be explained by a large increase in the resistance to radial current flow, interpreted as the resistance of the lumen of the tubules in the disk and hybrid circuit models. The fit of the lumped model is quite unsatisfactory.

be produced by some interaction of leakage around the microelectrode and anomalous rectification (Hodgkin and Nakajima, 1972 *a*).

There is one clear conclusion from our results in hypertonic solutions: the increase in radial resistance cannot be attributed to an increase in the access resistance. The failure of the lumped model to fit the phase plots and the low value of the variable *rare* rule out this possibility.

Measurements in Solutions of Varying Conductivity

Solutions were designed which had different conductivities but the same tonicity and concentration of KCl (Table I: solutions E-H) in order to vary the conductivity of the solution in the lumen of the tubules. In an attempt to hold the fiber membrane at constant conductance and constant potential, we held constant the concentration of conducting (that is, permeant) particles (hence the solutions are called CCCP). Unfortunately, and for reasons that are not entirely clear, the resting potentials and DC resistance were not the same in each solution, but at least they did not vary as drastically as in previous solutions in which the concentration of conducting particles was not held constant (Vaughan et al., 1972). Figs. 5 and 6 present phase plots from two of these four solutions. Comparison of these figures with each other and Fig. 1 shows that variations in the conductivity of the bathing solutions have profound effects reminiscent of those produced by changes in the variable *rer* (see Valdiosera et al., 1974 *b*, Fig. 3). The variable *gwg* was determined from the data of Eisenberg and Gage (1969) assuming that the membrane conductance to Cl^- is proportional to the extracellular concentration of Cl^- .

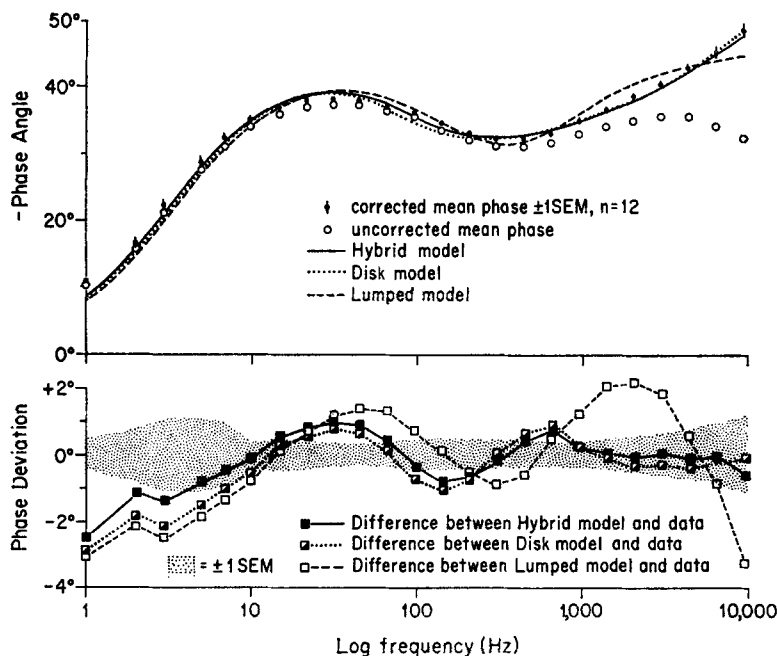


FIGURE 5. Phase and phase deviation plots of fibers in solution E. The plots are described in the legend of Fig. 2. The data from fibers in solution F, and the fit of the various circuit models, are qualitatively similar, but quantitatively distinct, from that shown here.

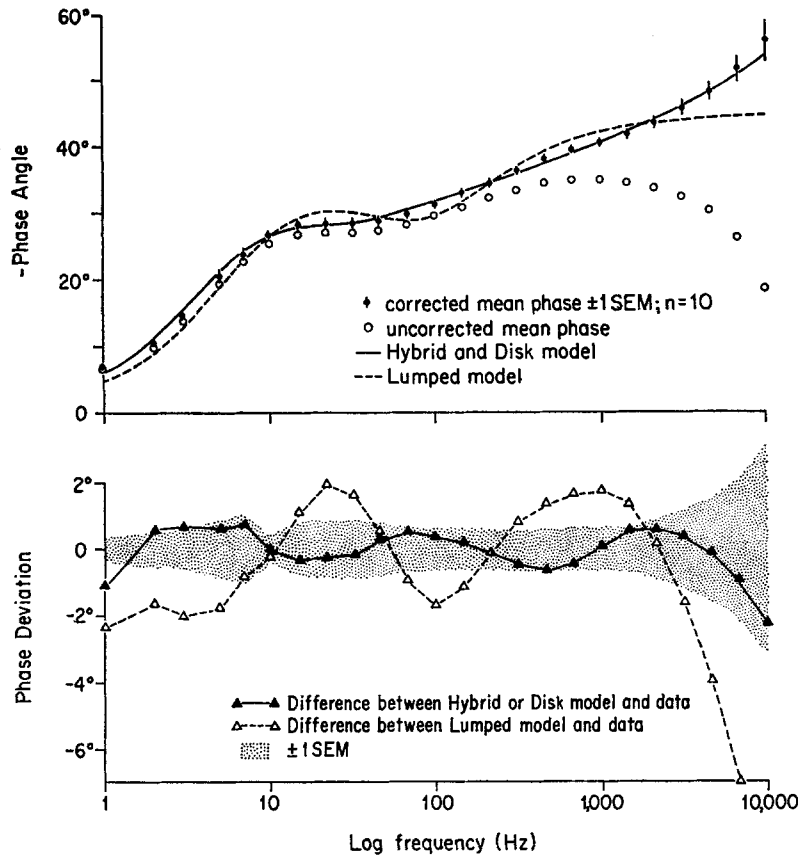


FIGURE 6. Phase and phase deviation plots of fibers in solution H. The plots are described in the legend of Fig. 2. The data from fibers in solution G, and the fit of the various circuit models, are qualitatively similar, but quantitatively distinct, from that shown here. The fit of the lumped model is quite unsatisfactory. The marked difference between these data and those shown in Figs. 2 and 5 illustrates the important effect of the conductivity of the bathing solution on the impedance of muscle fibers.

Solutions of varying conductivity drastically alter the shape of the phase characteristic and so serve as a check on the circuit models: some parameters, in particular the capacitance of the surface and tubular membranes, are not expected to vary with the conductivity or ionic strength of the bathing solution (at least in this range of ionic strengths) and so it is interesting to see how well the measured values of the parameters of the various circuit models agree with this prediction.

The capacitance of the tubular membrane varies somewhat with conductivity in both circuit models. It is not clear how much of this variation might be the result of experimental error or biological variation. The change in surface capacitance with conductivity separates the two circuit models more

clearly: in the hybrid model the surface capacitance hardly changes at all, whereas in the disk model there is a marked increase in surface capacitance. The behavior of the hybrid model is thus closer to our expectations, but unfortunately most of the difference between the models is in their fit to the data from fibers in solution H, which has the lowest conductivity, the largest correction for extracellular potential, and the greatest uncertainty caused by experimental error.

The variation of the conductivity of the lumen of the tubules with the conductivity of the bathing solution is shown in Fig. 7. It can be seen that there is a curvilinear relation, as might be expected if there were some 10–15 mM fixed charge in the lumen or on the membrane of the tubular system. Most of the curvature is produced by solution A, which has a different chloride concentration than the others, and so other interpretations are tenable. It is interesting to test the assumption that the ionic content and mobility of the solutions filling the lumen of the tubules is the same as in the extracellular bathing solution. Our results are inconsistent with this assumption if the

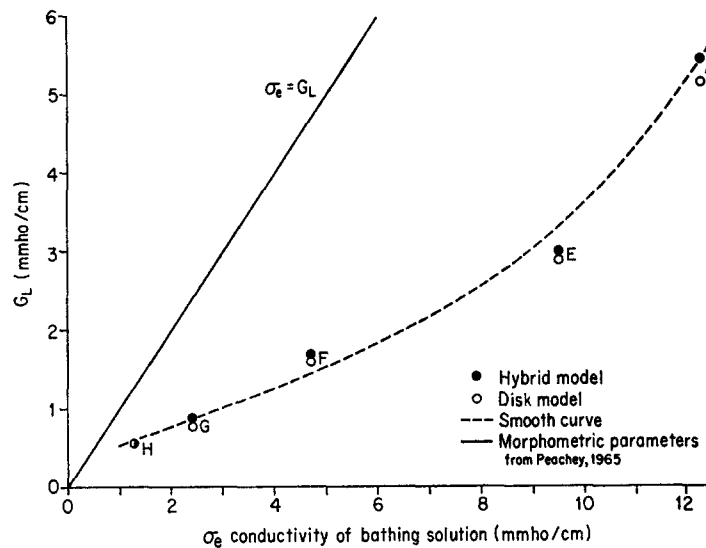


FIGURE 7. The variation in the conductivity of the lumen of the tubules. The points represent the observed values of the conductivity of the lumen (see Tables) computed using the morphometric data of Peachey (1965) in the form presented by Adrian et al. (1969). See text for discussion of uncertainty in the morphometric parameters. The letter beside each point identifies the solution in which the fiber was bathed during that set of measurements (see Table I). The dashed curve was drawn by eye. The straight line is the variation of conductivity predicted, using the morphometric data of Peachey, if the solution filling the lumen of the tubules has the same composition and conductivity as the solution bathing the muscle fiber. The data seem to show that the conductivity of the lumen is less than that of the bathing solution, either because of differences in mobility or composition of the solutions, and that there is a curvilinear relation between the conductivity of the two solutions.

morphometric parameters of Peachey (1965) are used (see solid line in Fig. 7). In order to reconcile our results with the assumption it would be necessary to use a value for the volume fraction of the tubular system much less than that reported, or to use a value for the network factor of Adrian et al. (1969) much less than one-half. Since we doubt that the morphometric data is in error to this extent (indeed it seems more likely that the volume fraction of the tubular system is larger than that observed in fixed material) and we have no reason to question the estimate of the network factor, we conclude that the ionic content and/or mobility of ions in the lumen of the tubules is much less than in the extracellular bathing solution, perhaps because the lumen of the tubules is occluded by some material like basement membrane.

It is important to realize that the increase in radial resistance observed in solutions of low conductivity cannot be explained by an increase in the access resistance presumably at the mouth of the tubules. The small value of the variable *rare*, and the failure of the lumped model to fit the data in these solutions rule out that possibility.

Measurements of Glycerol-Treated Muscle Fibers

Muscles were soaked in a Ringer solution containing 400 mM glycerol for 1 h and then returned to solution I or J. Measurements were made starting 1 h later. Because of the well-known fragility of glycerol-treated fibers, and their considerable variability from preparation to preparation and season to season, and in spite of the use of high concentrations of Ca^{++} as recommended by Eisenberg et al. (1971), we had difficulty in making reliable measurements on glycerol-treated fibers. Measurements made in solution J were reasonably easy, but the hypertonicity of the solution combined with the lack of knowledge of the physiological state of the treated muscle in this solution precludes rigorous interpretation of the experimental data. The data shown in Fig. 8 were taken from muscles returned to solution I. Measurements were made at half the number of frequencies used in other experiments in order to shorten the experiment, and with this modification successful experiments were completed on 14 fibers. Only the lumped model could be fit to the data using the standard curve-fitting procedure since the experimental information was insufficient to determine all the parameters of either the disk or the hybrid model. A different procedure, of problematical propriety, was used in these latter cases. The value of the time constant was constrained to the value appropriate for the lumped model, the justification being the near equality of the time constants of different models in all solutions in which the models fit reasonably well. The significance of parameters determined in this manner is not clear and so their values are relegated to the legend of Fig. 8 and the values of the lumped model are used in our further discussion.

It may seem that the properties of glycerol-treated fibers could be explained either by a large decrease in the tubular capacitance or by a large increase in

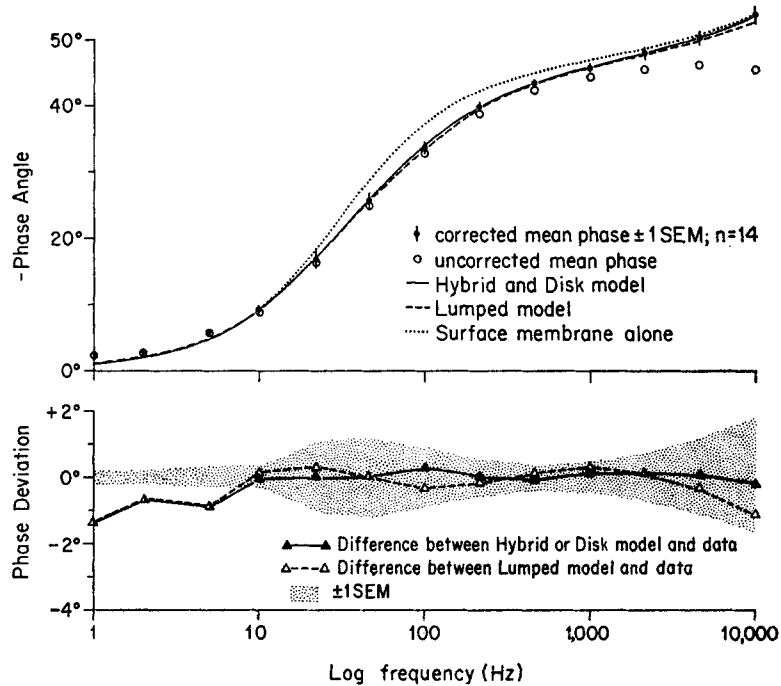


FIGURE 8. A phase and phase deviation plot of glycerol-treated fibers in solution I. The plots are described in the legend of Fig. 2. The data were taken at three frequencies per decade to decrease the duration of the experiment and so produce acceptable stability of the preparation. The phase plot expected if there were no tubular system in glycerol-treated fibers is shown; this plot has been computed by constraining the variable r_{er} to infinity, and allowing the curve-fitting procedure to determine the optimal value of the proximity parameter P (which turns out to be -0.01). The shape of the plot and its horizontal location show that there are few tubules present in glycerol-treated fibers (see Table VIII). The theoretical curves for the disk and hybrid model were computed by a modification of the standard curve-fitting procedure (the modification is described in the text) and the optimal parameter values are:

	r_c (ms)	c_{ec}	r_{ar}	r_{er}	P
Disk model	5.20	0.24	—	2.40	-0.012
Hybrid model	5.20	0.235	0.013	2.42	-0.012

the radial resistance since, of course, an infinite radial resistance and a zero tubular capacitance are indistinguishable. If these circuit elements have finite values, however, they can be distinguished. For instance, Fig. 10 shows that the experimental results from glycerol-treated fibers cannot be fit with normal values of the tubular and surface capacitance allowing arbitrarily large values of the radial resistance. Curve-fitting experiments with different values of radial resistance and different values of the surface capacitance show that

there is some ambiguity in the results but not much more than indicated by the estimates of the standard error of the parameters.

Derived Circuit Parameters of Glycerol-Treated Fibers

The interpretation of the electrical and mechanical properties of glycerol-treated fibers depends critically on the amount of tubular system which is functional, and in that sense "intact," in this preparation. For that reason the most important parameter of glycerol-treated fibers is the fraction of the tubular system which contributes to the electrical properties of such fibers, that is the fraction of the tubular system which still is a pathway for current flow from the sarcoplasm to the extracellular solution. The figures presented in Tables V, VI, VII, and VIII allow the calculation of the capacitance of

TABLE VIII
PROPERTIES OF GLYCEROL-TREATED FIBERS
(LUMPED MODEL)

Solution	n	$-V_m$	a	R	τ_c	c_{cc}	r_{er}	R^*	C_m^*	C_w^*	R_a^*
		mV	μm	%	ms	$\times 10^{-1}$		$\Omega\text{-cm}^2$	$\mu F/cm^2$	$\mu F/cm^2$	$\Omega\text{-cm}^2$
High Ca^{++}	14	74.3, 69.4 (1.8, 1.9)	33.5	1.0	5.20 (0.09)	1.99 (0.16)	2.87 (0.50)	2613	1.60	0.40	7473

R_i was taken as $169 \Omega\text{-cm}^2$.

The first number in the column labeled V_m is the resting potential after impalement with *both* microelectrodes; the second number is the resting potential on withdrawal.

The phase drift was $1.04 \pm 0.16^\circ$.

the tubular system referred to a unit volume of fiber (\bar{C}_w , see Adrian et al., 1969) in both glycerol-treated and normal fibers and the ratio of these figures is an estimate of the fraction of the tubular system which contributes to the passive electrical properties of these treated fibers. We thus estimate that 10% of the tubular system is intact in the glycerol-treated fibers studied here and that 90% of the tubular system makes no detectable contribution to the passive electrical properties measured here.

The electrical properties of the little tubular system remaining intact in glycerol-treated fibers are quite different from properties of the tubular system of normal fibers. The radial resistance (R_a^* , determined from the lumped model, see Table VIII) in series with the membrane of the residual tubules is remarkably high, being some 50–60 times higher than the normal series resistance. Thus, the few tubules remaining intact in glycerol-treated fibers are quite isolated from the external solution and would not be expected to have important effects in electrophysiological experiments, except those designed to measure a slow change in potential (Nakajima et al., 1973). Experiments on glycerol-treated fibers using solution J to disrupt the tubules gave similar

results to those reported above except that the value of the radial resistance was smaller and close to normal. It is not clear whether the different value of the radial resistance is caused by the hypertonicity or the high divalent cation concentration of the solution, or the (possibly) different sarcomere length of the preparation.

DISCUSSION

Comparison with Previous Results

Since the parameter values and circuit models used to describe the electrical properties of muscle fibers enter into so many analyses of the physiological properties of muscle, it is important to have as reliable a set of values as possible. We therefore will compare our experimental results with several of the circuit models and sets of parameter values used in the literature.

The lumped model of the tubular system was used in the pioneering work of Falk and Fatt (1964) and Freygang et al. (1967). Consideration of Figs. 2-6 and the relevant tables shows that the lumped model does not fit the experimental data nearly as well as the other models; indeed it fails to fit the data from fibers in solutions C, D, G, and H at all well. On these grounds we reject this model as a suitable description of muscle fibers. On the other hand, the model is relatively easy to handle mathematically and so may be useful in approximately describing the electrical properties of muscle fibers in the solutions in which it is not too bad a fit (Adrian and Almers, 1973). It is important to realize that the lumped model can represent the fiber reasonably well only if it is used with a set of circuit values which produce a phase plot similar to that observed, a set of values close to that given in Table VII.

Table IX shows the parameter values used by several authors. Figs. 9 and 10 show the corresponding phase characteristics, determined from the parameter values and the optimal values of the proximity parameter P . These plots have been computed by fitting our experimental data with the circuit model and parameter values reported, using our measured value of DC resistance and fiber radius, and allowing the curve-fitting procedure to determine the optimal value of P . This procedure is designed to produce the maximum possible agreement between data taken from different populations of frogs, with fibers of different diameter, and electrodes at different locations. The value of gwg is set somewhat arbitrarily in some cases but this is of little significance since it hardly changes the phase characteristic.

The phase characteristics corresponding to the circuit models and parameter values used by other authors are shown in Fig. 9. The characteristic determined from the data of Hodgkin and Nakajima (1972 *b*) is quite close to our observed characteristic, although still significantly different according to statistical tests on the value of R , the goodness of fit parameter. This result suggests that biological variation is not too great a problem. The characteristic

TABLE IX
COMPARISON WITH PREVIOUS RESULTS

	\bar{G}_L $\times 10^{-4}$	\bar{G}_w $\times 10^{-2}$	\bar{C}_w $\times 10^{-1}$	C_m^*	$r \times \epsilon$	cec $\times 10^{-1}$	<i>rare</i>	<i>gwg</i> $\times 10^{-1}$	<i>rer</i> $\times 10^{-2}$	<i>P</i> $\times 10^{-1}$	<i>R</i> %
	<i>mho/cm</i>	<i>mho/cm²</i>	<i>F/cm²</i>	$\mu F/cm^2$	<i>ms</i>						
Hodgkin and Nakajima, 1972	0.90	9	2.7	1.15	19.4	8.31	—	5.38	4.10	-1.41	2.7
Peachey and Adrian, 1973	1.50	15	3	1.0	20.8	8.63	0.588	8.97	5.87	-3.30	12.9
Adrian et al., 1969	1.50	15	3	1.0	20.8	8.63	—	8.97	2.46	-1.55	6.6
Schneider, 1970	0.415	—	2.55	0.97	18.0	8.47	—	—	8.89	-1.76	7.5
Freygang et al., 1967	***	***	***	—	13.8	6.43	1	—	12.1	-0.32	8.5
Falk and Fatt, 1964	***	***	***	2.6	19.1	6.12	1	—	11.6	0.30	8.7
Our hybrid model†	0.814	3.04	2.17	1.18	16.3	7.95	0.141	0.182	5.28	-1.55	1.6
Our disk model‡	0.766	3.04	2.35	0.73	16.1	8.72	—	0.182	4.81	-2.10	1.2

*** Not applicable to lumped model.

† These numbers differ from those derivable from Tables V, VI since the population of fibers is slightly different.

For corresponding plots see Fig. 9.

The phase characteristic is almost independent of the value of *gwg*, the fractional conductance of the tubular wall, and so does not depend on \bar{C}_w provided the DC resistance *r* is kept constant.

Variables defined in Adrian et al., (1969) and Valdiosera et al., (1974 *b*).

determined from the estimates of Adrian et al. (1969) is not such an adequate fit and that determined from the estimates of Peachey and Adrian (1973) is quite poor. In the latter case the deviation is so great that one would not expect calculations of action potential shape and conduction velocity to agree with experimental results, even if the nonlinear properties of the fiber were described correctly. The large deviation occurs because of the pronounced dip in the phase characteristic computed from the parameter values of Peachey and Adrian (1973). The dip is produced by the large value of *rare* which makes the model quite "lumpy." The misfit produced by the large value of *rare* could be ameliorated if the value of *cec* were reduced (see Figs. 4 and 5 of Valdiosera et al., 1974 *b*), and indeed this is why the totally lumped model can fit the experimental data better than the partially lumped model of Peachey and Adrian (1973), in which *cec* was held at a value appropriate for the disk model, and at the same time *rare* was made quite large.

Fig. 10 shows the phase characteristics corresponding to various models of glycerol-treated fibers. The lumped model was used and the estimates of the variable *cec* were taken from the papers indicated, scaled to the observed diameter of the fibers in our experiments. The DC resistance was taken to be our observed value but the curve-fitting procedure determined the optimal value of the parameter *rer* (the resistance in series with the tubular capacitance) and the proximity parameter *P*. The discrepancy with the phase characteristic generated by the data of Hodgkin and Nakajima suggests that

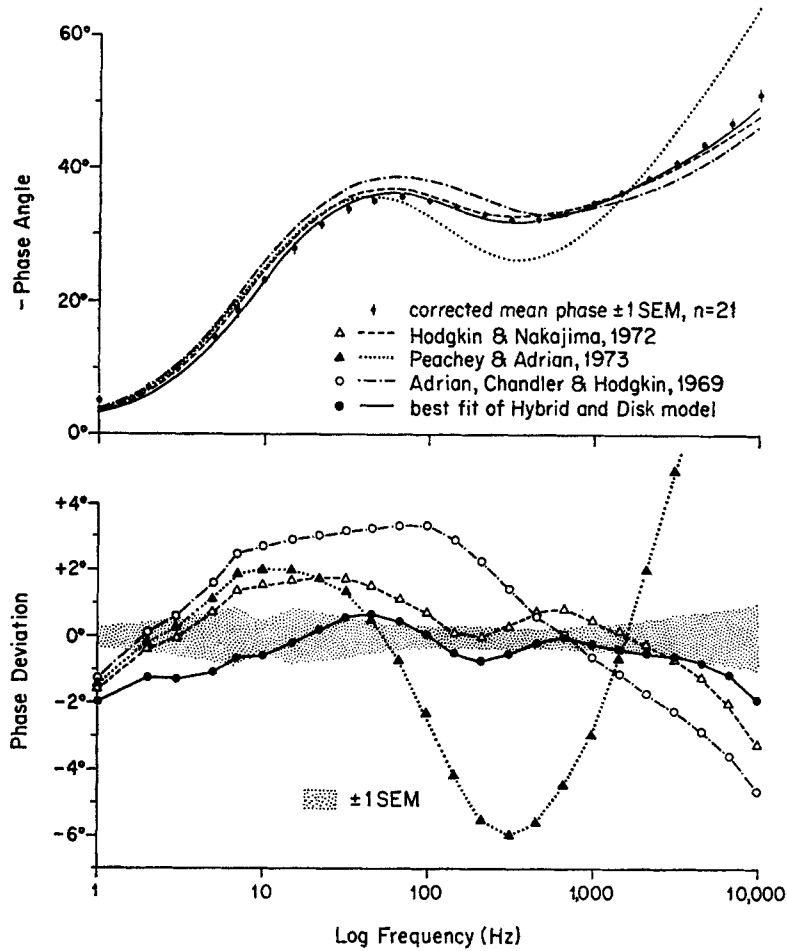


FIGURE 9. Phase and phase deviation plots to compare our data with previous circuit models and parameter values. The plots are described in the legend of Fig. 2, which also illustrates the experimental data in more detail. The theoretical curves have been computed from the parameter values and circuit models used by the references cited, adjusted to the values appropriate for fibers of the DC resistance and diameter we observed. In the curve-fitting procedure the parameters of the circuit models were constrained to the values shown in Table VIII, except the procedure was allowed to determine the optimal value of the proximity parameter P . While there is always uncertainty concerning the appropriate scaling of curves determined by one method from one group of frogs to another, we hope and believe that these curves show fairly the difference between previous circuit models and parameter values and our data.

their value of c_{cc} is too large to describe our results, perhaps because there is less tubular disruption in glycerol-treated fibers from *R. temporaria* than from *R. pipiens* (see Flitney, 1971). It is also possible that methods based on measurements from the foot of the action potential are subject to errors associated with the damaged state and low resting potential of glycerol-treated fibers

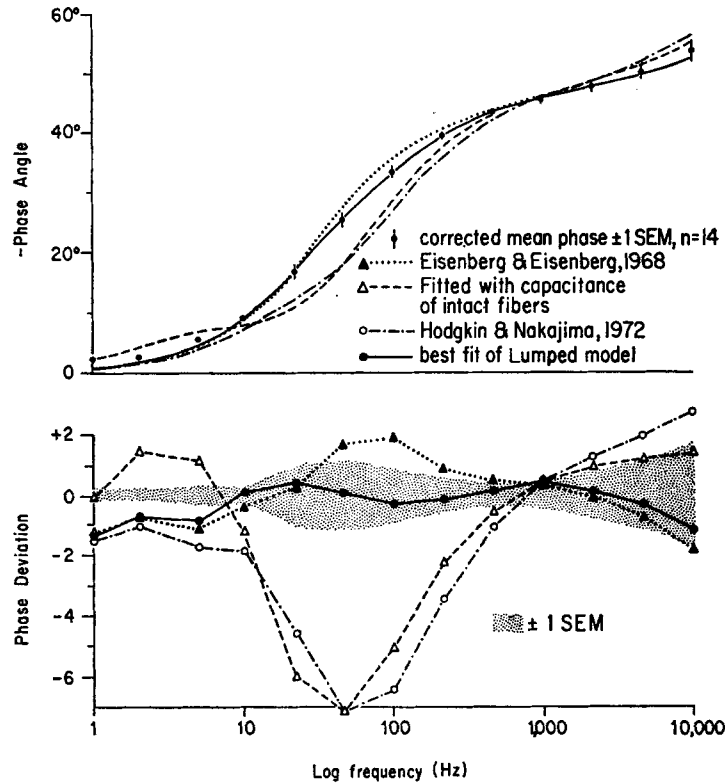


FIGURE 10. Phase and phase deviation plots to compare our data with previous circuit models and parameter values describing glycerol-treated fibers. The plots are described in the legend of Fig. 2 and the data are given in more detail in Fig. 8. The theoretical curves have been computed for the following parameter values of lumped model, which correspond to the parameter values given by the several references adjusted to be appropriate for the diameter and DC resistance we observed. While there is necessarily uncertainty in the scaling of data taken by one technique to data measured by another, we hope and believe that these curves show fairly the difference between the various results.

	r_c	c_{cc}	r_{cr}	P
	(ms)			
Hodgkin and Nakajima	4.34	0.42	3.32*	-0.019*
Eisenberg and Eisenberg	4.85*	0.076	3.04*	-0.009*
Capacitance of normal fibers	12.5	0.754	2.88*	-0.015

The * indicates parameters which were not constrained, but which were adjusted by the curve fitting procedure to their optimal values.

(perhaps the resting potential and therefore conduction velocity is not uniform down the length of glycerol-treated fibers). Recent results of Bastian and Nakajima (personal communication) give values of c_{cc} close to ours.

The value of c_{cc} from the morphological measurements of Eisenberg and

Eisenberg (1968) seems too small, suggesting that more tubules are intact in our preparation than they report. This discrepancy may be caused by different procedures for the selection of fibers (our figure is close to the highest they report) or to the difference in solutions (they did not use solution I to disrupt tubules) or to the failure of peroxidase to enter the intact tubules which appear to be in series with a large radial resistance. The latter alternative is supported by the recent findings of Franzini-Armstrong, Venosa and Horowitz (personal communication) that some 10% of the tubular system in glycerol-treated fibers is accessible to lanthanum applied in the bathing solution.

Computation of the Action Potential and Other Properties of the Tubular System

One of the most important determinants of the conduction velocity and shape of the action potential is the passive electrical properties of the muscle fiber. Our experimental results specify these passive properties, in the form of a phase characteristic, reasonably precisely and therefore in calculations of the action potential circuit models and parameter values should be used which predict a phase characteristic reasonably close to that observed. It is not sufficient simply to use the "correct" circuit model, or the "correct" values of the circuit parameters; rather *both* the circuit model and the parameter values must be correct, in the sense that together they produce a phase characteristic reasonably close to that observed.

This point becomes particularly important in theoretical studies of the effect of the access resistance on the expected properties of muscle fibers. In the extreme cases, in which the access resistance accounts for all the radial resistance of the muscle fiber (the lumped model), or none of the radial resistance (the disk model), it would be necessary to use quite different values of the circuit parameters if one wished to describe the passive electrical properties at all well (compare Tables VI and VII). If an access resistance were simply added into the disk model, leaving all the other parameters constant, a drastic distortion of the phase characteristic would occur (compare the curves in Fig. 9 labeled "Adrian, Chandler & Hodgkin, 1969" and "Peachey and Adrian, 1973") and computations based on such a procedure could not be expected to give results applicable to real muscle fibers.

Hypertonic Sucrose Solutions

The results from fibers in hypertonic sucrose solutions pose serious difficulties for both the disk and the hybrid model of the tubular system. The structure of the tubular system is apparently unchanged by these solutions (Birks and Davey, 1972) but the electrical parameters change in a perplexing way. The capacitance of the tubular system increases as if there were more area of internal membranes accessible to current flow; however, the radial resistance

to current flow also increases, although most models which would produce an increase in the area of accessible membrane would predict a decrease in the radial resistance. It is not possible to interpret these results using the disk or hybrid model without postulating specific actions of the sucrose solutions and so these results weaken our case that the disk or hybrid models can serve as an adequate general description of the electrical properties of frog skeletal muscle fibers. It is not impossible, however, that there are specific actions of the hypertonic sucrose solutions, perhaps associated with the low resting potentials of fibers in these solutions or with the apparent swelling of the sarcoplasmic reticulum (Birks and Davey, 1972), which might account for these results, or which might make it inappropriate to use any of the equivalent circuits we have proposed. If the equivalent circuit of fibers in hypertonic sucrose solutions were quite different from the circuits we consider, the parameter values we report would, of course, have little significance. Indeed, it has been suggested that the apparent radial resistance in hypertonic sucrose solutions might actually reside in the sarcoplasm or sarcoplasmic reticulum.

The results in $\times 2.5$ hypertonic sucrose show a small value of luminal conductivity, suggesting that the tubular membrane is quite isolated from the surface membrane in this solution. This isolation should affect excitation-contraction coupling (Gordon and Godt, 1970) and make it difficult to control the potential across the tubular membrane in voltage clamp experiments, especially in the presence of inward sodium current across the tubular membrane (Costantin, 1970; Bezanilla et al., 1972; Bastian and Nakajima, personal communication). The failure to control potential across the tubular membrane might result in serious difficulties in the interpretation of voltage clamp results, particularly in the shape of the early after potential.

Miscellaneous Conclusions

The large value of C_m^* in $\times 2.5$ hypertonic sucrose and in glycerol-treated fibers is difficult to explain. Fibers in these solutions have low resting potentials and so the large value may well be the result of experimental error associated with fiber damage, or it may represent a real dependence of the membrane capacitance (Schneider and Chandler, 1973) on DC potential. Finally, it is possible that these solutions induce some rearrangement of the caveoli and surface membrane itself (Dulhunty and Gage, 1973, Fig. 6).

Residual Uncertainties

A source of uncertainty in the interpretation of our results is the possibility that the longitudinal impedance of the fiber is reactive, not resistive as we have assumed (Mobley et al., 1973). Preliminary calculations show that if the longitudinal impedance is reactive only at low frequencies, where the evidence is best that this may be the case, the fit of our circuit models to the data from

fibers in normal Ringer is significantly improved. The only circuit parameter which changes significantly is the time constant, which is reduced somewhat. We thank Dr. B. Mobley for making this data available to us.

Choice of Circuit Model

The disk and the hybrid model both fit all the experimental data satisfactorily in all cases, although in several cases the fit of the hybrid model is significantly better (in the statistical sense) even taking into account the presence of an extra adjustable parameter. The optimal circuit values, however, particularly the capacitance of the surface and tubular membranes, are different in the two models. The variation of the capacitance of the surface membrane with conductivity of the external solution is more easy to explain if the values used are from the hybrid model. The value of the capacitance of the surface membrane from the hybrid models is easier to reconcile with the capacitance of the surface membrane of glycerol-treated fibers. The variation of surface capacitance with sarcomere length suggests that the value given by the disk model will be unreasonably small at longer sarcomere lengths, although we have not yet performed such experiments. On the other hand, the disk model gives the same figure for the specific capacitance of the tubular and surface membrane in a variety of solutions. Since we regard the latter finding as coincidental, in view of the uncertainty in the morphometric data, we regard the hybrid model as a somewhat better description of the electrical properties of muscle fibers. (Preliminary stereological measurements by B. Eisenberg support this view.)

There are obvious imperfections in the way either the disk or hybrid model describe the tubular system (Valdiosera et al., 1973 *b*) and so our results should not be taken as evidence for the existence of a morphologically identifiable access resistance at the mouth of the tubules, particularly one which would account for a large fraction of the resistance to radial current flow in the tubular system (Peachey and Adrian, 1973; Peachey, 1973). Rather, we feel that a circuit model which includes lumped resistance is a somewhat better description of the electrical properties of the fiber, probably because the lumpiness helps remedy some of the theoretical imperfections in the way the disk model describes the tubular system.

APPENDIX

The values of the circuit parameters presented in this paper are determined by fitting theoretical circuit models to composite phase data, each phase datum being the mean of the phase recorded from all fibers examined at that frequency. This procedure, which is less desirable than the alternative procedure of fitting the theoretical models to the data from each fiber individually, was adopted for reasons of economy.

TABLE X
COMPARISON OF FIBER BY FIBER ANALYSIS WITH THE ANALYSIS
OF A COMPOSITE FIBER

	τ_c	c_{cc}	r_{ars}	r_{er}	P	R_a^*	R_L^*	C_{10}^*	C_m^*
	$\times 10^{-2}$	$\times 10^{-1}$	$\times 10^{-1}$	$\times 10^{-2}$	$\times 10^{-2}$	$\Omega\text{-cm}^2$	$\Omega\text{-cm}^2$	$\mu F/\text{cm}^2$	$\mu F/\text{cm}^2$
Lumped model									
Composite results	1.91 (0.05)	6.02 (0.13)	—	4.43 (0.28)	-1.54 (0.91)	115	—	4.44	2.94
Mean results	1.91 (0.09)	6.15 (0.16)	—	4.04 (0.33)	+2.76 (1.34)	104 (11)	—	4.77 (0.44)	2.95 (0.26)
Disk model									
Composite results	1.91 (0.03)	8.28 (0.06)	—	3.55 (0.17)	12.6 (0.6)	—	92	6.11	1.26
Mean results	1.92 (0.09)	8.38 (0.20)	—	3.47 (0.35)	-14.8 (0.8)	—	89 (10)	6.53 (0.60)	1.23 (0.17)
Hybrid model									
Composite results	1.93 (0.03)	7.45 (0.06)	1.80 (0.33)	3.99 (0.19)	-8.71 (0.58)	19	85	5.56	1.90
Mean results	1.94 (0.09)	7.45 (0.23)	2.07 (0.26)	3.86 (0.36)	-9.73 (0.61)	22 (5)	78 (8)	5.90 (0.59)	1.93 (0.19)

The data is from 11 muscle fibers bathed in normal Ringer at a sarcomere length of 2.0 μm .

The "Composite results" are the average of the phase characteristics of the fibers, the average phase characteristic then being fit with the various circuit models.

The "Mean results" is the average result of fitting each fiber individually with the circuit models.

Numbers enclosed by parentheses are the standard error of the mean.

It seemed possible that the method of fitting the circuit models to composite data might introduce bias into our conclusions although on statistical grounds, and in view of the small standard error of the mean phase, this seemed unlikely (Papoulis, 1965: p. 212; Kendall and Stuart, 1969, pp. 231-235). In order to evaluate directly the possibility of bias, the data from 11 muscle fibers (bathed in normal Ringer: sarcomere length 2.0 μm) were analyzed by both methods, that is by fitting the circuit models both to composite results and to the data from each fiber. Table X shows the results of the analysis and it can be seen that the estimates of the circuit parameters do not differ significantly. There occasionally appears to be a significant difference between the estimates of the standard deviations of the circuit parameters, but these estimates have little physiological significance. We thank Professor A. F. Huxley for impressing upon us the importance of this check.

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Note added in Proof Mobley, Leung, and Eisenberg (*J. Gen. Physiol.*, 1974, in press) have recently shown that the longitudinal impedance of skinned muscle fibers is purely resistive.

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