

# Some Relations between Changes in the Linear Electrical Properties of Striated Muscle Fibers and Changes in Ultrastructure

W. H. FREYGANG, JR., S. I. RAPOPORT, and L. D. PEACHEY

From the Laboratory of Neurophysiology, National Institute of Mental Health, Bethesda, Maryland 20014, and the Departments of Biophysics and Biochemistry, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, 19104

**ABSTRACT** Some of the linear electrical properties of frog sartorius muscle have been investigated in Ringer's fluid and in a Ringer fluid made hypertonic by the addition of sucrose or NaCl. Electrical constants were determined from measurements of the phase angle of the admittance of a fiber for an applied alternating current, from measurements of the voltage induced by an inward pulse of current, and from measurements of the conduction velocity of the action potential and the time constant of its foot. The dilation of the transverse tubular system induced by the sucrose hypertonic Ringer fluid was correlated with the change in the electrical constants. From this it is concluded that a two time constant equivalent circuit for the membrane, as proposed by Falk and Fatt, is in good agreement with our results. Both the area of the membrane of the transverse tubular system, and the capacity ( $c_e$ ) attributed to it, increased in the sucrose hypertonic Ringer fluid. The resistance  $r_e$ , which is in series with  $c_e$ , did not fall when the transverse tubular system was dilated and probably is not located in that system.

One can often write an equation that describes tolerably well one's measurements of the electrical impedance or admittance of a cell. The equation, however, may describe more than one circuit. The simplest forms of all the circuits that can be described by the equation are called the canonical forms of the circuit. If a circuit which describes the biological system is composed of a greater number of circuit elements than appear in each canonical form, these extra elements cannot be determined by impedance measurements. A canonical form can contain no more than a limited number of circuit elements if each element is to be uniquely evaluated. Before one can assign the param-

eters in the equation to the electrical behavior of the various structures within the cell, one must guess which one of the canonical forms has the best chance of corresponding approximately to the cellular structure. Falk and Fatt (1964) have proposed the two time constant resistance-capacity circuit which is drawn in Fig. 1 in order to account for their measurements of the inside-to-outside admittance of frog fast muscle fibers. They suggested that a part ( $c_e$ ) of the capacity of unit length of muscle fiber was located across the membranous walls of the transverse tubular system (T system) and also that a resistance,  $r_e$ , was in series with  $c_e$ . The location of  $r_e$  was not defined exactly

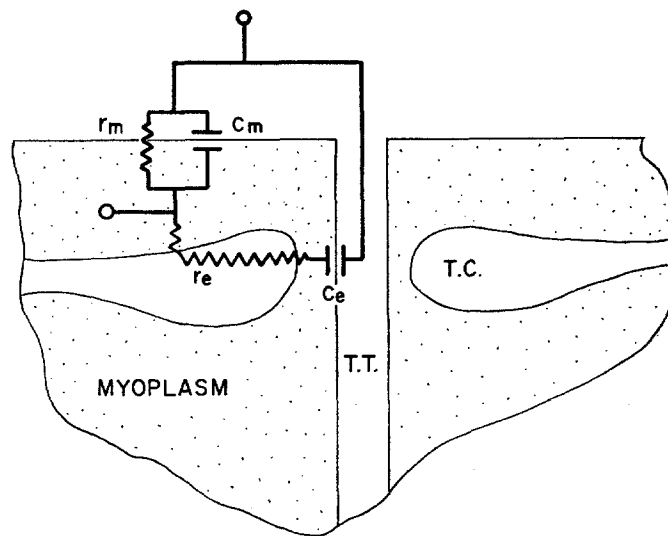


FIGURE 1. The equivalent circuit for muscle membrane proposed by Falk and Fatt (1964) superimposed on a schematic diagram of the ultrastructure of a muscle fiber in order to suggest a possible relation between them. The location of  $r_e$  is tentative. The resistances and capacities are those of a unit length of fiber. *T. T.* refers to the transverse tubular system and *T. C.* refers to the terminal cisternae of the sarcoplasmic reticulum.

but it seemed reasonable that  $r_e$  might be located between the T system and the terminal cisternae of the longitudinal reticulum. The surface membrane of the fiber was represented by a capacity and a resistance,  $c_m$  and  $r_m$ , in parallel.

In the experiments that are described in this paper, we dilated the T system of frog sartorius muscles by immersing them in Ringer's fluid that had been made hypertonic by the addition of sucrose, as reported by Huxley, Page, and Wilkie (1963) and Freygang, Goldstein, Hellam, and Peachey (1964 *b*). One would expect that the electrical parameters that are associated with the T system would be altered by this treatment while the other ones should be unchanged. In this way we were able to test the proposals of Falk and Fatt.

## METHODS

The experiments were performed on the sartorius muscle of *Rana pipiens* at room temperature, which was 22°C. The solutions in which the muscles were immersed were either Ringer's fluid, Ringer's fluid made hypertonic by the addition of sucrose, or Ringer's fluid made hypertonic by the addition of NaCl. The composition and relative tonicity, as measured by the depression of the freezing point of each of these solutions, are listed in Table I. At least a half-hour of immersion in each solution was allowed before starting an experiment. The length of the fiber was maintained constant throughout the experiments; it was between 4/3 and 3/2 slack length. The experiments were performed in the middle of the fibers.

The electron microscopy was performed in the way described by Freygang et al. (1964 *b*), except that an Associated Electrical Industries (White Plains, N.Y.) EM-6B electron microscope was used.

Micropipettes filled with 3 M KCl and having a flexible junction 1 to 2 mm from their tips were used even though the muscles did not twitch because it was our impression that the resting potentials fell less rapidly after impalement with these

TABLE I  
COMPOSITION OF SOLUTIONS

Solutions	K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>+2</sup>	Cl <sup>-</sup>	HPO <sub>4</sub> <sup>-2</sup>	H <sub>2</sub> PO <sub>4</sub> <sup>-1</sup>	Sucrose	Relative tonicity
Ringer's fluid, mM	2.5	120	1.8	121	2.15	0.85	—	(1)
Sucrose hypertonic, mM	2.5	120	1.8	121	2.15	0.85	232	2.0
NaCl hypertonic, mM	2.5	235	1.8	236	2.15	0.85	—	1.9

pipettes than with the rigid sort, perhaps because the tips were free to move with the fiber in response to vibration. Technical details not mentioned here can be found in earlier papers (Freygang et al., 1964 *a, b*).

*Measurement of the Phase Angle of the Admittance of the Fiber,  $\theta$*

We chose not to measure the current and voltage when a sinusoidal current was passed between the inside and the outside of a muscle fiber. Instead, only the phase angle between them was measured. This choice did not diminish the number of quantities that could be calculated from the data. The method for the measurements is illustrated in Fig. 2. We are indebted to Mr. Anthony Bak for his suggestions and for designing the circuit drawn in Fig. 3. The crux of the method is avoidance of the complications which arise from the capacity across the wall of the micropipette with which membrane potential is usually measured (the right one) by holding the voltage inside this pipette at the resting potential of the fiber. The amplifier  $A_2$  applies the negative feedback to do this. If there were no capacitative coupling between the insides of the two pipettes, the magnitude of the output of  $A_2$  would be proportional to the change in membrane potential. There is, however, coupling be-

tween the pipettes, and therefore the amplifier  $A_3$  with the circuit elements around it was employed to produce the same signal as the one that arose from the capacitive coupling between the pipettes. The cross-talk between the pipettes was hidden by subtracting the output of  $A_3$  from the output of  $A_2$ . An analysis of the circuit can be found in Appendix 1.

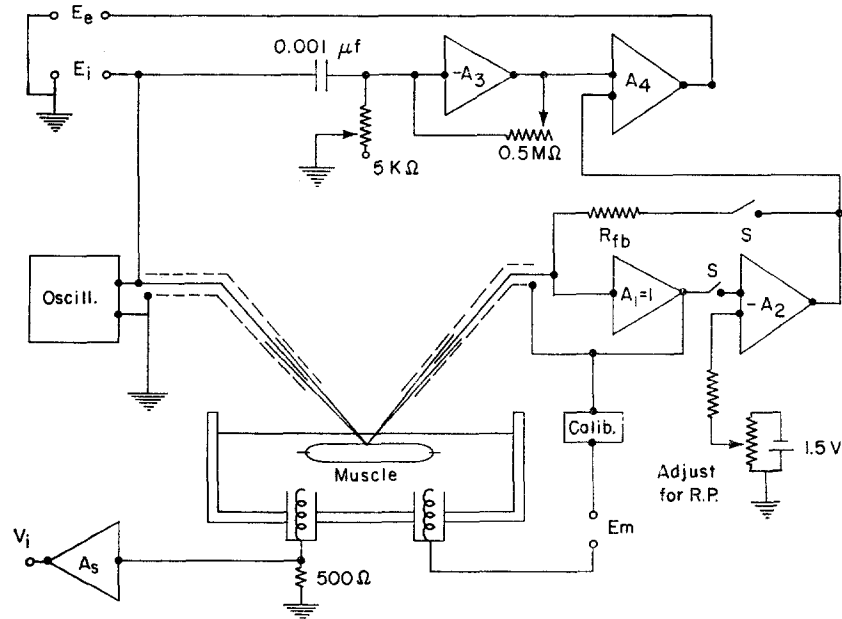


FIGURE 2. Diagram of the apparatus. The equipment between the input to  $A_1$  and the output of  $A_2$  is shown in Fig. 3. The oscillator was a Hewlett-Packard Co. (Palo Alto, Calif.) Model 202B low frequency oscillator which was capacitatively coupled to the circuitry.  $A_3$  was a Fairchild Camera and Instrument Co. (Syosset, N. Y.) Model 702A operational amplifier which was operated in the same way as the one shown in Fig. 3. The adjustment of the 5 K  $\Omega$  potentiometer changed the phase of the output of  $A_3$  and the adjustment of the 0.5 meg potentiometer controlled the amplitude of the output. These adjustments interact and this design is not recommended; a differentiating circuit with amplitude control would have been easier to adjust. Also, more extensive shielding between the micropipettes would have been helpful.  $A_4$  was a Tektronix Type 2A63 differential amplifier.  $A_5$  was an American Electronic Laboratories (Colmar, Pa.) Model 251 dc amplifier.

The experimental procedure was as follows: (1) Current was passed through the left pipette while the IR-drop across the 500 ohm resistor to ground was observed. Usually this showed rectification in the pipette. It was necessary to use pipettes with tip resistances of about 5 meg in order to find some that did not rectify. Pipettes with tip resistances of 10–20 meg and tip potentials less than 3 mv were chosen for the right one. (2) With both switches open, the tip of the right pipette was inserted in a fiber. The output of the unity gain cathode follower,  $A_1$ , showed the resting po-

tential. This voltage was backed off by the calibrated voltage. Approximately the same voltage, but with respect to ground, was set on the input to  $A_2$ . (3) Both switches were closed. The left pipette was moved into position for impalement of the fiber at a point within  $25 \mu$  of the site of puncture by the right one and a few microns above the membrane. (4) A large 10 kc signal was applied to the left pipette. The cross-talk was balanced by the adjustments around  $A_3$  so that the 10 kc signal was well below

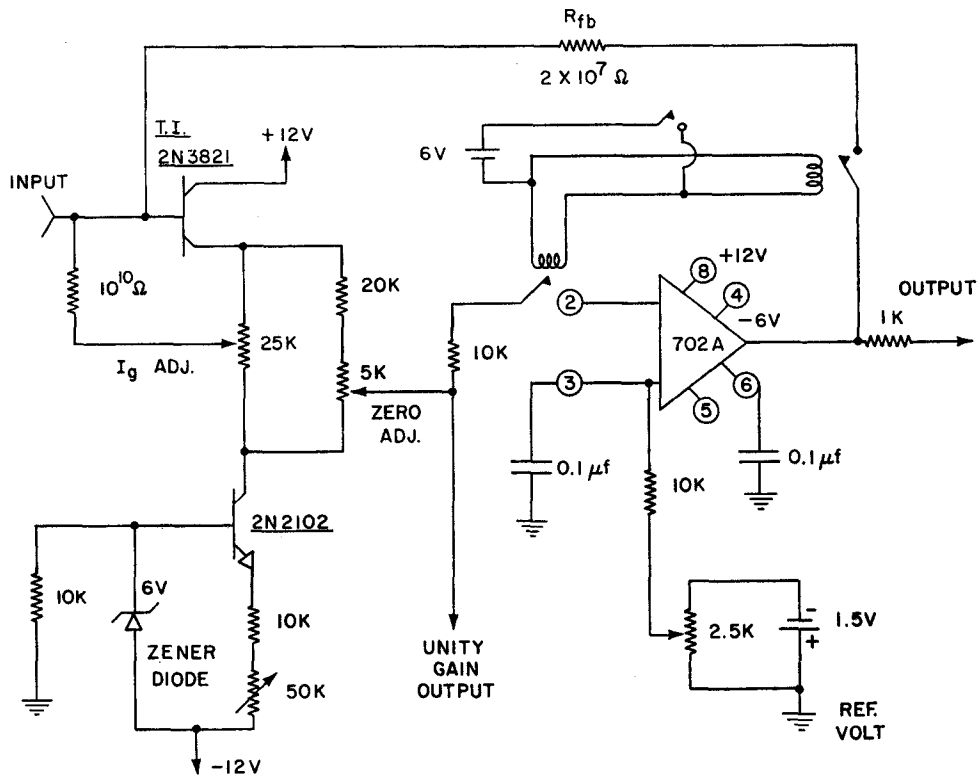


FIGURE 3. Schematic diagram of the unity gain cathode follower and a negative feedback device for holding the inside of a micropipette at the resting potential. The 702A is manufactured by Fairchild Camera and Instrument Co. (Syosset, N. Y.), the 2N3821 by Texas Instruments (Dallas, Tex.), and the 2N2102 by RCA.

the noise level in the output of  $A_4$ , which subtracted the signal of  $A_3$  from that of  $A_2$ . The subtraction was effective for lower frequencies too. (5) The signal from the oscillator was reduced before the left pipette was inserted in the fiber. Impalement was indicated by the sudden appearance of the sinusoidal signal in  $E_o$ . (6) Both switches were opened and the resting potential was measured again.  $E_o$  divided by the gain of  $A_4$  was usually close to  $E_m$  because  $R_{fb}$  was approximately the tip resistance of the right pipette. The sinusoidal component of  $E_m$  was less than 5 mv peak to peak. (7) After the switches were closed again, the phase angle between  $E_o$  and  $E_i$  was read for frequencies between 2 and 2,000 cps. These angles were read immediately by feeding

these signals into an Ad-Yu Electronics Type 405L Phase Meter (Passaic, N.J.) and were observed on an oscilloscope in some experiments as a Lissajous figure. (8) The switches were opened to measure the resting potential before the pipettes were withdrawn from the fiber.

#### *Determination of $b/g$ from $\theta$*

Thus far we have described how we measured the phase angle,  $\theta$ , of the admittance of a muscle fiber in which the electrical elements are distributed along its length. It is

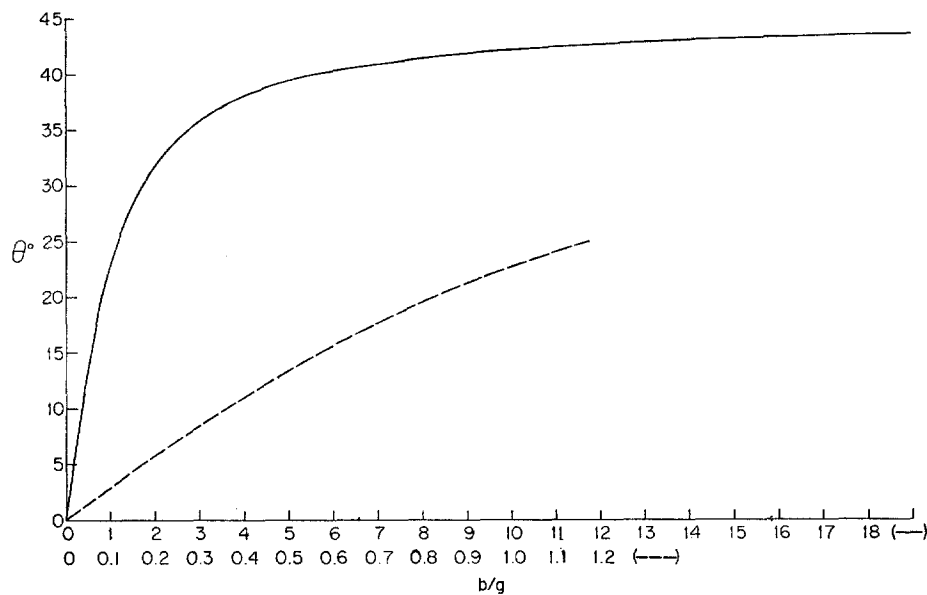


FIGURE 4.  $b/g$  as a function of  $\theta$ , calculated from equation 1. These curves were used to find the quantity  $b/g$  from the phase angle of the fiber admittance,  $\theta$ .  $b/g$  is plotted on two scales. The fiber was assumed to be a cable.

a convenience to deal with circuits in which the elements are lumped rather than distributed because the calculations are simpler. If  $y = g + jb$  represents the admittance of a unit length of muscle membrane, which is considered to have lumped elements such as in the circuit in Fig. 1, one can show from the cable equations (see Appendix 3) that

$$\tan \theta = \frac{\{[(b/g)^2 + 1]^{1/2} - 1\}^{1/2}}{\{[(b/g)^2 + 1]^{1/2} + 1\}^{1/2}} \quad (1)$$

Fig. 4 was calculated from equation 1 and was employed to obtain the quantity  $b/g$  from the measurements of  $\theta$ .  $\tan \theta$  corresponds to  $n/m$  in the notation of Tasaki and Hagiwara (1957) and Falk and Fatt (1964).

*Measurement of the Length Constant,  $\lambda$ , the Characteristic Resistance,  $R_o$ , and 85% Time of a Fiber*

These measurements were made in a conventional manner. Three pipettes were inserted in the same fiber. A square pulse of inward current of 100 msec duration was passed through the first pipette. A second pipette, at a distance  $x_1$  less than 0.3 mm from the first, recorded the change in membrane potential  $V_1$ . A third pipette a few millimeters,  $x_2$ , in the same direction away from the first recorded the change in membrane potential  $V_2$ . The length constant,  $\lambda$ , was obtained from the equation

$$\lambda = \frac{x_2 - x_1}{\ln(V_1/V_2)} \quad (2)$$

The current that flowed in the first pipette,  $I_o$ , was measured as the voltage across a 500 ohm resistor to ground.  $2R_o$ , the input resistance of a half length of fiber, was calculated from the equation

$$2R_o = \frac{2V_1 \exp(x_1/\lambda)}{I_o} \quad (3)$$

The changes in membrane potential were always less than 5 mv.

Measurements were made of the time required for  $V_1$  to complete 85% of its return to the resting potential after the current was switched off. When  $x_1/\lambda$  was 0.2, this time was slightly (3%) larger than the time for an 85% change when  $x_1/\lambda$  was zero. Since  $x_1/\lambda$  was always less than 0.2, a correction was not necessary. The 85% time was chosen because it corresponds to the product of the membrane resistance and capacity if the membrane is described simply as a resistance and a capacity in parallel and therefore has only one time constant (Hodgkin and Rushton, 1946).

*Measurement of the Time Constant of the Foot of the Action Potential and of Conduction Velocity,  $v$* 

Two pipettes, 2-3 mm apart, were inserted in the same fiber. An external electrode remote from the pipettes stimulated the fiber. The time constant was measured from semilogarithmic plots of the first 10 mv of the foot of the action potential vs. time. It was the time required for a 63% change in voltage on the semilogarithmic plot. Velocity was calculated as the speed of the peak of the action potential.

## RESULTS

*The Phase Angle of the Admittance of the Fiber,  $\theta$ , As a Function of Frequency*

Measurements of the angle,  $\theta$ , with the corresponding values of  $b/g$ , from a fiber in Ringer's fluid are illustrated in Fig. 5. Similar data from a fiber in the sucrose hypertonic solution are illustrated in Fig. 6.

For the circuit in Fig. 1, the relation between  $b/g$  and the elements in the circuit is given by the equation

$$b/g = \omega r_e c_e \frac{\frac{c_m}{c_e} + \frac{1}{\omega^2 c_e^2 r_e^2 + 1}}{\frac{r_e}{r_m} + \frac{\omega^2 c_e^2 r_e^2}{\omega^2 c_e^2 r_e^2 + 1}} \quad (4)$$

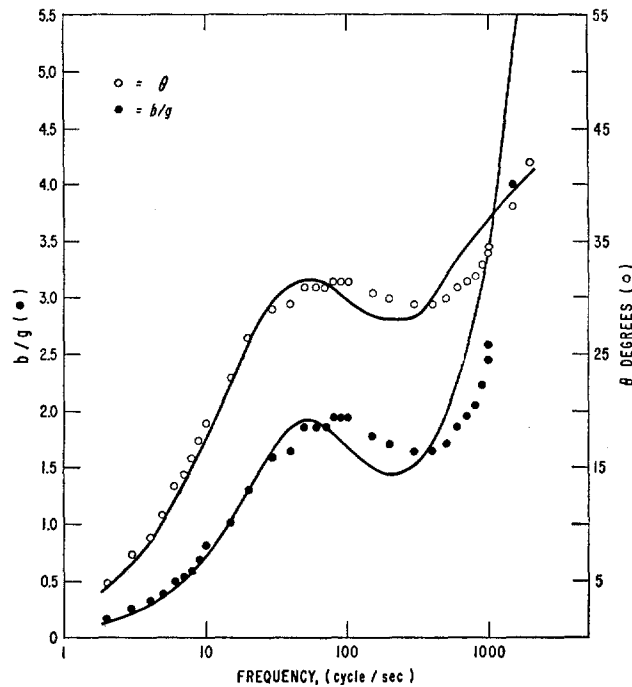


FIGURE 5. Measurements of the phase angle of the admittance of a fiber,  $\theta$ , as a function of frequency on a logarithmic scale. The fiber was in Ringer's fluid. Also drawn are values of  $b/g$ .  $b/g$  was obtained from  $\theta$  by use of the curves in Fig. 4. The lines through the curves are the best fit to equation 4 by the method of least squares. Fiber O, Table II.

in which  $\omega = 2\pi f$ , with  $f$  being the frequency in cycles/sec. Using a digital computer, the values of  $r_e/r_m$ ,  $c_e/c_m$ , and  $r_e c_e$  were obtained for each fiber from equation 4 by means of the method of least squares. These are listed in Table II.

It is assumed in these calculations that the resistances of the micropipettes were considerably larger than the characteristic impedance of the muscle fibers. The importance of this assumption is indicated in Appendix 1. In these experiments, current-carrying micropipettes having low tip resistances were chosen in order to avoid rectification. It is likely that some error was



introduced into our measurements of  $\theta$  in this way. It is estimated that  $\theta$  could be as much as  $3^\circ$  too high at about 15 cps and about  $1^\circ$  too high at 2 and 2000 cps.

The resting potentials of the fibers were dangerously close to the threshold of the action potential. This was caused by the relatively large size of the current-carrying pipette and the proximity of the two impalements. The low resting potentials introduce some uncertainty about  $r_m$  because the depolari-

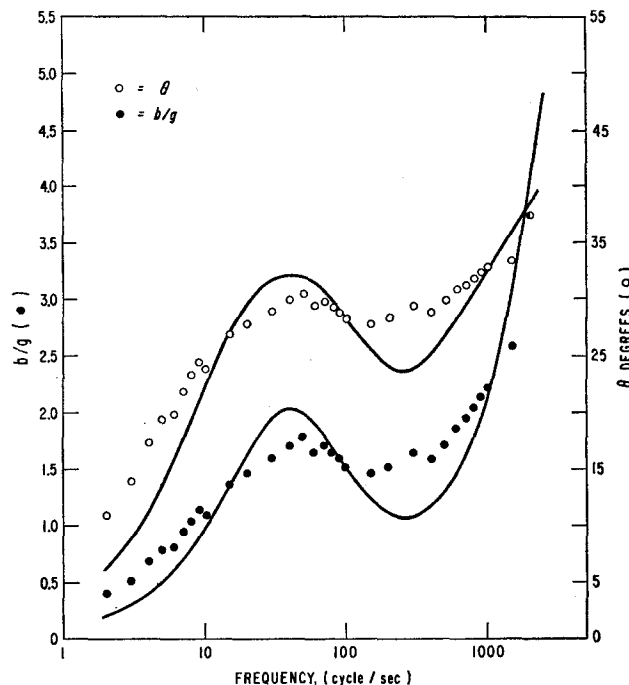


FIGURE 6. Measurements of the phase angle of the admittance of a fiber,  $\theta$ , as a function of frequency on a logarithmic scale. Fiber was in the hypertonic sucrose solution. Also drawn are values of  $b/g$ .  $b/g$  was obtained from  $\theta$  by use of the curves in Fig. 4. The lines through the curves are the best fit to equation 4 by the method of least squares. Fiber *P*, Table II.

zation should have lowered it, at least initially, but subsequently it may have increased (Nakajima, Iwasaki, and Obata, 1962). We estimated the effect on the values in Table II of a shunt across the membrane at the site of the impalements. A shunt as low in resistance as 10,000 ohms did not have an effect of serious magnitude. As pointed out by Falk and Fatt (1964), it is likely that the shunt resistance is very much greater than 10,000 ohms even when the resting potential is reduced by 20 mv as a result of the impalements.

The results obtained from fiber *K* are different from those from the other fibers. Possibly it was another physiological type of fiber. The mean values for

the fibers in Ringer's fluid listed in Table II are very close to the means of the values obtained by Falk and Fatt (1964). The ratio  $c_e/c_m$  was increased 66% by the immersion of the muscles in the sucrose hypertonic solution. The time constant  $\tau_{e_0}$  was less significantly increased, although the increase was 73%. The ratio  $\tau_e/\tau_m$  did not show a significant change. By themselves, these results suggest that an increase in  $c_e$  might be the major effect of the sucrose hypertonic solution because there is little reason to expect that  $c_m$  would decrease in this solution. Also, Fatt's (1964) transverse impedance measurements

TABLE II  
RESULTS FROM PHASE ANGLE MEASUREMENTS

Solution	Fibers	Resting potential (in-out)	$\tau_e/\tau_m$	$c_e/c_m$	$\tau_{e_0}$
		<i>mv</i>			<i>msec</i>
Ringer's fluid	K	80-63	0.042	1.28	0.427
	L	71-60	0.133	2.04	1.29
	M	72-62	0.148	1.79	1.17
	N	75-64	0.134	1.93	1.30
	O	75-64	0.147	1.96	1.17
Mean $\pm$ SE			0.121 $\pm$ 0.020	1.8 $\pm$ 0.136	1.07 $\pm$ 0.164
Sucrose hypertonic	P	77-71	0.094	3.49	1.21
	Q	79-60	0.198	2.84	1.36
	R	76-62	0.217	2.22	2.29
	S	69-66	0.194	3.00	2.15
	T	76-66	0.183	3.41	2.22
Mean $\pm$ SE			0.177 $\pm$ 0.022	2.99 $\pm$ 0.228	1.85 $\pm$ 0.23
Level of significance of change			<0.1	<0.01	<0.05

indicate that  $c_m$  is relatively unchanged when the muscle is immersed in the sucrose hypertonic solution.

*Membrane Resistance, Core Resistance, and 85% Time*

The core resistance of a unit length of fiber,  $r_i$ , was obtained from the equation

$$r_i = 2R_o/\lambda \quad (5)$$

Membrane resistance of a unit length of fiber,  $r_m$ , was obtained from the equation

$$r_m = \lambda^2 r_i \quad (6)$$

The 85% time was measured in the manner that has been described already. Means of the values that were found for each of the parameters are listed in Table III.

The mean value of  $r_m$  showed an 18% decline when the muscles were immersed in the sucrose hypertonic solution and a 26% decline when they were immersed in the NaCl hypertonic solution. Both these changes were reversed when the muscles were reimmersed in Ringer's fluid. Since it is likely that the fibers still had a higher internal chloride concentration than they would have had under conditions of Donnan equilibrium, the lowered membrane resistance probably was related to an increase in chloride conductance of the surface membrane.

The core resistance,  $r_c$ , increased in both the sucrose hypertonic and NaCl hypertonic solutions. Since the loss of potassium caused by immersion in the

TABLE III  
MEMBRANE RESISTANCE, CORE RESISTANCE  
AND 85% TIME DERIVED FROM EXPERIMENTS WITH  
SQUARE PULSES OF CURRENT

Solutions	No. of fibers	$r_m$ <i>megohm.cm</i>	$r_c$ <i>megohm/cm</i>	85% time <i>msec</i>
Ringer's fluid	44	0.293±0.013	6.41±0.31	25.98±0.93
Sucrose hypertonic	34	0.240±0.010	8.97±0.45	28.36±0.99
Return from sucrose	38	0.296±0.030	5.12±0.23	26.86±1.15
NaCl hypertonic	9	0.218±0.190	8.90±0.39	20.65±1.37
Return from NaCl	14	0.281±0.015	6.11±0.35	22.63±0.90

sucrose hypertonic solution is small (Freygang et al., 1964 *b*), one can calculate that the equivalent conductance of potassium, i.e. the conductivity of the myoplasm per unit potassium concentration of the myoplasm, fell about 30%. This fall might be explained by changes in potassium binding, by an increase of the viscosity of the myoplasm with dehydration, or by closing off of a small intracellular channel for current flow (perhaps within or adjacent to the Z line) due to fiber shrinkage.

The change in the 85% time between fibers immersed in Ringer's fluid and those in the sucrose hypertonic solution was not striking. Falk and Fatt (1964) give the following approximate relation between the 85% time (or  $1/H_2$  in their notation) and the electrical constants in Fig. 1.

$$85\% \text{ time} \doteq r_m(c_e + c_m) + r_c c_e \tag{7}$$

The lower values of  $r_m$  of the fibers in the sucrose hypertonic solution account for the failure of the 85% time to increase well above its value in fibers immersed in Ringer's fluid. Since  $c_e$  is only 1-2 msec (Table II and Falk and

Fatt, 1964), by neglecting it one can see that the term  $(c_e + c_m)$  was increased by approximately 33% in the fibers that were immersed in the sucrose hypertonic solution. Again, if we consider it unlikely that this solution could change  $c_m$ , we would conclude that  $c_e$  was raised by the exposure of the muscle to this solution.

In contrast to the 33% rise in  $c_e + c_m$  in fibers in the hypertonic sucrose solution, a similar approximate calculation for fibers in the NaCl hypertonic solution gives only a 7% rise. As will be described, the change in surface area of the T system in the NaCl hypertonic solution was much less, as estimated by electron microscopy.

#### *The Time Constant of the Foot of the Action Potential*

The minor deviations of the foot of the action potential from a straight line drawn through it on the semilogarithmic plot appeared to be random. Therefore the foot was assumed to have a single time constant. Conduction velocity and the time constant of the foot of the action potential are listed in Table IV. Both of these quantities were significantly altered by immersion of the muscle in the sucrose hypertonic solution. Velocity was reduced by 32% and the time constant was raised by 73%. These changes were reversed when the muscles were reimmersed in Ringer's fluid.

#### *Measurements from Electron Micrographs*

Electron microscopic measurements have shown that in frog sartorius fibers fixed after immersion in Ringer's fluid, the T system has a surface area of  $7.2 \times 10^2 \pi d^2$  cm<sup>2</sup> per cm length, where  $d$  is the fiber diameter (Peachey, 1965). 80% of this area is oriented in the transverse plane, and 20% is oriented longitudinally. Additional measurements were made on electron micrographs of sartorius muscles fixed after immersion in Ringer's fluid or in sucrose hypertonic Ringer's fluid and sectioned in the transverse plane. These show that the fraction of the transverse area of the fiber at the Z line occupied by the T system is increased from 0.3 in Ringer's fluid to 0.4 in sucrose hypertonic Ringer's fluid. At the same time, fiber diameter is reduced to 0.8 times its value in Ringer's fluid and therefore fiber transverse area is reduced to 0.64 times its size in Ringer's fluid. Thus the area of the T system in the transverse plane is altered by a factor of  $\left(\frac{0.4}{0.3}\right) (0.64) = 0.85$ . The length of the fibers remained unchanged and thus the number of sarcomeres and T systems per centimeter length of fiber remained unchanged in these experiments.

The longitudinal dimension of the T system increases by a factor of 3.8 in sucrose hypertonic Ringer's fluid (Freygang et al., 1964 *b*). Assuming fibril perimeter to decrease by the same factor as fiber diameter, i.e. 0.8, in sucrose

hypertonic Ringer's fluid, we estimate that the longitudinally oriented area of the T system changes by a factor of  $(3.8) (0.8) = 3.0$  when the fiber is placed in sucrose hypertonic Ringer's fluid. Multiplying each of these factors by the corresponding fraction of total area of the T system in Ringer's fluid one obtains  $(0.8) \times (0.85) + (0.2) \times (3.0) = 1.3$ , or a 30% increase in area of the T system in fibers in the sucrose hypertonic Ringer's fluid.

TABLE IV  
TIME CONSTANT OF FOOT OF ACTION  
POTENTIAL AND CONDUCTION VELOCITY

Solution	Fiber No.	Resting potential	Action potential	$v$	Time constant
		(in-out)	amplitude		of foot
		<i>mv</i>	<i>mv</i>	<i>cm/sec</i>	<i>sec</i> $\times 10^{-3}$
Ringer's fluid	1	73-73	105	226	0.205
	2	82-80	107	280	0.174
	3	86-80	104	272	0.125
	4	91-73	114	294	0.122
	5	80-75	108	275	0.142
Mean $\pm$ SE				269 $\pm$ 12	0.154 $\pm$ 0.016
Sucrose hypertonic	6	88-88	108	181	0.277
	7	90-80	108	175	0.346
	8	91-86	110	197	0.274
	9	88-86	108	177	0.259
	10	91-88	108	167	0.309
	11	87-81	107	188	0.244
	12	86-86	100	186	0.208
	13	82-80	105	189	0.213
Mean $\pm$ SE				183 $\pm$ 3	0.266 $\pm$ 0.016
Return to Ringer's fluid	14	87-83	108	256	0.180
	15	90-88	110	224	0.175
	16	93-93	112	244	0.139
	17	97-97	112	280	0.150
	18	92-92	114	243	0.133
Mean $\pm$ SE				249 $\pm$ 9	0.155 $\pm$ 0.009

Muscles that had been immersed in the NaCl hypertonic Ringer's fluid failed to show an increase in the longitudinal dimension of the T system (Fig. 7). 58 measurements on 3 muscles treated in this way gave a mean longitudinal dimension of the T system of  $200 \pm 50$  A (standard deviation), only slightly less than the value reported earlier (Freygang et al., 1964 *b*) for muscles that had been immersed in Ringer's fluid. It seems reasonable to conclude that the area of the T system was not enlarged in the NaCl hypertonic

solution. This finding taken with the small (7%) rise in  $c_e + c_m$  derived from the 85% time, serves as a check on the effect of hypertonicity by itself on membrane capacity. It appears that there was none.

When we recognize the inherent danger in measurements on electron micrographs of fixed and sectioned material, we must take these measurements cautiously.

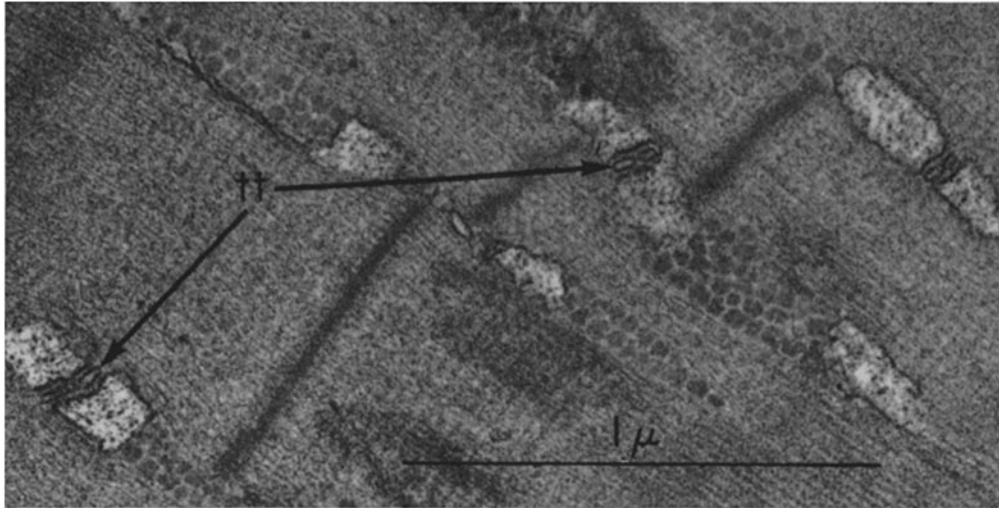


FIGURE 7. Longitudinal section of the *I* band region from a fiber that had been soaked in the NaCl hypertonic solution for 40 min before fixation. No swelling of the T system is evident. t.t. is cross-section of a transverse tubule.

## DISCUSSION

### *Effect of the Sucrose Hypertonic Solution on $c_e$ , $r_e$ , and $c_m$*

We shall mark the parameters which apply to muscle fibers that were immersed in the sucrose hypertonic solution with an asterisk and those that relate to fibers in Ringer's fluid will not have an asterisk. From the results listed in Tables II and III, the ratios  $c_e^*/c_e$ ,  $r_e^*/r_e$ , and  $c_m^*/c_m$  were calculated in the following way:

$$\frac{c_e^*}{c_e} = \frac{r_m}{r_m^*} \frac{r_e^* c_e^*}{r_e c_e} \frac{r_m^* r_e}{r_e^* r_m} = 1.4$$

$$\frac{r_e^*}{r_e} = \frac{r_e^* c_e^*}{r_e c_e} \frac{c_e}{c_e^*} = 1.2$$

$$\frac{c_m^*}{c_m} = \frac{c_m^* c_e}{c_e^* c_m} \frac{c_e^*}{c_e} = 0.87$$

Only the change in  $c_e$  is significant within 90% fiducial limits. The 40% increase in  $c_e$  correlates well with the 30% increase in the area of the T system which was estimated from measurements of electron micrographs. This is in accord with the proposal of Falk and Fatt (1964) that  $c_e$  represents the capacity of the walls of the T system. Further support for this proposal can be found in the 33% rise in  $c_e + c_m$  which was derived from the 85% time since  $c_m$  probably was not changed by the sucrose hypertonic Ringer fluid.

Some but not all of the increase in the longitudinally oriented area of the T system in sucrose hypertonic Ringer's fluid could come from reorientation of membrane previously oriented transversely, the area of which decreases under these conditions. However, the 30% increase in total area of the T system implies either that the membranes become stretched, or that new membrane material is recruited from some other source.

If  $r_e$  were located within the T system, as a core resistance along the axis of the tubules or as the resistance across the walls of the tubules for instance, one would expect it to be reduced by dilation of the T system. The results of these experiments indicate that it is either unchanged or perhaps it is increased in the fibers which were immersed in the sucrose hypertonic solution. It appears, therefore, that  $r_e$  is not located in the T system. The increase in  $r_e$  although not very significant, might be attributed to the effect of dehydration on some part of the longitudinal reticulum, which might also have produced the observed rise in the core resistance,  $r_i$  (Table III). Pugsley (1966), however, suggests that  $r_e$  may be located at the mouth of the T system because he found both  $r_e$  and the late afterpotential to be either absent or greatly reduced in toad sartorius muscle fibers. On the other hand, we do find<sup>1</sup> a late afterpotential in fibers from *Bufo marinus* of roughly the same magnitude and time course as that in sartorius fibers from *Rana pipiens*. Furthermore, preliminary electron microscopic observations<sup>2</sup> on *Bufo marinus* sartorius muscles soaked in ferritin solutions before fixation show a T system not obviously different from that of the frog.

The 13% decrease in  $c_m$  of the fibers immersed in the sucrose hypertonic solution is small and not significant.

#### *The Time Constant of the Foot of the Action Potential*

Another way to test the equivalent circuit of Falk and Fatt (1964) is to calculate the time constant of the foot of the action potential using their two time constant circuit to describe the properties of muscle membrane. The results of this calculation, as well as the results of a calculation in which a mem-

<sup>1</sup> Unpublished observations.

<sup>2</sup> Schild, R. Unpublished observations.

brane with a single time constant is assumed, can be compared with experimental data in order to see which is the closer to experimental reality.

The relation between membrane potential and time during the start of an action potential, while one can still assume linear electrical properties, is given by the equation

$$\frac{d^3V}{dt^3} + A \frac{d^2V}{dt^2} + B \frac{dV}{dt} + CV = 0 \quad (8)$$

in which

$$A = \frac{1}{(c_e r_e)} \left\{ 1 - \frac{v^2 r_m c_m}{\lambda^2 r_e c_e} (c_e r_e)^2 \right\}$$

$$B = \frac{-v^2}{\lambda^2} \left\{ 1 + \frac{r_m}{r_e} \left[ 1 + \frac{c_m}{c_e} \right] \right\}$$

$$C = \frac{-v^2}{\lambda^2 (c_e r_e)}$$

when the circuit of Falk and Fatt (Fig. 1) is assumed. Equation 8 is derived in Appendix 2. The general solution of this equation is

$$V(t) = a_1 e^{m_1 t} + a_2 e^{m_2 t} + a_3 e^{m_3 t} \quad (9)$$

in which  $a_i$  are constants. The roots  $m_1$ ,  $m_2$ , and  $m_3$  of equation 9 are reciprocal time constants of the foot. In order to obtain these roots, the constants  $A$ ,  $B$ , and  $C$  were calculated with the appropriate values in Tables II and III for fibers in each of the solutions. From these, the roots were calculated. Since, during the foot, the action potential goes positive with time, the reciprocal of the positive root is the calculated time constant. The two other roots, which are negative, may be discarded because  $V = 0$  when  $t = -\infty$  and therefore  $a_2$  and  $a_3$  must be zero.

If  $r_e$  is made infinite or zero, the equivalent circuit of Falk and Fatt reduces to a single time constant circuit of the resistance of the surface membrane and a capacity in parallel. Equation 8 then simplifies to a second order equation, the roots of which are

$$\frac{v^2 T_m}{2\lambda^2} \pm \frac{v}{2\lambda} \sqrt{4 + \frac{v^2 T_m^2}{\lambda^2}} \quad (10)$$

in which  $T_m = c_m r_m$  when  $r_e = \infty$  and  $T_m = r_m (c_e + c_m)$  when  $r_e = 0$ . This expression of the time constant of the foot has been published by Cole and Curtis (1938), Rosenblueth, Wiener, Pitts, and Garcia Ramos (1948), Tasaki and Hagiwara (1957), and Taylor (1963).



Table V shows the results of calculating the time constant of the foot of the action potential by three methods. The results can be compared with the experimental results in the fourth column. For the first two columns, the one time constant circuit described by equation 10 was assumed. Mean values from Tables III and IV for  $v$  and  $\lambda$  were used. For the first column, the value for  $T_m$  was the 85% time in Table III which was derived from the effect of a pulse of inward current. The agreement between the results of this calculation and the experimental result is very poor. A somewhat improved agreement was obtained when  $T_m$  was calculated from the mean data in Table II as equal to  $\tau_m c_m = (\tau_m/\tau_e) \times (c_m/c_e) \times \tau_e c_e$ . This is tabulated in the second column. In the third column the mean data in Tables II and IV were em-

TABLE V  
TIME CONSTANT OF FOOT OF  
THE ACTION POTENTIAL

	Calculated			Observed
	One time constant membrane circuit		Two time constant circuit	
	85% time from Table III	$c_m \tau_m$ from Table II*		
	<i>msec</i>	<i>msec</i>	<i>msec</i>	<i>msec</i>
Ringer's fluid	0.024	0.125	0.144	0.154
Sucrose hypertonic	0.028	0.215	0.224	0.266

$$* c_m \tau_m = \frac{\tau_m}{\tau_e} \frac{c_m}{c_e} \tau_e c_e$$

ployed to calculate the foot of the action potential from equations 8 and 9 which assume the two time constant circuit for the membrane. These results give the closest agreement with the experimental results.

The time constant of the foot of the action potential and its conduction velocity in fibers which were returned to Ringer's fluid, after immersion in the sucrose hypertonic solution, were not significantly different from those of fibers that had remained in Ringer's fluid. This suggests that the changes in the electrical properties induced by the sucrose hypertonic solution are reversible.

The significance of the calculations of the foot of an action potential by means of the two time constant circuit is that it provided an independent check on the estimates of the electrical properties which were made from the phase angle measurements and the effects of square pulses of current on membrane potential.

*Comments on the Two Time Constant Circuit*

On the basis of our results, it is likely that the equivalent circuit that describes the linear electrical properties of frog muscle fibers is more complicated than the circuit in Fig. 1. The relatively small scatter of the measurements that are illustrated in Figs. 5 and 6 indicates that they are probably reliable, yet the fit from least squares and equation 4 is poor. Furthermore, the form of the misfit was the same for all fibers tested in one solution, although different for Ringer's fluid and for sucrose hypertonic Ringer's fluid. In Ringer's fluid, the trough in the calculated curve was always deeper and at a lower frequency than that of the data points. In sucrose hypertonic Ringer's fluid, the peak was always too high and the trough was always too low but both were at roughly the right frequencies. The fault probably lies in the simplicity of the equivalent circuit that is described by equation 4, or in the nonlinearity of the circuit elements. It would seem reasonable, for example, for there to be a resistance across  $c_e$  and in series with  $r_e$  ( $r_{ce}$  of Falk and Fatt, 1964). Such a resistance would be an alternate path for direct current to the one through  $r_m$  and could not be determined by purely electrical measurements. There may be other elements in the true equivalent circuit of the muscle fiber which cannot be determined by our methods or by any other purely electrical procedure, since they are part of circuits not in canonical form.

Despite these reservations, a two time constant circuit fits the measurements of phase angle much better than the one time constant circuit and undoubtedly is a better approximation to the muscle membrane.

## APPENDIX 1

The diagram of the apparatus in Fig. 2 is drawn as a circuit in Fig. 8. If the relation between the input to the cathode follower,  $E_o$ , and the output of the operational amplifier,  $E_e$ , is simply  $E_e = -AE_o$  in which  $A$  is the amplification,

$$\frac{E_e}{E_i} = \frac{-A \left( \frac{Z_o}{R_i R_v + Z_o(R_i + R_v)} + j\omega C_c \right)}{\frac{1}{R_v} + \frac{1}{R_{fb}} (A + 1) - \frac{R_i}{R_v} \left( \frac{Z_o}{R_i R_v + Z_o(R_i + R_v)} \right) + j\omega(C_c + C_v)}$$

When  $A$  is very large, an approximation is

$$\frac{E_e}{E_i} \doteq \frac{-R_{fb}}{R_v} \left( \frac{Z_o}{R_i + Z_o \left( \frac{R_i}{R_v} + 1 \right)} + j\omega C_c R_v \right)$$

If  $R_i$  and  $R_v$  are about equal and  $R_i$  is sufficiently greater than  $Z_o$ , a further approximate relation is

$$\frac{E_o}{E_i} \doteq -R_{fb} \left( \frac{Z_o}{R_v R_i} + j\omega C_c \right).$$

After the electrical subtraction of the capacitive coupling between the pipettes,

$$\frac{E_o}{E_i} \doteq \frac{-R_{fb}}{R_v R_i} \cdot Z_o.$$

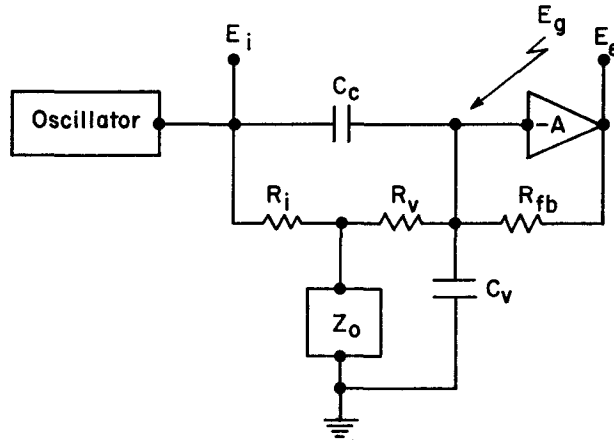


FIGURE 8. The output of the oscillator is  $E_i$ , that of the operational amplifier is  $E_o$ .  $R_i$  and  $R_v$  represent the tip resistances of the left and right micropipettes in Fig. 2, respectively, and  $C_c$  represents the coupling capacity between them.  $C_v$  represents the stray capacity between the inside of the right pipette and ground.  $Z_o$  represents the characteristic impedance of the muscle fiber.

#### APPENDIX 2

For the circuit in Fig. 1, the relation between the membrane potential,  $V(x, t)$ , and the membrane current,  $i(x, t)$ , in a unit length of cable is

$$c_e r_e c_m \frac{d^2 V(x, t)}{dt^2} + \left[ \frac{c_e r_e}{r_m} + c_e + c_m \right] \frac{dV(x, t)}{dt} + \frac{1}{r_m} V(x, t) = c_e r_e \frac{di(x, t)}{dt} + i(x, t).$$

From the cable equations

$$i(x, t) = \frac{1}{r_i} \frac{\partial^2 V(x, t)}{\partial x^2}.$$

For an action potential propagating at constant velocity,  $v$

$$v^2 \frac{\partial^2 V(x, t)}{\partial x^2} = \frac{\partial^2 V(x, t)}{\partial t^2}.$$

Combining the above to eliminate  $x$ , with  $\lambda = \sqrt{r_m/r_i}$ ,

$$\frac{d^3V(t)}{dt^3} + \frac{1}{(c_e r_e)} \left\{ 1 - \frac{v^2}{\lambda^2} \left( \frac{r_m}{r_e} \right) \left( \frac{c_m}{c_e} \right) (c_e r_e)^2 \right\} \frac{d^2V(t)}{dt^2} - \frac{v^2}{\lambda^2} \left\{ 1 + \left( \frac{r_m}{r_e} \right) \left[ 1 + \left( \frac{c_m}{c_e} \right) \right] \right\} \frac{dV(t)}{dt} - \frac{v^2 V(t)}{\lambda^2 (c_e r_e)} = 0,$$

which is equation 8 in the text.

### APPENDIX 3

The derivation of equation 1 is shown here for the convenience of the reader. It appears in less detail in the papers of Tasaki and Hagiwara (1957) and Falk and Fatt (1964).

Let us consider the muscle fiber as a cable with an inside to outside admittance across the membrane of  $y = g + jb$  mho/cm and a core resistance of  $r_i$  ohm/cm.

From the cable equations, the membrane current  $i(x, t)$  amp/cm is related to the membrane potential  $V(x, t)$  by

$$i(x, t) = \frac{1}{r_i} \frac{\partial^2 V(x, t)}{\partial x^2}$$

and by

$$i(x, t) = V(x, t)(g + jb).$$

Combining these equations and integrating

$$V(x, t) = A \exp(-x(r_i(g + jb))^{1/2}) + B \exp(x(r_i(g + jb))^{1/2}).$$

At  $V(\infty, t) = 0$ , therefore  $B = 0$ .

From the cable equation, the core current,  $i_i(x, t)$ , amperes

$$i_i(x, t) = \frac{-1}{r_i} \frac{\partial V(x, t)}{\partial x}$$

and the above give

$$i_i(x, t) = \frac{A}{r_i} (r_i(g + jb))^{1/2} \exp(-x(r_i(g + jb))^{1/2}).$$

At  $x = 0$ , the applied current  $I_o(t) = 2i_i(0, t)$ , therefore

$$A = \frac{I_o(t)}{2} r_i^{1/2} (g + jb)^{-1/2}$$

and the characteristic impedance,  $Z_o = V(0, t)/I_o(t)$

$$Z_o = \frac{(r_i/g)^{1/2}}{2(1 + jb/g)^{1/2}}$$

Let  $p = (m + jn)^2 = 1 + jb/g$ , therefore

$$p^2 = m^2 - n^2 + j2mn = 1 + jb/g.$$

Separating the real and imaginary parts

$$m^2 - n^2 = 1 \text{ and } n = b/2mg \text{ from which}$$

$$m^2 = \frac{1 \pm (1 + (b/g)^2)^{1/2}}{2}$$

and since  $m$  is real and positive

$$m = 2^{-1/2}(1 + (1 + (b/g)^2)^{1/2})^{1/2}$$

and

$$n = 2^{-1/2}(-1 + (1 + (b/g)^2)^{1/2})^{1/2}.$$

Substituting in the equation for  $Z_o$

$$Z_o = \frac{(r_i/g)^{1/2}}{2[(b/g)^2 + 1]^{1/2}} (m - jn)$$

Therefore the tangent of the phase angle of the characteristic admittance,  $\tan \theta$

$$\tan \theta = \frac{n}{m} = \frac{[(b/g)^2 + 1]^{1/2} - 1}{[(b/g)^2 + 1]^{1/2} + 1}$$

which is equation 1 in the text.

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