Capacity of Muscle Fiber Membrane[1]

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ABSTRACT

TASAKI, I. AND S. HAGIWARA. (Natl. Insts. Health, Bethesda, Md., and U. California, Los Angeles.) *Capacity of muscle fiber membrane.* Am. J. Physiol. 188(3): 423–429. 1957.—Using two or three microelectrodes inserted into single muscle fibers of the toad sartorius, the electric impedance of the muscle fiber membrane was measured with a.c. between 25 and 1500 cps. It was found that the capacity of the muscle fiber membrane is practically independent of the frequency of the measuring a.c. The membrane capacity determined from the time constant of potential rise in the foot of a propagated muscle action potential agrees with the value determined by a.c. impedance measurement. It was emphasized that the a.c. method of determining the cable constants of the muscle fiber (based on *eq. 1* under METHODS) is simple and gives satisfactory results.

RECENT observations with intracellular microelectrodes have revealed that the surface membrane of the muscle fiber of the frog or the toad has a capacity of 5–9 $\mu f/cm^2$ (1–3). In the present study, we have attempted to determine whether or not this large capacity of the muscle fiber membrane varies with the frequency of the measuring a.c.

We found that the membrane capacity of the muscle fiber is practically independent of the frequency in the range between 25 and 1500 cps. In addition, we have seen that the membrane capacity determined from the foot (the earliest part of the rising phase) of the action potential agrees with this large value determined by a.c.

METHODS

Material and Equipment. The material used was excised sartorius muscle of the toad, *Bufo marinus*. The technique and the experimental procedures are similar to those recently described by one of us (3). The muscle was fixed on a lucite platform in a Petri dish with its inner surface facing upward (fig. 1). Illuminating the muscle in Ringer from beneath, the connective tissue on the upper surface of the muscle was cleaned under a dissecting microscope.

Submicroscopic glass pipette electrodes filled with 3 M KCl solution were prepared by Mrs. I. Tasaki by her alcohol method (4, p. 455). With the aid of two or three micropositioners, the microelectrodes were inserted into a muscle fiber on the upper surface of the muscle under direct visual control. The fibers in the toad sartorius muscle are generally 60–120 μ in diameter; but it is not always easy to measure their diameter accurately under a dissecting microscope. The electrodes were fixed at approximately 45° to the length axis of the muscle fiber to be studied. The upper surface of the muscle was approximately 2 mm below the surface of the bathing fluid. The resistance of the microelectrodes used was between 7 and 15 megohms, when measured with their tips immersed in frog Ringer. The composition of the Ringer solution was, as a rule, 111 mM NaCl, 2 mM KCl, 1.5 mM $CaCl_2$, 2 mM $NaHCO_2$, 0.1 mM $NaII_2PO_4$ and 11 mM glucose.

The microelectrodes were shielded with metal tubing down to a point 5–10 mm above the tips. The metal shield of the recording electrode was driven by the output of a cathode-follower, which consisted of a GE 1620 tube in triode connection operated at low plate voltage. The effective input capacity of the cathode-follower was approximately 5 $\mu\mu f$, giving a time constant of voltage rise of about 50 μsec. for a square voltage pulse applied to the input through a 10-megohm resistor. The leading-in electrode, through which either a direct or an alternating current was sent into the muscle fiber, had a grounded metal shield. A Tektronix pulse generator (no. 161) or a General

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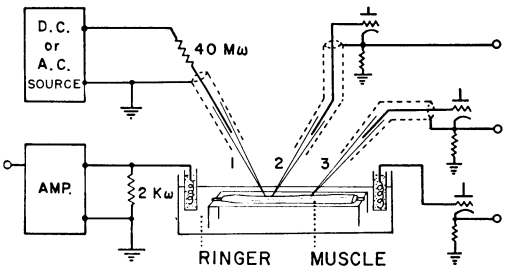

FIG. 1. Experimental arrangement used for determining the cable constants of a muscle fiber by the a.c. method and by the method based on the time constant of potential rise in the foot of a propagated action potential. Microelectrode #1 is for applying a d.c. pulse or a.c. to the muscle fiber, #2 and #3 for recording sinusoidal potential variations and action potentials. The large Ag-AgCl-agar electrode on the right in the diagram is the indifferent electrode for recording membrane potentials.

Radio beat-frequency oscillator (type no. 1304-A) was used as a voltage source. A 40-megohm resistor was connected between the voltage source and the microelectrode. The IR drop across the 2000 ohm resistor between ground and the bathing fluid in the Petri dish was a measure of the current sent into the muscle fiber. This was amplified 1000-fold by a Tektronix preamplifier (no. 122) before leading to the oscilloscope. A DuMont dual-beam oscilloscope and a Grass camera were used for recording.

The experiments were conducted at room temperature (24°C) and at low temperature (16°C).

Principle of the Method of Determining the Cable-Constants of the Muscle Fiber by A. C.[3] It is well known that a variation in the membrane potential along a muscle fiber immersed in a conducting fluid medium is described by the so-called cable equation

$$l^2 \frac{\partial^2 V(x,t)}{\partial x^2} = V(x,t) + \tau \frac{\partial V(x,t)}{\partial t} \qquad (1)$$

where $V(x, t)$ is the membrane potential at point x along the fiber at time t measured from the level of the resting membrane potential, τ the time constant of the membrane and l the space constant of the fiber. When an alternating current of amplitude I and frequency f is sent into a muscle fiber through a microelectrode inserted at $x = 0$, the distribution of the potential variation in the steady state is given by a solution of *equation 1* subject to the following boundary conditions:

$$-\frac{2}{s}\left[\frac{\partial V(x,t)}{\partial x}\right]_{x=0} = I \cos(2\pi f t)$$

and

$$V(\infty, t) = 0$$

where s is the resistance per unit length of the sarcoplasm. It is simple to demonstrate that the solution we want is

$$V(x,t) = \frac{Ils}{2\sqrt[4]{1 + 4\pi^2 f^2 \tau^2}} e^{-mx/l} \times \cos\left(2\pi f t - \frac{nx}{l} - \tan^{-1}\frac{n}{m}\right) \qquad (2a)$$

where

$$m = \frac{\sqrt{+1 + \sqrt{1 + 4\pi^2 f^2 \tau^2}}}{\sqrt{2}}$$

and (2b)

$$n = \frac{\sqrt{-1 + \sqrt{1 + 4\pi^2 f^2 \tau^2}}}{\sqrt{2}}$$

The cable property of a muscle fiber is completely defined by a set of three constants of the fiber, namely, by l, τ, and s, or by membrane resistance r, capacity c and s. If the amplitude of the potential variation, $V(x, t)$, is determined at three different frequencies, three equations necessary for determination of the three cable constants can be obtained.

Introducing $f = 0$ into *equation 2*, the well-known equation describing the spread of a steady (d.c.) potential along the fiber, namely,

$$V(x) = \frac{Ils}{2} e^{-x/l} \qquad (3)$$

is obtained. Comparing *equation 3* with *equations 1*, one finds that, with increasing frequency f, not only does the amplitude of potential variation at the site of current application ($x = 0$) decrease but the spatial distribution of the potential is also decreased. The factor m defined by *expression 2b* is the measure of shortening of the space constant for an a.c.

For frequencies greater than about 30 cps, $2\pi f\tau \gg 1$; therefore, *equation 1* becomes

$$V(x,t) \approx \frac{I\sqrt{s}}{2\sqrt{2\pi c f}} e^{-x\sqrt{\pi s c f}} \times \cos\left(2\pi f t - x\sqrt{\pi s c f} - \tfrac{1}{4}\pi\right), \qquad (4)$$

[3] Definitions and symbols: c: capacity per unit length of the muscle fiber (dimension: farad/cm); d: thickness of the nonaqueous layer in the muscle fiber membrane; f: frequency of a.c. sent into the muscle fiber through a microelectrode; I: amplitude of a.c.; h: slope of the straight line in fig. 4 related to the cable constant by *eq. 9*; l: space constant; k: value of $V\sqrt{f}/I$ for $f = 0$ obtained by extrapolation of the straight line in fig. 4; K: dielectric constant; m: factor indicating shortening of the space constant with increasing frequency of a.c.; p: time constant of potential rise in the foot of a propagated action potential; r: resistance of the muscle fiber membrane for a unit length; s: resistance of the sarcoplasm per unit length (dimension: ohm/cm); t: time; v: velocity of muscle impulse; V: amplitude of sinusoidal voltage $V(x, t)$ or $V(t)$; $V(x, t)$, $V(x)$ or $V(t)$: variation in membrane potential caused by the applied current regarded as a function of both x and t, of x, or of t alone; x: distance along the muscle fiber; τ: time constant of the membrane.

where c is the capacity of the membrane per unit length of the fiber. (Note that $\tau = cr$ and $l^2 = r/s$, r being the membrane resistance for a unit length of the fiber.) For this approximation, the resistance of the membrane, r, does not make any contribution to the impedance of the muscle fiber.

Introducing $x = 0$ into *equation 4*, the following equation describing potential variations at the site of application of a.c. is obtained:

$$V(0, t) \approx \frac{I\sqrt{s}}{2\sqrt{2\pi cf}} \cos(2\pi ft - \tfrac{1}{4}\pi). \qquad (5)$$

If both the capacity and the sarcoplasm resistance are independent of the frequency of the measuring a.c., the amplitude of the potential changes at $x = 0$ should vary inversely as the square root of the frequency f.

Principle of Determining the Membrane Capacity From the Foot of a Propagated Action Potential.

The start of activity at a point of a muscle fiber is preceded by the spread of electricity from the adjacent active region of the fiber. The rate of potential rise in this period of electrotonic potential rise should be determined by the cable properties of the muscle fiber at rest and by the velocity of impulse propagation; it is independent of the time course of the membrane potential during activity, in so far as one does not go into the mechanism whereby the velocity of impulse propagation is determined. The distribution of the membrane potential ahead of a propagating impulse, therefore, satisfies the equation for a uniform cable, i.e., *equation 1*.

Let us consider the case in which the impulse travels along the fiber at a uniform velocity v. Since the operator $\partial^2/\partial^2 x$ in *equation 1* can be replaced by $(1/v^2)\partial^2/\partial t^2$ in this case, the equation describing the potential changes $V(t)$ during the foot of an action potential is given by

$$\frac{l^2}{v^2}\frac{d^2V(t)}{dt^2} = V(t) + \tau\frac{dV(t)}{dt} \qquad (6)$$

The general solution of *equation 6* derived by Cole (5) and by Rosenblueth *et al.* (6) is

$$V(t) = Ae^{(v\tau+\sqrt{v^2\tau^2+4l^2})vt/(2l^2)} + Be^{(v\tau-\sqrt{v^2\tau^2+4l^2})vt/(2l^2)}, \qquad (7)$$

where A and B are arbitrary constants. The potential change $V(t)$ is zero a long time before the start of an action potential, namely, when $t = -\infty$; therefore the arbitrary constant B has to be equal to zero in the present case. The constant A depends only on our arbitrary choice of the origin of the time scale.

Introducing the known values (1) for the constants in the upper half of *equation 7*, namely, $v = 200$ cm/sec., $\tau = 0.03$ sec. and $l = 0.3$ cm, one finds that the second term under the square root can be ignored in face of the large first term. Thus, the approximate equation describing the foot of the action potential is

$$v(t) = A\, e^{\frac{2}{3}cv^2t}, \qquad (8)$$

in which c is the capacity of the membrane per unit length of the fiber, s the sarcoplasm resistance for the same length and v the velocity of the impulse. If the values of s and v are known for a given fiber, the capacity c can be determined from the time constant of potential rise during the foot of the action potential.

RESULTS

Capacity of the Muscle Fiber Membrane as a Function of the Frequency of the Measuring A.C. Two microelectrodes were introduced into a muscle fiber in normal Ringer at a distance of 30–50 μ. An alternating voltage of 1–5 volts peak-to-peak was applied to one of the microelectrodes through the 40-megohm resistor in figure 1. The potential difference across the membrane was recorded through the other microelectrode, connecting the output of the cathode-follower either directly to a d.c. channel of the oscilloscope or to a condenser-coupled differential amplifier before recording. The time course of the current sent into the fiber was recorded with a second beam of the dual-beam.

With subthreshold a.c. between 25 and 2000 cps, the amplitude of the potential variation observed was found to vary, at a given frequency, directly with the amplitude of the applied a.c. Since no appreciable potential variation was recorded when one of the two microelectrodes was placed outside the muscle fiber under investigation, it is evident

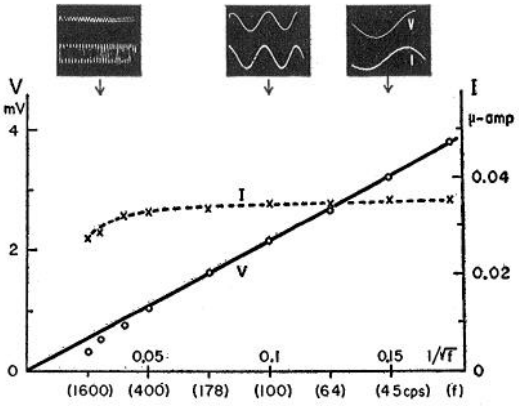

FIG. 2. Relationship between the amplitude of the membrane potential variation, V, at the site of application of sinusoidal currents of amplitude I and the frequency, f. Note that the phase difference between the two sine waves in the photographic records on the top is 45° as expected from *eq. 5* under METHODS. The resting potential of the fiber examined was approximately 90 mv, 24°C.

that the observed potential variation represents the potential drop across the muscle fiber membrane.

An example of the results of these measurements is presented in figure 2, together with some of the records on which the measurements were made. The amplitudes of the potential variation, V, and of the applied current, I, were plotted as ordinate against the reciprocal of the square root of the frequency of the applied a.c., $1/\sqrt{f}$, as abscissa. It can be seen in the figure that, in a wide range of frequency, the relationship between V and $1/\sqrt{f}$ is given by a straight line passing through the origin of the two axes. This is exactly what is expected from *equation 5* when the capacity c of the membrane is independent of the measuring a.c.

For frequencies above 700 cps, however, there was a slight (but systematic) divergence of the observed data from the expected straight line; the observed values of the membrane potential fell short of the expected values. In this frequency range, there is a slight decrease in the amplitude of the current, due to a leakage of the current through the grounded metal shield for the leading-in electrode (note a slight decline in line I in fig. 2). But, the reduction in the potential is more pronounced than that in I; as a consequence, the value of $V\sqrt{f}/I$, which is constant for frequencies below 700 cps, tends to decrease at higher frequencies. The reduction in the value of $V\sqrt{f}/I$ was between 5 and 25% at 900 cps.

The divergence just mentioned would not be surprising if one considers the effect of shortening of the space constant at high frequencies. *Equation 4* tells us that the apparent space constant measured with an a.c. of frequency f is only $1/m$ of the value determined by a d.c. Since the time constant of a fresh muscle fiber is around 30 msec., the value of m should be about 10 at 1000 cps and about 14 at 2000 cps. In the muscle fiber we have examined, the space constant for a d.c. is around 3 mm. At frequencies above 1000 cps, therefore, the diameter of the muscle fiber is no longer negligibly small compared to the space constant. In other words, the variation of the potential within the cross-sectional area at a given value of x becomes significant. This shortening of the space constant should naturally tend to lower the observed potential below the value expected from the one-dimensional approximation.

We have carried out 8 determinations of this type at room temperature and 14 determinations at about 15°C. In all cases there was a good linear relationship between the impedance V/I and the reciprocal of the square root of the frequency f in the range of frequency between 25 and 700 cps. The slope of the straight line, namely the constant $V\sqrt{f}/I$, was, in most cases, between 1 and 3 megohm·sec.$^{-\frac{1}{2}}$ at 15°C and slightly smaller at 24°C.

This type of impedance measurement on the muscle fibers indicates that, for a.c. of which the period extends from the duration of the rising phase of the action potential up to several times the time constant of the resting membrane, the capacity of the muscle fiber membrane is independent of the frequency of the measuring a.c. If the effective capacity of the membrane decreases at high frequencies, then the observed values of V should diverge upwards from the straight line passing through the origin of the axes as the frequency goes up. In fact, such a divergence has never been observed.

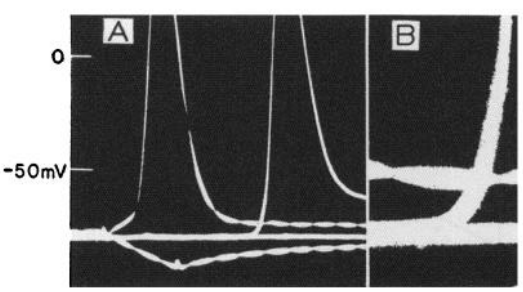

FIG. 3. Action potentials of a muscle fiber recorded by the arrangement of fig. 1. The distance between microelectrode #1 and #2 was 0.95 mm and that between #2 and #3 was 9.8 mm. One of the oscillograph beams was blanking at 1000 cps. *B*: Enlarged photograph of the initial portion of the second action potential in photograph *A*. 16°C.

Membrane Capacity Obtained From the Foot of a Propagated Muscle Action Potential. It has been pointed out by A. F. Huxley in England (personal communication) and others that a propagated muscle action potential is preceded by a short, exponentially rising prepotential, or, a foot, and that the time con-

stant of this potential rise can be used to determine the capacity of the muscle fiber membrane. We carried out a series of experiments to see if the capacity determined from the foot of the action potential agrees with the value obtained by impedance measurements with a.c.

In order to determine the membrane capacity from the foot of the action potential, it is necessary to know the velocity of the muscle impulse with fair accuracy. Both the impulse velocity and the rate of potential rise in the foot can be measured by exciting a muscle fiber at one point and recording the action potential at two different points of the fiber. An example of the records obtained in this manner is presented in figure 3. The distance between the two recording microelectrodes divided by the time interval between the two recorded action potentials gives the average rate of impulse propagation. The rate of potential rise at the beginning of the second response is not contaminated by the applied (stimulating) current and can be used to determine the rate of potential rise.

The time constant of our recording system was not short enough to register the potential changes which progress within a fraction of a millisecond. Fortunately, however, the time constant of an exponentially rising voltage is not affected by the input capacity of the recording device. No correction for the effect of the input capacity was introduced in our measurement of the time constant of potential rise in the foot of the action potential.

In 12 determinations carried out at 15°C, the time constant of potential rise in the foot varied between 0.10 and 0.27 msec., the average value being 0.16 msec. The impulse velocity varied between 1.8 and 2.5 m/sec.

The accuracy of determination of the membrane capacity by this technique is seriously limited by the difficulty of measuring both the sarcoplasm resistance per unit length, s, and the size and shape of the cross section of the fiber. We have circumvented this difficulty by limiting our interest to a comparison of the data obtained from the foot with those determined by a.c. In *equation 4* mentioned under METHODS, the exponential term describing the spatial distribution of the membrane potential contains the product sc, just as the time constant of potential rise for the foot given by *equation 8* does. A comparison of the product sc determined by an a.c. impedance measurement with the value of this product obtained from the foot of the action potential in the same fiber would be expected to clarify whether or not the two different methods give consistent results.

The experiments designed to examine the consistency of the two different methods of determining the membrane capacity were carried out in the following manner: Two microelectrodes were inserted into a muscle fiber at a distance of 0.6–0.8 mm. Subthreshold sinusoidal currents of 30–700 cps were sent into the fiber through one of the electrodes, and the variation in the membrane potential caused by these a.c. was recorded with the other electrode. Then, the third microelectrode was introduced into the fiber at a distance of about 5 mm from the second (recording) electrode (see fig. 1). If the resting potential of the fiber observed at the second electrode was still normal at this moment, a short, suprathreshold current pulse was applied to the fiber through the first microelectrode and a record of a propagated action potential was taken at two points of the fiber.

An example of the results of the impedance measurement of this type is furnished in figure 4. On the photographic records of which a few

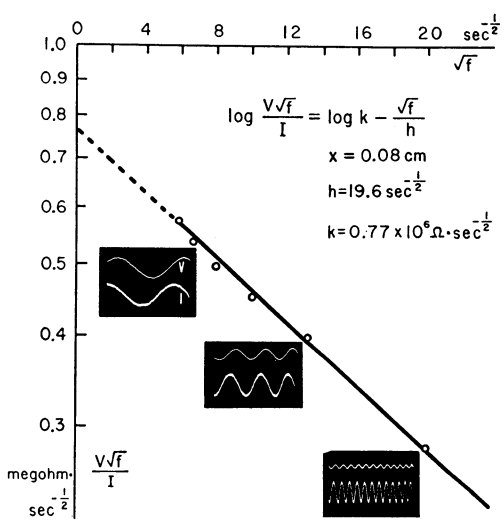

FIG. 4. Relationship among the amplitude of a sinusoidal current, I, sent into a muscle fiber at $x = 0$, the variation in the membrane potential, V, observed at $x = 8$ mm and the frequency of the a.c., f. The logarithm of $V\sqrt{f}/I$ was plotted against $\sqrt{f}$. Further detail in text. 24°C.

TABLE I. COMPARISON OF THE PRODUCT OF THE MEMBRANE CAPACITY AND THE SARCOPLASM RESISTANCE DETERMINED FROM THE FOOT OF THE ACTION POTENTIAL, $1/(pv^2)$, WITH THE CORRESPONDING VALUE DETERMINED BY THE A.C. METHOD, $1/(\pi x^2 l^2)$

Muscle Fiber No.	Time Constant for Foot, p	Impulse Velocity, v	k	xh	$\frac{1}{pv^2} : \frac{1}{\pi x^2 h^2}$
	sec. $\times 10^{-4}$	*cm/sec.*	*ohm·sec.*$^{-\frac{1}{2}}$ $\times 10^{6}$	*cm.* *sec.*$^{-\frac{1}{2}}$	
1	1.2	210	1.2	1.1	0.19:0.26
2	2.7	180	0.77	1.7	0.11:0.11
3	2.5	180	0.49	2.0	0.12:0.08
4	1.6	180	0.85	1.7	0.19:0.11
5	1.5	190	0.54	1.4	0.18:0.16

Measurements were made on two sartorius muscles of a toad, 16°C.

samples were shown in the figure, the ratio of the amplitude of the sinusoidal membrane potential, V, to the amplitude of the current, I, was determined as a function of the frequency f. Then, the values $V\sqrt{f}/I$ were calculated from the data and were plotted on a semi-logarithmic paper against $\sqrt{f}$. As can be seen in the figure, the observed points lay on a straight line.

The linear relation between log $(V\sqrt{f}/I)$ and $\sqrt{f}$ observed is expressed by the equation given in the figure, which is nothing but *equation 4* simplified by the following substitution:

$$k = \frac{\sqrt{s}}{2\sqrt{2\pi c}}, \quad h = \frac{1}{x\sqrt{\pi sc}} \qquad (9)$$

and $|V(x, t)| = V$. Since the values of k and h are directly measurable by experiments of this type, the a.c. method of determining the cable constants is simpler and more accurate than the previous method using only d.c. pulses. On several occasions, it has been shown (by introducing three microelectrodes into a muscle fiber) that the value of k determined by the type of experiment of figure 2 (i.e. with the leading-in and recording electrodes inserted at one point on the fiber) agrees with the value determined by the technique of figure 4 (with the recording electrode introduced at some distance away from the leading-in).

From *equation 8*, is is clear that the time constant of potential rise in the foot, p, is related with the cable constants of the fiber by

$$p = \frac{1}{scv^2}. \qquad (10)$$

The value of $1/(pv^2)$, which is directly measurable in the record of figure 3, therefore, represents the product sc obtainable from the foot. The second expression in *equation 9* indicates that the value of $1/(\pi x^2 h^2)$ obtainable directly from the observation of figure 4 represents the product sc too. The product sc has a dimension of sec·cm^{-2}.

In table 1 are presented the data from five different muscle fibers, on which both the a.c. measurements and the recording of the action potential at two points were successfully carried out on each individual fiber. In all these fibers, the resting potential observed at the two recording electrodes were normal (80–90 mv), and there was no significant difference in the shape and the size of the two action potentials. (The data from one fiber which showed a prolonged action potential at one of the recording electrodes were excluded in the table.) In the last column of the table, the values of the product sc determined by the two different methods were compared. Considering the possibility of a slight injury at one of the three microelectrodes, the agreement between the two values is very satisfactory. It safely follows from this result that the large capacity of the muscle fiber membrane is actually charged by the local current that flows around the active-inactive boundary ahead of a propagated muscle impulse.

DISCUSSION

The capacity of the muscle fiber membrane in toad's sartorii obtained in the present study was, in good agreement with the previous data (1–3), between 5 and 9 μf/cm^2. The accuracy of these measurements is limited mainly by the difficulty of estimating the circumference of individual muscle fibers in an intact sartorius muscle. This capacity is large, compared to that of ordinary physical condensers or of the squid axon (7). It does not seem improbable, however, that this capacity is determined, like the capacity of the myelin sheath (8, 9) or that of the squid axon membrane (10), by the thickness of the dielectric material on the surface of the living cell.

The capacity of a parallel-plate condenser is given by $C = K/(4\pi d)$, where C is the capacity per unit area, K the dielectric constant and d the thickness of the dielectric between the plates. Since one c.g.s. electrostatic unit of capacity is 1.1 $\mu\mu$f in practical units, and since the dielectric constants of most of liquid and solid fatty

compounds (including myelin) are known to be 5–10, the thickness of the nonaqueous layer in the muscle fiber membrane is probably of the order of 10 Ångstroms. This figure is still far larger than the internuclear distance of a carbon-carbon bond, which is known to be approximately 1.5 Ångstroms with a C—C—C bond angle of about 111° (see Pauling, 11, pp. 167 and 84). It is possible that the dielectric constant of this layer of a molecular dimension is very different from that of a thick myelin sheath.

The frequency dependence of the membrane capacity and the nonlinear behavior of the membrane resistance has been demonstrated in the squid giant axon (10). These complicating factors were not observed in the muscle fiber of the toad within the range of intensity and frequency of the a.c. employed in the present investigation. In the muscle fiber immersed in normal Ringer, the threshold membrane potential was in general 30–40 mv above the resting potential. Within this limit of membrane depolarization (by a d.c. or a.c.), the surface membrane of a normal muscle fiber behaved as an ideal condenser with a finite leakage resistance, the time constant being 30–50 msec.

In the muscles immersed in low-sodium or sodium-free Ringer (generally saturated with a mixture of 95% oxygen and 5% carbon dioxide), many muscle fibers were found to behave almost linearly up to 80 and 120 mv in membrane depolarization, showing an approximate proportionality relationship between the intensity of the applied current (d.c. pulses of 20 msec. in duration) and the change in the membrane potential. The difficulty of explaining this finding in terms of the sodium theory of action potential production (13) has been discussed elsewhere (14).

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