Capacitance of the Surface and Transverse Tubular Membrane of Frog Sartorius Muscle Fibers

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ABSTRACT The passive electrical properties of glycerol-treated muscle fibers, which have virtually no transverse tubules, were determined. Current was passed through one intracellular microelectrode and the time course and spatial distribution of the resulting potential displacement measured with another. The results were analyzed by using conventional cable equations. The membrane resistance of fibers without tubules was 3759 ± 331 ohm-cm^2 and the internal resistivity 192 ohm-cm. Both these figures are essentially the same as those found in normal muscle fibers. The capacitance of the fibers without tubules is strikingly smaller than normal, being 2.24 ± 0.14 μF/cm^2. Measurements were also made of the passive electrical properties of fibers in a Ringer solution containing 400 mM glycerol (which is used in the preparation of glycerol-treated fibers). The membrane resistance and capacitance are essentially normal, but the internal resistivity is somewhat reduced. These results show that glycerol in this concentration does not directly affect the membrane capacitance. Thus, the figure for the capacitance of glycerol-treated fibers, which agrees well with previous estimates made by different techniques, represents the capacitance of the outer membrane of the fiber. Estimates of the capacitance per unit area of the tubular membrane are made and the significance of the difference between the figures for the capacitance of the surface and tubular membrane is discussed.

The capacitance of cell membranes has often been measured and interpreted in terms of the dielectric constant and thickness of the membrane substance. In nerve, the membrane capacitance has been found to be about 1 μF/cm^2 (Cole and Curtis, 1950) which seems a reasonable value for a membrane about 80 A thick and composed mostly of lipid. However, muscle membrane has a much higher capacitance (Katz, 1948; Fatt and Katz, 1951) although

the thickness of the membrane appears much the same and there is no reason to believe the lipid is different. The estimates of capacitance in both nerve and muscle fibers have generally been based on the assumption that the membrane area of 1 cm length of such fibers is πd cm² (where d is the diameter of the fiber in centimeters). If the surface area were greater than this, the above assumption would give an overestimate of the capacitance per unit area. Muscle fibers have, in fact, a system of invaginations of the surface membrane, the transverse tubular system (Andersson-Cedergren, 1959; Peachey, 1965), which increases the membrane area severalfold, whereas nerve fibers do not. Falk and Fatt (1964) have separated the capacitance of frog sartorius muscle fibers into two components by measuring the impedance of muscle fibers over a range of frequencies. The capacitance attributed to the transverse tubular system was 4.1 μF/cm². The other capacitance, of 2.6 μF/cm², was assigned to the surface membrane. Although the distribution of the capacitances seems very reasonable (see also Freygang et al., 1967), it has been difficult to test and to reconcile with the estimates of the areas of the surface and transverse tubular membranes. The ratio of the area of the transverse tubular membrane to surface membrane (in a 50 μ fiber) has been estimated to be 4.5 (Peachey and Schild, 1968) whereas the ratio of respective capacitances is less than 2.

A method has recently been described (Howell and Jenden, 1967) which causes selective disruption of the transverse tubular system. The number of transverse tubules left in these preparations has been measured using horseradish peroxidase as an extracellular marker and found to be 1.6% (Eisenberg and Eisenberg, 1968). This technique therefore offers the opportunity to determine the separate capacitances of the surface and transverse tubular membranes. In this paper the capacitance of muscle fibers with and without transverse tubules has been measured: the average capacitances were found to be 2.1 μF/cm² for the surface membrane and 4.0 μF/cm² for the transverse tubular system. Some of these results have been reported briefly elsewhere (Eisenberg and Gage, 1967).

METHODS

Measurements were made on the surface fibers of frog sartorius muscles (*Rana pipiens*) from June to September, 1967. Two microelectrodes filled with 3 M KCl (resistance 10–20 megohms) were inserted into a fiber, 30–100 μ apart, and the exact electrode separation measured. A rectangular current pulse was passed through one electrode and the resulting displacement of membrane potential measured with the other. The current electrode was then reinserted into the fiber at a more distant point, and the procedure repeated. Measurements were taken at several different electrode separations. The diameter of a fiber was measured optically using a Wild (Wild Heerburg Instruments, Farmingdale, N. Y.) stereomicroscope. These measurements were aided

by the use of oblique illumination of the muscle and high magnification. It was often found that moving a fiber with a microelectrode helped to define its edges.

All solutions used in these experiments contained curare (10^{-5} g/ml) to block possible synaptic effects and tetrodotoxin (10^{-7} g/ml) to stop spontaneous twitching (see Results). The composition of the Ringer solution was (mM) Na^+ 120, K^+ 2.5, Ca^{++} 1.8, Cl^- 121, HPO_4^{--} 2.15, and $H_2PO_4^-$ 0.85 (pH = 7.2). The glycerol-Ringer solution contained 400 mM glycerol in addition to the above ions. Muscles were either transferred from solution to solution in beakers or were mounted in a

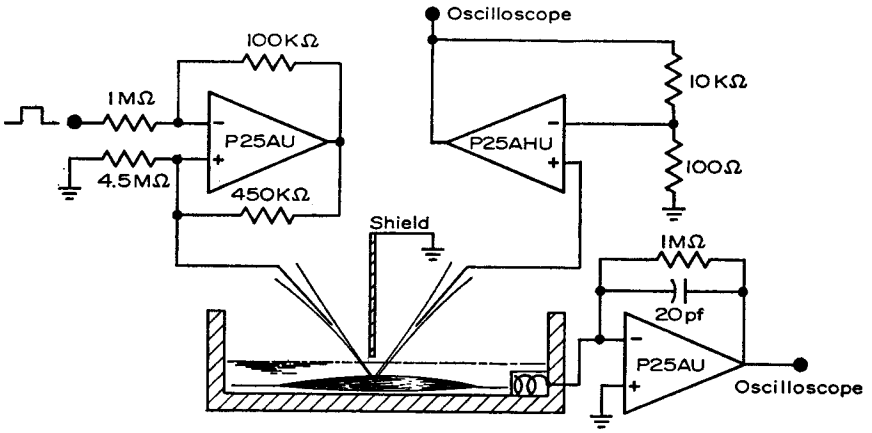

FIGURE 1. A diagram of the recording apparatus. The operational amplifier (*P25AU*) in the upper left corner is connected as a constant current generator. The operational amplifier in the middle of the diagram (*P25AHU*) is connected as a follower with gain. The amplifier in the lower right corner (*P25AU*) is connected as a current monitor. A shield is placed between the electrodes to decrease capacitive artifact.

bath at approximately ⅔ rest length and the solutions changed by draining the bath and adding a new solution. Room temperature was maintained at 18–21°C.

Three electronic systems, constructed with operational amplifiers (Philbrick, Dedham, Mass.), were used in these experiments.

A constant current generator, suggested by Dr. E. A. Johnson, was used for passing current (Fig. 1). That is, the output current was constant no matter what the resistance of the microelectrode and was proportional to the voltage applied to the current generator. The circuit is described elsewhere (Philbrick, 1966, p. 66). This system was particularly helpful since it allowed the use of higher resistance electrodes which produced less damage in fibers subjected to multiple penetrations. The use of such microelectrodes is often limited by their poor current-passing characteristics. The current passed was initially measured with an operational amplifier connected in an ammeter configuration (Moore, 1963) (Fig. 1). Use of a 1 megohm feedback resistor conveniently gave a 1 mv output for 1 namp input. The system for measuring potential consisted of a high input impedance operational amplifier connected as

a "follower with gain" (Philbrick, 1966, p. 81) as shown in Fig. 1. The output of this amplifier was displayed on an oscilloscope with two time bases. Thus, the signal could be displayed simultaneously at fast and slow sweep speeds, which greatly improved the accuracy of time course measurements while still giving information about steady-state values.

RESULTS

Surface muscle fibers without transverse tubules were obtained by soaking a sartorius muscle in glycerol-Ringer solution for 1 hr and then transferring the muscle into a Ringer solution containing no glycerol (Howell and Jenden

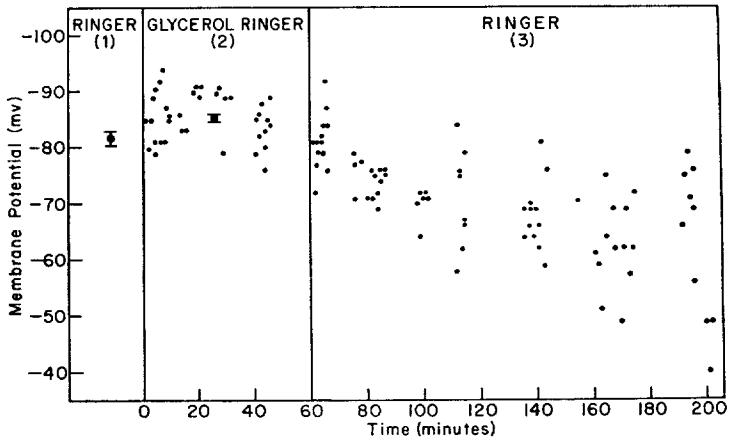

FIGURE 2. A plot of resting potential against time in the appropriate solutions. Each small point represents one measurement. The larger points represent the mean membrane potentials in the different solutions. The bars represent ± 1 SEM. Condition (1) represents a muscle in normal Ringer; condition (2), a muscle in Ringer to which 400 mM glycerol had been added; condition (3), a muscle after return to Ringer. The tubular system is virtually absent in condition (3) but intact in (2)

1967). 5 or 10 min after return to the Ringer solution, muscles became cloudy and opaque and sometimes twitched spontaneously for up to 1 hr. This twitching could not be prevented by adding curare (10^{-5} g/ml) to solutions but was blocked by tetrodotoxin (10^{-7} g/ml). Intracellular recording when successful, revealed action potentials accompanying these twitches. Thus, it seems likely that these spontaneous twitches were produced by action potentials which perhaps were associated with transient leaks produced by the disruption of the transverse tubules. To prevent such movement during the first hour, tetrodotoxin (10^{-7} g/ml) was often added to the Ringer solutions.

One microelectrode was inserted into a surface fiber of treated preparations and the membrane potential recorded. Many fibers had low resting potentials and the fraction of such fibers increased with time (Fig. 2). However, many

fibers had resting potentials greater in magnitude than −70 mv (inside potential with respect to outside) for more than 2 hr. It will be shown later (Eisenberg and Gage, 1969) that fibers with low resting potentials are often "leaky" whereas fibers with high resting potentials are not. In those fibers with resting potentials greater in magnitude than −70 mv another microelectrode was inserted within 100 μ of the first and the separation of the electrodes measured. A small hyperpolarizing rectangular pulse of current was passed into the fiber and the voltage displacement recorded. The current electrode was then withdrawn and inserted in the same fiber several times, at accurately measured separations of up to 2 mm. The current, the steady-state potential, and the time to reach half this potential ($T_{1/2}$) were recorded at each separation. In each fiber the transfer resistance $R(x)$ (the ratio of

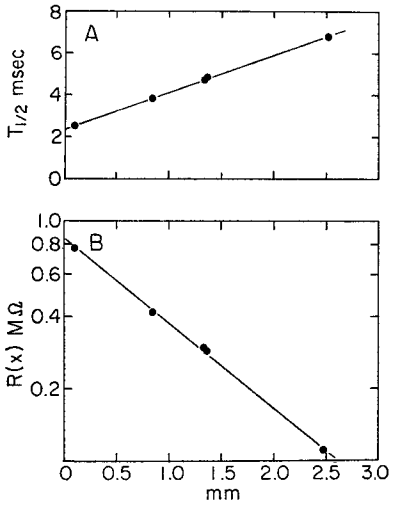

FIGURE 3. (A) plot of time to half-maximum against distance. The y intercept of this curve determines the over-all time constant of the membrane. (B) A plot of transfer resistance against potential. Note the logarithmic scale of the ordinate. The length constant of the fiber is determined from this plot.

the potential at electrode separation x to the current applied) was plotted against electrode separation. One of these graphs is illustrated in Fig. 3 B (Note the logarithmic scale on the ordinate.) The "y intercept" of the line drawn gives the input resistance (R_o) of the fiber. If it is assumed that the muscle fiber behaves like a uniform cylindrical cable, the equations of one dimensional cable theory (Hodgkin and Rushton, 1946) can be used to determine membrane resistance and capacitance. In the steady state,

$$\frac{V(x)}{I_o} = R(x) = \tfrac{1}{2}\sqrt{r_m r_i}\, e^{-x/\sqrt{r_m/r_i}} = R_o e^{-x/\lambda} \qquad (1)$$

Where r_m is the resistance of 1 cm length of fiber membrane, r_i is the resistance of 1 cm length of the intracellular fluid.

$\sqrt{r_m/r_i}$ = the length constant λ

$\frac{1}{2}\sqrt{r_m r_i}$ = the input resistance R_o; i.e. V/I_o at zero electrode separation

I_o is the current applied (at the point $x = 0$)

The meaning of the space constant can be made clear by rewriting equation (1). When the electrode separation equals the space constant; i.e., when $x = \lambda$

$$\frac{V(x = \lambda)}{I_o} = \tfrac{1}{2}\sqrt{r_m r_i}\, e^{-1} \doteq 0.37R_0$$

In other words the length constant is the distance at which the observed resistance has dropped to 37% of its value at zero electrode separation. Similarly, it is useful to describe the input resistance in physical terms: the input resistance is the ratio of voltage recorded (at a very close electrode separation) to the current applied.

It is possible to derive the parameters of the system (r_m, r_i) from measurements of the space constant and input resistance. Thus, the resistance per unit length is given by

$$r_i = 2\left[\frac{1}{2} \cdot \frac{\sqrt{r_m r_i}}{\sqrt{r_m/r_i}}\right] = \frac{2R_o}{\lambda} \tag{2}$$

and the resistance of a unit length of membrane is

$$r_m = 2[\sqrt{r_m/r_i}][\tfrac{1}{2}\sqrt{r_m r_i}] = 2\lambda R_o \tag{3}$$

The lower case parameters (r_m, r_i) refer to the properties of a unit length of fiber, but it is often more useful to refer the properties to 1 cm² area of the structure in which they arise. The resistivity of the intracellular fluid can be determined from r_i if the fiber is assumed to be a circular cylinder of radius a:

$$R_i = r_i(\pi a^2) \tag{4}$$

Similarly, the specific resistance of the membrane is

$$R_m = r_m(2\pi a) \tag{5}$$

Thus, by plotting the decrement of potential with distance, the input resistance and space constant can be directly determined, and then, from these parameters, the specific membrane resistance and internal resistivity.

There are several methods for determining the specific capacitance of the membrane, all depending on measurements of the time course of potential changes following application of a step of current. The method used here consists of measuring the time the electrotonic potential took to reach half its steady-state value. This time to half-maximum ($T_{1/2}$) depends on the electrode separation. Hodgkin and Rushton (1946) have shown empirically that in nerve axons a plot of $T_{1/2}$ against x approximates a straight line, with a slope of $\tau/2$. The time constant, τ, is an abbreviation for the product $R_mC_m = r_mc_m$. We have made such plots of $T_{1/2}$ against electrode separation but four or five measurements did not permit a reliable estimate of slope. One of these graphs is shown in Fig. 3 A. Fortunately, however, the "y intercept" of the curve, that is to say the time to half-maximum at zero electrode separation, $T^0_{1/2}$, is also a measure of the membrane time constant. This can be seen by writing the solution (Hodgkin and Rushton, 1946) of the full time-dependent cable equation for the case where $x = 0$:

$$V(t, x = 0) = V(t = \infty, x = 0) \operatorname{erf} \sqrt{t/\tau} \tag{6}$$

where $V(t = \infty, x = 0)$ is the steady-state potential at $x = 0$ and erf is an error function (defined and tabulated in Abramowitz and Stegun, 1964). Now, when $t = T^0_{1/2}$

$$\frac{V(t = T^0_{1/2})}{V(t = \infty)} = \frac{1}{2}; \text{ so } \frac{1}{2} = \operatorname{erf} \sqrt{T^0_{1/2}/\tau} \tag{7}$$

From the tables we find that erf (0.4769) = 0.50 and thus

$$\frac{T^0_{1/2}}{\tau} = (0.4769)^2 = 0.2274 \tag{8}$$

and

$$\tau = R_mC_m = 4.398\ T^\circ_{1/2} \tag{9}$$

The time constant was sometimes also determined by measuring (at small electrode separation) the time the voltage took to reach 84% of its final value (Hodgkin and Rushton, 1946). The method of extrapolation had the advantage over this latter method in that it combined data taken at a variety of electrode separations.

Analyses of both capacitance and resistance were done in many glycerol-treated fibers in which the transverse tubular system was disrupted; the results are shown in Table I. The steady-state parameters (space constant and input resistance) lie within the range found in normal muscle fibers.

The derived values of membrane resistance and internal resistivity are normal also. The time-dependent properties, however, differ strikingly from those of normal fibers. The membrane time constant is about one-third of normal and the mean value of the membrane capacitance (2.24 $\mu F/cm^2$) is similarly reduced.

The largest source of error in these results is in the measurement of fiber diameter and the assumption that the fiber is a cylinder of circular cross section. In order to avoid these errors it has been common practice to assume

TABLE I

CABLE ANALYSIS IN GLYCEROL-TREATED FIBERS

Fiber code	V_m	Diameter	R_{input}	λ	R_m	R_i	C_m
	mv	*μ*	*kohms*	*mm*	*ohm cm²*	*ohm cm*	*μF/cm²*
3–8	−82	55	553	1.71	3266	154	2.0
3–9	−82	64	530	1.46	3110	233	2.3
8–4	−81	63	460	1.72	3150	169	2.0
8–9	−86	55	600	1.35	2808	213	1.9
8–10	−93	64	960	1.90	7376	329	1.9
8–11	−93	59	870	1.86	6057	261	2.3
8–12	−83	54	570	1.42	2780	188	2.3
8–13	−86	55	830	1.36	3899	290	2.8
8–14	−89	59	390	2.18	3188	100	1.5
8–15	−79	73	455	1.80	3755	211	1.7
8–16	−89	36	910	2.20	4614	88	2.7
8–17	−89	68	590	1.65	4212	267	2.1
8–18	−93	55	520	1.77	3179	140	2.3
15–18	−71	78	370	1.48	2682	239	1.9
15–19	−82	50	390	1.40	1731	112	3.9
15–20	−74	55	650	2.18	4903	142	1.6
15–21	−89	50	560	1.80	3200	125	2.9
				Mean	3759	192	2.24
				SEM	331	17	0.14

a value for internal resistivity and then calculate fiber diameters from equations (2–4) above. If an internal resistivity of 200 ohm-cm is assumed for each fiber (Fatt, 1964), and the data recalculated, the mean fiber properties are not significantly changed. If the internal resistivity is taken as 250 ohm-cm (Katz, 1948) the data are changed somewhat, R_m is raised and C_m decreased 12%. These differences tend to exaggerate the difference between the capacitance of glycerol-treated fibers and normal fibers.

In order to insure that glycerol itself does not have a direct effect on the membrane, perhaps by changing the thickness, dielectric constant, or resistivity of the muscle membrane, similar cable analyses were done in fibers while they were still in glycerol-Ringer when the transverse tubular system,

in electron micrographs, is still intact (Howell and Jenden, 1967; Eisenberg and Eisenberg, 1968). Table II shows the results from nine of these fibers. Most of these results were taken from fibers that had been immersed in glycerol-Ringer for more than 20 min. The mean membrane potential was higher (inside more negative) than in Ringer solution and the internal resistivity significantly lower. These observations suggest that glycerol acts, at least to some extent, like an impermeant solute and thus causes shrinkage of the fibers and consequent concentration of the intracellular contents.[1] Although we saw no change in fiber diameter, the intracellular volume could still have decreased. Blinks (1965) has shown that frog muscle fibers have a cross section in the shape of an indented triangle, so that changes in volume

TABLE II

CABLE ANALYSIS IN GLYCEROL

Fiber code	V_m	Diameter	R_{input}	λ	R_m	R_i	C_m
	mv	μ	*kohms*	*mm*	*ohm cm²*	*ohm cm*	$\mu F/cm^2$
4	−98	59	448	1.98	3326	127	4.9
5	−90	45	730	1.97	4146	123	4.8
6	−95	43	520	1.74	2443	87	7.3
7	−88	55	510	1.92	3383	126	6.2
11	−96	41	430	1.68	1874	69	11.7
12	−98	55	700	2.07	5014	161	4.2
13	−99	59	580	2.42	5262	134	6.1
14	−92	68	640	1.98	5483	241	4.0
15	−96	59	480	2.13	3793	126	6.0
				Mean	3858	133	6.1
				SEM	416	16	0.8

may cause little change in apparent diameter. Thus, the increase in membrane potential in the fibers in glycerol-Ringer could be caused by an increase in intracellular potassium concentration and the decrease in internal resistivity by an increase in the total concentration of electrolytes in the intracellular medium. Since the membrane resistance and capacitance are within the normal range for fibers of 50–60 μ diameter, it seems clear that glycerol does not change the resistive or capacitive properties of the membrane. If fiber diameters are calculated assuming that the mean internal resistivity (133 ohm-cm) applies to all the fibers, the membrane resistance and capacitance are not significantly changed.

The striking difference in the time course of electrotonic potentials in fibers with, in contrast to those without, tubules provides an easy method of

[1] Freygang et al. (1967) have shown, however, that hypertonic sucrose solutions raise the internal resistivity.

qualitatively determining whether disruption of the tubular system has occurred and has been so used in other experiments (Eisenberg and Gage, 1969; Gage and Eisenberg, 1969).

DISCUSSION

It has been suggested previously that the apparently large capacitance per unit length of muscle can be explained by the large amount of membrane area included in the transverse tubular system (Falk and Fatt, 1964). Since it has been clearly demonstrated that only a very small fraction of the transverse tubular system is intact in glycerol-treated fibers (Eisenberg and Eisenberg, 1968), it is very likely that the smaller capacitance of glycerol-treated fibers reflects the disruption of the transverse tubules. The capacitance of the remaining surface membrane is 2.2 μF/cm^2 giving a capacitance for the tubular membrane of 3.9 μF/cm^2. These figures are referred to 1 cm^2 of a simple cylinder of membrane, without tubular infoldings or invaginations; the figures will be further discussed in order to refer them to the area of the structures in which they are presumed to arise.

It is interesting to compare our figures with those reported earlier. Falk and Fatt (1964) investigated the capacitance of frog sartorius fibers by analyzing the potential changes produced by sinusoidal currents of different frequencies and reported a figure of 2.6 μF/cm^2 for the capacitance of the surface membrane and 4.1 μF/cm^2 for the tubular membrane. The agreement between the results of the two different types of experiment is quite striking.

The value of 2.2 μF/cm^2 for the surface membrane is more than double that for nerve membrane. It occurred to us therefore that a sufficient number of transverse tubules might be left intact in glycerol-treated fibers to give an overestimate of the "true" membrane capacitance. The results of an examination of the fine structure of similar fibers similarly treated (Eisenberg and Eisenberg, 1968) give some estimate of the percentage of tubules which might be left, though it should be pointed out that the fibers used in the present experiments were selected for high membrane potential whereas those used in the structural studies were not. If the figure of 1.6% is used as the best estimate of the fraction of the tubular system left in glycerol-treated fibers and the figure of 4 as the area of the transverse tubules relative to the surface, the true membrane area may calculated to be about 6% of the apparent surface area. This figure can be used to determine the capacitance of the surface membrane: $C_m^s = 2.2 \times (1 - 0.06) = 2.1$ μF/cm^2.

It is interesting to note the difference between Falk and Fatt's figure of 2.6 μF/cm^2 and ours of 2.1 μF/cm^2. If both these figures are taken as correct, the difference between the two enables a speculative estimate to be made of the depth to which high frequency current penetrates the sarcotubular system. If at the frequencies used by Falk and Fatt (from 3–10 kc/sec),

some of the tubular system is still contributing to the observed capacitance, a high figure for the surface capacitance would be obtained and the discrepancy would give some measure of the amount of tubular system through which current was still flowing. If the difference in the two figures is interpreted in this manner, it seems that the tubular system is contributing 0.5 $\mu F/cm^2$ of capacitance at these high frequencies. This 0.5 $\mu F/cm^2$ represents about 10% of the total tubular capacitance. In order to estimate the space constant of the tubules it is only then necessary to determine what depth of the tubular system includes 10% of the tubular area. If the tubular system is treated as a disc, the annulus which would contain 10% of the tubular system turns out to be very small, only a few micra deep. This suggests that rapid potential changes are greatly attenuated deep in the muscle fiber (see Falk, 1968). However, if there is any active conductance change which "propagates" down the tubules, the above argument would lose most of its usefulness.

The figure for the capacitance of the surface membrane of 2.1 $\mu F/cm^2$ is significantly higher than that for nerve (about 1.0 $\mu F/cm^2$, Taylor, 1965). A discussion of the difference may not be very meaningful since the experimental situations in the two cases are so different. The major source of uncertainty in our figure is the actual amount of membrane in 1 cm length of fiber. This figure is subject to systematic error due to such factors as wrinkling of the surface membrane, deviation from circular cross section of the fiber, and to random error in the measurements involved. The figure for the capacitance of nerve is subject to a different source of error, namely that caused by the apparent frequency dependence of the measured capacitance (Cole and Curtis, 1950; Taylor, 1965). These factors probably do not account for all of the difference between the capacitance of the two membranes and thus the possibility remains that the membrane of muscle has a higher specific capacitance than that of nerve.

The specific capacitance of biological membranes depends on their average thickness and dielectric constant. If the membrane of the sarcolemma were thinner than that of nerve, or if the average dielectric constant greater, the higher value of the specific capacitance of muscle membrane would be explained. The average dielectric constant could be greater than that of nerve for two reasons: either the lipid composition of the membranes might differ, the lipid of the sarcolemma being more polar or more polarizable, or the muscle membrane might have a higher fraction of very high dielectric constant regions, like those which would constitute aqueous channels for the flow of ions. Given the present state of knowledge of the molecular structure of membranes it is impossible to decide between these hypotheses.

Finally, to obtain the specific capacitance of the tubular membrane, estimates of the ratio of tubular to surface membrane area can be used. The best figure for the capacitance of the tubular system is given by the difference

between the total capacitance of a normal fiber and the figure obtained here for the capacitance of the sarcolemma. Thus, the capacitance of the tubular system is 6.1 − 2.1 = 4.0 μF/cm^2. The area implicit in this measurement is the area of the outer membrane. The data of Peachey (1965) and of Peachey and Schild (1968) suggest that the appropriate figure for the ratio of areas in a 50 μ diameter fiber is 4.5. If this represents an overestimate of the area of tubular membrane (Eisenberg and Eisenberg, 1968), the ratio could be somewhat less than this. When the ratio is taken as approximately 4 the capacitance of the tubular membrane becomes 1.0 μF/cm^2. If this figure is correct, it suggests that the tubular membrane has a different thickness

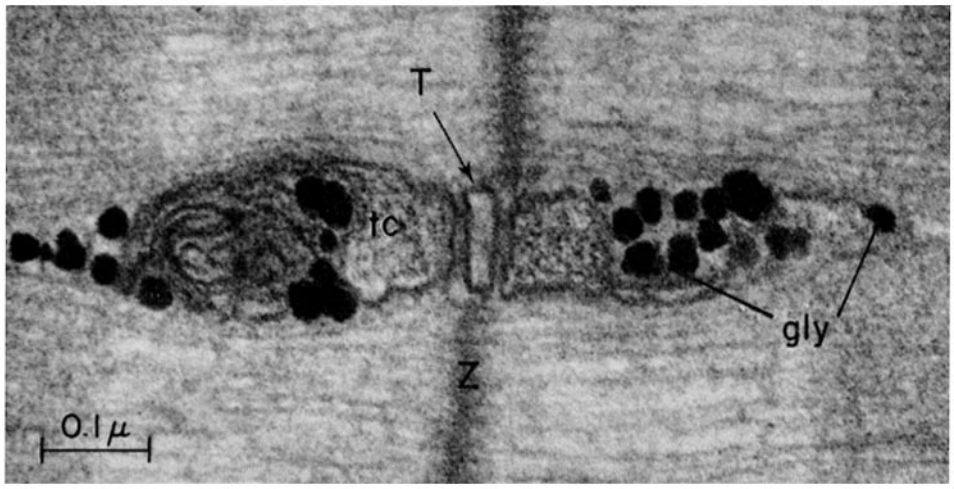

FIGURE 4. Longitudinal section of frog sartorius muscle: The triadic elements are shown cut in cross-section. The arrow points to the zone of the transverse tubule (*T*) which is not covered by terminal cistena (*tc*). *Z* line (*Z*) and glycogen granules (*gly*) are shown. Prepared for examination in the electron microscope as described by Peachey (1965). The micrograph was graciously supplied by Brenda Eisenberg.

or dielectric constant than the outer membrane. This is not totally implausible since the two membranes have different permeabilities to ions (Eisenberg and Gage, 1969). Another explanation for the apparent difference between the specific capacitance of the tubular membrane and the surface membrane is possible, however. Fig. 4 shows a micrograph of a triad, that is to say of the characteristic structure found where the transverse tubule forms an intimate relation with the membranes of the sarcoplasmic reticulum. It should be noted that in the triad there are really two types of tubular membrane: that which is "covered" by reticular membrane and that which is bare. If it is assumed that the junction between the two membranes is what has been called a capacity-coupled tight junction (Martin and Pilar, 1964),

then the specific capacitance of that region of membrane would be less than that elsewhere. Physically, this is because the junction is behaving, in such a case, like a single membrane of about double or triple normal thickness. Peachey (1965) estimates that 27% of the tubule is uncovered and 73% in close apposition to the reticular membrane. If the specific capacitance of the latter is assumed to be one-third that of the rest of the membrane, the figure of 1 $\mu F/cm^2$ for the capacitance of the tubular membrane becomes compatible with the 2.1 $\mu F/cm^2$ for the surface. The 73% of the membrane would contribute $\frac{1}{3} \times (0.73\ K)$ to the capacitance where K is the specific capacitance of the tubular membrane. The bare membrane would contribute $0.27\ K$ to the capacitance. If these two terms are added and the sum set equal to the figure of 1.03 $\mu F/cm^2$, the specific capacitance of the tubular membrane becomes 2.0 $\mu F/cm^2$, a figure which is in reasonable agreement with our figure for the specific capacitance of the surface membrane. In this manner the difference between the figures for the capacitance of the tubular membrane and surface membrane can be explained without invoking hypothetical differences in molecular structure.

Note Added in Proof It has recently been brought to our attention that Dr. S. A. Krolenko has published several papers on glycerol-treated muscle fibers. The most recent paper is in *Tsitologiya.* 1968. **10:** 803.

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REFERENCES

ABRAMOWITZ, M., and I. A. STEGUN. 1964. Handbook of Mathematical Functions. National Bureau of Standards, Washington, D. C.

ANDERSSON-CEDERGREN, E. 1959. Ultrastructure of motor end plate and sarcoplasmic components of mouse skeletal muscle fiber as revealed by three dimensional reconstructions from serial sections. *J. Ultrastruct. Res.* Suppl. 1. 1.

BLINKS, J. R. 1965. Influence of osmotic strength on cross-section and volume of isolated single muscle fibers. *J. Physiol. (London).* **177:**42.

COLE, K. S., and H. J. CURTIS. 1950. Bioelectricity: Electric Physiology. *In* Medical Physics. Otto Glaser, editor. Year Book Publishers, Chicago. **2:**82.

EISENBERG, B., and R. S. EISENBERG. 1968. Selective disruption of the sarcotubular system in frog sartorius muscle. *J. Cell Biol.* **39:**451.

EISENBERG, R. S., and P. W. GAGE. 1967. Changes in the electrical properties of frog skeletal muscle fibers after disruption of the transverse tubular system. *Science.* **158:**1700.

EISENBERG, R. S., and P. W. GAGE. 1969. Ionic conductances of the surface and transverse tubular membranes of frog sartorius fibers. *J. Gen. Physiol.* **53:**279.

FALK, G. 1968. Predicted delays in the activation of the contractile system. *Biophys. J.* **8:**608.

FALK, G., and P. FATT. 1964. Linear electrical properties of striated muscle fibers observed with intracellular electrodes. *Proc. Roy. Soc. Ser. B. Biol. Sci.* **160:**69.

FATT, P. 1964. An analysis of the transverse electrical impedance of striated muscle. *Proc. Roy. Soc. Ser. B. Biol. Sci.* **159:**606.

FATT, P., and B. KATZ. 1951. An analysis of the end plate potential recorded with an intracellular electrode. *J. Physiol. (London).* **115:**320.

FREYGANG, W. H., S. I. RAPOPORT, and L. D. PEACHEY. 1967. Some relations between changes in the linear electrical properties of striated muscle fibers and changes in ultrastructure. *J. Gen. Physiol.* **50**:2437.

GAGE, P. W., and R. S. EISENBERG. 1969. Action potentials, afterpotentials, and excitation-contraction coupling in frog sartorius fibers without transverse tubules. *J. Gen. Physiol.* **53**:298.

HODGKIN, A. L., and W. A. H. RUSHTON. 1946. The electrical constants of a crustacean nerve fiber. *Proc. Roy. Soc. Ser. B. Biol. Sci.* **133**:144.

HOWELL, J. N., and D. J. JENDEN. 1967. T-tubules of skeletal muscle: morphological alterations: which interrupt excitation-contraction coupling. *Fed. Proc.* **26**:553. (To be published in full 1969. *J. Physiol. (London).*)

KATZ, B. 1948. The electrical properties of the muscle fiber membrane. *Proc. Roy. Soc. Ser. B. Biol. Sci.* **135**:506.

MARTIN, A. R., and G. PILAR. 1964. An analysis of electrical coupling at synapses in the avian ciliary ganglion. *J. Physiol. (London).* **171**:454.

MOORE, J. W. 1963. Operational amplifier. *In* Physical Techniques in Biological Research. W. L. Nastuk, editor. Academic Press, Inc., New York. **6B**:77.

PEACHEY, L. D. 1965. The sarcoplasmic reticulum and transverse tubules of the frog's sartorius. *J. Cell Biol.* **25**(3,Pt. 2):209.

PEACHEY, L. D., and R. F. SCHILD. 1968. The distribution of the T-system along the sarcomeres of frog and toad sartorius muscle. *J. Physiol.* **194**:249.

PHILBRICK, 1966. Applications Manual for Computing Amplifiers, Philbrick Researches, Dedham, Mass.

TAYLOR, R. E. 1965. Impedance of the squid axon membrane. *J. Cell and Comp. Physiol.* **66**:21.