# The Capacitance of Skeletal

# Muscle Fibers in Solutions of

# Low Ionic Strength

P. C. VAUGHAN, J. N. HOWELL, and R. S. EISENBERG

From the Department of Physiology, University of California at Los Angeles, Los Angeles, California 90024. Dr. Vaughan's present address is the Department of Physiology, University of British Columbia, Vancouver, British Columbia, Canada. Dr. Howell's present address is the Department of Physiology, University of Pittsburgh, Pittsburgh, Pennsylvania 15213.

ABSTRACT  The capacitance of skeletal muscle fibers was measured by recording with one microelectrode the voltage produced by a rectangular pulse of current applied with another microelectrode. The ionic strength of the bathing solution was varied by isosmotic replacement of NaCl with sucrose, the [K] [Cl] product being held constant. The capacitance decreased with decreasing ionic strength, reaching a value of some 2 $\mu F/cm^2$ in solutions of 30 mM ionic strength, and not decreasing further in solutions of 15 mM ionic strength. The capacitance of glycerol-treated fibers did not change with ionic strength and was also some 2 $\mu F/cm^2$. It seems likely that lowering the ionic strength reduces the capacitance of the tubular system (defined as the charge stored in the tubular system), and that the 2 $\mu F/cm^2$ which is insensitive to ionic strength is associated with the surface membrane. The tubular system is open to the external solution in low ionic strength solutions since peroxidase is able to diffuse into the lumen of the tubules. Twitches and action potentials were also recorded from fibers in low ionic strength solutions, even though the capacitance of the tubular system was very small in these solutions. This finding can be explained if there is an action potential–like mechanism in the tubular membrane.

## INTRODUCTION

The volume of the transverse tubular (T) system of frog skeletal muscle is sensitive to changes in the osmolarity of the bathing medium (Freygang et al., 1964): increasing the osmolarity produces swelling of the tubules. This swelling seems to involve an increase in the total area of the membranes of the tubular system, as well as a change in the shape of the tubules, and thus it is not surprising that the capacitance of muscle fibers in these solutions is increased (Freygang et al., 1967). The T system also swells in isotonic solutions of low ionic strength (Rapoport et al., 1969), perhaps because of osmotic effects

associated with fixed charges located within the lumen of the tubules (Rapoport, 1969). We have investigated the capacitance of muscle fibers in isotonic solutions of low ionic strength, expecting to find an increase in capacitance produced by the swelling of the T system.

We have found, however, that the total capacitance of muscle fibers (capacitance of the tubular system plus the capacitance of the surface membrane measured by rectangular current pulses applied with a microelectrode) decreases in solutions of low ionic strength. Since ionic strength has no effect on the capacitance of glycerol-treated preparations, which have little intact tubular system (Howell, 1969; Krolenko, 1969; Eisenberg and Eisenberg, 1968; Gage and Eisenberg, 1969), the effect of lowering ionic strength appears to be a reduction in the capacitance associated with the tubular system. This reduction is not produced by a break in the connection between the tubules and the extracellular space since an extracellular marker can diffuse into the lumen of the tubules and since a normal twitch is present in these low ionic strength solutions.

The presence of a normal twitch in solutions of low ionic strength, in which the tubular capacitance is virtually absent, can be explained by regenerative electrical properties of the tubular membrane.

An abstract concerning these results has been presented (Vaughan et al., 1970).

## METHODS

### Preparation

The experiments described were performed on the semitendinosus and sartorius muscles from both small and large frogs (Rana pipiens: body length about 4 or 7 cm, respectively). Usually a "bundle" of some 100 fibers was dissected from the semitendinosus muscle, but in early experiments, in which the best measurements of fiber diameter were made, the bundles were much smaller (10–40 fibers). Sartorius muscles were obtained from large frogs only, and were not further dissected. Microelectrodes were inserted into surface fibers on the deep side of the sartorius.

### Measurement of Fiber Diameter

In each preparation an estimate of the mean fiber diameter was made by visual inspection at a magnification of 100 using an eyepiece graticule in a stereoscopic microscope. The most accurate measurements were probably those made on small bundles of semitendinosus fibers where edge fibers could be seen clearly. Errors in this measurement, which are probably the largest uncertainty in our results, appear most directly in the computed value of the internal resistivity $R_i$. The average value of $R_i$ (calculated assuming that the muscle fibers were circular in cross-section) from all measurements made at normal [K] [Cl] product is 162 ± 16 ohm cm (mean ± standard error [SE] of the mean) which is in good agreement with the more precise figure of 169 ohm cm determined by Nakajima and Hodgkin (1970). The experimental

data were not significantly changed if diameters were calculated from an assumed value of $R_i$ = 169 ohm cm and indeed the data from the muscles in the "0.24 Ringer" solution were analyzed in this manner.

### Electrical Measurements

The electrical properties of the fibers were measured by applying rectangular pulses of current to the fiber with one microelectrode (filled with 3 M KCl, resistance between 5 and 15 M $\Omega$) and recording the change in potential with another electrode. Measurements of the linear properties were made with small hyperpolarizing currents (5 $\times$ $10^{-9}$ amp) which produced potential changes of less than 5 mv. The circuit used for recording potential has been described previously (Gage and Eisenberg, 1969). Rectangular voltage pulses, obtained from a conventional stimulator, were converted into current pulses by a circuit with an effective output resistance of greater than $10^{11}$ ohms (New, 1972).[1]

The electrical parameters of the cell (namely, the input resistance, the length constant, and the time constant) were measured by the methods of Fatt and Katz (1951), except that the membrane time constant was determined from half-decay times as described by Gage and Eisenberg (1969).

### Solutions and Tension Measurements

Table I lists the solutions used in these experiments. The solutions were designed according to two principles. (a) The ionic strength and conductivity of the solution were altered by replacing NaCl with osmotically equivalent amounts of sucrose. All solutions were adjusted to an osmolarity of 230 mosmol/liter as measured by freezing point depression. (b) Solutions were usually made with normal [K] [Cl] product in order to avoid possible changes in the volume of intracellular compartments associated with bulk movements of KCl. In the solution of lowest conductivity ("0.12 IS") it was not possible to maintain constant [K] [Cl] product and some experiments were performed in solutions made by simple substitution of sucrose for NaCl ("0.24 Ringer"). However, the change in fiber volume would be expected to be less than 2.5% and no changes in diameter could be observed. Resting potentials were in general those that would be predicted from the external potassium concentration (Hodgkin and Horowicz, 1959). Tension measurements were made "isometrically" with an RCA 5734 transducer.

### Glycerol Treatment

Muscle fibers were soaked for 1 hr in a Ringer solution to which 400 mM glycerol had been added. They were then transferred to a Ringer solution containing elevated $Mg^{++}$ and $Ca^{++}$ concentrations since such solutions disrupt the tubular system while preserving high resting potentials (solution G of Table I in Eisenberg et al., 1971).

### Electron Microscope

Small bundles dissected from the semitendinosus were exposed to low ionic strength solutions containing 0.05% horseradish peroxidase (type II, Sigma Chemical Co.,

[1] New, W., Jr. 1972. A simple wideband constant current source. Manuscript submitted.

St. Louis, Mo.) for 40 min before being fixed for 1 hr with 8 % glutaraldehyde buffered at pH 7.2 with 100 mM sodium cacodylate and containing 2 mM $CaCl_2$. The bundles were then rinsed for 1 hr in 100 mM sodium cacodylate plus 2 mM $CaCl_2$ and then exposed to the incubation solution for 1 hr, which solution was the same as that used by Eisenberg and Eisenberg (1968), and contained 0.05 % 3,3'-diaminobenzidine tetrahydrochloride, 0.01 % $H_2O_2$, and 0.05 M tris(hydroxymethyl)amino methane (Tris)–maleate buffer, pH 7.6. The bundles were postfixed with 1 % $OsO_4$ in 100 mM sodium cacodylate at pH 7.2 and then dehydrated in alcohol and transferred to propylene oxide and then to Epon for embedding. Sections were either left unstained or were doubly stained with uranyl acetate and lead citrate and examined under a Siemens Elmiskop 1A electron microscope. Survey micrographs were examined to determine the fraction of potential T-tubule sites where peroxidase reaction product could be detected. No attempt was made to examine only superficial

TABLE I

SOLUTIONS

| Name | $Na^+$ | $K^+$ | $Ca^{++}$ | $Cl^-$ | Sucrose | $HPO_4^-$ | $H_2PO_4^-$ | Ionic strength |
|---|---|---|---|---|---|---|---|---|
| | meq | meq | meq | meq | mm | meq | meq | mm |
| Ringer | 118 | 2.50 | 1.08 | 119.7 | 0 | 1 | 1 | 125 |
| 0.50 IS | 53 | 5.20 | 1.08 | 57.4 | ~125 | 1 | 1 | 63 |
| 0.24 IS | 13 | 12.25 | 1.08 | 24.4 | ~190 | 1 | 1 | 30 |
| 0.24 Ringer | 22.8 | 2.50 | 1.08 | 24.4 | ~190 | 1 | 1 | 30 |
| 0.12 IS | 3 | 7.5 | 1.08 | 9.66 | ~221 | 1 | 1 | 15 |

fibers and it was clear that the reaction product often did not penetrate uniformly throughout the bundle. Counting of T-tubule sites was restricted to fibers which had a conspicuous layer of reaction product at the cell edge.

RESULTS

*Capacitance in Solutions of Normal* [K] [Cl] *Product*

Analyses were made of the passive electrical properties of muscle fibers in solutions of low ionic strength. The solutions were made with normal [K] [Cl] product in order to prevent movements of potassium and chloride across the cell membranes or changes in the concentration of these ions in the sarcoplasm or subcellular structures. Table II presents the results of these analyses. The membrane resistance $R_m$ (measured in ohm centimeters²) and the total capacitance $C$ (in microfarads per square centimeter) were calculated for each fiber from the measured length constant, input resistance, and time constant using either a measured or calculated value of the fiber diameter. (See the Methods for references to more detailed discussion of these techniques.) The values shown are the mean ± standard error of the mean ($n$ = number of fibers measured). Figures for the membrane resistance are given

for the sake of completeness, but the variation in potassium and chloride concentration makes the interpretation of these measurements depend on indirect argument. Furthermore, large variations in $R_m$ were found (compare the values for 0.24 IS in Tables II and III) which were not random and seemed to depend on some uncontrolled variable, perhaps the season of the year or the reproductive state of the frogs. Capacitance clearly declines as ionic strength is reduced. It was important to determine whether the capacitance continued to decline as ionic strength was lowered, or whether it reached some minimum value. In order to further reduce ionic strength it was neces-

TABLE II

CHANGES IN CAPACITANCE OF NORMAL MUSCLE

| Solution | $n^*$ | $\bar{R}_m\ddagger$ | $\bar{R}_i$ | $a$ | $\bar{C}$ |
|---|---|---|---|---|---|
| | | *ohm cm²* | *ohm cm* | *μ* | *μF/cm²* |
| Ringer | 59 | 3311 | 151 | 23, 38§ | 4.75 |
| | | (177)‖ | (9) | | (0.23) |
| 0.5 IS | 35 | 4576 | 167 | 23, 38 | 3.65 |
| | | (464) | (22) | | (0.17) |
| 0.24 IS | 11 | 2973 | 207 | 23 | 2.08 |
| | | (285) | (30) | | (0.37) |
| 0.12 IS | 14 | 8810 | 151 | 38 | 2.19 |
| | | (967) | (53) | | (0.21) |

* $n$ is the number of observations.
‡ A superscript bar denotes mean value.
§ Two figures for the radius are given when experiments were performed on two populations of fibers.
‖ Numbers in parentheses are standard errors of the mean.

sary to reduce the [K] [Cl] product. The 0.12 IS solution was designed to maintain a reasonably high [K] [Cl] product while lowering the ionic strength by another factor of two. It can be seen that the capacitance in this solution was essentially the same as that in the 0.24 IS solution, indicating that the capacitance had reached a minimum value.

### Capacitance in Solutions of Normal K Concentration

Since it seemed possible that the changes in capacitance shown in Table II might be caused by the changes in potassium concentration used to maintain normal [K] [Cl] products, further experiments were made to test the effects of ionic strength at normal K concentration. Table III shows the results of capacitance measurements in the solution "0.24 Ringer." These results do not seem to differ substantially from the results measured with different potassium concentrations. The complete data from two sets of measurements (made in solutions Ringer, 0.24 IS and 0.24 Ringer) are shown in order to

TABLE III

CAPACITANCE MEASUREMENTS IN THREE SOLUTIONS

| Solution | Diameter* | Input resistance | Length constant | Time constant | $R_m$ | $C$ |
|---|---|---|---|---|---|---|
| | $\mu$ | kohm | mm | msec | ohm cm² | µF/cm² |
| Ringer | 45.4 | 1110 | 2.13 | 28.6 | 6756 | 4.23 |
| | 68.1 | 556 | 2.40 | 24.5 | 5701 | 4.31 |
| | 55.8 | 839 | 2.43 | 25.21 | 7153 | 3.52 |
| | 66.9 | 498 | 2.10 | 22.6 | 4327 | 5.21 |
| | 65.2 | 628 | 2.48 | 25.0 | 6381 | 3.93 |
| | **60.3‡** | **726** | **2.31** | **25.18** | **6064** | **4.24** |
| | (±4.3)§ | (±112) | (±0.08) | (±0.97) | (±496) | (±0.28) |
| 0.24 IS | 44.0 | 942 | 1.70 | 13.2 | 4398 | 3.00 |
| | 50.4 | 703 | 1.66 | 10.1 | 3719 | 2.72 |
| | 58.5 | 620 | 2.00 | 7.5 | 4491 | 1.70 |
| | 46.4 | 786 | 1.57 | 7.0 | 3684 | 1.91 |
| | 51.3 | 717 | 1.75 | 10.6 | 4046 | 2.62 |
| | 53.9 | 645 | 1.74 | 8.7 | 3803 | 2.29 |
| | 69.2 | 501 | 2.23 | 17.5 | 4862 | 3.60 |
| | 59.2 | 682 | 2.22 | 11.9 | 5639 | 2.11 |
| | 89.1 | 416 | 3.10 | 12.5 | 7140 | 1.75 |
| | 74.8 | 487 | 2.50 | 15.1 | 5803 | 2.61 |
| | 78.3 | 442 | 2.50 | 12.1 | 5470 | 2.21 |
| | 46.1 | 1005 | 1.98 | 16.0 | 5760 | 2.79 |
| | **60.1** | **662** | **2.08** | **11.9** | **4901** | **2.44** |
| | (±4.2) | (±54) | (±0.13) | (±1.0) | (±310) | (±0.16) |
| 0.24 Ringer | 66.9 | 702 | 2.87 | 20.5 | 8383 | 2.44 |
| | 60.0 | 793 | 2.66 | 20.0 | 7972 | 2.51 |
| | 63.9 | 756 | 2.87 | 29.4 | 9993 | 2.94 |
| | 53.8 | 1045 | 2.81 | 25.4 | 11348 | 2.24 |
| | 80.4 | 493 | 2.96 | 16.8 | 9600 | 1.75 |
| | 78.1 | 450 | 2.55 | 15.8 | 6418 | 2.46 |
| | 66.8 | 1175 | 4.87 | 27.8 | 24010 | 1.16 |
| | 47.3 | 1001 | 2.08 | 27.9 | 6175 | 4.53 |
| | 81.8 | 626 | 3.90 | 38.0 | 12498 | 3.04 |
| | 33.0 | 2489 | 2.50 | 64.4 | 13063 | 4.93 |
| | 73.9 | 838 | 4.30 | 32.2 | 16598 | 1.94 |
| | 79.0 | 647 | 3.76 | 37.4 | 12076 | 3.10 |
| | **65.4** | **918** | **3.18** | **29.6** | **11511** | **2.75** |
| | (±4.3) | (±156) | (±0.24) | (±3.8) | (±1426) | (±0.31) |

* The diameter was calculated from an assumed value of $R_i$ = 169 ohm cm² (Nakajima and Hodgkin, 1970).

‡ Numbers in boldface type are the mean values.

§ Numbers in parentheses are standard errors of the mean.

illustrate the scatter of the data. It is interesting to note that the measured parameters (input resistance, length constant, and time constant) differ substantially in the two solutions, presumably reflecting the different ionic constituents, yet there is not a large difference in the value of the capacitance.

## Capacitance of Glycerol-Treated Fibers

Since the previous experiments showed that a minimum value of capacitance of about 2 $\mu F/cm^2$ was reached in solutions of low ionic strength, it seemed likely that the effect of ionic strength might be on just one component of the fiber capacitance, the capacitance of the tubular system. In order to test this possibility capacitance was measured on glycerol-treated preparations, which have little intact T system. The value of the capacitance of glycerol-treated fibers (Table IV) is in good agreement with that reported by Gage and Eisenberg (1969) and does not vary significantly with ionic strength.

The capacitance measurements are summarized in Fig. 1. The filled circles are means of measurements on normal fibers, the open circles means of

### TABLE IV
### CAPACITANCE OF GLYCEROL-TREATED FIBERS

| Solution | $n^*$ | $\bar{R}_m\ddagger$ | $\bar{R}_i$ | $a$ | $\bar{C}_m$ |
|----------|-------|---------------------|-------------|-----|-------------|
|          |       | *ohm cm²*           | *ohm cm*    | $\mu$ | *$\mu F/cm^2$* |
| Ringer   | 14    | 3516                | 113         | $\begin{cases} 25 \\ 35 \\ 45 \end{cases}$ | 2.31 |
|          |       | (464)§              | (12)        |     | (0.37) |
| 0.12 IS  | 8     | 9474                | 129         | 33  | 2.26 |
|          |       | (1286)              | (20)        |     | (0.24) |

\* $n$ is the number of observations.
‡ A superscript bar denotes mean value.
§ Numbers in parentheses are standard errors of the mean.

measurements on glycerol-treated fibers. The vertical lines represent ±1 standard error (SE) of the mean. The scale on the right-hand side of the figure represents the capacitance of the tubular system $(C_e)$, assuming that 2 $\mu F/cm^2$ of capacitance arises in some other system of membranes (primarily the surface membrane). Thus, the distance between the points and the dotted line is meant to represent the capacitance of the tubular system.

### Localization of Exogenous Peroxidase in Muscles Bathed in Solutions of Low Ionic Strength

It seemed possible that solutions of low ionic strength might seal off the openings of the tubular system, thus preventing current from entering the T system or crossing the tubular membranes. One way to test this hypothesis is to apply an extracellular marker to a muscle and then to determine the location of this marker. Eisenberg and Eisenberg (1968) have shown that horseradish peroxidase is a reliable marker of extracellular space, filling almost all of the tubular system with an electron-opaque reaction product. We have therefore soaked bundles of muscle fibers in solutions of low ionic strength

(0.24 IS) for 30 min, then for 40 min in the same solution containing 0.05% peroxidase, and then fixed the muscle in 8% glutaraldehyde. The other preparative procedures are described in Methods.

In two control preparations, exposed to horseradish peroxidase in normal Ringer, 447 tubular sites were counted; of these 364, or 81%, contained reaction product. This agrees closely with the figures of Eisenberg and Eisenberg (1968) who reported finding T tubules at 83.7% of the potential sites. In three low ionic strength preparations, 1666 sites were counted; of these 1425, or
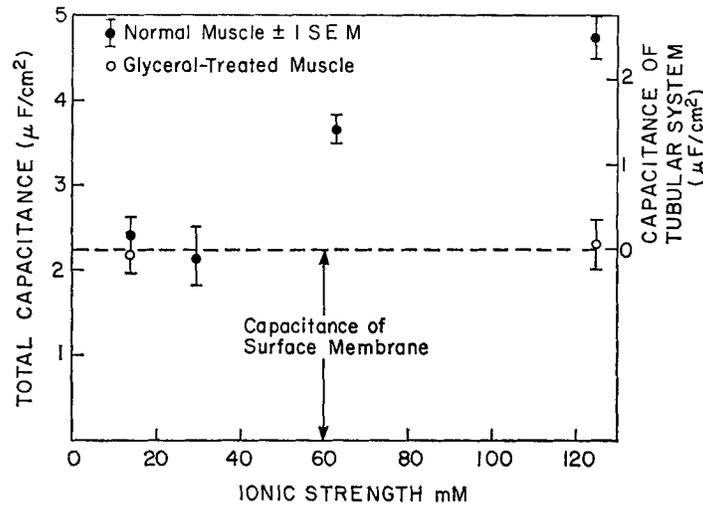


FIGURE 1. The capacitance of muscle fibers in solutions of low ionic strength. The filled circles represent the capacitance of normal muscle fibers and the vertical lines show ±1 SE of the mean. The open circles show the capacitance of glycerol-treated fibers, which capacitance presumably represents the capacitance of the surface membrane.

86%, contained reaction product. These figures suggest that the T tubules were not closed off from the extracellular space by the low ionic strength solutions.

The observation of Rapoport et al. (1969) that the T tubules swell in low ionic strength solution was confirmed. However, the swelling was less obvious in the peroxidase-treated fibers than in fibers exposed to low ionic strength solutions which were devoid of peroxidase.

*Mechanical and Electrical Responses of Fibers Bathed in Solutions of Low Ionic Strength*

A series of experiments were performed to determine the mechanical responses of small bundles of fibers bathed in low ionic strength solutions. Solution 0.24 Ringer was chosen since it had a high enough sodium concentration

to produce action potentials (Fig. 2) and yet had almost no tubular capacitance (Fig. 1). The action potentials recorded in these solutions had a reduced rate of rise and decreased overshoot as would be expected from the "sodium hypothesis" (Hodgkin and Katz, 1949). Twitches, however, were essentially normal (Fig. 2). The length of the bundle was adjusted to give maximum twitch tension, and the stimulus strength set to a just supramaximal value. The ratio of peak twitch tension in the solution of 0.24 Ringer to that in normal Ringer was 1.2 ± 0.1 (three bundles).
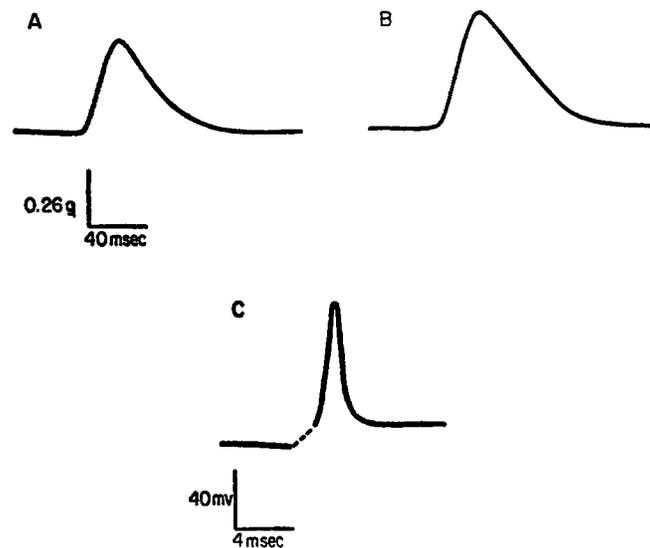
FIGURE 2. Twitch and action potential in low ionic strength solutions. (A) shows the twitch with a just supramaximal stimulus of a muscle fiber in normal Ringer. (B) shows a twitch with a just supramaximal stimulus of a muscle fiber in a low ionic strength Ringer (solution 0.24 Ringer). (C) shows an action potential recorded in this low ionic strength Ringer. The resting potential was 88 mv.

Muscle fibers often did not seem to survive repetitive stimulation in this solution so measurements were made with as few stimuli as possible, just enough to insure that the stimulus strength was supramaximal. Overton (1902) has made an extensive study of the effect of low ionic strength solutions on twitch tension. Our results support his conclusion that "Until the concentration of NaCl is lowered to 0.15% [20 mM] one cannot detect, at least without graphic methods, any difference in excitability or contraction strength from normal muscles" (our translation).

DISCUSSION

*Separation of the Total Capacitance into Its Components*

A discussion of the capacitance of muscle fibers requires the separation of this capacitance into its components, each component presumably representing

the capacitance of some system of membranes within the fiber. The total capacitance of a muscle fiber is written as the sum of the capacitance of the surface membrane $C_m$ and the tubular system $C_e$ :

$$C = C_m + C_e .\tag{1}$$

One method of determining $C_m$ has been to measure the total capacitance of glycerol-treated preparations. Eisenberg and Eisenberg (1968) conclude that the tubular system in these preparations is almost entirely disrupted, about 2% of the tubular system remaining intact. Thus, if we assume that the glycerol treatment has no effect on the capacitance of the surface membrane, the total capacitance of glycerol-treated fibers can be represented as

$$C_{glyc} = C_m + \epsilon C_e\tag{2}$$

with $\epsilon \simeq 0.02$. Using this type of analysis, Gage and Eisenberg (1969) concluded that $C_m = 2.1 \ \mu F/cm^2$ and $C_e = 4.0 \ \mu F/cm^2$. Recent measurements of capacitance of glycerol-treated fibers by Nakajima and Hodgkin (1970) have given a probably more precise figure of $1.9 \ \mu F/cm^2$ for $C_m$ determined by rectangular pulse measurements, and $0.9 \ \mu F/cm^2$ for the capacitance determined from the foot of the action potential. The difference between these two figures suggests that there is a component of capacitance, about $1 \ \mu F/cm^2$, present in glycerol-treated fibers, which is measurable at the relatively long times used in rectangular pulse measurements, but which is not measurable at the short times used in measurements from the foot of the action potential. This behavior would occur if there were a component of the capacitance of glycerol-treated fibers which is in series with some resistance. The anatomical correlate of this component of capacitance is puzzling; it could of course come from tubules remaining in the glycerol-treated preparation, but this would imply that at least 25% of the tubular system is intact ($\epsilon = 0.25$) and accessible to current flow in glycerol-treated fibers, a figure hard to reconcile with the figure of 2% ($\epsilon = 0.02$) for the fraction of the tubular system which is accessible to peroxidase (Eisenberg and Eisenberg, 1968) or with many of the qualitative properties of glycerol-treated fibers. Another possibility is that there is some system of membranes, other than the surface membrane, which has resistance in series with it and which accounts for about $1 \ \mu F/cm^2$ of capacitance. Finally, an extra component would appear if the phase angle of the membrane capacitance were significantly different from $90°$. Further discussion of these points can be found in Eisenberg (1971).

The other method for separating the total capacitance into two components involves measurements of impedance over a wide range of frequencies (Falk and Fatt, 1964; Freygang et al., 1967; Schneider, 1970; Eisenberg, 1971) and the subsequent fitting of these measurements by different equivalent circuits. The two circuits usually considered represent the electrical behavior

of the tubular system in different ways: one, the four-element or lumped circuit, places all the tubular membrane in series with a single resistor; the other, the distributed circuit, treats the T system as a dense network of tubules, the core of which offers significant resistance to the flow of current.

Unfortunately, the different circuits when applied to the same data give different values for $C_m$ and $C_e$ ; the lumped model gives roughly $C_m = 2.6$ $\mu F/cm^2$, $C_e = 3.4$ $\mu F/cm^2$ (Falk and Fatt, 1964; Freygang et al., 1967) whereas the distributed model gives $C_m = 1$ $\mu F/cm^2$, $C_e = 5$ $\mu F/cm^2$ (Schneider, 1970). The two models give different figures because one (the lumped model) attributes almost all the capacitance present at frequencies of some 1–10 kcycle/sec to the surface membrane, whereas the other, the distributed model, attributes some of the capacitance measured at these frequencies to the tubular system. Thus, impedance measurements presently available do not provide an unambiguous determination of the capacitance of the surface and tubular membranes.

### The Effects of Ionic Strength

The effects of ionic strength on the total capacitance strongly suggest that there are two components of membrane capacitance. Our experiments show that lowering the ionic strength can decrease the total capacitance to about 2 $\mu F/cm^2$; thus, 2 $\mu F/cm^2$ of the total capacitance seems to be independent of ionic strength, whereas the rest of the capacitance (about 3 $\mu F/cm^2$ in these experiments) depends strongly on ionic strength. The experiments on glycerol-treated preparation show that the capacitance of those fibers (also about 2 $\mu F/cm^2$) is insensitive to ionic strength. It is natural to conclude from these results that the effect of ionic strength is entirely on the tubular capacitance, reducing it to essentially zero in solutions below 30 mM ionic strength. The following paper analyzes this effect in more detail and attempts to interpret this result in terms of a distributed model of the tubular system. Similarly, it is natural to identify the 2 $\mu F/cm^2$ of capacitance of glycerol-treated fibers (which is independent of ionic strength) with the 2 $\mu F/cm^2$ of capacitance of intact fibers which is independent of ionic strength, and to attribute this capacitance to structures associated with the surface membrane.

There is at least one alternate explanation of these results. It is possible that the capacitance which is insensitive to ionic strength in normal fibers includes a component due to the tubular system and that this component happens to be about the same size as the component of capacitance of glycerol-treated fibers which is due to the remaining tubules. It seems difficult to test this hypothesis with the techniques used here.

### Factors Which Might Account for the Ionic Strength Effect

The decrease in tubular capacitance with a decrease in ionic strength seems particularly difficult to explain since the structures presumably responsible

for the capacitance swell (Rapoport et al., 1969) in these solutions. (A quantitative description of the change in shape and increase in area found by Rapoport et al. [1969] is given in the following paper.) It is thus clear that some other process must account for the decrease in capacitance.

One process which would decrease the capacitance would be a sealing off of the tubular system from the extracellular space. Then, current would not be able to enter the tubular system and measurements of the total fiber capacitance would not measure the capacitance of the tubular sytem. Two of our results, however, make this hypothesis untenable, at least in its simple form: the presence of a normal twitch and the diffusion of an extracellular marker into the lumen of the tubules both suggest that the tubules remain open to the extracellular space. Our experiments do not rule out the possibility that the tubules are sufficiently collapsed near the fiber surface to restrict the flow of current significantly, and yet are still sufficiently open to allow the diffusion of peroxidase to occur.

Another class of explanations is based on the idea that increasing the internal resistance of the lumen of the tubules limits the radial distance to which current can flow into the fiber. The following paper is devoted to an analysis of this effect.

*Implications for Electromechanical Coupling*

It was quite surprising to find an essentially normal twitch in muscles bathed in solutions of low ionic strength, although we subsequently learned that such mechanical responses were well described some time ago (Overton, 1902). Presumably, a normal twitch implies that most of the tubule membrane is depolarized beyond mechanical threshold for a substantial time. Thus, a normal twitch in solutions of low ionic strength implies that the profile of tubular potential (as a function of both time and radial location) produced by an action potential is much the same as in normal Ringer. The results of the capacitance experiments, on the other hand, suggest that in solutions of low ionic strength there is a drastic alteration in the profile of the tubular potential produced by the currents used to measure the capacitance; in particular, there seems to be no flow of these currents into the tubular system, and thus no change in tubular potential.

It is difficult to reconcile these two findings without invoking a regenerative change in the properties of the T system during the course of an action potential. This regenerative change could conceivably be structural, involving a transient swelling or opening of the mouth of the tubule, but it is tempting to ascribe it to the electrical properties of the tubular membrane, in particular to a mechanism resembling an action potential. The recent findings of Costantin (1970) (see also Eisenberg and Costantin, 1971) concerning the propagated radial spread of contraction make this idea particularly attractive.

BIBLIOGRAPHY

COSTANTIN, L. L. 1970. The role of sodium current in the radial spread of contraction in frog muscle fibers. *J. Gen. Physiol.* **55**:703.

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. 1971. The equivalent circuit of frog skeletal muscle. *In* Contractility of Muscle Cells. R. Podolsky, editor. Prentice-Hall, Inc., Englewood Cliffs, N.J.

EISENBERG, R. S., and L. L. COSTANTIN. 1971. The radial variation of potential in the transverse tubular system of skeletal muscle. *J. Gen. Physiol.* **58**:700.

EISENBERG, R. S., J. N. HOWELL, and P. C. VAUGHAN. 1971. The maintenance of resting potentials in glycerol-treated muscle fibres. *J. Physiol. (London).* **215**:95.

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

FREYGANG, W. H., JR., D. A. GOLDSTEIN, D. C. HELLAM, and L. D. PEACHEY. 1964. The relation between the late after-potential and the size of the transverse tubular system of frog muscle. *J. Gen. Physiol.* **48**:235.

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 P. HOROWICZ. 1959. The influence of potassium and chloride ions on the membrane potential of single muscle fibers. *J. Physiol. (London).* **148**:127.

HODGKIN, A. L., and B. KATZ. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (London).* **108**:37.

HOWELL, J. N. 1969. A lesion of the transverse tubules of skeletal muscle. *J. Physiol. (London).* **201**:515.

KROLENKO, S. A. 1969. Changes in the T-system of muscle fibres under the influence of influx and efflux of glycerol. *Nature (London).* **221**:996.

NAKAJIMA, S., and A. L. HODGKIN. 1970. Effect of diameter on the electrical constants of frog skeletal muscle fibres. *Nature (London).* **227**:1053.

OVERTON, E. 1902. Beiträge zur allgemeinen Muskel- und Nervenphysiologie. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **92**:346.

RAPOPORT, S. I. 1969. A fixed charge model of the transverse tubular system of frog sartorius. *J. Gen. Physiol.* **54**:178.

RAPOPORT, S. I., L. D. PEACHEY, and D. A. GOLDSTEIN. 1969. Swelling of the transverse tubular system in frog sartorius. *J. Gen. Physiol.* **54**:166.

SCHNEIDER, M. F. 1970. Linear electrical properties of the transverse tubules and surface membrane of skeletal muscle fibers. *J. Gen. Physiol.* **56**:640.

VAUGHAN, P. C., J. N. HOWELL, and R. S. EISENBERG. 1970. Changes in the capacitance of frog skeletal muscle. *Fed. Proc.* **29**:656.