Voltage Clamp Experiments on Single Muscle Fibers of *Rana pipiens*

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ABSTRACT A voltage clamp for single muscle fibers has been developed. Stability of the system was achieved when an artificial node was created by enclosing a single muscle fiber in a petroleum jelly seal which served as an analogue of the myelin sheath. Typical voltage clamp records were obtained with large inward transient currents followed by a delayed rectification of the outward currents. These currents looked qualitatively similar when the transverse tubular system was destroyed. Errors in current measurement, especially those due to anomalous rectification, are discussed.

INTRODUCTION

Voltage clamp experiments on skeletal muscle fibers (Adrian and Freygang, 1962; Henček et al., 1969; Rougier et al., 1968) have been particularly difficult to accomplish due to a number of technical difficulties. Frankenhaeuser et al. (1966) have shown that the system developed by Dodge and Frankenhaeuser (1958) is useful on skeletal muscle for potentiometric studies and steady-state voltage clamp measurements. Adrian et al. (1966, 1968, 1970) have utilized an elegant microelectrode technique to voltage clamp a point near the end of a single muscle fiber. This paper presents a modification of the technique used by Frankenhaeuser et al. (1966), providing improved resolution of the transient inward currents under voltage clamp conditions. An analysis of these experiments showed results consistent with those obtained with a different technique by Adrian et al. (1970). A preliminary account of this work has been previously reported (Moore, 1968).

METHODS

Single muscle fibers from the frog (*Rana pipiens*) were dissected and mounted in a Lucite chamber similar to that described by Dodge and Frankenhaeuser (1958) with the exception that pool A, schematically shown in Fig. 1, had a width of about 150 μ. Large fibers with diameters of approximately 80 μ were selected. The seals were generally applied to increase somewhat the length of the EA and AB seals and reduce the length of fiber in pool A. The dimensions stated in the text and in the
legend of Fig. 1 are the chamber dimensions and thus represent the upper and lower limits for the width of pool A and the EA seal, respectively. The deviations from the stated dimensions varied from fiber to fiber but were no greater than 20%. In most experiments the fibers were depolarized in a solution with a high potassium concentration, (K)_o, before mounting, in order that the regions under the petroleum jelly seals would be inexcitable. As shown in Fig. 1 the seals were applied in such a way that petroleum jelly enclosed the entire fiber in pool B adjacent to the recording pool A, thereby creating an analogue of the myelin sheath. After mounting the fiber, the solution in pool A was changed to a high sodium solution and the amplifiers were balanced so that the holding potential, V_H, was about -80 mv. The voltage clamp technique used was similar to that described previously (Moore, 1967). In all of the figures the current is expressed in terms of V_H, the output of the clamping amplifier (Moore, 1967).

In order to estimate the seal resistances in these experiments, experiments were done using the oil gap technique of Cole and Hodgkin (1939). These measurements, which are described in the Appendix, showed that the upper limit for the seal re-
sistance from E to A, $R_{EA}$, was about 2 MΩ. The seal resistance per unit length was 10 MΩ/mm and the internal longitudinal resistance was approximately 0.5 MΩ/mm, which gives an internal specific resistivity of about 300 Ω cm at 20°-24°C. This figure is somewhat higher than that reported by Nakajima and Hodgkin (1970).

The electrical parameters determined by the oil gap technique indicate that the impedance pathway between pool E and region D inside the fiber, $Z_{ED}$, is of the order of $10^6$ Ω. The effective resistance, $R_{ef}$ (Katz, 1949), of the fiber in pool E was about $7 \times 10^4$ Ω and the axial resistance was about $2 \times 10^6$ Ω where the distance from region D of Fig. 1 to the edge of the EA seal was taken to be 300 μ. These estimates demonstrate that the axial resistance constitutes a significant part of the impedance, $Z_{ED}$, and will therefore tend to reduce the discrepancy at high frequencies between the true current of the controlled membrane and that calculated by equation 1 below.

Since the various impedance values for a particular experiment were not established it is difficult to evaluate the errors in current measurement and potential control. However, the criteria of adequate potential control established by Julian et al. (1962) for a gap method were essentially met. These criteria are (a) a short pool length, (b) a nonconducting solution at the boundaries, (c) a low impedance pathway for current injection, and (d) sufficient gain and bandwidth in the control amplifier (Julian et al., 1962). The preparation developed here just meets these criteria since the pool length is about the same as the fiber diameter but not less and the boundaries of the pool are petroleum jelly with a thin film of conducting fluid surrounding the fiber. The closeness with which these criteria are met is evident from the finding that in a number of well-dissected fibers it was not possible to achieve potential control. This difficulty was generally due to seals which were too long or not applied firmly. Insufficient potential control was manifested by oscillatory behavior similar to that observed by Julian et al. (1962) for wide gaps. Only fibers which showed a uniform potential trace ($V_A$) in pool A and nonthreshold, graded currents for increasing potential steps were used. The uniformity of $V_A$ does not necessarily demonstrate a constant potential in pool A; however, the microelectrode experiments of Frankenhaeuser et al. (1966) suggest a static error of a few per cent and a dynamic error possibly approaching 10%. Frankenhaeuser et al. (1962) suggest that the errors are in part due to regenerative currents under the seals. In the experiments reported in this paper there was a high potassium solution under the seals and the length of pool A was less than in the above paper. Both of these factors should reduce the errors in potential control. However, the discrepancy in the recorded action potentials recorded by the gap method and with microelectrodes is probably due to a small variation in the potential at D (see Fig. 1).

All fibers were stimulated before mounting to determine the existence of a propagated twitch. The glycerol-treated single fibers (Eisenberg and Gage, 1967) did not contract on electrical stimulation.

**Solutions**

The solutions used had the compositions, in millimoles per liter, given in Table I. All solutions were buffered at pH 7 with either 2 mM tris(hydroxymethyl)amino methane (Tris)-maleate or 1.0 mM Na$_2$HPO$_4$ and 0.4 mM NaH$_2$PO$_4$. A concentration of 8 mM CaSO$_4$ was present in all of the solutions containing sulfate ions.
Nomenclature

The potentials, $-V_A$ and $V_E$, refer to the potentials recorded in pools A and E, respectively, with reference to ground potential, $V_B$. $V_A$ is assumed to be essentially equal to $E$, the inside potential minus the outside potential, and under resting conditions is referred to as the holding potential, $V_H$.

**TABLE I**

**SOLUTION COMPOSITIONS**

| Solution | NaCl | KCl | RbCl | CaCl₂ | Na₂SO₄ | K₂SO₄ | Sucrose |
|----------|------|-----|------|-------|--------|-------|---------|
| I        | 120  | 2.5 | -    | 1.0   | -      | -     | -       |
| II       | -    | 122.5 | -    | 1.0   | -      | -     | -       |
| III      | -    | -   | -    | -     | 40     | 1.75  | 120     |
| IV       | -    | -   | -    | -     | 40     | 1.75  | 120     |
| V        | 20   | -   | 100  | 1.8   | -      | -     | -       |

**RESULTS**

**Action Potential Measurements**

Action potentials recorded from single fibers in sulfate Ringer fluid (solution III) are seen in Fig. 2 to be similar to those reported by Frankenhaeuser et al. (1966). It appears that in general the insulation with petroleum jelly in pool B does not significantly modify the shape of the recorded action potential. However, action potentials with notched peaks as reported by Frankenhaeuser et al. (1966) were not seen in these experiments. It should be noted that these membrane action potentials characteristically have a relatively slow rate of fall which leads into the afterpotential, as compared to action potentials recorded with microelectrodes which have a faster rate of fall and a more distinct afterpotential (Frank, 1957; Hutter and Noble, 1960; Nastuk and Hodgkin, 1950; Perrson, 1963).

It has been shown that treatment with glycerol will destroy most of the transverse tubular system of frog skeletal muscle (Howell and Jenden, 1967; Howell, 1969) and correspondingly abolishes the afterpotential as recorded with microelectrodes (Gage and Eisenberg, 1967). A membrane action potential which was recorded from a single fiber pretreated with glycerol (Fujino et al., 1961) according to the method described by Eisenberg and Gage (1967) is shown in Fig. 3. It is clear that the afterpotential has been reduced despite a continued slow rate of fall. This effect is less pronounced in fibers equilibrated in chloride Ringer fluid (Hutter and Noble, 1960). These experiments thus confirm the finding of Gage and Eisenberg (1967) that the afterpotential of frog skeletal muscle is diminished after glycerol treatment.
Voltage Clamp Experiments

Fig. 4 illustrates a typical series of voltage clamp runs in which the end pools contained isotonic potassium chloride (solution II) and pool A contained normal Ringer solution (solution I). The settling time of the voltage in all the experiments reported in this paper was about 200 μsec. The first run of Fig. 9 shows a typical voltage trace recorded from pool A.

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Figure 2. Membrane action potential in sulfate Ringer fluid (solution III). $V_A = -70$ mv; temperature, 7°C; left scale, $V_E$; right scale, $V_A$ (lower trace). Fiber No. 5.

Figure 3. Membrane action potential from glycerol-treated fiber in sulfate Ringer fluid. $V_A = -80$ mv; temperature, 7°C; left scale, $V_E$; right scale, $V_A$ (lower trace). Fiber No. 7.
The ionic currents recorded from single muscle fibers generally showed large inward transient currents and a distinct outward steady-state current. There were a number of fibers which showed large currents at the beginning of an experiment; however, at later times smaller outward currents were seen despite the maintenance of large inward currents. In general it appeared that the steady-state outward current was more labile than the inward transient current. Similar findings were reported by Adrian et al. (1970).

![Graph showing voltage clamp currents in chloride Ringer solution.](image)

**Figure 4.** Voltage clamp currents in chloride Ringer solution. The six successive membrane current recordings ($I_m$) were measured at step cathodal polarizations of 41, 47, 59, 65, 71, and 82 mV, respectively. Temperature, 12°-13°C; fiber No. 26.

A current-voltage plot of the transient and steady-state currents is seen in Fig. 5. The currents are not corrected for leakage because of the complicated current injecting pathway used with this technique. Referring to Fig. 1, the current path is across the membrane in pool E, which contains a high potassium solution, through the myoplasm to pool A, and then through the membrane in pool A which contains normal Ringer solution. Thus, when outward current is flowing through the membrane in pool A, inward current is flowing through the membrane in pool E and vice versa. The membrane current is given by the relationship

$$I_m = \frac{V_{R}}{Z_{ED}A_E}$$

(1)
where $I_m$ is in amperes per square centimeter, $V_E$ is the potential in pool E, $Z_{ED}$ is the impedance from pool E to region D inside the fiber of pool A, and $A_N$ is the area of the membrane in pool A. Using a nodal area, $A_N$, of $2 \times 10^{-4}$ cm$^2$ and $5 \times 10^4$ Ω for the resistance, $Z_{ED}$, for the case of high (K)$_o$ in pool E the maximal steady-state current ($I_m$) is about 1-5 ma/cm$^2$.

Since the membrane in pool E is depolarized by a high (K)$_o$ it is necessary to consider the nature of the anomalous rectification expected from this portion of the current injecting path. It has been shown that a significant proportion of the potassium current can flow easily in an inward direction; however,
small currents near the holding potential and the current at the reversal potential for the transient current. Currents corrected for leakage in this manner were then used to determine the voltage-dependence rate constants of the ionic conductances.

The transient current measurement is also subject to the above considerations as well as the possible error introduced by the capacitance of the membrane in pool E. Using the potentiometric system it was shown by Frankenhaeuser et al. (1966) that a step function in E did not inject a step current across the membrane in A.

Two experiments were done which suggest that the error in the current measurement introduced by the capacitance in pool E was tolerable. One experiment, shown in Fig. 9, was a voltage clamp run of a fiber pretreated with glycerol. Since the membrane capacitance was smaller in the detubulated fibers (Eisenberg and Gage, 1967) the errors due to large capacitative currents should be reduced. In all the experiments using pretreated fibers the transient currents appeared qualitatively the same as in the untreated fibers. These results suggest that the distortion of the transient currents, as measured by monitoring $V_E$, was not qualitatively significant. The second experiment was done by stretching the muscle fiber to one and one-half its resting length and allowing only 150–200 μ of one end of the fiber in pool E. The stretched fiber and closed cable structure significantly reduced the total capacitance of the membrane in pool E without any qualitative effect on the voltage clamped currents. In another experiment it was still possible to record significant inward current after the fiber in pool E had been cut.

Advantage was taken of the anomalous rectification of the membrane in pool E to record large inward transient currents. It has been shown that muscle fibers placed in isotonic potassium sulfate show a very high resistance to outward current (Katz, 1949; Adrian, 1964). The rectification is so great that a reasonable approximation to the data would be a constant current for most positive internal potentials. In the experiment shown in Fig. 6 the fiber was bathed in a K₂SO₄ solution (solution IV) except for pool A which contained Na₂SO₄ (solution III). Large inward currents (large $V_E$) compared to the small outward steady-state currents (small $V_E$) were seen. This result reflects the high impedance of the current pathway for the inward currents in pool A and outward currents in pool E compared to the lower impedance of the current path for outward currents in pool A and inward currents in pool E. The current-voltage plot of Fig. 7 demonstrates the extreme rectification frequently found in the sulfate solutions.

The application of the petroleum jelly seals in these experiments is absolutely critical. In the experiment illustrated in Fig. 8 a notch in the initial current was observed which probably reflects a space-clamp problem (Taylor
et al., 1960). In other experiments where the seals were poorly made it was impossible to achieve stability in the voltage clamp system.

A similar rectification of the voltage clamp currents for a fiber pretreated with glycerol is shown in Fig. 9. Fibers treated with glycerol were more sensitive to damage by the passage of current under voltage clamp conditions than normal fibers. Since prolonged depolarizing pulses generally damaged such treated fibers it was not possible to observe the effect of tubular destruction on inactivated delayed currents (Nakajima et al., 1962). However, the limited data from two treated fibers indicate that the membrane currents for short depolarizing pulses are similar to those recorded from untreated fibers.

One method to eliminate the variable impedance in the current injecting path was to remove the anomalous rectification of the membrane in pool E. This was accomplished by bathing the fiber in 100 mM RbCl (solution V), which effectively prevents the muscle membrane from passing large inward currents (Adrian, 1964). Fig. 10 illustrates the delayed rectification of the outward potassium current in such an experiment. The current-voltage plot...
of Fig. 11 shows that the resting or leakage conductance is relatively constant up to the sodium equilibrium potential.

Since the initial inward current recorded in these experiments was totally blocked by $4 \times 10^{-6}$ g/ml of tetrodotoxin it seems likely that the current is carried by sodium ions (Adrian et al., 1970). Consistent with this view is the increased inward current after a short step cathodal polarization. Fig. 12 illustrates a series of short pulse experiments showing the rapid exponential decay of the sodium conductance.
Experiments were also done in high potassium solutions which showed increased inward currents on repolarization during the steady-state current. These results are consistent with the hypothesis that potassium ions are principal current carriers for the steady-state current.

**Figure 9.** Voltage clamp runs from a glycerol-treated fiber in sulfate Ringer solution. In the first record of the left column $V_E$ (membrane current) and $V_A$ (membrane potential, lower trace) are superimposed. The ten subsequent runs are membrane current recordings ($V_E$, scale to the left) measured at increasing step cathodal polarizations corresponding to 40, 46, 51, 58, 66, 74, 81, 91, 101, and 114 mv, respectively. The holding potential was $V_A = -90$ mv and the prepulse value of $V_E$ is $-10$ mv (holding current); temperature, 7°C. Fiber No. 6.
The steady-state inactivation of the transient current is shown in Fig. 13. These data are similar to those reported by Adrian et al. (1970) with the exception that the $h_x$ curve of Fig. 13 was slightly steeper.

In order to reduce the error in the membrane current measurement two experiments were done with EA seals the length of which was 10-fold greater than in the above experiments. Such an increase in the seal is analogous to the myelin sheath of nerve and serves to increase greatly the axial resistance pathway through the myoplasm in comparison to the input resistance of the fiber in pool E. For reasons described in the Methods section, it was not possible to voltage clamp this preparation as effectively as before; however, graded currents were observed and analyzed. A kinetic analysis of the inward current showed a small decrease of questionable significance in the time con-
stants compared to those from experiments with short EA seals. The analysis was analogous to the Hodgkin and Huxley (1952) formalism (Moore, 1971) and showed results similar to those reported by others (Adrian et al., 1970; Ildefonse et al., 1971; Stanfield, 1970). Experiments were also done by injecting current through microelectrodes impaled in pool E; however, it was difficult to maintain adequate preparations under these conditions. In conclusion it appears that, although the potential control achieved by this technique is adequate, the uncertainties of the current measurement require an alternative method to assess the errors involved.

**DISCUSSION**

A complication of voltage clamp experiments on skeletal muscle compared to nerve axons is the presence of the complex sarcotubular system. The possibility that the tubular membrane is an excitable structure raises the question of whether or not adequate potential control is possible for the muscle membrane (Adrian et al., 1969; Falk, 1968). The experiments on detubulated fibers reported here suggest that the currents flowing through the tubular membrane do not qualitatively distort the voltage clamp records. However, significant quantitative differences between normal and glycerol-treated fibers

![Figure 11. Current voltage relationships of the initial and steady-state currents. Abscissa, cathodal polarization from the holding potential; ordinate, $V_E$; temperature 8°C. Fiber No. 24.](image-url)
may exist, possibly due to a series resistance effect similar to that observed in giant axons.

Previous investigations have suggested a distinct threshold for the delayed current (Adrian et al., 1969; Heistracher and Hunt, 1969; Kao and Stanfield, 1970). The current voltage plots of Figs. 5 and 11 do not indicate a distinct change in slope for the delayed current; however, no significant outward current occurs until a potential is reached which gives transient inward currents. Similar results were shown in Fig. 7 of the paper by Adrian et al. (1970).

Because of the lack of sufficient experimental data it is not possible to suggest a kinetic model for the permeability mechanism; however, the analysis does suggest that the muscle membrane is similar to the node of Ranvier. In addition, the analysis clearly indicated that two time constants were required to describe the turning off of the inward transient current and the turning on of the outward delayed current. In some experiments the difference in time

![Figure 12. Inward current tails after short cathodal pulses. The points shown were measured with an analogue to digital converter and superimposed to demonstrate the decreasing sodium permeability with time. The duration of each of the four pulses was from \( t_a \), represented by the break in the resting current, to 0.2 msec before the peak tail current for each pulse. The continuous curve shows the inward transient current interrupted by the faster tail transients. Abscissa, time; ordinate, \( V_E \); temperature, 8°C. Fiber No. 20.](image-url)
FIGURE 13. \( h_\infty \) versus voltage curve. The experimental points were plotted assuming that the level of inactivation at \(-79\) mV was 0.86. The smooth curve represents the equation

\[
h_\infty = \frac{1}{1 + \exp \left( \frac{E + 70}{4} \right)}.
\]

Abscissa, membrane potential \( E \); ordinate, level of inactivation \( h_\infty \); temperature, \( 8^\circ \text{C} \). Fiber No. 20.

constants was so large that it appeared as a distinct bend in voltage clamp current vs. time. The voltage clamp results on skeletal muscle therefore support the hypothesis that the sodium and potassium permeability mechanisms are different and possess properties very similar to those observed in axonal membranes.

APPENDIX

Single fibers were dissected and mounted vertically in an oil gap similar to that described by Cole and Hodgkin (1939). Only fibers which responded to an electrical stimulus with a propagated twitch were used in these experiments. The total DC resistance, \( R \), across the oil gap was measured as a function of length. All measurements were made at room temperature. The equation used to interpret the data (Cole and Hodgkin, 1939) was

\[
V_o = \frac{r_1 r_2 i_o}{r_1 + r_2} x + \frac{i_o \pi^2 \lambda}{(r_1 + r_2) \left( \frac{r_1 + r_2}{r_2} + \coth \frac{x}{\lambda} \right)}
\]

where

\[
\lambda = r_4 / (r_1 + r_2),
\]

\( V_o \) is the potential difference between the external electrodes,

\( i_o \) is the total current flowing between the electrodes,

\( x \) is a measure of the width of the gap where the center of the gap is defined as \( x = 0 \) and the electrode regions are considered infinite,
\( r_1 \) is the external longitudinal resistance (\( \Omega/cm \)), 
\( r_2 \) is the internal longitudinal resistance (\( \Omega/cm \)), and 
\( r_4 \) is the membrane resistance (\( \Omega cm \)).

The method of analysis used was identical to that developed by Cole and Hodgkin (1939) and is described here for the convenience of the reader. Equation 1 a is of the form

\[
R = ms + Y = \frac{2V_o}{i_o}
\]

where

\[
s = 2x,
\]

\[
m = \frac{r_1r_2}{r_1 + r_2}, \text{ and}
\]

\[
Y = \frac{2r_1^2\lambda}{(r_1 + r_2) \left( \frac{r_1r_2}{r_1} + \coth \frac{s}{2\lambda} \right)}
\]

For \( s = \infty \), \( Y = Y_w \),

\[
Y_w = \frac{2r_1^2}{(r_1 + r_2) \left( \frac{r_1 + r_2}{r_2} + 1 \right)}
\]

The parameter, \( h \), is defined by the relation

\[
(h + 1)^2 = \frac{r_1 + r_2}{r_2}
\]

so that

\[
\frac{Y}{Y_w} = \frac{2 + h}{1 + h + \coth \frac{s}{2\lambda}} = \frac{2 + h}{1 + h + \coth \frac{msh}{Y_w}}
\]

Furthermore,

\[
r_1 = m(h + 1)^2
\]

\[
r_2 = \frac{m(h + 1)^2}{h^2 + 2h}
\]

\[
\lambda = \frac{Y_w}{2mh}
\]

\[
r_4 = \lambda^2(r_1 + r_2).
\]
Equation 2a predicts a curve for low values of \( s \) and a straight line for high values. It was found that the initial points for short oil gaps of 1-2 mm or less were highly variable and not reproducible. This result was probably due to the meniscus which formed around the fiber and prevented the formation of a well-defined oil gap. Since no reproducible curvature was observed for any fiber, the assumption was made that all the curvature predicted by equation 2a occurred within the first millimeter of the gap. The curvature is determined by the term with \( msh/Y_0 \) which is essentially constant for values of \( msh/Y_0 = 2 \). For \( s = 1 \), let \( msh/Y_0 = 2 \) and \( h = 2Y_0/m \). This assumption sets the value of \( \lambda \) equal to 0.25 mm since \( \lambda = Y_0/2mh = 1/4 \). With these relations the values of \( h \) and \( Y_0 \) can be experimentally determined and \( r_1 \), \( r_2 \) and \( r_4 \) calculated by equations 6a, 7a, and 9a. Thus, the only measurements used in the calculations were the slope, \( m \), of the resistance vs. gap length curve and the intercept at zero length.

The values of \( r_2 \) and \( r_4 \) were not initially sensitive to the rate of curvature. For fiber 1 of Table II calculations were made for different values of \( h \) corresponding to the complete curvature of equation 2a occurring in the first 0.5, 1.0, and 1.5 mm of the oil gap. The calculated values of \( r_2 \) were 0.45, 0.47, and 0.50 M\( \Omega \)/mm, respectively. The correlating values of \( r_4 \) were 0.97, 0.80, and 0.73 \( \Omega \) cm \( \times \) 10\(^6\). However, the value of \( r_1 \) is more sensitive to the degree of curvature. The corresponding values of \( r_1 \) were 62, 12, and 5 M\( \Omega \)/mm. Thus, the estimation of \( r_1 \) is only within an order of magnitude.

All the results found in Table II were calculated on the assumption of 1 mm of curvature. The value of the internal specific resistivity, \( \rho_2 \), is similar to that found by Katz (1949). The value of \( \rho_2 \) was calculated by the relation

\[
\rho_2 = r_2 \pi r^2
\]

where \( r \) is the radius of the fiber.

Using a value of \( r_1 = 10 \) M\( \Omega \)/mm, a fiber diameter, \( d \), of 80 \( \mu \), and \( \rho_0 = 80 \) \( \Omega \) cm for the specific resistivity of the Ringer solution (Katz, 1949), the thickness, \( b \), of the

\[
\begin{array}{cccccccccc}
\text{Fiber} & \text{Solution} & \text{Diameter} & r_1 & r_2 & r_4 \times 10^{-6} & \rho_2 & \rho_n & K_m \\
\hline
1 & I & 12 & 0.47 & 0.80 & - & - & - & - \\
2 & I & 20 & 0.23 & 1.26 & - & - & - & - \\
2 & III & 20 & 1.64 & - & - & - & - & - \\
3 & I & 60 & 13 & 0.89 & 0.84 & 251 & 1580 & - \\
3 & III & 13 & 3.08 & - & - & - & - & - \\
4 & I & 85 & 3 & 0.80 & 0.25 & 466 & 670 & - \\
4 & III & 3 & 2.5 & - & - & - & - & - \\
5 & I & 94 & 18 & 0.49 & 1.11 & 323 & 3280 & - \\
6 & I & 90 & 13 & 0.47 & 0.86 & 300 & 2420 & - \\
7 & I & 80 & 9 & 0.41 & 0.64 & 202 & 1510 & -
\end{array}
\]
Ringer fluid layer surrounding the fiber in the oil gap was 1.6 \( \mu \) employing the relation

\[
b = \frac{\rho_o}{\pi d_1}.
\]

In three experiments shown in Table II the membrane resistance in sulfate Ringer solution (solution III) was found to be increased about three-fold over the chloride Ringer value. In these experiments the slope, \( m \), of the resistance versus length curve was unchanged compared to the control value, which means that the internal specific resistivity was unaltered by the sulfate Ringer. Since the ionic strength and the tonicity of these solutions were the same this result was expected. The only measured experimental difference in the sulfate solution was an increase in \( Y_r \). The values of \( r_1 \) in sulfate solution shown in Table II were calculated on the basis of an unchanged \( r_1 \) and \( r_2 \), consequently a constant value of \( h \) and thus decreasing the value of \( s \) for which complete curvature occurred.

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REFERENCES

ADRIAN, R. 1964. The rubidium and potassium permeability of frog muscle membrane. J. Physiol. (Lond.). 175:134.

ADRIAN, R. H., W. K. CHANDLER, and A. L. HODGKIN. 1966. Voltage clamp experiments in skeletal muscle of fibers. J. Physiol. (Lond.). 186:51P.

ADRIAN, R. H., W. K. CHANDLER, and A. L. HODGKIN. 1968. Voltage clamp experiments in striated muscle fibers. J. Gen. Physiol. 51:1883.

ADRIAN, R. H., W. K. CHANDLER, and A. L. HODGKIN. 1969. The kinetics of mechanical activation in frog muscle. J. Physiol. (Lond.). 204:207.

ADRIAN, R. H., W. K. CHANDLER, and A. L. HODGKIN. 1970. Voltage clamp experiments in striated muscle fibers. J. Physiol. (Lond.). 208:607.

ADRIAN, R. H., and W. H. FREYGANG. 1962. Potassium conductance of frog muscle membrane under controlled voltage. J. Physiol. (Lond.). 163:104.

COLE, K. S., and A. L. HODGKIN. 1939. Membrane and protoplasm resistance in the squid giant axon. J. Gen. Physiol. 22:571.

DODGE, F. A., and B. FRANKENHAUSER. 1958. Membrane currents in isolated frog nerve fibre under low voltage clamp conditions. J. Physiol. (Lond.). 143:176.

EISENBERG, R. S., and P. W. GAGE. 1967. Frog skeletal muscle fibers: changes in electrical properties after disruption of transverse tubular system. Science (Wash. D.C.). 158:1700.

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

FRANK, G. H. 1957. Negative after potential of frog's skeletal muscle. J. Neurophysiol. 20:602.

FRANKENHAUSER, B., B. D. LINDLEY, and R. S. SMITH. 1966. Potentiometric measurement of membrane action potentials in frog muscle fibres. J. Physiol. (Lond.). 183:152.

FUJINO, M., T. YAMAOUCHI, and K. Suzukir. 1961. Glycerol effect and the mechanism linking excitation of the plasma membrane with contraction. Nature (Lond.). 192:1159.

GAGE, P. W., and R. S. EISENBERG. 1967. Action potentials without contraction in frog skeletal muscle fibers with disrupted transverse tubules. Science (Wash. D.C.). 158:1702.
HEISTRACHER, P., and C. C. HUNT. 1969. The relation of membrane changes to contraction in twitch muscle fibres. J. Physiol. (Lond.). 201:589.

HEN6EK, M., W. NONNER, and R. STXMPFLI. 1969. Voltage clamp of a small muscle membrane area by means of a circular sucrose gap arrangement. Pfluegers Arch. Eur. J. Physiol. 313:71.

HODGKIN, A. L., and P. HOROWICZ. 1959. The influence of potassium and chloride ions on the membrane potential of single muscle fibers. J. Physiol. (Lond.). 148:127.

HODGKIN, A. L., and A. F. HUXLEY. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. (Lond.). 117:500.

HOLLOW, J. N. 1969. A lesion of the transverse tubules of skeletal muscle. J. Physiol. (Lond.). 201:515.

HOLLOW, J. N., and D. J. JENDEN. 1967. T-tubules of skeletal muscle: morphological alterations which interrupt excitation-contraction coupling. Fed. Proc. 26:553.

HUTTER, O. F., and D. NOBLE. 1960. The chloride conductance of frog skeletal muscle. J. Physiol. (Lond.). 151:189.

ILDEFONSE, M., O. ROUGIER, and G. Roy. 1971. Quantitative analysis of the initial sodium current in twitch skeletal muscle fibre of the frog. Proc. 25th Int. Congr. Physiol. Sci. 9:268.

JULIAN, F. J., J. W. MOORE, and D. E. GOLDMAN. 1962. Current-voltage relations in the lobster giant axon membrane under voltage clamp conditions. J. Gen. Physiol. 45:1217.

KAO, C. Y., and P. R. STANFIELD. 1970. Actions of some cations on the electrical properties and mechanical threshold of frog sartorius muscle fibers. J. Gen. Physiol. 55:620.

KATZ, B. 1949. Les constantes electriques de la membrane du muscle. Arch. Sci. Physiol. 3:285.

MOORE, L. E. 1967. Membrane currents at large positive internal potentials in single myelinated nerve fibers of Rana pipiens. J. Physiol. (Lond.). 193:433.

MOORE, L. E. 1968. Membrane currents in single muscle fibers of Rana pipiens. Proc. 24th Int. Congr. Physiol. Sci. 7:304.

Moore, L. E. 1971. Effect of temperature and calcium ions on rate constants of myelinated nerve. Am. J. Physiol. 221:151.

Nakajima, S., and A. L. HODGKIN. 1970. Effect of diameter on the electrical constants of frog skeletal muscle fibers. Nature (Lond.). 227:1053.

Nakajima, S., S. IWASAKI, and K. OBATA. 1962. Delayed rectification and anomalous rectification in frog's skeletal muscle membrane. J. Gen. Physiol. 46:97.

NASTUK, W. L., and A. L. HODGKIN. 1950. The electrical activity of single muscle fibers. J. Cell. Comp. Physiol. 35:39.

Persson, A. 1953. The negative after-potential of frog skeletal muscle. Acta Physiol. Scand. Suppl. 58:205.

ROUGIER, O., G. VASSORT, and M. ILDEFONSE. 1968. Analyse quantitative en voltage imposé du courant de membrane de la fibre musculaire squelettique, C. R. Hebod. Seances Acad. Sci. Ser. D Sci. Nat. (Paris). 266:1754.

STANFIELD, P. R. 1970. The effect of tetraethylammonium ion on the delayed currents of frog skeletal muscle. J. Physiol. (Lond.). 209:209.

TAYLOR, R. E., J. W. MOORE, and K. S. COLE. 1960. Analysis of certain errors in squid axon voltage clamp measurements. Biophys. J. 1:161.