Electrical Properties and Excitation-Contraction Coupling in Skeletal Muscle Treated with Ethylene Glycol

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ABSTRACT The contractility of the frog sartorius muscle was suppressed after treatment with a Ringer solution added with ethylene glycol (EGR). No contraction was elicited by nerve stimulation when the muscle was brought back to normal Ringer solution after having been soaked in 876 mM EGR for 4 hr or in 1095 mM EGR for 2 hr. However, the action potential of normal amplitude was generated and followed by a depolarizing afterpotential. The resting membrane potential was slightly decreased from the mean normal value of -91.1 mV to -78.8 mV when 1095 mM EGR was used, and to -82.3 mV when 876 mM EGR was used, but remained almost constant for as long as 2 hr. The afterpotential that follows a train of impulses and a slow change in membrane potential produced by a step hyperpolarizing current (so-called "creep") were suppressed after treatment with ethylene glycol. The specific membrane capacity decreased to about 50% of the control values while the specific membrane resistance increased to about twice the control values. Therefore, the membrane time constant remained essentially unchanged. The water content of the muscle decreased by about 30% during a 2 hr immersion in 1095 mM EGR, and increased by about 30% beyond the original control level after bringing the muscle back to normal Ringer. The intracellular potassium content did not change significantly during these procedures. Some differences between the present results and those obtained with glycerol are discussed.

INTRODUCTION

It has long been known that the skeletal muscle loses its ability to contract when treated with hypertonic solutions (Fenn, 1936; Hodgkin and Horowicz, 1957; Howarth, 1958; Yamaguchi et al., 1962; Dydynska and Wilkie, 1963; Caputo, 1968). Recently it was found that the contractility of the muscle was suppressed when it was brought back to normal Ringer solution after having been soaked in a solution made hypertonic with solutes capable of penetrating the muscle membrane; these solutes include glycerol (Howell and Jenden, 1967).
After treatment with a Ringer solution added with glycerol at a concentration of 400 mM, the muscle was still able to elicit action potentials by nerve stimulation. The action potentials were normal in amplitude but lacking the depolarizing afterpotential (early afterpotential) which normally follows each spike (Gage and Eisenberg, 1967, 1969). The resting membrane potential declined steadily after excitation-contraction (E-C) uncoupling (Howell, 1969; Howell et al., 1970; Eisenberg et al., 1971). The late afterpotential that follows a train of impulses (Freygang et al., 1964) and the slow potential change produced by a hyperpolarizing pulse of current (so-called “creep”) were also suppressed (Gage and Eisenberg, 1969). Both the late afterpotential and the creep were related to the existence of an intracellular compartment in which the potassium ions may accumulate during a train of impulses or may be removed by a hyperpolarizing current (Adrian and Freygang, 1962). It was proposed that the intracellular compartment might correspond to the sarcotubular system (Hodgkin and Horowicz, 1960). Furthermore, after returning to normal Ringer following glycerol treatment, the sarcotubular system became vesiculated and could not be reached by electron-opaque markers such as horseradish peroxidase (Graham and Karnovsky, 1966; Eisenberg and Eisenberg, 1968) or ferritin (Krolofenko, 1969), suggesting that the normal connection of the T system with the extracellular space was impaired.

Since the synaptic transmission is not impaired in the glycerol-treated muscles, such preparations have been conveniently used for studies of the end-plate membrane (Kordas, 1969, 1970; Maeno et al., 1971; Deguchi and Narahashi, 1971; Deguchi et al., 1971). However, the glycerol treatment has disadvantages in that the resting membrane potential declines steadily, that internal sodium concentration increases, and that internal potassium concentration decreases (Henderson, 1970). Solutions made hypertonic with substances having a low permeability across the membrane such as sucrose, glucose, or sodium chloride also inhibit the E-C coupling (Hodgkin and Horowicz, 1957; Howarth, 1958) but block the neuromuscular transmission (Fatt and Katz, 1952; Furshpan, 1956). We have found that ethylene glycol inhibits the E-C coupling without blocking the neuromuscular transmission and without causing a great depolarization. Therefore, electrical properties of the muscle treated with ethylene glycol were analyzed.

METHODS

Material Experiments were carried out with the sartorius muscle of the frog (Rana pipiens). To facilitate diffusion and uncoupling, small frogs and muscles (about
25 mg) were used. Only the experiments for determining water and potassium content were carried out with larger muscles (about 150 mg) to make measurements more accurate. In either case muscles were dissected with the nerve (about 1 cm) attached, and the proximal and distal ends were tied off with thin silk threads. Special care was taken not to damage the muscle at the proximal tendon. The muscles were soaked in hypertonic solution at 5°C for a certain period of time, and then mounted in a Lucite chamber containing normal Ringer solution at room temperature (22°C). The low temperature was chosen for soaking in hypertonic solution to prolong the survival time of muscle.

**Solutions** Frog Ringer solution was used as the normal bathing medium and contained K⁺ 2.4 mM, Na⁺ 116 mM, Ca²⁺ 1.7 mM, Cl⁻ 122 mM. The pH was maintained at 7.4 by addition of 3 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid). Ethylene glycol, glycerol, or sucrose was added to the Ringer. Two concentrations of ethylene glycol were tried: 1095 mM (called 6X EGR) and 876 mM (5X EGR). The osmolarity of these solutions was determined by measurements of lowering of the freezing point.

**Recording Techniques** Glass microelectrodes filled with 3 M KCl solution and having an electric resistance of 5–10 MΩ were inserted in the muscle fiber either to inject current or to record the membrane potential. The recording microelectrode was connected to a high input impedance preamplifier, and the time constant of the recording system was estimated to be 33 μsec. The nerve was stimulated by means of a suction electrode.

In order to monitor the base line on the screen of the oscilloscope, the input was short-circuited for less than 2 msec duration at the beginning of each sweep by means of an electronic switch triggered by a square pulse. The diagram of the switch is shown in Fig. 1. It consists of two field effect transistors (FET), one P-channel FET (Texas Instruments Inc., Dallas, Tex.; 2N3820) in series with the preamplifier, and an N-channel FET (Texas Instruments Inc., 2N3819) in parallel with the oscilloscope. If the gates of both transistors are kept negative, the amplifier will be con-
nected through a resistance of a few ohms with the oscilloscope. When a positive pulse is applied to the gates, a resistance of the order of 10⁹ Ω is built up in series with the amplifier and at the same time the oscilloscope input is shunted by a resistance of less than 100 Ω to the ground. The error introduced in the voltage measurements by the use of this circuit with the transistors properly biased was of the order of 0.1%.

To inject square pulses of current into the muscle fibers, an operational constant current supply was employed (Gage and Eisenberg, 1969).

**Determination of Intracellular Water and Potassium Content**

The sartorius muscles were dissected from both legs of a frog and weighed. One of the muscles was soaked in normal Ringer solution as control, and the other was immersed in 6X EGR. After 2 hr both muscles were carefully blotted on a filter paper, weighed, and dried for 2 hr in an oven at 150°C. The difference between the first and second weighings corresponds to the change in water content of the muscle. The dry weight of the muscle was also recorded. To determine the potassium content the dried muscles were digested in a mixture of 17 ml of concentrated nitric acid and 3 ml of 70% (v/v) perchloric acid. To accelerate the process and insure better digestion, it was carried out in an oven at 75°C. Finally the macerate was dissolved in a deionized water to make a final volume of 100 ml. The potassium concentration was determined by means of a Perkin-Elmer 303 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.). Several values have been proposed for the volume of the extracellular space in the skeletal muscle, ranging from 8 to 22.4% (see Ling et al., 1969). In the present study, a value of 12% was used because this value is close to most of the measurements. This value was assumed to remain constant in all solutions used, because Dydynska and Wilkie (1963) found it unchanged upon increasing the tonicity of solution up to twice the normal value (6X EGR is about 1.5X hypertonic).

**RESULTS**

**Resting and Action Potentials**

While being soaked in 5X or 6X EGR, the muscle remained semitransparent, and contractions observed under a X30 dissection microscope could hardly be distinguished from those of the normal muscle. In a few cases, the isometric twitch tension was monitored and a transient decrease in tension was observed immediately after immersion of the muscle in the hypertonic solution. Neuromuscular transmission was not impaired, but large spontaneous miniature end-plate potentials (up to 5 mv by overlapping) were often observed. The membrane potential in muscles soaked in normal Ringer was estimated to be $-91.12 \pm 1.41$ mv (mean ± standard error of mean of 50 fibers). The membrane was hyperpolarized to $-95.4 \pm 0.4$ mv (149 fibers) while soaking in 6X EGR, the difference being highly significant ($P < 0.01$) as determined by Student's $t$ test (Guttman and Wilks, 1965).

Muscles were soaked in 6X EGR for 2 hr or in 5X EGR for 4 hr at 5°C,
and then returned to normal Ringer at 22°C. Vigorous twitches occurred spontaneously several times upon returning to Ringer, and then the contraction elicited by nerve stimulation gradually decreased in amplitude and eventually disappeared in 15-30 min. Such an uncoupling was sometimes incomplete, especially when large muscles isolated from large frogs or 5X EGR was used. The mean resting potential was estimated to be $-78.9 \pm 0.81$ mv (279 fibers) for seven muscles treated with 6X EGR, and $-82.1 \pm 0.87$ mv (214 fibers) for seven muscles treated with 5X EGR, the difference between the

| TABLE I
| MEMBRANE POTENTIAL OF MUSCLE FIBERS
| UNCOUPLED WITH ETHYLENE GLYCOL RINGER (EGR) |

| Experiment number | Membrane potential* (mv) | Slope† (mv/min) | $V(t=0)$† (mv) | Number of fibers | Recording period (min) |
|-------------------|--------------------------|-----------------|----------------|------------------|------------------------|
| Fibers uncoupled with 6X EGR |
| 22                | $-87.5 \pm 4.1$          | $-0.140 \pm 0.29$ | $-76.4 \pm 24.8$ | 16               | 131                    |
| 26                | $-86.1 \pm 1.4$          | $-0.003 \pm 0.08$ | $-86.0 \pm 5.1$  | 48               | 127                    |
| 29                | $-66.2 \pm 2.1$          | $0.104 \pm 0.11$ | $-73.5 \pm 8.6$  | 28               | 120                    |
| 31                | $-78.3 \pm 1.3$          | $-0.015 \pm 0.12$ | $-77.4 \pm 9.1$  | 48               | 141                    |
| 77                | $-83.1 \pm 1.2$          | $0.010 \pm 0.06$ | $-83.4 \pm 4.94$ | 65               | 142                    |
| 79                | $-86.6 \pm 1.7$          | $-0.096 \pm 1.73$ | $-80.1 \pm 47.18$ | 38               | 114                    |
| 24                | $-59.2 \pm 1.8$          | $-0.453 \pm 0.28$ | $-40.4 \pm 12.0$ | 37               | 56                     |
| Fibers uncoupled with 5X EGR |
| 15                | $-84.1 \pm 1.5$          | $0.139 \pm 1.45$ | $-99.2 \pm 156.4$ | 14               | 112                    |
| 16                | $-80.1 \pm 2.1$          | $-0.0002 \pm 0.42$ | $-80.3 \pm 20.8$ | 12               | 15                     |
| 17-A              | $-83.3 \pm 3.7$          | $-0.204 \pm 1.03$ | $-80.3 \pm 17.7$ | 12               | 24                     |
| 19                | $-78.5 \pm 2.2$          | $+0.202 \pm 0.22$ | $-82.9 \pm 11.1$ | 40               | 74                     |
| 23                | $-86.8 \pm 1.1$          | $-0.018 \pm 0.23$ | $-87.3 \pm 7.2$  | 22               | 48                     |
| 25                | $-78.0 \pm 1.6$          | $-0.012 \pm 0.10$ | $-77.0 \pm 9.3$  | 66               | 148                    |
| 27                | $-92.4 \pm 1.3$          | $-0.035 \pm 0.09$ | $-90.1 \pm 6.8$  | 48               | 100                    |

* Mean ± SEM.
† Mean ± 0.95 confidence limits.

means being highly significant ($P < 0.001$). Table I gives the results of experiments in which the decline of the resting potential was measured as a function of time. The slope of decline after treatment with 6X EGR was estimated to be $-0.044$ mv/min (279 fibers) and that after treatment with 5X EGR $-0.008$ mv/min (214 fibers), the difference between the slopes being insignificant ($P > 0.05$). Sometimes variability between fibers in the same muscle is large, as can be seen in the 0.95 confidence limits.

After treatment with 6X EGR in another series of experiments, the mean amplitudes of the action potential and resting potential were estimated to be $116.8 \pm 0.84$ and $-84.5 \pm 0.56$ mv, respectively (271 fibers). The spike was
followed by a depolarizing afterpotential provided that the membrane was
not greatly depolarized. Fig. 2 shows the action potential recorded from a fiber
after treatment with $6 \times \text{EGR}$, and the action potential from another fiber
still immersed in the hypertonic solution. The early depolarizing afterpoten-
tial was clearly present in both cases.

**Figure 2.** Action potentials elicited by nerve stimulation in a muscle fiber soaked in
$6\times$ ethylene glycol Ringer (EGR) (right) and in another muscle fiber in normal Ringer
after having been soaked in $6\times$ EGR (left). The action potential in $6\times$ EGR is followed
by a depolarizing afterpotential and contraction that dislodges the microelectrode and
that, after $6\times$ EGR, is also followed by a depolarizing afterpotential but lacks contraction.

**Figure 3.** Relationship between the initial amplitude of the early afterpotential and
the resting potential in muscle fibers uncoupled with $6\times$ ethylene glycol Ringer. The
initial amplitude was measured by extrapolating the afterpotential back to the moment
when the action potential attained the peak. The regression line calculated by the least
square method follows the equation $y = -33.05 - 0.52x$. Concentric circles indicate the
number of observations with the same value.

The amplitude of the early afterpotential (EAP) in normal muscle fibers is
a linear function of the resting membrane potential (Persson, 1960, 1963;
Gage and Eisenberg, 1969 a). Fig. 3 shows the relationship between the initial
amplitude of EAP's and the resting membrane potential in the uncoupled
muscles. The initial amplitude was measured by extrapolating the EAP to
the time when the action potential reaches its peak. The regression line was
calculated by the least square method. It shows a reversal potential of $-64$
mv, which is close to the value obtained by Persson (1963) for normal muscle fibers. In the uncoupled muscle the measurements of the amplitude of the EAP at a 5 msec interval starting at the time when the action potential reaches its peak fell on a straight line rather than on an exponential curve. This is interesting because in the normal muscle the EAP decays exponentially with a time constant similar to that of the membrane (Ishiko and Sato, 1956).

After a train of impulses, the membrane potential does not return to the resting level immediately but is followed by a slow repolarizing phase. The half-decay time of the late afterpotential (LAP) is about 300 msec and its amplitude is a function of the duration and frequency of repetitive stimuli (Freygang et al., 1964). Freygang et al. (1964) attributed this afterpotential to an accumulation of potassium ions in the sarcotubular system, and Gage and Eisenberg (1969) found that the LAP was suppressed after the functional disruption of the T system with 400 mM glycerol Ringer. A train of action potentials produced in an ethylene glycol uncoupled muscle at a frequency of 100 pulses/sec is shown in Fig. 4. A similar record from another muscle immersed in a Ringer solution added with 200 mM sucrose is also illustrated in Fig. 4. The LAP is present in the muscle immersed in sucrose Ringer which possesses a functional sarcotubular system but is absent in the muscle uncoupled with ethylene glycol.

Another electrical property of the muscle fiber that disappears after uncoupling with glycerol (Eisenberg and Gage, 1967; Gage and Eisenberg, 1969) is the so-called creep. When a strong inward step current lasting for about 1 sec was applied to the normal muscle fiber, the resultant potential change, after having risen with a time-course determined by the membrane time constant, further increased more slowly and eventually attained a steady state.
(Fig. 5, control). A similar record from a fiber immersed for 2 hr in 6X EGR and that from another fiber after uncoupling with 6X EGR are also shown in Fig. 5. The creep was absent in the last case. Anodal break excitation was produced in about 10% of the fibers uncoupled either with ethylene glycol or glycerol (Fig. 5). None of the normal control fibers or the fibers soaked in the hypertonic Ringer produced anodal break excitation.

**Figure 5.** Creep and anodal break excitation. The initial phase of the potential change produced by a strong and long-lasting inward step current is followed by a slowly rising phase (creep) in normal control muscle (upper left). The creep is present in a muscle fiber soaked in 6X ethylene glycol Ringer (EGR) (upper right), but disappears after uncoupling with 6X EGR (middle) or with 400 mM glycerol (lower right). Anodal break excitation is observed in about 10% of the muscle fibers uncoupled with either EGR or glycerol (lower left and right).

**Cable Properties**

As described in the preceding sections, the membrane potential is higher and more stable in the ethylene glycol uncouple muscle than in the glycerol uncoupled muscle, and the early afterpotential is not changed in amplitude and duration. This raises a question as to whether these two treatments produce uncoupling by different mechanisms. It has been shown that the glycerol treatment is able to disrupt functionally the sarcotubular system (Eisenberg and Eisenberg, 1968; Krolenko, 1969) and that the uncoupled fibers have a smaller membrane capacity and shorter time constant (Eisenberg and Gage, 1967; Gage and Eisenberg, 1969; Howell, 1969). The magnitude of the change in membrane capacity is in good agreement with the membrane capacity of the sarcotubular system (Falk and Fatt, 1964), suggesting that the sarcotubular space and its membrane are disrupted by the glycerol treatment.
To compare further the two methods and to clarify the mode of action of ethylene glycol, the cable properties of muscle fibers were studied. Square pulses of inward current of about $10^{-8}$ amp in strength and 25 msec in duration were applied through a microelectrode inserted in a fiber while another microelectrode was successively inserted in the same fiber at various distances from the current microelectrode (50–1500 μm, four to five impalements). The time for the resultant potential to reach 85% of the steady state at an interelectrode distance of less than 50 μ (Hodgkin and Rushton, 1946) was taken as a measure of the membrane time constant. The potential change at the zero interelectrode distance and the length constant were estimated from the regression line relating the potential to the interelectrode distance according to the following equation:

$$\ln V_x = \ln (V_0) - x/\lambda$$

where $V_x$ is the steady-state potential at the point of current injection, $V_0$ is the steady-state potential at the distance $x$, and $\lambda$ is the length constant of the fiber. Then the input resistance, $R_i$, is given by

$$R_i = V_0/I$$

where $I$ is the intensity of applied current. It follows that

$$R_i = V_0/I = 0.5\sqrt{r_m/r_i} \exp \left(-x\sqrt{r_i/r_m}\right) = R_e \exp \left(-x/\lambda\right)$$

where $R_e$ is the input resistance at the distance $x$, $r_m$ is the membrane resistance of a unit length of fiber, and $r_i$ is the longitudinal resistance of the cytoplasm in a unit length of fiber. Thus we obtain

$$r_i = \frac{2R_e}{\lambda}$$

and

$$r_m = 2R_e \cdot \lambda.$$
The above equations may be solved only when the fiber diameter is measured or $R_i$ is known. The measurement of the fiber diameter involves some uncertainty owing to polygonal cross-section of the fiber and low magnification of the microscope (X 60). Therefore, we first calculated data assuming a constant value for $R_i$ (200 $\Omega$ cm) (Falk and Fatt, 1964). The results of calculations are given in the upper three rows of Table II. It is seen that the diameter of the fibers significantly ($P < 0.001$) increases when the muscle is soaked for 2 hr in the hypertonic solution. This is opposite to what would be expected. The result may be due to the erroneous assumption that the internal resistance is kept constant while the muscle fibers are soaked in 6X EGR. Therefore, calculations were made using a value of 100 $\Omega$ cm for the internal resistivity while the muscle fibers are soaked in 6X EGR, and the results are given in the middle row of Table II. Notice that with this value of internal resistivity, the calculated diameter for the fibers in 6X EGR is the same as that for the control fibers for which $R_i$ is assumed to be 200 $\Omega$ cm. A decrease of the internal resistivity of this order of magnitude may be expected if the intracellular water content is reduced during the immersion in the hypertonic solution.

To examine this possibility, the electrical properties were calculated using the fiber diameters measured with an accuracy of $\pm 12 \mu$ in the same group of fibers as in the previous calculations, and the results are given in the lower three rows of Table II. No significant difference ($P > 0.2$) was found between the fiber diameters of the three groups, i.e. control, 6X EGR, and uncoupled. The internal resistivity was reduced to 42.5% control in the hypertonic solution; this decrease is in the same order of magnitude as that predicted in the previous calculation.

From the results given in the lower three rows of Table II, it is apparent that the only changes in the cable properties of the fibers soaked in 6X EGR are a decrease in $R_i$ and the resultant increase in the length constant, both being statistically significant ($P < 0.05$ and $P < 0.01$, respectively). After uncoupling two major changes in the cable properties of the fibers occurred: a decrease in specific membrane capacity to 60% of the controls ($P < 0.05$) and an increase in specific membrane resistance to 168% of the controls ($P < 0.05$). As the internal resistivity returned to the normal value upon uncoupling, the length constant also returned to the normal level. No difference in the values for the membrane time constant was found among the three experimental conditions.

**Intracellular Water Content and Potassium Concentration**

Since the internal resistivity decreases while the muscle is soaked in 6X EGR, a reduction of the water content of the fiber and a simultaneous increase in intracellular potassium concentration are expected to occur. In order to examine such changes, one sartorius muscle was kept in normal Ringer solution
**TABLE II**

**CABLE PROPERTIES OF MUSCLE FIBERS BEFORE AND AFTER E-C UNCOUPLING WITH ETHYLENE GLYCOL RINGER (EGR)**

| Condition       | Specific membrane resistance | Specific membrane capacitance | Internal resistivity | Diameter | Length constant | Time constant | Input resistance | Number of fibers |
|-----------------|------------------------------|------------------------------|----------------------|----------|-----------------|---------------|-----------------|------------------|
|                 | Ω cm²                        | μF/m²                        | Ω cm                | μ        | μ               | μsec          | MΩ              |                  |
| 1 Control       | 2076±359                     | 4.18±0.73                    | (200)                | 53±5     | 1152±122        | 7.70±0.51     | 0.56±0.08       | 5                |
| 2 In 6× EGR     | 3197±204                     | 2.31±0.18                    | (200)                | 72±4     | 1693±85         | 7.15±0.42     | 0.43±0.04       | 10               |
| 3 After 6× EGR  | 3079±640                     | 2.21±0.18                    | (200)                | 50±3     | 1328±100        | 6.65±1.39     | 0.71±0.1        | 5                |
| 4 In 6× EGR     | 2261±640                     | 3.18±0.26                    | (100)                | 51±3     | 1693±85         | 7.15±0.42     | 0.43±0.04       | 10               |
| 5 Control       | 2576±498                     | 3.58±0.83                    | 398±124              | 64±5     | 1152±122        | 7.70±0.51     | 0.56±0.08       | 5                |
| 6 In 6× EGR     | 2536±260                     | 3.08±0.45                    | 148±30               | 60±4     | 1693±85         | 7.15±0.42     | 0.43±0.04       | 10               |
| 7 After 6× EGR  | 4324±754                     | 1.69±0.24                    | 296±134              | 57±8     | 1330±129        | 7.34±1.53     | 0.76±0.1        | 4                |

Data of lines 1–4 were calculated assuming the internal resistivity as indicated in the parenthesis. Data of lines 5–7 were from the same sets of experiments as 1–3, but calculations were made using the measured fiber diameters. Membrane potentials were: controls (from four muscles), −81.2 ± 6.34 mV; in 6× EGR (from two muscles), −84.6 ± 1.59 mV; after uncoupling (from four muscles), −74.6 ± 4.74 mV.
as the control, while the other muscle from the same frog was treated with 6\times EGR. Fig. 6 summarizes the results of measurements of potassium content. There was no significant change in potassium content during and after soaking in 6\times EGR.

Changes in water content and potassium concentration are shown in Fig. 7. The water content decreased while soaking the muscle in 6\times EGR and increased after uncoupling, whereas the potassium concentration changed in the opposite direction during these treatments. Hyperpolarization observed in 6\times EGR and depolarization after uncoupling may be at least partly related to these changes in potassium concentration. However, it should be noted that the measurement of intracellular potassium and water content were made on large muscles. Therefore, uncoupling may not have been completed in deep fibers causing some underestimate in the magnitude of the changes.

**DISCUSSION**

The coupling between electrical activity and concentration was completely blocked after treatment of the muscle with a Ringer solution made hypertonic.
with ethylene glycol. The muscle was first soaked in 6× or 5× EGR for 2 or 4 hr and brought back to normal Ringer solution. Electrical stimulation failed to produce contraction some 15–30 min after return to Ringer. The resting membrane potential declined by about 10 mv, but the depolarization was much less than that observed after treatment with glycerol (14–35 mv depolarization) (Howell, 1969; Henderson, 1970; Eisenberg et al., 1971). However, Eisenberg et al. (1971) have recently reported that the depolarization is slowed by an addition of magnesium at a concentration of 5 mM and an increase in calcium concentration to 5 mM. One of the possible mechanisms involved in the depolarization after ethylene glycol treatment is a decrease in internal potassium concentration as a result of water movement.

The active membrane potential underwent no change after uncoupling with ethylene glycol. The mean value of 32 mv is almost exactly the same as that obtained with normal muscle fibers by Nastuk and Hodgkin (1950). In the muscle fibers uncoupled with glycerol, the active membrane potential also remained essentially unchanged (Gage and Eisenberg, 1969 a).

However, a difference is noted between glycerol and ethylene glycol treatments in their effects on early afterpotential. Although EAP was modified in amplitude and duration in the muscle fibers uncoupled with glycerol (Gage and Eisenberg, 1967, 1969 a), it was present in normal amplitude and duration in the EGR-treated fibers. This difference appears to be due at least in part to different resting membrane potentials. When the membrane was depolarized, the amplitude of EAP was decreased and eventually reversed its polarity, the reversal potential being estimated to be -64 mv, or 27 mv less negative than the normal resting potential. This reversal potential is the same as that obtained with normal muscle fibers by Persson (1960, 1963). In addition, Henderson (1970) did observe the EAP in the muscle fibers in which the membrane potential was restored to near normal value some 4 hr after uncoupling with glycerol.

The specific membrane capacity and membrane time constant obtained in the present study with normal muscle fibers are smaller than those reported previously (Fatt and Katz, 1951; Ishiko and Sato, 1960; Falk and Fatt, 1964), the capacity being about one-half and the time constant about one-third. However, the differences virtually disappear when the data by Ishiko and Sato (1960) are recalculated using the internal resistivity of 200 Ω cm. In addition, Hodgkin and Nakajima (1972) have recently found that in frog skeletal muscle fibers the specific membrane capacity is a function of the fiber diameter and reported a value of 4.6 ± 0.17 μF/cm² (n = 9) for 50-μ fibers. This value is not significantly different from 3.58 ± 0.83 μF/cm² (n = 5) described in the present paper (P > 0.10).

Drastic changes were observed in the specific membrane capacity and the specific membrane resistance after uncoupling with ethylene glycol. The ca-
Capacity decreased from the normal control value of 3.58 µF/cm², while the membrane resistance increased from the control value of 2576 to 4324 Ω cm². Because of these opposite changes, the membrane time constant remained essentially unchanged. In addition, the late afterpotential and the creep of anelectrotonic potential also decreased after uncoupling with ethylene glycol. The LAP and creep are interpreted as being due to changes in potassium concentration in the T-system lumen (Adrian and Freygang, 1962). Therefore, these changes in membrane capacity, membrane resistance, LAP, and creep are compatible with the notion that the T system is functionally eliminated in the muscle uncoupled with ethylene glycol. It should be noted that the uncoupling using glycerol results in similar changes in those electrical parameters except for the membrane time constant which decreases.

It has been shown that after treatment with glycerol the T system forms swollen vesicles (Howell and Jenden, 1967; Eisenberg and Eisenberg, 1968; Krolenko, 1969; Howell, 1969). Furthermore, electron microscope marker molecules such as ferritin or horseradish peroxidase can no longer enter the T system (Eisenberg and Eisenberg, 1968). It should be noted that such structural changes in T system produced by 200 mM glycerol are reversible (Krolenko, 1969). This suggests that a narrowing of the lumen or a coalescence of the sarcotubular membranes rather than a complete disruption of the T system is more likely to occur. The fact that the resting membrane potential after uncoupling with ethylene glycol is relatively stable is in favor of this view. However, we cannot exclude the possibility that the T system is actually disrupted after ethylene glycol treatment but the resultant holes in the muscle membrane seal by themselves quickly.

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REFERENCES

Adrian, R. H., and W. H. Freygang. 1962. The potassium and chloride conductance of frog muscle membrane. J. Physiol. (Lond.). 163:61.

Caputo, C. 1968. Volume and twitch tension changes in single muscle fibers in hypertonic solutions. J. Gen. Physiol. 52:793.

Deguchi, T., and T. Narahashi. 1971. Effects of procaine on ionic conductances of end-plate membranes. J. Pharmacol. Exp. Ther. 176:423.

Deguchi, T., T. Narahashi, and H. G. Haas. 1971. Mode of action of nereistoxin on the neuromuscular transmission in the frog. Pestic. Biochem. Physiol. 1:196.

Dydyńska, M., and D. R. Wilkie. 1963. The osmotic properties of striated muscle fibres in hypertonic solutions. J. Physiol. (Lond.). 169:312.

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. Frog skeletal muscle fibers: changes in electrical properties after disruption of transverse tubular system. Science (Wash. D.C.). 158:1700.

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

FALK, G., and P. FATT. 1964. Linear electrical properties of striated muscle fibres observed with intracellular electrodes. Proc. R. Soc. Lond. B Biol. Sci. 160:69.

FATT, P., and B. KATZ. 1952. Spontaneous subthreshold activity at motor nerve endings. J. Physiol. (Lond.). 117:109.

Fenn, W. O. 1936. The role of tissue spaces in the osmotic equilibrium of frog muscles in hypotonic and hypertonic solutions. J. Cell. Comp. Physiol. 9:93.

Freygang, W. H., Jr., D. A. Goldstein, and D. C. Hellam. 1964. The after-potential that follows a train of impulses in frog muscle fibers. J. Gen. Physiol. 47:929.

Fursphan, E. G. 1956. The effect of osmotic pressure on the spontaneous activity at motor nerve endings. J. Physiol. (Lond.). 134:689.

Gage, P. W., and R. S. Eisenberg. 1969. Action potentials without contraction in frog sartorius fibers without transverse tubules. J. Gen. Physiol. 53:298.

Gage, P. W., and R. S. Eisenberg. 1969. Capacitance of the surface and transverse tubular membrane of frog sartorius muscle fibers. J. Gen. Physiol. 53:265.

Graham, R. C., and M. J. Karnovsky. 1966. The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney. Ultrastructural cytochemistry by a new technique. J. Histochem. Cytochem. 14:291.

Gutman, I., and S. S. Wilks. 1965. Introductory Engineering Statistics. John Wiley and Sons Inc., New York.

HENDERSON, E. 1970. Potassium exchange and afterpotentials in frog sartorius muscles treated with glycerol. J. Gen. Physiol. 56:692.

Hodgkin, A. L., and P. Horowicz. 1957. The differential action of hypertonic solutions on the twitch and action potential of a muscle fibre. J. Physiol. (Lond.). 136:17P.

Hodgkin, A. L., and P. Horowicz. 1960. The effect of sudden changes in ionic concentrations on the membrane potential of single muscle fibres. J. Physiol. (Lond.). 153:370.

Hodgkin, A. L., and S. Nakajima. 1972. The effect of diameter on the electrical constants of frog skeletal muscle fibres. J. Physiol. (Lond.). 221:105.

Hodgkin, A. L., and W. A. H. Rushton. 1946. The electrical constants of a crustacean nerve fibre. Proc. R. Soc. Lond. B Biol. Sci. 133:444.

Howarth, J. V. 1958. The behavior of frog muscle in hypertonic solutions. J. Physiol. (Lond.). 144:167.

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

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

Howell, J. N., P. C. Vaughan, and R. S. Eisenberg. 1970. Maintenance of resting potentials in glycerol treated muscle fibres. Biophys. J. 10:75a.

Ishiko, N., and M. Sato. 1956. The negative after-potential of skeletal muscle fibres. Kuma- moto Med. J. 9:190.

Ishiko, N., and M. Sato. 1960. The effect of stretch on the electrical constants of muscle fibre membrane. Jap. J. Physiol. 10:194.

Korda, M. 1969. The effect of membrane polarization on the time course of the end-plate current in frog sartorius muscle. J. Physiol. (Lond.). 204:493.

Korda, M. 1970. The effect of procaine on neuromuscular transmission. J. Physiol. (Lond.). 208:1.

Krolenko, S. A. 1969. Changes in the T-system of muscle fibres under the influence of influx and efflux of glycerol. Nature (Lond.). 221:966.
Krolenko, S. A., and S. Y. Adamyan. 1967. Permeability of muscle fibers to nonelectrolytes. Tsitologiya. 9:185.

Krolenko, S. A., S. Y. Adamyan, and N. E. Shvinka. 1967. Vacuolization of skeletal muscle fibers. I. Vacuolization of fibers after discharging low-molecular nonelectrolytes. Tsitologiya. 9:1346.

Ling, G. N., M. C. Neville, S. Will, and P. Shannon. 1969. Studies on insulin action. II. The extracellular space of frog muscle. Demonstration of D-mannitol and sucrose entry into isolated single muscle fibers and intact muscles. Physiol. Chem. Physics. 1:85.

Maeno, T., C. Edwards, and S. Hashimura. 1971. Difference in the effects on the end-plate potentials between procaine and lidocaine as revealed by voltage-clamp experiments. J. Neurophysiol. 34:32.

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

Persson, A. 1960. The negative after-potential of frog skeletal muscle fibres. Acta Physiol. Scand. Suppl. 175:117.

Persson, A. 1963. The negative after-potential of frog skeletal muscle fibres. Acta Physiol. Scand. Suppl. 205:1.

Yamaguchi, T., T. Matsushima, M. Fujino, and T. Nagai. 1962. The excitation-contraction coupling of the skeletal muscle and the “glycerol effect.” Jap. J. Physiol. 12:129.