Action Potentials, Afterpotentials, and Excitation-Contraction Coupling in Frog Sartorius Fibers without Transverse Tubules

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ABSTRACT In frog sartorius muscle fibers in which the transverse tubular system has been disrupted by treatment with glycerol, action potentials which are unaccompanied by twitches can be recorded. These action potentials appear to be the same as those recorded in normal fibers except that the early afterpotential usually consists of a small hyperpolarization of short duration. After a train of action potentials no late afterpotential is seen even when the membrane potential is changed from the resting level. In fibers without transverse tubules hyperpolarizing currents do not produce a creep in potential. The interruption of excitation-contraction coupling, the changes in the afterpotentials, and the disappearance of creep are all attributed to the lack of a transverse tubular system.

Many phenomena which have no counterpart in nerve fibers have been observed in skeletal muscle. Until the transverse tubular system was discovered, these phenomena remained without explanation. The discovery in muscle fibers of an extracellular space consisting of a network of narrow tubules lined with membrane has provided the structural basis for many hypotheses which attempt to explain these phenomena. The transverse tubular system is so inaccessible, however, that direct investigations of its influence on the properties of muscle have been impossible. We have used glycerol-treated fibers in which the transverse tubular system is virtually absent (Howell and Jenden, 1967; Eisenberg and Eisenberg, 1968; Gage and Eisenberg, 1969; Eisenberg and Gage, 1969) to determine whether several phenomena of muscle do in fact reflect properties of the transverse tubular system.

It is generally believed that the transverse tubular system forms an essential part of the system by which an action potential on the surface membrane

causes contraction deep in the muscle fiber. Because localized contraction can be produced by depolarizing current applied extracellularly at localized sites along the Z line (Huxley and Taylor, 1958), it has seemed likely that the tubular system acts as a pathway into the fiber for the currents associated with the action potential. By this hypothesis action potentials should not be accompanied by contraction in fibers with a disrupted tubular system.

The shape of an action potential in skeletal muscle is characterized by an afterdepolarization which is called here the early afterpotential. The time course of the later part of this early afterpotential corresponds to the time constant of the membrane (Falk, 1961; Persson, 1963). Because the transverse tubular system is responsible for a large part of the capacitance of muscle fibers (Fatt, 1964; Falk and Fatt, 1964; Eisenberg, 1967; Gage and Eisenberg, 1969), its removal should reduce the decay time of the early afterpotential.

Other electrical properties of muscle fibers have been attributed to the transverse tubular system. The slow potential changes (late afterpotential and creep) which are present in skeletal muscle fibers have been attributed to prolonged changes in the potassium concentration of the solution filling the tubular lumen. Because of the very high ratio of the surface area of the membranes of the tubular system to the volume of the lumen of the tubules, relatively small current flows would be expected to produce significant changes in the concentration of ions. Thus, the prolonged slowly declining potential change which follows a train of action potentials (here called the late afterpotential) has been attributed (Freygang, Goldstein, and Hellam, 1964; Freygang, Goldstein, Hellam, and Peachey, 1964) to an accumulation in the lumen of the transverse tubules of the potassium which presumably crosses the tubular membrane during the train. Similarly, a slow potential change occurs when a sufficiently large inward (hyperpolarizing) current is passed across the membrane of muscle fibers: the potential does not approach a steady state with the normal time constant but continues to increase as if the membrane resistance were increasing (Adrian and Freygang, 1962). This slow increase in potential (called "creep") was attributed to a progressive decline in the potassium concentration in the tubular lumen. In the present investigation prolonged hyperpolarization and trains of action potentials have been recorded in glycerol-treated muscle fibers in order to determine whether the transverse tubular system is involved in these phenomena.

Some of these results have been briefly described (Eisenberg and Gage, 1967; Gage and Eisenberg, 1967).

METHODS

The preparation and solutions have been fully described (Gage and Eisenberg, 1969), but in most of these experiments the solutions did not contain tetrodotoxin. In order

to elicit action potentials, surface muscle fibers were depolarized with an intracellular microelectrode. A constant current generator (Gage and Eisenberg, 1969) was used to control the current through the microelectrode. Potential was recorded with a second intracellular microelectrode connected to an operational amplifier in the potential-recording configuration described previously (Eisenberg and Gage, 1969). This circuit allowed accurate recording of fast transients without the necessity for capacity neutralization.

RESULTS

Action Potentials

In glycerol-treated surface fibers with resting potentials greater than −70 mv, action potentials could always be elicited. These action potentials had

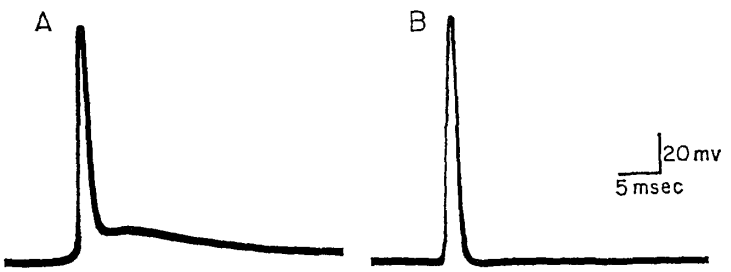

FIGURE 1. Action potentials in normal (A) and glycerol-treated (B) muscle fibers The "normal" fiber was immersed in a hypertonic Ringer solution which contained 300 mM NaCl in order to reduce movement. Both fibers had resting potentials of −82 mv. Note the pronounced early afterpotential in the normal fiber (A) and the virtual absence of an afterpotential in the glycerol-treated fiber (B).

a distinct threshold and were propagated. When a fiber was depolarized with a long current pulse of suprathreshold strength, repetitive action potentials were observed. The amplitude of action potentials in glycerol-treated fibers was the same as in fibers with intact transverse tubular systems providing the resting potentials were the same (Fig. 1). The action potential in Fig. 1 A was recorded from a fiber which was immersed in hypertonic Ringer (containing 300 mM NaCl) to prevent contraction (Hodgkin and Horowicz, 1957) whereas the action potential in B was recorded from a glycerol-treated fiber. The height of the overshoot was not significantly changed by the glycerol treatment.

Excitation-Contraction Coupling

In fibers in which the transverse tubular system had been disrupted, action potentials never caused twitches. Even long trains of action potentials caused no discernible movement. Depolarization of the surface membrane with current also caused no contraction. On several occasions when the whole

sartorius was stimulated with platinum electrodes it was evident that the deeper fibers were contracting, thus suggesting that these fibers still had an intact transverse tubular system. This observation is consistent with the relatively normal appearance (in the electron microscope) of the transverse tubular system in deep fibers (Eisenberg and Eisenberg, 1968) in glycerol-treated muscles. Occasionally, the membrane time constant was normal in glycerol-treated surface fibers suggesting that the transverse tubular system was intact and in these fibers, action potentials were accompanied by contractions. It was sometimes found that a slight stretch applied to these fibers caused a short burst of contractions; immediately afterwards the time constant of the membrane became markedly shorter and action potentials did not produce contraction.

These observations show that following the glycerol treatment, action potentials in the surface membrane cannot produce twitches, presumably because of the disruption of the tubular system.

Early Afterpotential

The most striking change in the action potentials in glycerol-treated fibers was the absence of the normal early afterpotential. This potential normally consists of a relatively slow repolarization of the membrane at the end of an action potential (Fig. 1 *A*). The rate of decay of the later part of the early afterpotential corresponds to the time constant of the fiber. Because the time constant of glycerol-treated fibers has been reduced to about one-third of normal (Gage and Eisenberg, 1969), the early afterpotential in these fibers should also be less prominent if the afterpotential is, in fact, caused by the slow recharging of the membrane capacitance. In glycerol-treated fibers, however, not only was the early afterpotential reduced in duration, but also its sign was usually reversed. Action potentials are shown (Fig. 1) in a normal (A) and glycerol-treated fiber (B). The "normal" action potential (A) was recorded from a fiber in a hypertonic solution (containing 300 mM NaCl) in order to prevent twitching (Hodgkin and Horowicz, 1957) and the early afterpotential can be clearly seen. The action potential in the glycerol-treated fiber is not followed by the normal early afterpotential but rather by a small hyperpolarizing afterpotential (Fig. 1 B) with a relatively rapid time course.

In normal fibers the first part of the early after potential often appears as a downward notch immediately after the spike (Fig. 1 A). This "notch" is known to have a reversal potential at about −68 mv and to be accompanied by an increase in membrane conductance (Persson, 1963). The hyperpolarization seen in glycerol-treated fibers also has a reversal potential but at several millivolts more negative than the resting potential. The reversal potential was found by recording action potentials in fibers in which the membrane potential was varied by passing current from an intracellular

electrode. A typical experiment is illustrated in Fig. 2. Action potentials were elicited at different membrane potentials and it can be seen that reversal of the early afterpotential occurred close to the resting potential. A graph of the maximum amplitude of the early afterpotential against membrane potential is shown in Fig. 3. In two fibers with resting potentials of −68 mv (circles) and −84 mv (squares) the membrane potential was set at a more depolarized or hyperpolarized level by passing current. A plot of the amplitude of the early afterpotential against membrane potential was approximately linear

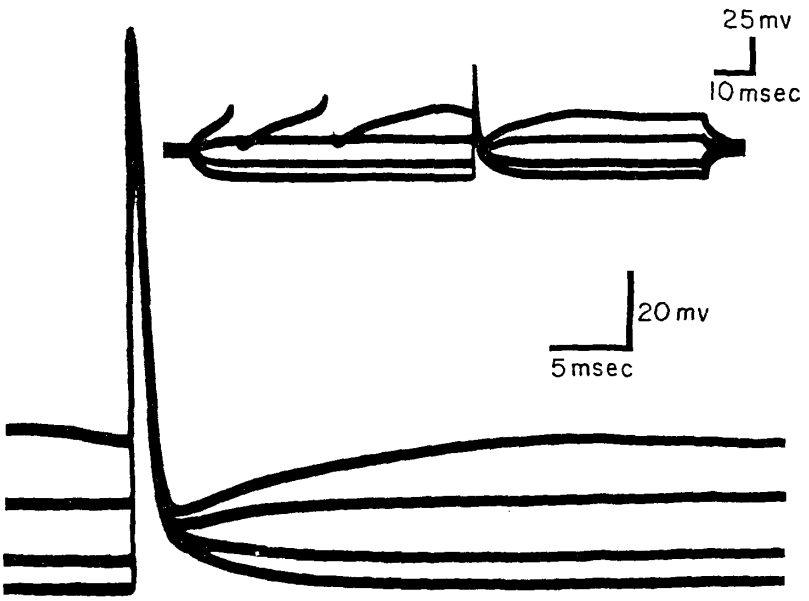

FIGURE 2. Early afterpotentials in glycerol-treated fiber at different membrane potentials which were set by passing constant current from an intracellular electrode. The main figure shows action potentials and early afterpotentials at the different levels of membrane potential. Inset is the same record at a slower sweep speed and lower voltage gain to show the resting membrane potential, the polarized membrane potential, and the early afterpotential following the spike. At the level of depolarization in the uppermost trace, repetitive action potentials occurred.

in the range close to the resting potential. The intercepts of the lines and the abscissa give the reversal potentials; in both cases these were at a more hyperpolarized level than the membrane potential. This reversal potential differs from the reversal potential in normal fibers in that it occurs at a slightly more negative potential than the resting membrane potential whether the latter is at −68 mv or −84 mv.

It is tempting to speculate that the level of the first phase of the early afterpotential has an equilibrium potential set mainly by the potassium equilibrium potential (E_K). Any accumulation of potassium ions in the

transverse tubules during an action potential would make the net E_K more positive than would be predicted from the distribution of potassium across the surface membrane. When the transverse tubules are disrupted, however, potassium ions would not accumulate and the net E_K, now equivalent to the surface E_K, would remain unchanged during an action potential. Another explanation might be that following or during an action potential the transverse tubules become permeable to another ion with an equilibrium potential

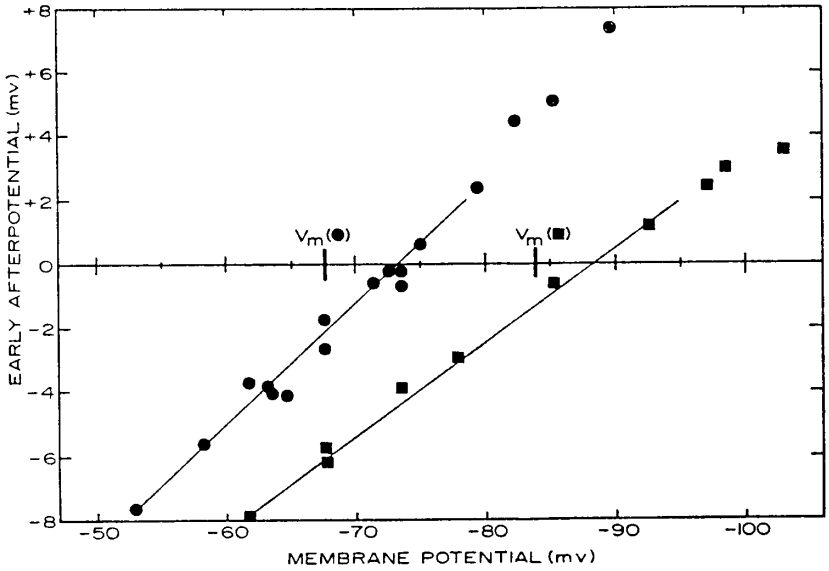

FIGURE 3. A plot of the maximum amplitude of the first part of the early afterpotentia against the membrane potential (immediately before and after the action potential) The results from two experiments are shown, the data in one case being shown as filled circles, in the other case as filled squares. The resting potentials (V_m) of the two fibers are shown. Note that the absolute values of the reversal potential (the "x intercept" of the line) are quite different, but that the difference between resting potential and the reversal potential is about the same in the two cases. The points above the line were difficult to measure and are shown mainly for the sake of completeness.

more positive than E_K during the first phase of the early afterpotential. Without more evidence the question remains open.

Late Afterpotential

After repetitive action potentials in frog sartorius fibers the membrane remains depolarized for some time (Freygang, Goldstein, and Hellam, 1964; Freygang et al., 1964). The amplitude of this depolarization and the time constant of decay of the afterpotential depend on the number of action potentials in the train. Following a train of 10 action potentials at a frequency of 100/sec, the peak amplitude of the late afterpotential is about 13 mv and the

time constant of the decay is about 300 msec (Freygang, Goldstein, and Hellam, 1964; Freygang et al., 1964). It has been proposed that these late afterpotentials are the result of accumulation in the transverse tubules of potassium ions which leave the fiber during the action potentials (see also Hellam et al., 1965). Because of the diffusion delays caused by the long narrow tubular system, this concentrated pool of potassium takes some time to be diluted to the normal potassium concentration; during this time the tubular membrane remains depolarized. This hypothesis is based of necessity on indirect evidence but would predict that fibers without a transverse tubular system should not have late afterpotentials. This is indeed what we found. A standard train of 10 or 11 action potentials at 100/sec was used to

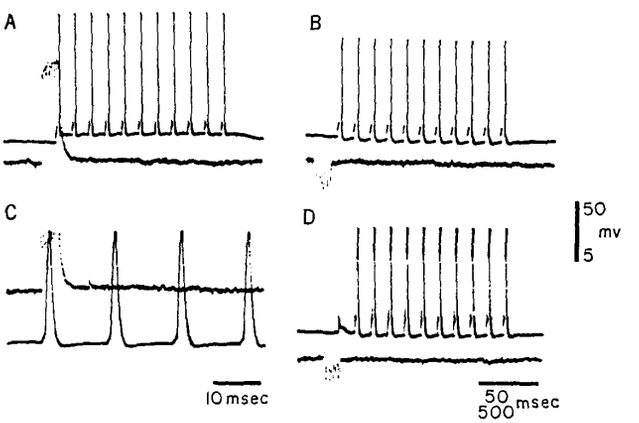

FIGURE 4. Trains of action potentials not followed by late afterpotentials in glycerol-treated muscle fibers. Each pair of records, taken from different fibers, shows repetitive action potentials at low gain and high sweep speed (above in A, B, and D; below in C) and at high gain and slow sweep speed (noisier traces). Note the absence of the late afterpotential.

allow comparison with previous results obtained in normal fibers. The late afterpotential found in normal fibers was not present in fibers without transverse tubules. This is illustrated in Fig. 4. Records were taken at two different gains and sweep speeds: one trace at high gain and a slow sweep speed (noisier record, below in A, B, and D, above in C) provided accurate records of the late afterpotential, the other at a lower gain and a faster sweep speed gave a good record of the action potentials. It can be seen (Fig. 4) that there was no late afterpotential in any of these four fibers (A, B, C, and D) and this was invariably found.

If in normal fibers the late afterpotential has an equilibrium potential more positive than the resting potential whereas in glycerol-treated fibers the reversal potential is the same as the resting potential, the lack of a late afterpotential in the latter case could be simply explained by the absence of a

driving force ($E_m - E_K$). In order to determine whether this is so, an attempt was made to elicit late afterpotentials in glycerol-treated fibers in which the membrane potential was displaced from the resting level with polarizing currents. If the equilibrium potential of the late afterpotential corresponded to the resting membrane potential, polarization should reveal an afterpotential. Records from two such experiments are shown in Fig. 5. The upper traces (at high gain and slow sweep speed) show the membrane polarization level and the potential after each train. The lower traces (at low gain and faster sweep speed) were used to monitor the action potentials. It is clear that there is no late afterpotential in glycerol-treated fibers even when the

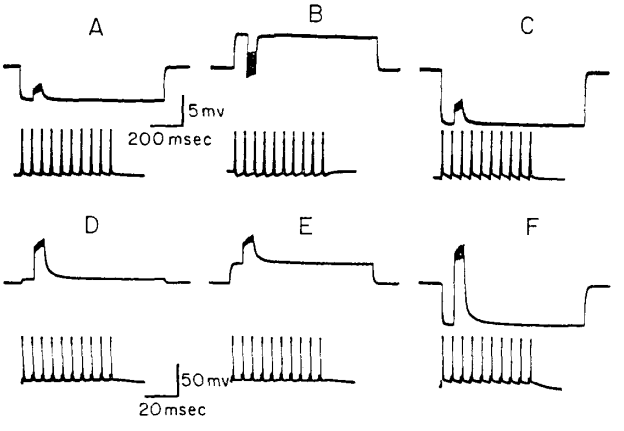

FIGURE 5. Trains of action potentials not followed by late afterpotentials at different levels of membrane potential in glycerol-treated muscle fibers. In these experiments (A, B, C from one fiber; D, E, F, from another) the lower sweep was recorded at low gain and fast sweep speed to show the presence of action potentials and the upper traces were recorded at high gain and slow sweep speed to permit better examination of the afterpotential, if any. Note the absence of a late afterpotential in all cases.

membrane potential is displaced from the resting level. These results show that the phenomenon of a late afterpotential can be attributed to the transverse tubular system but do not distinguish between the alternative explanations of accumulation of potassium in the tubules or a slow change in the conductance of the tubular membrane following a train of action potentials.

Creep

As mentioned in the Introduction, the slow creep in potential produced by inward current in muscle fibers has been explained by postulating a progressive decline in the potassium concentration in the tubules. Such an explanation would predict that no creep would occur in muscle fibers without transverse tubules and this was what we found. Some typical results are illustrated in

Fig. 6. In A electrotonic potentials were recorded in response to hyperpolarizing current pulses of varied intensity applied to a normal fiber in Ringer solution (pH 7.2). Creep becomes apparent when the hyperpolarization exceeds 15 mv and is pronounced at 30 mv. In glycerol-treated fibers in the same solution (Fig. 6 B) the records show no creep. Because creep is more pronounced when the chloride conductance is reduced (Adrian and Freygang, 1962), some experiments were done in solutions at pH 5.6 since in acid solutions, the chloride conductance is very low (Hutter and Warner, 1967 *a*, *b;* Moore, 1966; Eisenberg and Gage, 1969). In normal fibers creep occurs at a smaller hyperpolarization in these acid solutions (Fig. 6 C). The "bel-

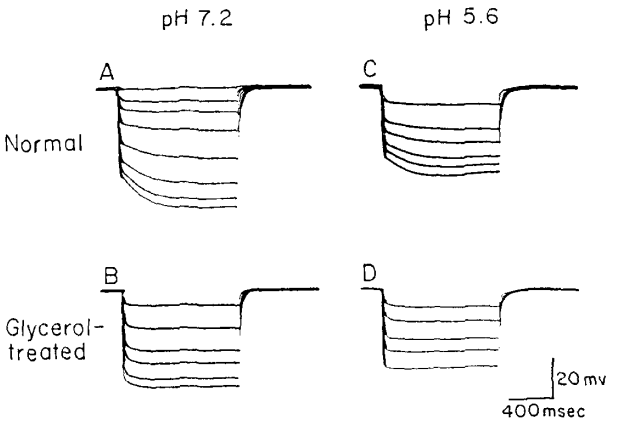

FIGURE 6. The time course of hyperpolarization of normal (A, C) and glycerol-treated muscle fibers (B, D) at pH's of 7.2 (A, B) and 5.6 (C, D). The potential displacements were produced by rectangular current pulses. Note the progressive increase in potential (creep) in the normal fiber (especially for large polarizations) and the absence of this creep in glycerol-treated fibers. Note also the maxima (bellying) during the larger hyperpolarizations in the normal fibers.

lying" present in the bottom traces in A and C was often seen in normal fibers and will be discussed later. Even under conditions of lowered chloride conductance creep was never observed in glycerol-treated fibers (Fig. 6 D). It would seem therefore that the phenomenon of creep is also associated with the presence of an intact transverse tubular system.

DISCUSSION

It is evident from these results that muscle fibers without tubules do not twitch in response to action potentials or depolarization. Our results do not show, however, whether the contractile apparatus in these treated fibers is capable of producing contraction. Since caffeine produces contractures in glycerol-treated fibers (Howell and Jenden, 1967; Sandow and Geffner, personal communication), it would seem that these fibers are indeed capable of

contracting. The absence of twitches in glycerol-treated muscle fibers thus shows that the transverse tubular system is an essential link between excitation of the surface membrane and contraction. The presence of relatively normal action potentials in glycerol-treated fibers reveals some of the properties of these fibers. First, the very existence of the action potential shows that the sodium and potassium conductance systems presumably involved must lie on the surface membrane. Our results, however, do not rule out the possibility that these systems are also present in the tubular membrane. Second, the normal overshoot of the action potential shows that the internal sodium concentration of these fibers is not greatly altered and further suggests that the internal potassium concentration is probably not grossly changed.

The only part of the action potential which is appreciably changed is the early afterpotential. The later part of this potential is greatly shortened in glycerol-treated fibers supporting the suggestion that the time course of the early afterpotential is determined by the membrane time constant. Because the early afterpotential has a reversal potential within a few millivolts of the resting membrane potential and the time course is so shortened, the early afterpotential is virtually absent in glycerol-treated preparations (Gage and Eisenberg, 1967). The polarization experiments (Figs. 2 and 3) show, however, that the mechanism underlying the early afterpotential is present, though with different properties from normal, in glycerol-treated fibers. The change in the reversal potential upon disruption of the tubules suggests that in normal fibers the level is governed to some extent by the tubular system as well as the surface membrane. As mentioned in the Results the mechanism responsible may be the potassium equilibrium potential in the tubules but the possibility that other ions may be involved has not been excluded.

In order to analyze the slow potential changes of muscle, it is convenient to use an equation (Hodgkin and Horowicz, 1959) which describes the membrane potential in terms of the conductance and equilibrium potential of various ions and the total current flow

$$V_m = \frac{I}{g_K^s + g_K^t + g_{Cl}^s} + \frac{g_K^s}{g_K^s + g_K^t + g_{Cl}^s} E_K^s + \frac{g_K^t}{g_K^s + g_K^t + g_{Cl}^s} E_K^t + \frac{g_{Cl}^s}{g_K^s + g_K^t + g_{Cl}^s} E_{Cl}^s \quad (1)$$

Where V_m denotes membrane potential; I, membrane current; and E, with the appropriate sub- and superscripts, the equilibrium potential for the conductance system indicated. This equation has been written in terms of the potassium conductance of the surface and tubular membranes (g_K^s and g_K^t, respectively), which in the resting state are in the ratio of about

1:2; and in terms of the chloride conductance of the surface g_{Cl}^{s} alone, since there is no resting chloride conductance in the tubular system (Eisenberg and Gage, 1969). Currents which are small during the processes of interest, such as the sodium and capacity current, have been neglected.

During the late afterpotential there is presumably little if any net current flowing through the membrane and the first term of the right side of equation (1) can be neglected. The membrane potential is then set only by the equilibrium potentials and relative conductances of the various systems involved. Freygang et al. (1964) have proposed that the late afterpotential is caused by a slow change in $E_K{}^t$, associated with a change in the potassium concentration in the tubular lumen. Our results support this hypothesis in two ways. First, it is clear that if the potassium concentration in the tubules is to influence the membrane potential, there must be an appreciable potassium conductance in the tubular membranes. In the preceding paper the results suggest that most of the resting potassium conductance system is in fact in the tubular system (Eisenberg and Gage, 1969). Second, the results reported here indicate that the late afterpotential is absent in fibers without tubules and thus imply that the late afterpotential is associated with the tubular system. Another explanation of the late afterpotential consistent with our data is possible, however. If even at rest $E_K{}^s$ were not the same as $E_K{}^t$, then a slow change in $g_K{}^t$ following a train of action potentials would produce a late afterpotential. Such an afterpotential could occur in the absence of any change in the concentration of ions in the tubular lumen and would, of course, be absent in fibers without tubules. Furthermore, in view of the close proximity of the membrane of the terminal cisternae of the sarcoplasmic reticulum and much of the tubular membrane (see, for instance, Fig. 4, Gage and Eisenberg, 1969), it is not inconceivable that the potassium ion concentration on the "inside" of the tubular membrane (i.e. inside the sarcoplasmic reticulum) might be different from that of the sarcoplasm, thus making $E_K{}^s$ different from $E_K{}^t$. It is possible, of course, that both of the proposed mechanisms are involved in the late afterpotential; or that the afterpotential is produced by a process involving some ion as well as, or other than, potassium.

The slow creep of potential which occurs during inward current flow is somewhat more difficult to analyze since in this case the first term of equation (1) is not necessarily negligible. Adrian and Freygang (1962) have suggested that this hyperpolarization is caused primarily by a reduction in the potassium concentration of the solution in the lumen of the tubules. Our results support this hypothesis in that we have shown that there is no creep when there are no tubules, and that there is potassium conductance in the tubular membrane (Eisenberg and Gage, 1969). It is possible, however, to explain the phenomenon of creep in terms of a slow change in the conductance $g_K{}^t$,

as suggested in the discussion of the late afterpotential (see also Adrian, Chandler, and Hodgkin, 1968). In the analysis of creep, the presence of a term which involves the ratio of I (current) and $g_K{}'$ (tubular potassium conductance) means that a difference in the equilibrium potential for potassium in the tubules and surface does not have to be invoked.

In normal muscle fibers when rather large inward currents were passed, the potential displacement passed through a distinct maximum and then declined (bellying) (Fig. 6 A and C). This phenomenon was reversible and was not associated with the deterioration of the fiber which occurs when very large hyperpolarizing currents are passed. This maximum in the creep potential was not reported by Adrian and Freygang (1962) and may be associated with a difference in technique. In their experiments, "conductance" was recorded under conditions when the membrane potential did not vary appreciably with distance whereas we recorded potentials under conditions when membrane potential did vary with distance. Another explanation of the maximum is possible. It is known (Hodgkin and Horowicz, 1959; Adrian & Horowicz, personal communication) that in muscle fibers the potassium conductance is reduced at very low potassium concentrations with the result that the membrane depolarizes. A decline in g_K has also been found when the potassium concentration is reduced to very low levels under different conditions and it has been suggested that the potassium concentration affects potassium permeability by determining the number of K carriers in the membrane (Horowicz, Gage, and Eisenberg, 1968). If strong prolonged inward currents were so to deplete the potassium concentration in the tubular lumen that $g_K{}'$ was reduced in a similar way, the maxima caused by the stronger hyperpolarizing currents (Fig. 6 A and C) might then be explained.

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