THE EFFECTIVENESS OF THE LONGITUDINAL FIELD, COUPLED WITH DEPOLARIZATION IN ACTIVATING FROG TWITCH MUSCLES

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ABSTRACT

Effective excitation, preceding the mechanical response in frog twitch muscles, involves two distinct events: depolarization of the excitable membrane and the flow of internal currents. To distinguish between the effects of these two potential factors in activation of the contractile machinery, experiments ought to be conducted in which one or the other is excluded. Our experiments are designed to distinguish between these effects by indirect methods.

Depolarization in a longitudinal electric field can be expected to be greatest at the ends where current leaves the muscle fibers (space constant at [K] = 16 mM/liter is >1 mm.), whereas the internal longitudinal current is known to be greatest in the middle portion. Depolarization, therefore, should affect the ends more strongly and internal current the middle portion.

Our experiments show that non-propagating frog twitch muscles shorten, during isotonic work, along their whole length and not only at their ends, when effectively stimulated in a longitudinal A.C. field. At a field strength about twice the new threshold value (at $[K]_o$ = 16 mM) shortening is distinctly greater in the middle portion of the muscle than at the ends. The muscles, although temporarily non-propagating, remain *intact* throughout the experiment, as demonstrated by complete recovery after repolarization.

These findings may be taken as an indication that internal currents are more directly linked to activation than is depolarization, but the latter is an essential priming step, which must precede or coincide with effective current flow.

When the potential difference of 60 to 100 mv. across the resting muscle membrane is quickly reduced to a "critical" value (52 mv., winter frog, Jenerick and Gerard, 1953), the potential falls to zero and becomes actually reversed (see for references, Hodgkin, 1951). This brief ($\cong$ 0.6 msec.) reversal of the membrane potential, *i.e.* the action potential, propagates itself with high velocity (2 m./sec., 20°C., Sten-Knudsen 1954) by a mechanism similar to the conduc-

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tion of a nerve impulse. "Activation" of the contractile system, in intact normal muscle, follows this self-propagated activity of the fiber membrane.

The exceedingly short time lag between the initiation of the action potential and "active state" ($\cong$ 3 msec.) in frog twitch muscles, and also the short time requirement for full activity ($\cong$ 30 msec.), raise the question of how the contractile system is influenced, from a distance of $\cong$ 50 μ, by an action potential generated at the fiber membrane (Hill, 1949 *a*, *b*, 1950, 1951; Katz, 1950; Sandow, 1952; Huxley, 1956).

Hill (1950) stated that "no known link exists at present between the events of excitation and those of contraction. It is impossible . . . to suppose that the active state is propagated inwards from the surface by some actual substance diffusing in. A self-propagating process of some kind, started at the surface must be looked for."

When the action potential propagates along the fiber, not only is the potential difference across the membrane eliminated (depolarization), but also a longitudinal field is generated inside the fiber, carrying considerable current. It is significant that the time integral of this field is uniform over the whole cross-section area of the fiber (Bay, Goodall, and Szent-Györgyi, 1953). Extensive studies with simplified muscles (glycerinated psoas) and extracted muscle proteins lead to the conclusion that a process which upsets the ionic balance, *i.e.* the distribution of charges on the contractile proteins, brings about contraction (Szent-Györgyi, 1953; Weber and Portzehl, 1954). The suggestion was also advanced, considering the periodic distribution of divalent cations (Draper and Hodge, 1949) and their drastic effects on the contractile system, that Ca and Mg may play a special role in activation (Sandow, 1952; Weber and Portzehl, 1954; Morales and Botts, 1956). Bay, Goodall, and Szent-Györgyi proposed (1953) that the longitudinal ("window") field activates the contractile system by charge displacement.

The time limitations justly emphasized by Hill (1950), based on elasticity and heat measurements in the frog sartorius muscle during mechanical latency, demand that any mechanism proposed for activation in this muscle must become effective within 3 msec. after stimulation and must complete its action within 30 msec. The maximum longitudinal potential gradient which occurs during a propagated spike (at 20°C) is 3v/cm. (Sten-Knudsen, 1954) which, if effective, can displace ions (for example Ca) by a distance of about 2 μ in 30 msec.

However attractive the conjecture that the longitudinal component of the action potential is more directly linked to activation than membrane depolarization, it completely lacks experimental support and the general view is that the longitudinal field is ineffective. According to Katz (1950) "the stimulus arises not from the current which circulates through the interior of the muscle fiber, but from the change of electric charge at the fibre surface." He only in-

sists, however, that "the disturbance of the contractile matter must originate at the fibre surface," and for the effectiveness of the internal currents he uses the phrase "doubtful," without claiming that this possibility had been ruled out. A more firm attitude has been recently expressed by Huxley (1956). He states that "during the action potential, considerable currents flow along the interior of the fibre and it has been suggested that the fibrils are stimulated to contract by these currents flowing through them. This has been shown definitely not to be the case (Sten-Knudsen, 1954) and it is fairly clear that the essential thing is the change in the potential difference across the membrane itself."

Clearly if Sten-Knudsen's conclusions (1954) are correct concerning the ineffectiveness of the longitudinal external field, the participation of internal currents in the activation process is ruled out. We have repeated and extended his experiments and arrived at the conclusion that the externally applied A.C. longitudinal field is effective in eliciting muscle response in non-propagating frog twitch muscles (Csapo and Suzuki, 1957). Being able to study the mechanical response of non-propagating frog muscles to electrical stimulation, for periods of 1 hour without loss of tension, we devised experiments to show that the effect of the external longitudinal field is associated with internal longitudinal current and not with electrotonic membrane potential change, or change in the longitudinal resistance of the fiber due to irregularities.

It should be pointed out that the conclusion of Huxley and Taylor (1955), that "the influence of membrane depolarization is conveyed to the interior of the fibre by spread along the 'Z' bands," is by no means in contradiction to the notion that internal currents effectively participate in the activation process, since depolarization is always accompanied by internal current flow. These experiments (Huxley, 1956) could be interpreted as demonstrating that the excitable membrane is inhomogeneous and its various portions within one sarcomere, in different muscles, respond differently to depolarizing current. The questions remain open: what is spreading along cross-structures, how the contractile system is activated between two Z bands 2.5 μ apart, and how muscles not having such cross-structures are activated?

It must be remembered that during normal excitation not only longitudinal currents flow, but also the membrane is completely depolarized, in fact the membrane polarity is reversed. Experiments designed to show the effect of the longitudinal field must imitate this condition as closely as possible. In most muscles and specifically in the frog twitch muscle, however, it is impossible to study the effect of the longitudinal field on a completely depolarized muscle because at a steady membrane potential of about 35 mv. the muscle completely fails to respond to electrical stimulation. To keep the mechanical response in frog muscle close to its normal value the membrane potential must be kept, therefore, around 40 mv., a value at which the effective external longitudinal

field requirement is still very high, probably because of considerable membrane resistance. Nevertheless, it is possible to obtain close to maximum response in a longitudinal A.C. field of sufficient intensity, provided that the membrane is depolarized to this value of about 40 mv.

On the other hand the demonstration that activity in muscles coincides with depolarization, and furthermore, that contraction is localized to the depolarized region, do not rule out the participation of internal currents in the effect. For this it must be shown that internal currents were not generated when activation occurred, not even transiently. This is necessary because it is conceivable that once internal currents exerted their respective effects in the series of events leading to activation, their participation would be needed no longer.

In this paper experiments are presented in support of the conclusion that activation in frog twitch muscles results from a combined effect of depolarization and that of internal currents. That depolarization is an essential first step in the series of events leading to activation (Kuffler, 1946) is also indicated by our observation that the longitudinal field is only effective if the muscle is sufficiently depolarized. That the external longitudinal A.C. field is associated with internal longitudinal currents is indicated by the fact that shortening in the non-propagating (K depolarized) muscle is the greatest in the middle portion of the muscle, even when a stimulus, slightly exceeding the new threshold (in excess K), is used. This finding is in good agreement with the observations of Rushton (1930), Hodgkin and Rushton (1946), and Katz (1948), that the voltage gradient is the greatest in the middle portion of nerve and muscle.

Methods

The sartorius and the toe muscle (musculus extensor longus digiti IV) of the frog (*Rana pipiens*) were used during October and November, 1956. The muscles were prepared under a dissecting microscope at a magnification of 10 in normal Ringer (mM/liter: 111 NaCl, 11.9 $NaHCO_3$, 1.8 $CaCl_2$, 1.3 KCl; and 100 mg. per cent dextrose). The muscles were mounted horizontally in a square lucite chamber of 5 cm. × 5 cm., in 40 ml. Ringer, the height of which was 1.8 cm. Platinum plate electrodes covered two opposite internal walls of the chamber. This arrangement is very similar to that used by Sten-Knudsen (1954), except that no metal besides the electrodes was immersed in the bath.

The sartorius was mounted horizontally, one of the flat surfaces of the muscle looking upward. The pelvic end of the sartorius was held by a thin J-shaped glass hook with a slot about 1 mm. wide and 6 mm. long, the pelvic end being prevented from pulling through the slot by a small piece of its cartilaginous origin. The tibial end was tied to a thin and flat lucite lever of minimum friction, which transmitted the tension to a Grass strain gauge, No. FT10, the output of which was recorded on paper by a Grass ink writer, type IIID. For the toe muscle the same set-up was used, except that the tendons were tied at both ends and the Grass gauge was type FT02. When shortening was recorded photographically, during isotonic work, the sartorius was after-loaded with 5 gm., and the toe muscle with 0.5 to 0.8 gm.

The muscles were immersed in normal Ringer and oxygenated by 97 per cent O_2 + 3 per cent CO_2 (pH, 7.3). They were tested for maximum isometric tension in a 1 second tetanus (lv./cm., 60 c./s., A.C.). Sartorius muscles producing less than 70 gm., and toe muscles producing less than 4 gm. tension were discarded. If tension at the peak of the tetanus was not steady the muscle was also discarded. After having been depolarized by high K in order to abolish propagation, the muscles were always repolarized in normal Ringer at the end of the experiment. Only those experiments are included here in which recovery (as indicated by maximum tension in a propagated response) was complete.

After having been tested for maximum force the muscles were marked by small graphite particles or by thin black nylon threads. Generally seven marks were placed on the upper surface of the muscle, dividing the whole length into six, about equal, segments. The two tendinous ends of both sartorius and toe muscles were omitted from the experiment by placing the last marks at the two ends of the muscle where muscle tissue gives place to tendon. After the muscles were marked, they were tested again for maximum tension to make certain that no damage was done during the marking procedure.

Shortening during isotonic work was recorded photographically. In some experiments only one picture was taken at the peak of isotonic work (about 200 msec. after the beginning of stimulation). In other experiments a motion picture camera was used which was capable of taking pictures repeatedly at time intervals of about 20 msec. This technique was employed to increase the accuracy of the measurements.

Arguments

The experiments to be described were designed to establish whether the sartorius and toe muscle (musculus extensor longus digiti IV), rendered non-propagating by excess K, shorten during isotonic work along their whole length or only at their ends, when stimulated slightly above the new threshold (at $[K]_o$ = 16 mM/liter) in a longitudinal A.C. field. If the muscle shortens along its whole length, is the shortening greatest at the ends or in the middle portion, or does the shortening vary in different segments along its length? If the field strength is increased in steps, will it be the relative shortening of individual segments which changes, or rather the extent of shortening of each segment?

In addition, some experiments were carried out to reinvestigate the question whether depolarization alone, without the participation of internal currents, can fully activate frog muscles.

The justification for carrying out these experiments came from an extensive study (partial report, Csapo and Suzuki, 1957) which provided evidence that non-propagating frog muscle can be effectively activated in an external longitudinal A.C. field of sufficient strength and optimum frequency. These results, however, could not be used as evidence that the effect of the external longitudinal field is associated with internal longitudinal currents. As pointed out to us by Mr. Andrew Huxley (personal communication) it is possible to argue that the effect of the external longitudinal field (4 to 20 v./cm.) is associated with

electrotonic membrane potential changes, which are known to be greatest at the ends where current leaves the fibers. It could also be argued that the external longitudinal field is effective through depolarization of the fiber membrane due to changes in the longitudinal resistance of the individual fibers along their length, or of the space between the fibers.

It seemed possible for us to test experimentally the validity of these just arguments. If the external longitudinal A.C. field effect is associated with electrotonic membrane potential change, then shortening should be localized entirely or ought to be greater at the ends than at the middle portion, because of the small space constant. As the field strength is increased in steps, one can expect more and more shortening toward the middle portion due to a spreading of the electrotonic membrane potential change toward the middle of the muscle, but the ends ought to be always more affected. If, therefore, the order of the individual segments along the length of the muscle is plotted against their respective shortening, a family of *concave* curves should be obtained which would gradually straighten out as the field strength is increased due to an increasing spread of the electrotonic membrane potential change toward the middle of the muscle.

If, on the other hand, depolarization arises from changes in the longitudinal resistance of individual fibers or from irregularities in the space between fibers, one should get a set of irregular curves or possibly straight lines in a similar plot.

If, on the other hand, the effect of the external longitudinal A.C. field on non-propagating frog muscle is associated with internal longitudinal currents, one can expect maximum shortening in the middle portion of the muscle, diminishing toward the ends. This expectation is justified by the observations of Rushton (1930), Hodgkin and Rushton (1946) on nerve, and by the findings of Katz (1948) on muscle, demonstrating that "the voltage gradient along the inside of the fibers is the smallest at the ends and the greatest along the middle portion" (quoted from Sten-Knudsen, 1954). With a minimum effective stimulus which depolarizes the whole length of the muscle one may expect already more shortening in the middle portion than at the ends. Increasing the field strength should result in a family of *convex* curves in a plot as described before, moving upwards on the shortening axis as the field strength is increased, and more and more fibers are affected.

RESULTS

Fig. 1 illustrates an experiment on the toe muscle. The muscle is marked off into six segments by seven graphite particles. The non-propagating muscle (16 mM K/liter) is photographed at rest (*A*) and at the peak of isotonic work, induced by a longitudinal A.C. field (60c./s.) of 4 v./cm. (*B*), 8v./cm. (*C*), and 12v./cm. (*D*) respectively. One can see that shortening involves the *whole*

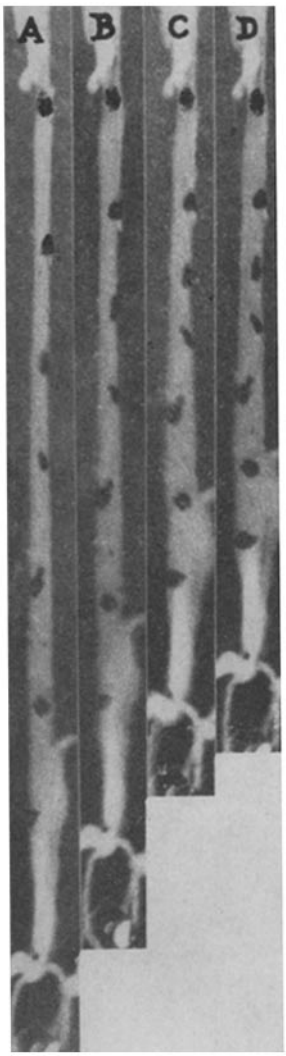

FIG. 1. Shortening during isotonic work, at different portions along the length, in non-propagating frog toe muscle. The muscle is rendered non-propagating by excess K (16 mM/liter) in an otherwise normal Ringer. Marks are graphite particles. Muscle is photographed at rest (A), and at the peak of isotonic work (load = 0.8 gm.), *i.e.* 200 msec. after the application of a longitudinal A.C. field (60 c./s.) of 1 sec. duration and of different strengths: 4 v./cm. (B), 8 v./cm. (C), and 12 v./cm. (D) respectively. Note that shortening involves the whole length and *not* only the ends.

length and is greatest in the middle portion of the muscle. Similar results were obtained in over ten similar experiments on the toe and sartorius muscles.

The sartorius and the toe muscles were rendered non-propagating by high K(16 mM/liter) in an otherwise normal Ringer, and thus depolarized to about 42 mv. (as measured by Jenerick and Gerard, 1953). Previous experiments (partial report, Csapo and Suzuki, 1957) demonstrated that under these conditions close to maximum tension can be obtained in a longitudinal A.C. field (20 v./cm. 60c./s.), repeatedly, in a steady state for a period of at least 60 minutes. At $[K]_o$ = 12 mM/liter the muscle no longer propagates. The higher $[K]_o$ = 16 mM/liter is used here to lower the membrane potential significantly below the "critical value" so as to be certain that the muscle is truly non-propagating, and furthermore because tension is greater at $[K]_o$ = 16 than at 12 mM/liter, probably because of better penetration of the stimulating current, provided by the lowered membrane resistance. We photographed the normal and non-propagating muscles at the peak of isotonic work, induced by a longitudinal A.C. field of different strength, and demonstrated that the middle portion shortens more than the ends.

It could still be argued, however, that maximum relative shortening in the middle, as was observed to be the case, was actually due to asymmetry of the muscle. Indeed the sartorius has its greatest cross-sectional area at about the middle of the muscle, and clearly at the ends the muscle fibers are mixed with connective tissue elements. It is necessary, therefore, to correct for this asymmetry if one is to claim that maximum relative shortening in the middle is a genuine effect of the internal longitudinal field. The correction can be made by relating the shortening of each segment in the non-propagating condition to maximum shortening of the same segment in the normal propagating muscle, calling this latter 100 per cent.

At the threshold (4 v./cm.) the ends of the sartorius are only slightly or not all contracted as contrasted to the middle portion. In the very slender toe muscle, 4 v./cm. is clearly well above the threshold strength, indicating that the relatively strong currents required in a non-propagating muscle are needed for adequate penetration of the current to the deeper layers of the muscle.

Although we found these data convincing, we attempted further improvements of the techniques. Our measurements were based on a single picture taken some 200 msec. after stimulation. We felt that a series of pictures taken at about 20 msec. intervals would increase confidence in our data, since for each contraction phase we would have some ten pictures rather than one, and thus a series of points would show the relative shortening of individual segments more accurately.

The result of such an experiment, using a motion picture camera, is illustrated by Fig. 2. A longitudinal segment in the middle of the muscle was photographed during isotonic work in the intact sartorius propagating (A) and non-propagating (B). Pictures were taken at about 20 msec. intervals.

From experiments of the type illustrated by Fig. 2, Fig. 3 is plotted. The measurements were made at the peak of isotonic work, at about 200 msec. after the beginning of stimulation. Graph A illustrates the shortening of differ-

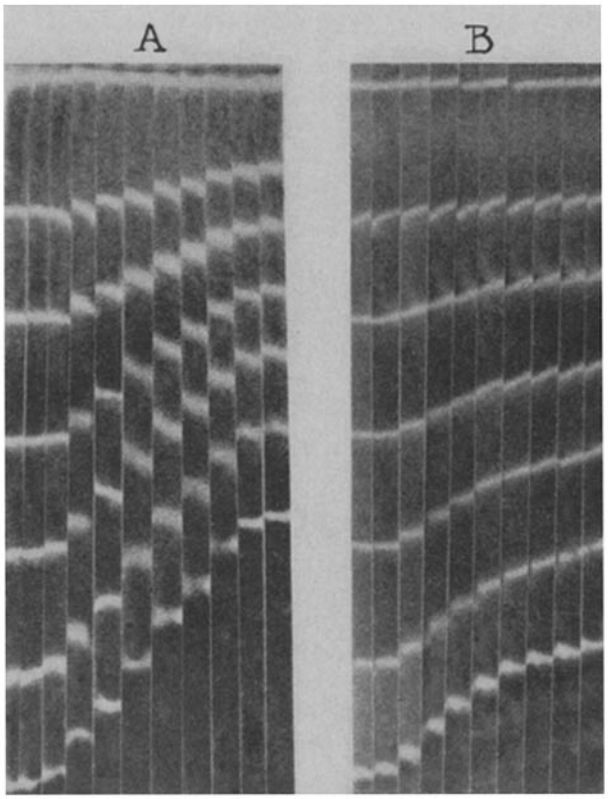

FIG. 2. Shortening during isotonic work, at different portions along the length, in the propagating and non-propagating frog sartorius muscle. The sartorius is marked off by black nylon threads. In a maximum tetanus (1 v./cm., 60 c./s., 1 sec. duration) the propagating muscle (A) is photographed during isotonic work (load = 5 gm.) in 20 msec. intervals by a motion picture camera. The muscle is then rendered non-propagating by excess K (16 mM/liter) and the experiment is repeated, using a strong longitudinal A.C. field (8 v./cm.) for stimulation (B). Note that the non-propagating sartorius shortens along its whole length and *not* only at its ends, and that shortening is greater in the middle position than at the ends.

ent segments in the intact sartorius, propagating (1 v./cm.) and non-propagating (8 to 12 v./cm.), plotted against the order and relative length of the individual segments along the length of the muscle. Graph B illustrates shortening in the non-propagating sartorius relative to maximum shortening of the propagating muscle. It can be seen that shortening is clearly greatest in the

middle of a non-propagating muscle after cross-section asymmetry along the length is corrected for. We hope to elaborate on this point further by recording shortening continuously with a constant speed movie camera, and by measuring depolarization along the length of the muscle in the A.C. field. Further experiments are also called for by our notion that in the K-depolarized muscle the middle portion begins to shorten first.

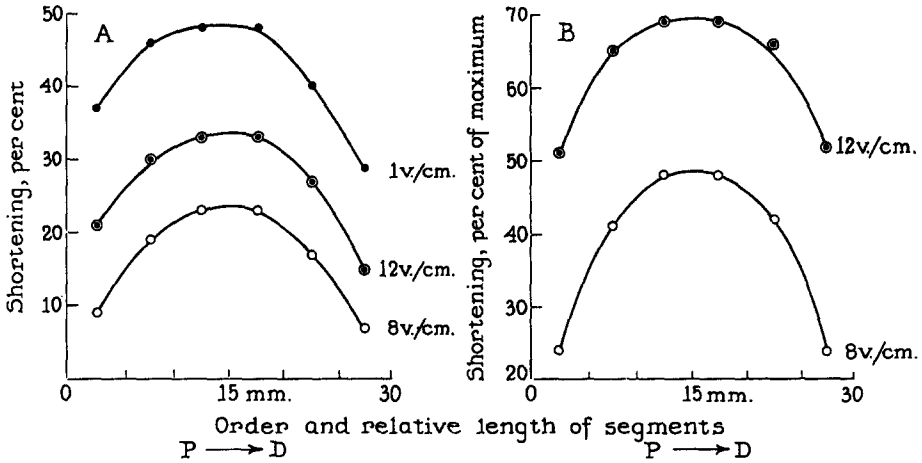

FIG. 3. The relative shortening of different portions in the non-propagating sartorius along its length Fig. 3 is plotted from the data illustrated in Fig. 2. Shortening of different segments along the length of the muscle is plotted at (A). Maximum tetanus in the propagating muscle is induced by a field strength of 1 v./cm., whereas the non-propagating muscle is stimulated at a field strength of 8 v./cm. and 12 v./cm. respectively. Note that shortening in both the propagating and non-propagating muscle is greater at the middle portion than at the ends.

Since in a maximum tetanus, in the propagating muscle, shortening is maximal in every segment, the asymmetry of the muscle can be corrected for by plotting shortening $\frac{\text{Non-propagating}}{\text{Propagating}} \times 100$. Such a plot is shown, illustrating that preferential shortening in the middle portion is a genuine effect.

A question of significance for the present discussion is the following: Can depolarization alone, without the participation of internal currents, fully activate frog twitch muscles? The view is often expressed that it can, yet we know of no experiment in which the participation of internal currents in the effect was clearly ruled out. Sten-Knudsen (1954) states that high frequency transverse field at $[K]_o$ = 12 mM is effective in stimulating muscle without the intervention of any longitudinal potential gradient. We found (partial report, Csapo and Suzuki) that this effect of the high frequency transverse field can be obtained only close to the critical potential, and that the addition of 1 to 2

mM KCl, to the Ringer abolishes the effect without changing the contractile capacity of the muscle. The question may be raised, therefore, whether or not these muscles are not in some kind of "transient" state between propagating and non-propagating conditions. Furthermore, in a transverse field internal currents are generated, although the longitudinal component may be slight.

We observed that intact sartorius or toe muscle, when suddenly immersed in high K Ringer solution (25 to 100 mM/liter) develops only fractional tension in contracture. Fractional tension may be explained by the slow diffusion of K to the extracellular space, relative to the rate of a secondary process following depolarization which results in the cessation of the mechanical response. Increasing the rate of K penetration, by increasing the external K does not eliminate the disturbing effect of "inactivation," because the rate of this process is also proportional to the external [K].

We reduced the diameter of intact sartorius and toe muscles by reducing the number of fibers and compared the tetanic tension with contracture tension in these muscle bundles. We found that the former is always greater than the latter, although the difference between the two was somewhat reduced with the reduction in muscle diameter.

Hodgkin and Horowicz (personal communication) obtained about equal tension with the maximum tetanus during contracture in single fibers, depolarized by excess K (up to 100 mM/liter). This indicates that depolarization by excess K can elicit maximum force, when diffusion is eliminated. This experiment also suggests that fractional tension in intact whole muscle is due to the relatively fast inactivation process following depolarization. This explanation, however, is not fully convincing. There are methods by which inactivation can be delayed, *i.e.* contracture, elicited by excess K, prolonged, without comparable change in the kinetics of free diffusion of K to the extracellular space. These methods are (*a*) lowering the temperature, (*b*) increasing the $[Ca]_o$. Both of these procedures result in a substantial increase in contracture duration, without significant change in the magnitude of contracture. If, during contracture, of such long duration, a longitudinal A.C. field (of 4 to 12 v./cm.) is applied repeatedly to the muscle it is possible to superimpose upon contracture a series of mechanical responses, similar in shape and magnitude to a normal tetanus. This is evidence that low temperature and high Ca did not alter significantly the capacity of the muscle to develop more tension than obtained in contracture and also that contracture tension is only a fraction of that which the muscle can develop.

It is significant to point out that in the single fiber experiments of Hodgkin and Horowicz the participation of internal currents in eliciting contracture, has not been ruled out. Since the front of their high K solution moves along the length of the muscle with a velocity of about 60 cm./sec. effective internal currents must be generated. These currents, although of short duration, may

effectively participate in the activation process, since after they have accomplished their task in the series of events their participation may not be needed in the subsequent steps.

DISCUSSION

The studies described in this paper lead to the conclusion that the external longitudinal field effectively activates non-propagating frog sratorius and toe muscles, provided that sufficient depolarization precedes the application of an effective stimulus. Experiments are presented which support the view that this effect of the external field is associated with internal longitudinal currents and not with electrotonic membrane potential change. This conclusion is in contradiction to those of others, previously expressed (see for references Sten-Knudsen, 1954). It is necessary, therefore, to review briefly the evidence which supports our interpretation, as well as that on which opposite conclusions were based.

Our own interest in this problem was started off by the observation (Csapo, 1954) that rabbit uterine strips, rendered non-propagating by high K (120 mM/liter, replacing Na), developed maximum tension in a longitudinal A.C. field (12 v./cm., 60 c./s.) for a period of more than 60 minutes. The same field, applied transversely, gave less than 15 per cent tension (Csapo, unpublished data). Since the length of the individual cells in this muscle is not more than 300 μ (with a 30:1 axial ratio, however) and the electrical properties of the cell membrane are not well established, our studies were continued on the electrophysiologically much better characterized frog sartorius and toe muscles. This experiment with the uterus, nevertheless appears to be of interest. The uterus is different from frog twitch muscles at least in one important respect. It can be drastically depolarized (120 mM K) *without* loss in mechanical response. This unique property allows one to compare the effect of the longitudinal and transverse fields in a muscle in which the $\frac{[K]_i}{[K]_o}$ is close to 1. This cannot be done with frog twitch muscles, which soon cease to respond to electrical stimulation if the $[K]_o$ exceeds 24 mM.

In the next step of our experiments (partial report, Csapo and Suzuki) we used excess K, Na lack, and procaine for blocking propagation in frog muscle. We observed that tension developed in the blocked muscle was different depending on the blocking agent used, being greatest in K and smallest in procaine block. In all three cases, tension *increased*, within limits, in the non-propagating muscle with gradual depolarization, brought about by excess K; tension and membrane potential within limits were inversely related. This inverse relation could be exaggerated by increasing the [Ca] in the perfusion Ringer. In contrast to the rabbit myometrium, frog twitch muscles failed to respond altogether to electrical stimulation when the [K] of the normal Ringer (at

$[Ca]_o$ = 1.8 mM) exceeded 24 mM. Mechanical response returned, however, in this 24 mM K-Ringer if the [Ca] was increased. By a gradual increase in the $[Ca]_o$ we could show that in high K (24 mM) tension is a function of the $[Ca]_o$. This effect of Ca could not be explained by its known effect on the membrane potential (Jenerick and Gerard, 1953).

This finding is in agreement with the recent observation of Niedergerke (1956), that heart strips depolarized by excess K do not develop tension if the Ringer contains no Ca. When Ca is added to such a depolarized muscle, tension develops without further change in membrane potential.

These experiments in all strongly suggest that Ca ion is more directly linked to activation than is depolarization.

The question is: how could we find the longitudinal field effective when others found it ineffective? Sten-Knudsen (1954) states that "when a parallel fibered muscle is placed in a longitudinal field, and provided propagation is blocked, contraction is localized at the cathodic end. In this region . . . current leaves the fibers and establishes a potential difference (a depolarization) across the surface membrane. The experiments . . . agree with the view that surface depolarization is an indispensable agent (Kuffler, 1946; Hill, 1950)." We agree with the statement, since in our own experiments depolarization with excess K appeared to be an essential "priming" step. But then Sten-Knudsen (1954) states "that the longitudinal component of the current has little or no effect in eliciting muscle contraction." We believe that this conclusion has no clear experimental support. The observation that in a D.C. field only the cathodal end[1] of the non-propagating muscle contracts merely shows the significance of depolarization as a first step in the series of events leading to contraction. But it does not serve as evidence for the direct effect of depolarization on activation, nor does it exclude the significance of the longitudinal field as a subsequent step. It can be argued that it is the joint action of depolarization and the longitudinal field which produces the effect at the cathodal region and there only, because this region alone is sufficiently depolarized; the rest is not, or is even hyperpolarized.

The same argument is valid concerning the conclusiveness of the experiments of Watanabe and Ayabe with microelectrodes (1956). Two microelectrodes were placed in a frog muscle fiber rendered non-propagating by procaine or by Na lack. No contraction was observed when a longitudinal current was produced between the electrodes unless they were far apart, when current could have leaked out, resulting in depolarization. These results again only show that depolarization is indispensable as a first step in activation, but do not rule

[1] While this paper was being considered for publication, experiments in our laboratory (Mashima and Csapo, data to be published) showed that the D.C. effect is not localized to the cathodal region, but that the muscle shortens at its whole length with the exception of the extreme anodal end.

out the possibility that activation is more directly linked to the longitudinal current than to depolarization.

Sten-Knudsen (1954) depolarized the toe muscle with 12 mM KCl-Ringer, slightly below the "critical potential" (Jenerick and Gerard, 1953). He compared the maximum contractile force, of the normal muscle, with that of the non-propagating muscle in the transverse field, when the whole muscle contracts, and in the longitudinal field "when only the ends contract." He found 80 per cent tension in transverse application of the field (2 v./cm., 1200 c./s.) and 2 per cent steady force in the longitudinal field (2 v./cm., 200 c./s.).

We (Csapo and Suzuki, 1957, partial report) repeated these experiments of Sten-Knudsen (1954). We found that if optimum stimuli (both for strength and frequency) are used in the transverse and longitudinal fields, tension is not significantly different. It is simple to establish the optimum stimulus in a non-propagating muscle. Both the field strength and frequency optima are indicated by the saturation of the tension/field strength or frequency curves. If the muscle is overstimulated relaxation is instantly prolonged and recovery, following repolarization, becomes incomplete. We obtained 100 per cent recovery at the end of our experiments in which the muscle, when depolarized, was repeatedly stimulated in a 16 v./cm. longitudinal A.C. field. We also found that the addition of 1 to 2 mM excess K to the Ringer, in which a K-blocked muscle was suspended, abolished altogether the high frequency effect of the transverse field. We wonder whether in 12 mM K (slightly below critical potential) the muscle is not in a "transitory" state with respect to propagation. We also found that the longitudinal field becomes more effective when the non-propagating toe or sartorius muscle is depolarized stepwise (within limits) by excess K. We have shown (see above) not only that the non-propagating toe and sartorius muscle contracts along its whole length in a longitudinal A.C. field, but that the middle contracts more than the ends. We demonstrated that this is true at a field strength value of 4 v./cm.

We suggest, therefore, that Sten-Knudsen's results (1954) are due to ineffective stimulation (both for frequency and for strength) in the longitudinal field. The effectiveness of the high frequency transverse field, on the other hand, should be evaluated with caution, since it occurs only *at* or *just below* the critical potential and disappears if the membrane is further depolarized by only a few millivolts. Yet tension in the longitudinal field increases by stepwise depolarization (within limits), indicating that the contractile system is not losing contractile capacity by this procedure. The fact, furthermore, that optimum frequency in the transverse field shifts to lower and lower values as the membrane is depolarized only slightly below the critical potential (between $[K]_o$ = 11 to 13 mM/liter) is a further indication that the membrane is in a transient condition. A $[K]_o$ higher than 12 mM/liter, permits maximum tension for low frequency only (50 c./s. to 100), both with transverse and longitudinal stimulation.

Taking all the information discussed above into account, it appears that depolarization has a multiple function in the excitation process in muscle. It is responsible for starting off the propagated action potential and for the generation of currents inside the muscle fiber; in addition, depolarization seems to serve as an essential "priming" step in the series of events leading to activation. It is conceivable that the equilibrium of ions, periodically distributed and bound to cross-structures (such as the Z membranes), becomes disturbed by depolarization. There is also much evidence that Ca is a key ion in activation (see for review, Sandow, 1952) and that this ion is periodically distributed. But the release of ionized Ca resulting from the effect of depolarization at cross-structures does not overcome the time limitations of Hill, set for the coupling process. This ion still has to move great distances if it is to be used for the activation of the contractile system throughout the space between one Z membrane and the next.

It is conceivable that Ca, for example, which is not available for the actomyosin system during rest, becomes gradually available during the development of full activity and the opposite may be true for the "relaxing factors" (see for references Weber and Portzehl, 1954; Morales and Botts, 1956). It is also conceivable that it is the movement of Ca ion which transforms a resting muscle into an active one. Depolarization may shift the Ca equilibrium toward ionized Ca, which has to move then between the zones, where it is held during rest. This may be achieved effectively by the contribution of the longitudinal field in the process of the excitation contraction coupling.

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