The Mode of Transverse Spread

of Contraction Initiated by Local

Activation in Single Frog Muscle Fibers

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ABSTRACT   Isolated single frog muscle fibers were locally activated by applying negative current pulses to a pipette whose tip was in contact with the fiber surface. In contrast to the graded inward spread of contraction initiated by a moderate depolarization, the contraction in response to a strong negative current was observed to spread transversely around the whole perimeter but not through the center of the fiber. This response was elicited only with pipettes of more than 6 μ diameter. The response was still present if the sodium of the Ringer solution was replaced by choline, or the chloride was replaced by nitrate or propionate. The duration of the response appeared to be independent of the duration of stimulating current in fresh fibers, while the contraction lasted as long as the current went on in deteriorated fibers. The contraction was first initiated at the area of fiber surface covered by the pipette, and spread around the perimeter of the fiber with a velocity of 0.8–6 cm/sec. Possible mechanisms of the response are discussed in connection with the properties of the transverse tubular system, the possibility of some self-propagating process along the walls of the tubules being suggested.

INTRODUCTION

In the preceding paper (Sugi and Ochi, 1967), it was shown that two different types of local contraction may be initiated by local activation of a crayfish muscle fiber; one was a graded inward or transverse spread of contraction initiated by moderate depolarization, while the other was elicited by strong negative current and was observed to spread around the whole perimeter but not through the center of the fiber (see their Fig. 11). These results led us to do local activation experiments on frog fast muscle fibers to ascertain whether the two types of response mentioned above are common to both vertebrate and invertebrate striated muscle fibers. It will be shown that the response of frog muscle fibers to local activation is essentially the same as that of crayfish muscle fibers. Preliminary accounts of this work have appeared (Sugi and Ochi, 1965 a, b).

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METHODS

Single muscle fibers were isolated from the semitendinosus muscles of the frog (*Rana japonica* or *Rana nigromaculata*). The fiber (diameter 40–100 μ) was mounted horizontally in a glass trough (3 × 5 × 0.6 cm deep) by tying a piece of tendon at each end to a glass hook fixed to the bottom of the trough. The sarcomere length of the fiber mounted in this way was 2.5–3 μ.

The experimental methods used were identical with those previously described (Sugi and Ochi, 1967), except that pipettes for local activation (external tip diameter 3–40 μ) were made with a de Fonbrune microforge. The values of contact resistance of the pipette ranged from 100 to 500 KΩ, varying inversely with tip diameter.
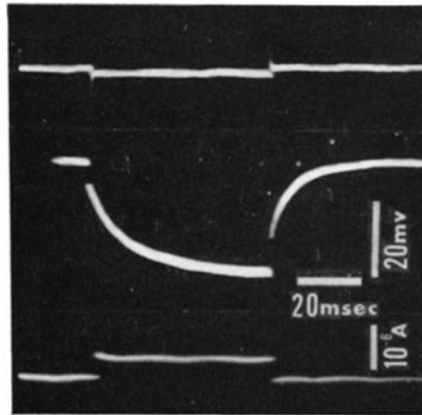

FIGURE 1. Change in membrane potential of the fiber during local activation. Upper and middle traces are membrane potential and lower trace is current for middle trace. For further explanation see text.

The normal Ringer solution had the following composition (mM): NaCl, 115; KCl, 2.5; CaCl$_2$, 1.8 (pH 7.2 by NaHCO$_3$ or Tris-HCl buffer). Tubocurarine chloride (10^{-5} g/ml) was also added to the solution. In some experiments the sodium of the solution was replaced by choline, and nitrate or propionate was used to substitute for chloride in others.

All experiments were performed from February to May, 1965; i.e., on winter frogs, at room temperature (16–26°C).

RESULTS

Localization of Membrane Potential Changes

Fig. 1 shows the change in membrane potential of the fiber during local activation as examined with an intracellular microelectrode. As is shown in the upper trace, little or no change in membrane potential was recorded during the application of a current pulse producing a membrane depolarization up to 100 mv, indicating the localization of membrane depolarization under the pipette. A stronger current pulse produced an irreversible reduction of membrane resistance which was indicated by a sudden appearance of hyperpolariz-

ing electrotonic potential as shown in the middle trace. As was the case in crayfish fibers, the reduction of membrane resistance could occur without any visible damage to the fiber.

Transverse Spread of Contraction Initiated by Moderate Depolarization

Fig. 2 is an example of local contractions initiated by moderate depolarizations of less than 100 mv. It can be seen that the contraction is confined to the sarcomeres under the pipette, no longitudinal spread being observable. In
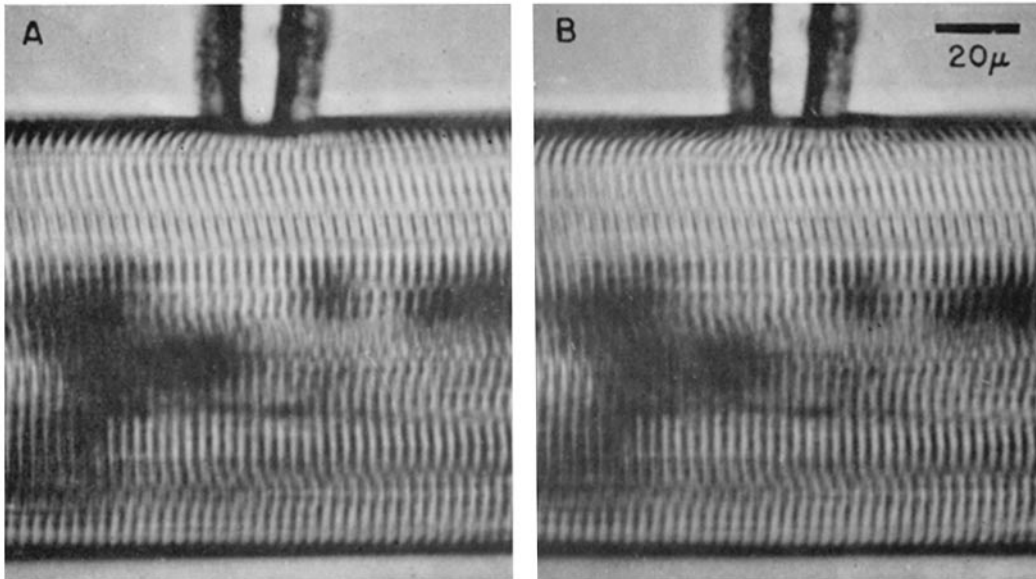

FIGURE 2. Selected frames from a cinefilm showing a muscle fiber before (A) and during (B) the application of a current pulse producing a membrane depolarization of 50 mv and of 100 msec duration.

agreement with the findings of Huxley and Taylor (1958), the distance of inward spread of contraction was continuously graded according to the stimulus strength, increasing with the increase in both the magnitude and duration of depolarization. The critical depolarization for producing a just perceptible contraction was 20–50 mv for the pipettes of 6–40 μ diameter. For a given amount of depolarization, the distance of inward spread was increased with the increase in diameter of the pipette. If a prolonged (more than 500 msec) depolarization was applied by a pipette having a diameter half as large as the diameter of the fiber, the contraction spread not only inwards but further transversely passing through the center of the fiber. These results are similar to those obtained on crayfish fibers.

Transverse Spread of Contraction Initiated by Strong Negative Current

NATURE OF THE RESPONSE   It was found that, with the application of strong negative current pulses producing membrane depolarization of more than 100 mv, the contraction spreading around the whole perimeter of the fiber could also be initiated in frog muscle fibers. Fig. 3 is a typical example showing the nature and reversibility of the response. It will be seen that the contraction is initiated not only at the upper edge of the fiber opposite the pipette, but also at the lower edge to which the striations from the upper contracted region extend, while the length of sarcomeres at the center of the fiber remains unchanged. It was ascertained by changing the focus of the microscope that the contraction spread around the whole perimeter but not through the center of the fiber, the contraction at the upper or lower surface being observable if the upper or lower surface was in focus under the microscope.

This type of local contraction could be initiated in all the fibers used. It appeared that the smaller the diameter of the fiber, the more readily the response was elicited with a given pipette. The critical potential difference across the contact resistance of the pipette for the initiation of the response was 100–250 mv for current pulses of 50–200 msec duration, being equal to or above the value necessary to produce the reduction of membrane resistance. However, the response was reproducible at least several times without visible damage to the fiber. Meanwhile, positive current pulses never produced this type of response; strong positive pulses caused contractions, which were in most cases irreversible, only under the pipette.

It was not possible to elicit the response confined to a single sarcomere with a pipette of 3 μ diameter. The smallest diameter of the pipette with which the response could be initiated was about 6 μ. An example of the response confined to a few sarcomeres is presented in Fig. 4. This suggests that it may be necessary to stimulate at least a few sarcomeres simultaneously to initiate the response.

It was frequently observed that the sarcomeres opposite the pipette did not contract but moved quickly in the longitudinal direction. This may be regarded as being analogous with the longitudinal displacement of a contracting part described in the preceding paper (Sugi and Ochi, 1967), since this movement of a stimulated part was minimized if the pipette was placed at the middle part of the fiber.

EFFECT OF IONS   The response was still elicited when the sodium of the Ringer solution was replaced by choline, indicating that the action potential mechanisms are not necessarily involved in the response. The response was also present if either nitrate or propionate was substituted for chloride. In nitrate-Ringer's solution, the critical strength of current for the initiation of
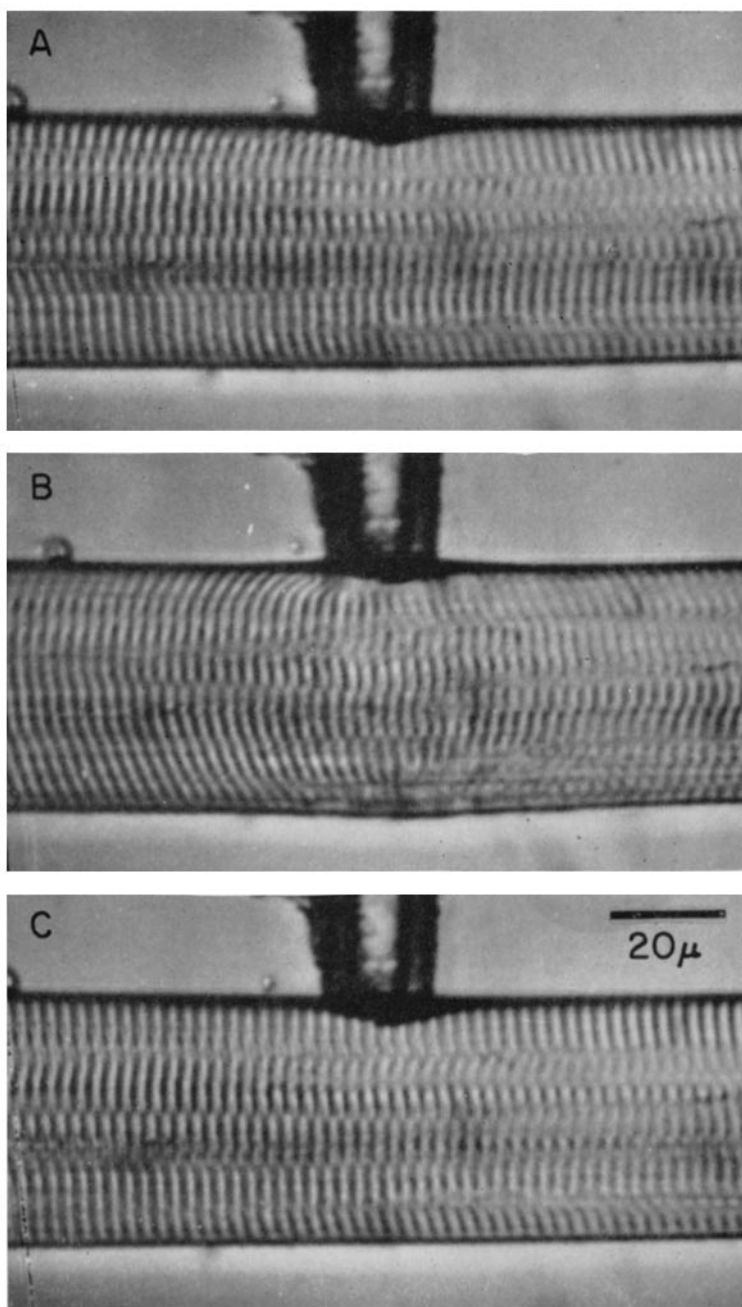
Figure 3.   Selected frames from a cinefilm showing a muscle fiber during (A, B) and after (C) a local contraction initiated by a strong current pulse of 100 msec duration producing a potential difference of 150 mv across the contact resistance of the pipette. Note that the contraction is first initiated at the upper edge of the fiber (A) and spreads to the lower edge (B).

the response was decreased appreciably, though this effect of nitrate was not studied quantitatively.

CONTRACTION CURVE AND VELOCITY OF TRANSVERSE SPREAD   Cinematographic records indicated that, as with crayfish muscle fibers, the contraction was first initiated at the area of fiber surface covered by the pipette, and then spread circumferentially along the striation pattern (Fig. 3 A and B). The
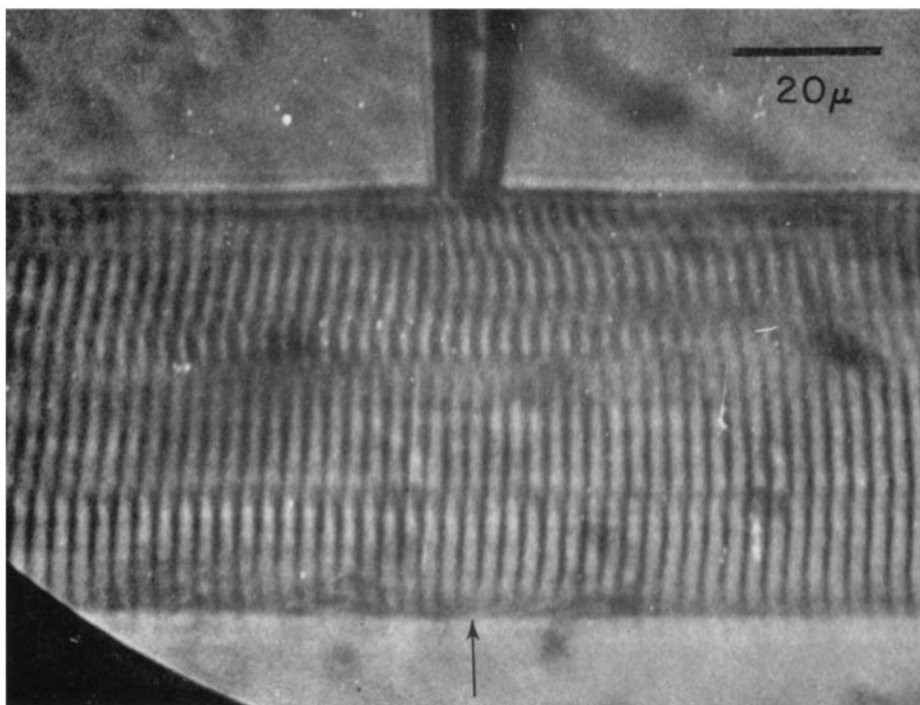

FIGURE 4.   Photomicrograph of a muscle fiber during a local contraction elicited by a strong current pulse of 100 msec duration producing a potential difference of 250 mv across the contact resistance of the pipette. The contraction at the lower edge indicated by arrow is just barely perceptible.

time course of the response and the velocity of the spread of contraction around the perimeter of the fiber were studied by a method similar to that used in the previous work (Sugi and Ochi, 1967). Two examples of the records are shown in Fig. 5.

In fresh fibers, the duration of the response, i.e. the time between the onset of contraction and the completion of relaxation, was 50–100 msec, and the duration remained constant if the duration of stimulating current was changed from 50–300 msec. When the response was elicited more than several times, an irreversible contraction was usually produced under the pipette. However,
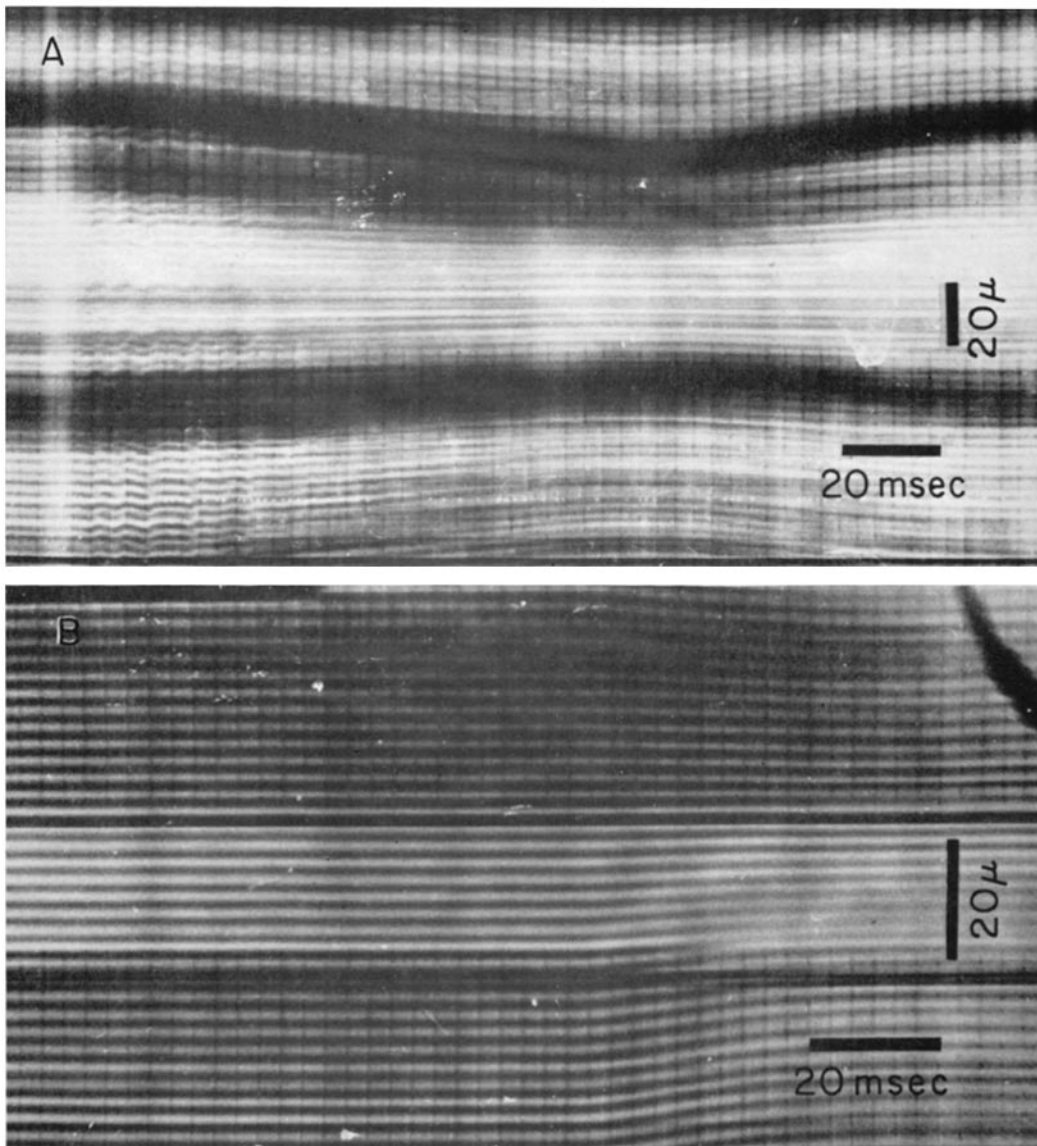
FIGURE 5. Two examples of records showing the time course of contraction. The onset of stimulating current is indicated by a flash of light from the xenon lamp in A (vertical white line at the beginning of the record), and by an upward deflection of the electromagnet in B (black line at the top of the record). In both A and B, parallel white lines are striations of the fiber. In A, no carbon particles were attached to the fiber.

the contraction spreading around the whole perimeter of the fiber could still be initiated in such deteriorated fibers. The contraction in deteriorated fibers lasted as long as the current went on as did the contraction initiated by moderate depolarization.

The average velocity of the spread of contraction around the whole perimeter of the fiber was determined from the difference in the reaction time between two different parts across the fiber. The values obtained from six experiments on four different fibers were found to be 0.8–6 cm/sec at room temperatures of 24–26°C. These values are in good agreement with the values of crayfish muscle fibers. The velocity of the spread was maximum in fresh fibers, and decreased gradually with continuing deterioration of the fiber.

DISCUSSION

The present experiments have shown that the response of frog fast muscle fibers to local activation is essentially the same as that of crayfish muscle fibers as summarized in Fig. 11 of the preceding paper (Sugi and Ochi, 1967). Since the properties of graded contractions initiated by moderate depolarizations have already been described and discussed in that paper, the discussion will hereafter be concerned exclusively with the contractions initiated by strong negative current pulses.

The main features of the contractions initiated by strong negative current pulses in both types of muscle fibers were as follows: (a) the contraction was confined to the sarcomeres in contact with the tip of the pipette; (b) the contraction spread around the perimeter but not through the center of the fiber with a velocity of the order of 1 cm/sec; (c) the response was accompanied by the reduction of membrane resistance; and (d) the response could be initiated even with brief pulses of sufficient strength. The only remarkable difference between crayfish and frog fibers was the duration of the response (several seconds, crayfish muscle fibers; 50–100 msec, fresh frog muscle fibers). This difference might be due, at least partly, to the difference in the rate of calcium uptake by the sarcoplasmic reticulum (Podolsky and Costantin, 1964), and it may be reasonable to suppose that the underlying mechanism for the initiation of the response is common to both types of muscle fibers.

A possible explanation for the response may be as follows: some self-propagating process might be set up at part of the transverse tubular system as a result of a large outward flow of current across its membrane, and propagate more readily along the tubules located in the vicinity of the fiber surface than towards the center of the fiber, thus activating the neighboring myofibrils to form a ring-shaped contracted region around the whole perimeter of the fiber. It seems necessary to suppose some self-propagating process, since the response was initiated in a nearly all-or-none manner in contrast with the graded contraction depending on the magnitude, duration, and area of moderate

depolarization. It has been assumed by some authors that the impulse similar to that at the fiber surface might travel along the tubular membranes (Porter and Palade, 1957; Peachey and Porter, 1959; Huxley, 1964) without experimental evidence. However, Natori (1955) showed that a wave of contraction spreading along the length of a skinned fiber preparation can be initiated by electrical stimulation, indicating that some kind of impulse actually may be conducted along the membrane of the sarcoplasmic reticulum. Although the membrane constants of the transverse tubules are unknown, the velocity of the spread of 0.8–6 cm/sec found in this work seems to be compatible with the fact that the diameter of the tubules is about 0.03 μ (Porter and Palade, 1957; Andersson-Cedergren, 1959; Peachey, 1965). Gonzales-Serratos (1966) observed with an ingenious method that the shortening of myofibrils within a frog muscle fiber begins near the fiber surface and spreads inwards with a velocity of about 8 cm/sec at 20°C during a twitch. This is a value nearly equal to the velocity of the spread around the perimeter of the fiber.

Then, it becomes necessary to explain why this hypothetical self-propagating process travels more readily along the tubules near the fiber surface than inwards. One possibility is that only the tubules in the surface region are capable of conducting this process. Another and more likely possibility is that there is no regional difference in the properties of the tubules, but only the tubules in the surface region provide a favorable condition for the conduction. If this conduction is accompanied by the eddy current flowing through the tubular and the surface membranes, the tubules near the fiber surface will be more favorable for the conduction than those in the interior of the fiber, since the pathway for the eddy current is shortest at the surface region. In this connection, it is of interest that the transverse tubules increase in diameter and become irregular in shape near the fiber surface (Peachey, 1965), though it is not possible to correlate this to either of the above possibilities. The only evidence concerning the nature of this conduction is that it may be different from the propagated action potential at the fiber surface, as the response was initiated in frog muscle fibers in choline-Ringer's solution and in crayfish muscle fibers which showed no propagated action potential (Sugi and Ochi, 1967).

The question arises whether such a self-propagating process is involved in the normal inward spread of activation triggered by the all-or-none action potential. It may be argued that the condition for the initiation of the response differs too much from the physiological condition, as the critical strength of the current was equal to or above the value that caused the reduction of membrane resistance. However, only a small area of surface membrane was stimulated in this work, and therefore it seems possible that the current strong enough to elicit the self-propagating process might flow across the tubular membrane when the whole perimeter of the fiber was depolarized with a transient reduction of resistance for unit area of membrane in each action

potential. In such a case, the conduction might occur not only around the primeter but also towards the center of the fiber to activate all the myofibrils within the fiber.

Recently, Strickholm (1966) reported that the contraction involving the whole sarcomeres can be initiated by local activation of frog muscle fibers. Although his cinematographic records seem to be inadequate to disprove the possibility that the contraction is localized around the perimeter of the fiber, it does seem possible that the whole transverse tubular network may be excited under some conditions. Much more experimental work is needed to determine the actual mechanism of inward spread of activation which is believed to occur along the transverse tubular membrane of a muscle fiber.

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