The Mode of Transverse Spread of Contraction Initiated by Local Activation in Single Crayfish Muscle Fibers

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ABSTRACT   Isolated single crayfish muscle fibers were locally activated by applying negative current pulses to a pipette whose tip was in contact with the fiber surface. The contraction initiated by a moderate depolarization spread inwards in a graded manner according to the magnitude and duration of depolarization. Increase of the depolarized area increased the distance of the inward spread for a given amount of depolarization. If a large area of the surface membrane was depolarized with a large pipette for a sufficiently long time, the contraction spread not only inwards, but further transversely passing through the center of the fiber. Successive brief depolarizations given at an appropriate interval could produce contraction more effectively for a given amount of total current than did a prolonged depolarization. On the other hand, the contraction initiated by a strong negative current was observed to spread around the whole perimeter but not through the center of the fiber. Each type of local contraction always spread along the striation pattern and not longitudinally. Possible mechanisms of these responses are discussed in connection with the transverse tubular system of the muscle fibers.

INTRODUCTION

It is generally agreed that the first step leading to contraction in a striated muscle fiber is normally a reduction of membrane potential; i.e., depolarization (Kuffler, 1946; Sten-Knudsen, 1954, 1960; Huxley and Taylor, 1958; Watanabe, 1958; Orkand, 1962 a). Concerning the question how the membrane depolarization reaches the myofibrils to bring them into activity, it has been suggested by A. F. Huxley and his colleagues (Huxley and Straub, 1958; Huxley and Taylor, 1958; Huxley and Peachey, 1964) that the transverse tubular system forming a tubular network in the transverse plane of each sarcomere (Porter and Palade, 1957; Andersson-Cedergren, 1959; Peachey and Huxley, 1964; Brandt, Reuben, Girardier, and Grundfest, 1965; Peachey, 1965) may be the structure along which the influence of membrane depolarization is conducted inwards. Recently it has been shown physiologically

2145

(Girardier, Reuben, Brandt, and Grundfest, 1963; Endo, 1964) and electron microscopically (H. E. Huxley, 1964; Brandt et al. 1965; Peachey, 1965) that the membrane of the transverse tubules is continuous with the surface membrane, providing strong support for the above hypothesis. However, the mechanism of the inward spread of activation is not as yet clear. According to Huxley (1957, 1959), the depolarization of surface membrane may be conducted electrotonically along the membrane of the transverse tubules to activate neighboring myofibrils. Falk and Fatt (1964) suggested another scheme for inward conduction which is based on the analysis of the electrical properties of striated muscle fibers. Girardier et al. (1963) are of the opinion that, in crayfish muscle fibers, the current loop between the surface membrane and the anion-permselective membrane of the transverse tubular system might cause passive accumulation of cations in the vicinity of the tubules to trigger contraction.

As to the nature of the inward spread, however, the only evidence provided experimentally is that the spread is not of an all-or-none type, but graded according to the stimulus strength (Huxley and Taylor, 1958). The aim of the present work was to study the mode of spread of contraction initiated by local activation in isolated single crayfish muscle fibers to give information about the mechanism of inward spread of activation. It will be shown that the contraction initiated by local activation of the fiber may spread not only inwards, but further transversely through the center or around the whole perimeter of the fiber without spreading longitudinally. A preliminary report of this work has appeared elsewhere (Sugi and Ochi, 1965 a).

METHODS

Preparation

Most of the experiments were performed with the medial head of the superficial abdominal extensor muscle of the crayfish, *Cambarus clarkii* (Pilgrim and Wiersma, 1963). In a few cases, the claw adductor muscle was used with similar results. Single fibers were isolated free of injury with a piece of shell attached to each end serving as a support. Fibers of small or medium size (major diameter 70–200 μ) were selected, since fibers of larger size did not provide good optical conditions for microscopic observation of local contraction. The single fiber preparation was mounted horizontally in a glass trough (2.5 × 5 × 0.5 cm deep) filled with the bathing solution. One end of the preparation was supported with a glass hook fixed to the bottom of the trough, while the other end was held with another glass hook connected with a micromanipulator or pinned to a cork plate attached to the bottom of the trough so that the fiber was stretched to about 1.2 times the resting length in the body (to 0.7–1.2 cm) with the sarcomere spacings of about 10 μ. The bathing solution was changed from time to time by a water vacuum suction tube.

Local Activation

The method of local activation of the muscle fibers was essentially the same as that used by previous authors (e.g., Huxley and Taylor, 1958; Strickholm, 1962). The surface membrane was depolarized in a limited area by placing the tip of a glass pipette in contact with the fiber surface and applying current pulses to the pipette to make its interior go negative with respect to the indifferent electrode in the surrounding medium. The rectangular current pulses from an electronic stimulator were applied through an isolation circuit to a Ag-AgCl wire electrode inserted into the pipette, while the indifferent electrode was a Ag-AgCl plate in the trough. Usually a 1 MΩ resistor was included in series with the electrodes to keep current constant. Current pulses were monitored as the potential difference across a 15 KΩ resistor on one beam of a dual beam oscilloscope.

PIPETTES The external tip diameter of the pipette used for local activation of the fiber was 20–120 μ. First, micropipettes for intracellular recording were made by drawing glass tubes of 3 mm diameter on a vertical pulling machine. These were then shortened to provide the required tip diameter by plunging them into gelatin mixed with carborundum. The tip of the pipette was made smooth by heating with a nichrome wire so as not to injure the surface membrane when the pipette was placed in contract with the fiber surface. Pipettes were filled with the bathing solution.

CALCULATION OF MEMBRANE POTENTIAL CHANGES The contact resistance of the pipette, i.e. the increase of the resistance of the pipette when its tip was brought into contact with the fiber surface from a distant position, was measured by means of a Wheatstone bridge arrangement analogous to that used by Huxley and Taylor (1958), balance of the bridge being monitored with a differential DC amplifier and the oscilloscope. The values of the contact resistance ranged from 20 to 150 KΩ, varying inversely with tip diameter. The magnitude of the potential change across the area of surface membrane covered by the pipette was calculated by multiplying the contact resistance by the applied current. This method of approximation of local membrane depolarization rests on the assumption that the magnitude of membrane potential change is almost equal to the potential difference across the contact resistance of the pipette; the electrotonic potential produced within the fiber is negligible as compared with the potential difference across the contact resistance of the pipette, since the input resistance of the fiber is negligibly small as compared with the total resistance of the patch of surface membrane covered by the pipette. The validity of this calculation will be discussed later.

Optical Apparatus

MICROSCOPE The fibers were observed under an ordinary light microscope with a condenser having a long focal length and a dry objective having a long working distance (Nikon, 20×, NA0.4, working distance 5.72 mm or Leitz, 30×, NA0.6 working distance 6.5 mm). The trough was mounted on the mechanical stage of the microscope so that any part of the fiber could be brought into the field. The pipette

held in a micromanipulator was brought into contact with the edge of the fiber in focus under the microscope. The diameter and the sarcomere spacings of the fiber were measured with an eyepiece scale.

PHOTOGRAPHIC ARRANGEMENTS   The contractions were recorded with a 16 mm cinecamera at 35–50 frames/sec on Fuji Minicopy film (Fuji Photofilm Co., Ltd., Kanagawa-ken, Japan). A low voltage tungsten lamp was used with a green filter. The field was observed continuously during photography. Photomicrographs of the contraction at any chosen moment after the onset of a depolarizing current pulse were also taken with a 35 mm camera and a xenon lamp flash device (duration of a flash, less than 1 msec) driven by the electronic stimulator. Fuji Neopan 3S film was used. The flash was displayed on one beam of the oscilloscope with a phototube together with the depolarizing pulse on the other beam.

Solutions

The normal bathing solution was van Harreveld's solution (van Harreveld, 1936) containing (mM): NaCl 230, KCl 6, CaCl$_2$ 15, MgCl$_2$ 3. Buffering was provided by Tris-HCl buffer at pH 7.2–7.4. The bathing solution containing manganese (1–10 mM) was made by adding manganous chloride, with an osmotically equivalent amount of sodium chloride removed. Solutions were changed by overflowing the trough with solution several times the volume of the trough within 30 sec.

All the experiments were performed at room temperature (16–28°C).

RESULTS

Preliminary Experiments

1. ELECTRICAL PROPERTIES OF MUSCLE FIBERS   The electrical properties of the superficial abdominal extensor muscle fibers were examined with two intracellular glass microelectrodes of the Ling-Gerard type; one for passing current while the other recorded the potential difference across the membrane. The muscle fibers showed a uniform membrane potential of 60–80 mv when a microelectrode was inserted at different sites along the fiber surface or passed right through the fiber. The recording electrode always picked up the electrotonic potential wherever the two electrodes were situated within the same fiber, whereas no potential change could be detected when the two electrodes were inserted into different fibers. The contraction produced by an intracellularly applied outward current was not confined to a definite local region, but spread longitudinally with decrement according to the stimulus strength. These results indicate that the fibers behave as functionally and electrically independent units.

The membrane constants were measured on fibers having a diameter of less than 200 μ in which the electrotonic potential declined exponentially with distance from the current electrode. The average values in six experiments at 27–28°C were: length constant 2.0 mm, time constant 17 msec, specific mem-

brane resistance 980 Ω cm^2, specific membrane capacity 20 μ f/cm^2, input resistance 26 KΩ. The response of the fibers to a depolarizing current was a local potential change including an early hump or oscillatory component, with no propagated spikes observed. These results were analogous to those described for other crustacean muscle fibers (Fatt and Katz, 1953; Fatt and Ginsborg, 1958; Werman and Grundfest, 1961; Orkand, 1962 a; Atwood, 1963; Atwood and Dorai Raj, 1964; Dorai Raj, 1964).
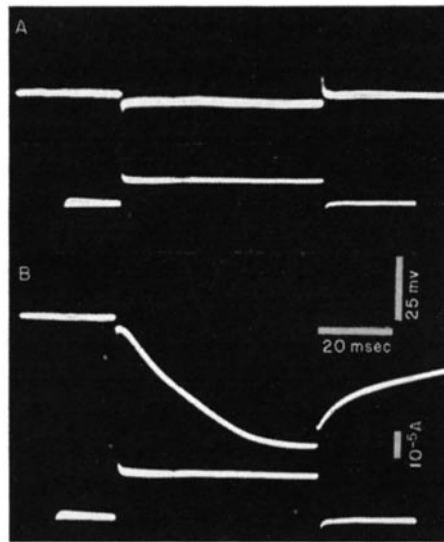
FIGURE 1.   Change in membrane potential of the fiber during the application of current to the pipette in contact with the fiber surface. Upper trace is membrane potential and lower trace is current. Record A was obtained during the application of a current pulse producing a local membrane depolarization of 150 mv, no change in membrane potential being produced. Slight downward deflection of the potential trace still remains when the electrode is withdrawn from the fiber, and therefore is not due to the change in membrane potential. Record B was obtained during the application of a current pulse producing a potential difference of 300 mv across the contact resistance of the pipette. A hyperpolarization is produced as a result of a reduction of membrane resistance.

2. LOCALIZATION OF MEMBRANE POTENTIAL CHANGES   It seemed desirable to ascertain whether the change in membrane potential produced by the current applied to the pipette was actually localized within the area of surface membrane covered by the pipette. A microelectrode was therefore inserted into the fiber at a point 10–15 μ distant from the tip of the pipette in contact with the fiber surface so as to detect the change in membrane potential during the application of a negative current pulse to the pipette. The results are shown in Fig. 1. Little or no change in membrane potential was recorded during the application of a current pulse producing local membrane depolari-

zation up to 200–300 mv as calculated by the above-mentioned method (Fig. 1 A), indicating that the change in membrane potential was actually localized under the pipette without appreciably changing the membrane potential elsewhere. Therefore, the method of calculation of local membrane potential change may be valid in this case.

On the other hand, a hyperpolarization was recorded during the application of a stronger negative current pulse which produced a potential difference of more than 200–300 mv across the contact resistance of the pipette (Fig. 1 B). Once such a hyperpolarization was produced, the microelectrode always picked up hyperpolarizations for subsequent application of currents regardless of whether the current was strong or weak, the magnitude of hyperpolarization varying according to the current strength. This result may be explained as being due to a reduction of membrane resistance at the fiber surface covered by the pipette caused by applying too large a potential difference across the membrane, providing a low resistance path from the interior of the fiber to the interior of the pipette (Gelfan, 1933; Huxley and Taylor, 1958). The degree of reduction of membrane resistance could be estimated from the values of the specific membrane resistance, the input resistance of the fiber, the magnitude of hyperpolarization, and the area of fiber surface covered by the pipette, and it was shown that the membrane resistance was reduced to less than one-hundredth of the initial value. Consequently a considerable current flows in through the surface membrane over a large area thus producing a hyperpolarization, but flows out only where the membrane is covered by the pipette, and the above calculation of local membrane potential change is invalid though the inside positive direction of potential difference across the membrane is still localized under the pipette.

3. CHANGE IN CONTACT RESISTANCE OF PIPETTE DURING LOCAL CONTRACTION   It was also a question whether the contact resistance; i.e., the resistance of the seal formed by the tip of the pipette against the fiber was kept constant during a local contraction under the pipette. To examine this point, a glass capillary microelectrode was inserted axially into the pipette so that the tip of the microelectrode came just in contact with the fiber surface when the pipette was pressed against the fiber. Then the time course of potential difference across the contact resistance of the pipette was recorded with this microelectrode during the application of a current pulse and the resulting local contraction. An example of the results is shown in Fig. 2. If the pipette was distant from the fiber, only little potential change was produced during the application of a current pulse to the pipette (Fig. 2 A). When the pipette was brought into contact with the fiber surface, a large potential change consisting mainly of the potential difference across the contact resistance was recorded (Fig. 2 B), while a marked local contraction was observed to occur under the pipette. It will be seen that the magnitude of the potential change is

kept fairly constant in spite of the resulting local contraction under the pipette, indicating that the contact resistance remains fairly constant during local contraction.

Transverse Spread of Local Contraction Initiated by Moderate Depolarization

1. NATURE OF THE RESPONSE   When the magnitude of local membrane depolarization exceeded some critical value, a reversible local contraction was initiated under the pipette. Fig. 3 (see also Figs. 4–6) is an example showing
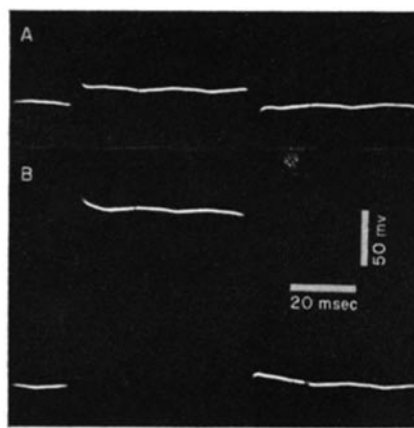

FIGURE 2.   Change in contact resistance of the pipette during local contraction. Each record shows the potential difference between the microelectrode inserted axially into the pipette and the indifferent electrode in the surrounding medium. In A, the pipette was kept distant from the fiber. In B, the pipette was brought into contact with the fiber surface. Diameter of the pipette was 70 μ and the contact resistance was 65 KΩ. The magnitude of potential difference obtained by subtracting record A from record B was actually equal to the value obtained by multiplying the contact resistance by the applied current (2 $\times$ 10^{-6} amp).

the nature of the local contraction. It can be seen that the contraction, i.e., the shortening of sarcomere length is produced at the edge of the fiber opposite the tip of the pipette, and spreads inwards along the striation pattern, while no longitudinal spread of contraction is observable. The critical depolarization for producing a just perceptible response varied with the duration of depolarization; the longer the duration the smaller the critical value. With depolarizations of 200–500 msec duration, the critical value was 20–50 mv.

The distance to which the contraction was observed to spread inwards was increased in a graded manner with the increase in the magnitude and duration of depolarization. The contraction lasted as long as the depolarization went on (at least up to 10 sec). The distance of inward spread for a given amount of depolarization was also increased if a larger pipette was used to increase the area of depolarization. Thus, if a large part of the fiber surface was depolarized

by more than 150 mv for 0.2–1 sec with a pipette having a diameter nearly half as large as the major diameter of the fiber, the contraction was observed to spread not only inwards but further transversely passing through the center of the fiber without spreading longitudinally. Fig. 4 shows an example of such a marked transverse spread of contraction. It was not possible, however, to make the above graded type of local contraction spread across the whole diam-
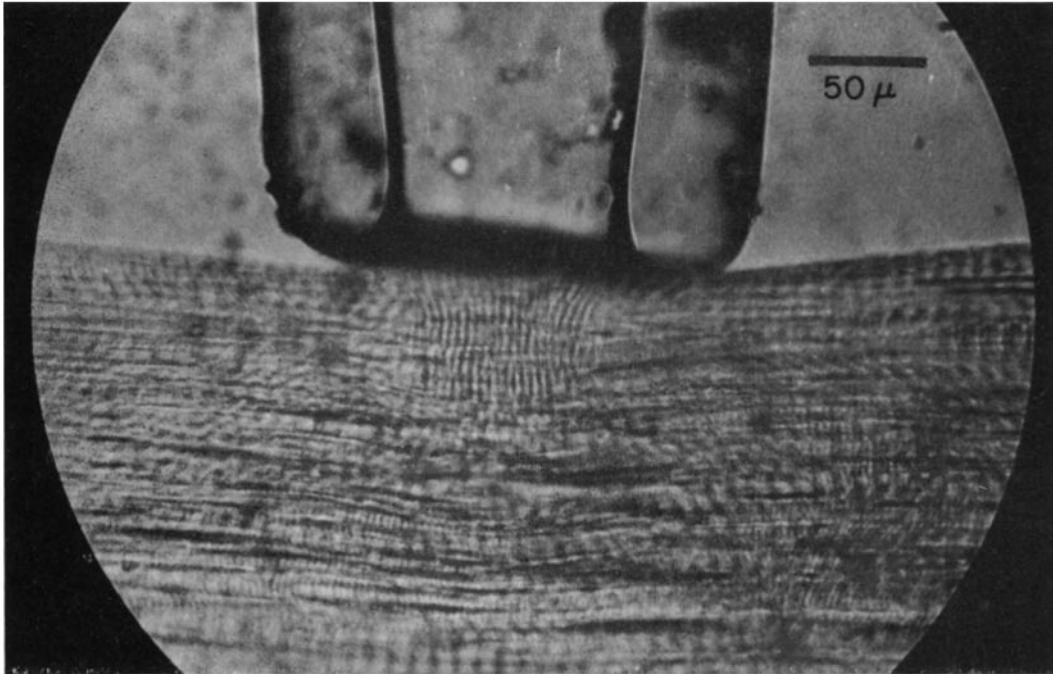

FIGURE 3. Photomicrograph of the fiber during a local contraction initiated by a depolarization of 200 mv and 500 msec duration. The pipette for local activation is in contact with the upper edge of the fiber.

eter of the fiber; the transverse spread produced by a prolonged depolarization appeared to reach its upper limit within a few seconds and subsequent application of depolarization was not effective for making the contraction spread further. The maximum distance of transverse spread attained by a prolonged depolarization was dependent on the diameter of the pipette; roughly speaking it was nearly equal to the diameter of the pipette.

In a few cases, the spread of contraction in the circumferential direction was examined with the tip of a pipette obliquely in contact with the upper surface of the fiber. It appeared that the distance of circumferential spread in each direction along the striation pattern was nearly equal to the distance of transverse spread.

2. SUMMATION   Fig. 5 shows local contractions produced by a 200 msec depolarization (B), by a 50 msec depolarization (C), and by two successive 50 msec depolarizations given at an interval of 100 msec (D), the magnitude of each depolarization being 150 mv. It will be seen that the contraction produced by two successive 50 msec depolarizations is just as large as that attained by a 200 msec depolarization with respect to both the extent of shorten-
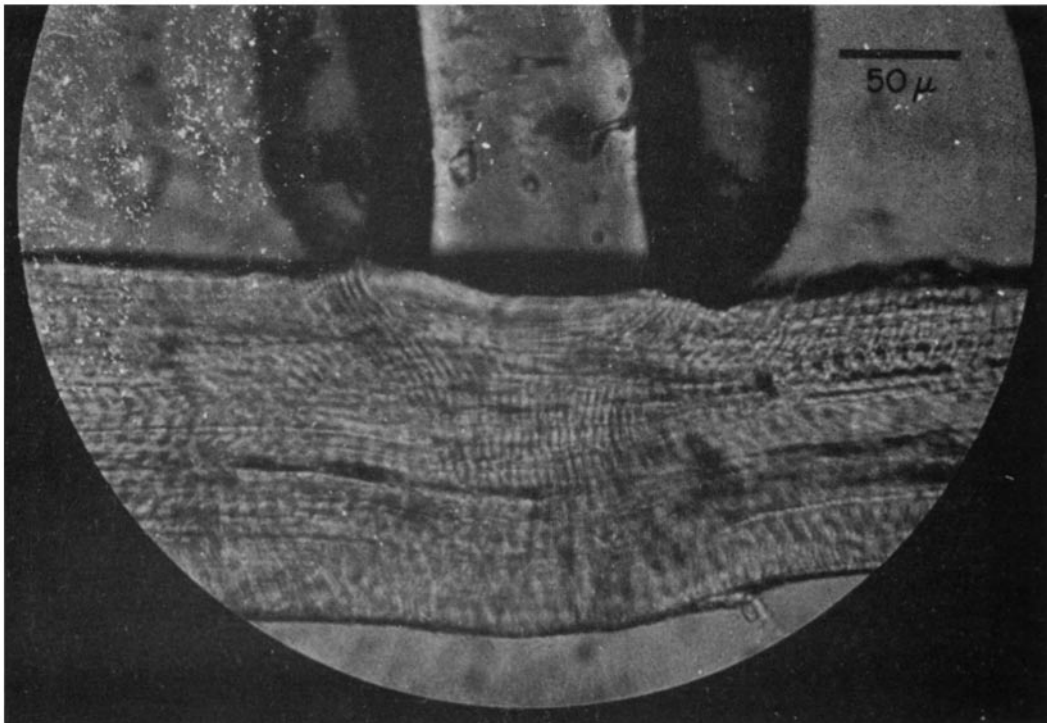

FIGURE 4.   Photomicrograph of the fiber during a local contraction spreading across the center of the fiber. The contraction was initiated by a depolarization of 180 mv and 1 sec duration.

ing and the distance of transverse spread, whereas the contraction produced by a 50 msec depolarization is much smaller than those produced by the two other types of stimulation. It follows from this that local contractions may summate to produce a much larger contraction. The summation of local contraction is composed, therefore, not only of a temporal summation, i.e. the increase in the extent of shortening of the already contracted part, but also of a spatial summation; i.e., the increase in the distance of transverse spread due to the increase in the number of activated myofibrils running in parallel within the fiber. It should also be noted that successive brief depolarizations
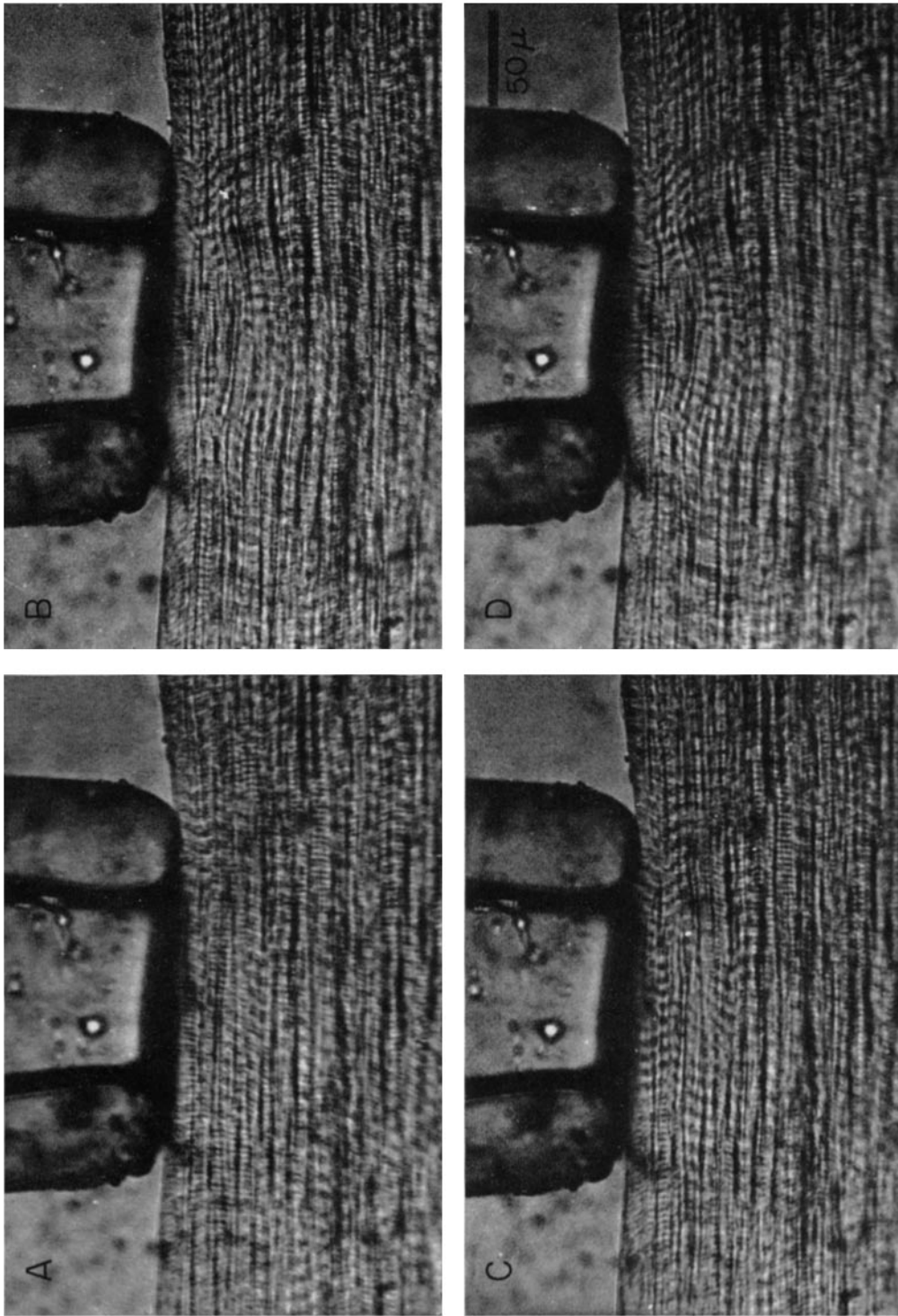
FIGURE 5. Effect of two successive brief depolarizations as compared with a prolonged depolarization. A, resting fiber. B, C, and D are local contractions produced by a 200 msec, a 50 msec, and two successive 50 msec depolarizations of the same magnitude (150 mv).

given at an appropriate interval can produce contraction more effectively for a given amount of total current than does a prolonged depolarization, since the time between the onset of the first and the removal of the second 50 msec depolarization is equal to the duration of a 200 msec depolarization.

3. EFFECT OF MANGANESE   It has been shown that manganese reduces the coupling between membrane potential and contraction in crayfish muscle fibers (Orkand, 1962 *b*). Therefore, the effect of manganese on the contraction initiated by local membrane depolarization was examined. First, the relation between depolarization and isometric tension was examined with the aid of intracellular stimulation and recording techniques analogous to those used by Orkand (1962 *a*, *b*), the tension response being recorded by hooking one end of the preparation to the tip of a glass capillary lever attached to the anodal pin of a mechanoelectric transducer (RCA 5734). In agreement with Orkand (1962 *b*), the tension response for a given amount of intracellularly applied outward current decreased markedly in the presence of maganese (1–10mM) in spite of the increase of the effective membrane resistance (Fig. 6 A and B). The resting membrane potential was increased by only a few millivolts with the highest concentration of manganese used. The local contraction initiated by a given amount of depolarization also became much smaller in the presence of manganese (1–10 mM) (Fig. 6 D) than in the normal bathing solution (Fig. 6 C). It is noteworthy that manganese reduces markedly not only the extent of shortening but also the distance of transverse spread. This result seems to suggest that the change in the coupling between membrane potential and contraction might be due, at least partly, to the change in the number of myofibrils activated for a given amount of depolarization.

Transverse Spread of Contraction Initiated by Strong Negative Current

1. NATURE OF THE RESPONSE   When a strong negative current producing local membrane depolarization of more than 200–300 mv was applied to the pipette, some but not all the fibers showed another type of local contraction different from that initiated by moderate depolarization. Fig. 7 is a typical example showing the nature and the reversibility of the response. It will be seen that the contraction spreads transversely all the way across the fiber without spreading longitudinally. This type of local contraction could be initiated even with brief current pulses of less than 1 msec duration provided its strength was sufficient, though it was necessary to use a pipette with tip diameter of more than 50 μ; the response could not be initiated with smaller pipettes. It appeared that this type of response could be elicited only in fibers obtained from animals in good condition; otherwise, the response was not initiated even with so strong a current as to cause irreversible damage to the fiber. The response was never initiated by a strong positive current applied to the pipette.
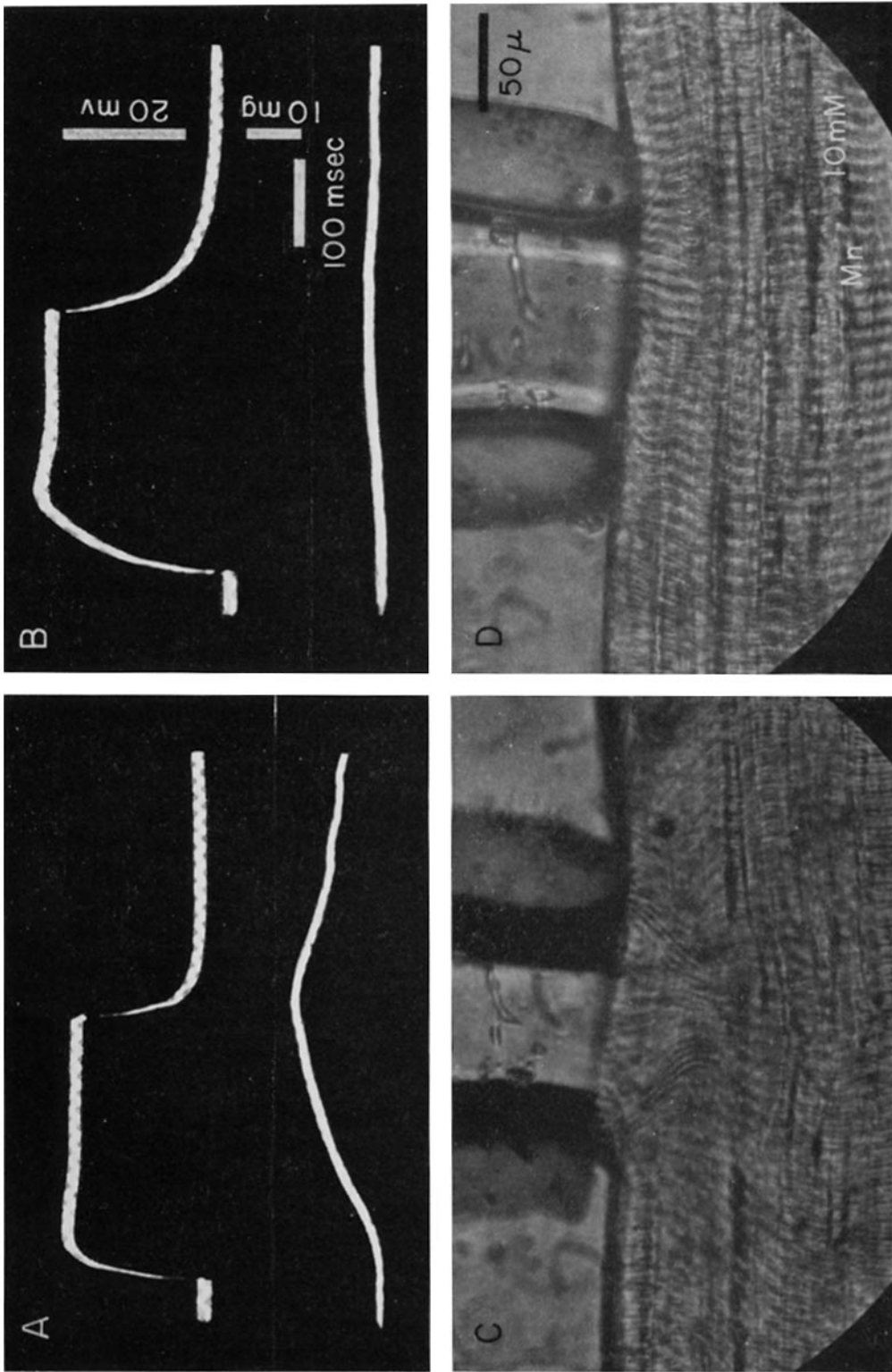
FIGURE 6. Effect of manganese on tension response and local contraction. In A and B, the upper trace is membrane potential and the lower trace is tension response elicited by intracellular stimulation. Current strength was 7×10^{-7} amp. Local contractions in C and D were produced by a depolarization of 150 mv and 500 msec duration. A and C in normal solution, and B and D in solution containing 10 mM manganese.
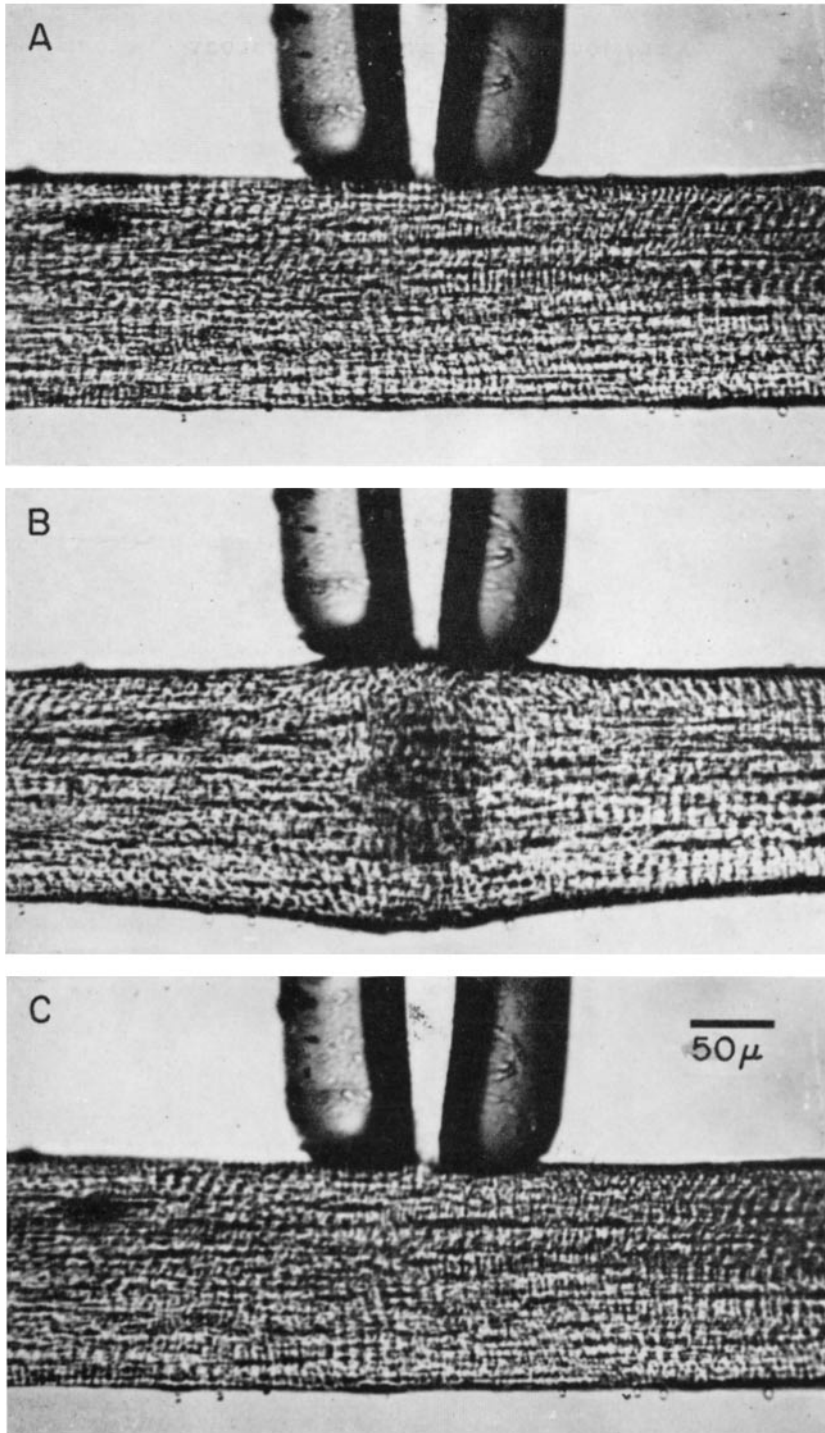
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FIGURE 7. Selected frames from a cinefilm showing the fiber before (A), during (B), and after (C) a local contraction initiated by a strong current pulse of 5 msec duration producing a potential difference of 450 mv across the contact resistance of the pipette. Note that the contraction spreads all the way across the fiber.

As mentioned above, the application of a strong current producing local membrane depolarization of more than 200–300 mv caused a reduction of membrane resistance under the pipette. As a matter of fact, it was found that, once the response was elicited, the critical current strength for subsequent initiation of the response became appreciably smaller than the initial value, indicating the reduction of membrane resistance by the first application of a strong current. With brief pulses of 2–10 msec duration, the critical potential difference across the contact resistance of the pipette for the initiation of the response was 200–250 mv. However, the response could be elicited many times without visible damage to the fiber.

2. LOCALIZATION OF CONTRACTION AROUND FIBER PERIMETER   Closer microscopic observations revealed a conspicuous feature of the above local contraction. Namely, the contraction was more or less localized around the whole perimeter of the fiber during the response. This localization of contraction was most marked if the response was initiated by a very brief pulse of less than 1 msec duration. Fig. 8 is a photomicrograph showing the localization of contraction. It will be seen that the contraction, i.e. the shortening of sarcomere length, is produced not only at the upper edge of the fiber opposite the tip of the pipette but also at the lower edge to which the striations from the upper contracted region extend, while the sarcomere length at the central part remains uniform. Such an appearance results from the fact that most of the contracted part forming a ring around the fiber is out of focus except for both edges. This finding indicates that the contraction may spread transversely around the whole perimeter but not through the center of the fiber. The possibility that some remainder of the motor nerve at the fiber surface may be involved in the above transverse spread was disproved, since tetrodotoxin (10^{-9}-10^{-6} g/ml) blocking nervous conduction in crayfish (Ogura and Mori, 1963; Ozeki and Grundfest, 1965) did not affect the response.

3. CONTRACTION CURVE AND VELOCITY OF THE TRANSVERSE SPREAD   In the early experiments, the contraction curve of the response was obtained by projecting a cinerecord of the response on the oscillographic paper (Oriental) rotating on a kymograph through a slit parallel to the long axis of the fiber. Fig. 9 is an example of the records obtained in this way. As is seen in the record, the contraction occurs symmetrically with respect to the axis of the pipette and is followed by the relaxation lasting for several seconds. The extent of shortening was increased with the increase in the duration of current pulse. By changing the position of the slit with respect to the projected image of the fiber, contraction curves at various parts across the fiber were obtained. It was noticed that the closer the part was to the tip of the pipette, the earlier its movement began after the onset of current, indicating that the contraction was first initiated at the fiber surface covered by the pipette and spread transversely around the perimeter of the fiber.

Experiments were further performed in which the image of the fiber was projected, also through a slit parallel to the length of the fiber after an appropriate (ca. 100 X) magnification of the microscope, on a 35 mm film (Fuji X-ray film) moving at a velocity of about 1 m/sec so as to measure more ac-
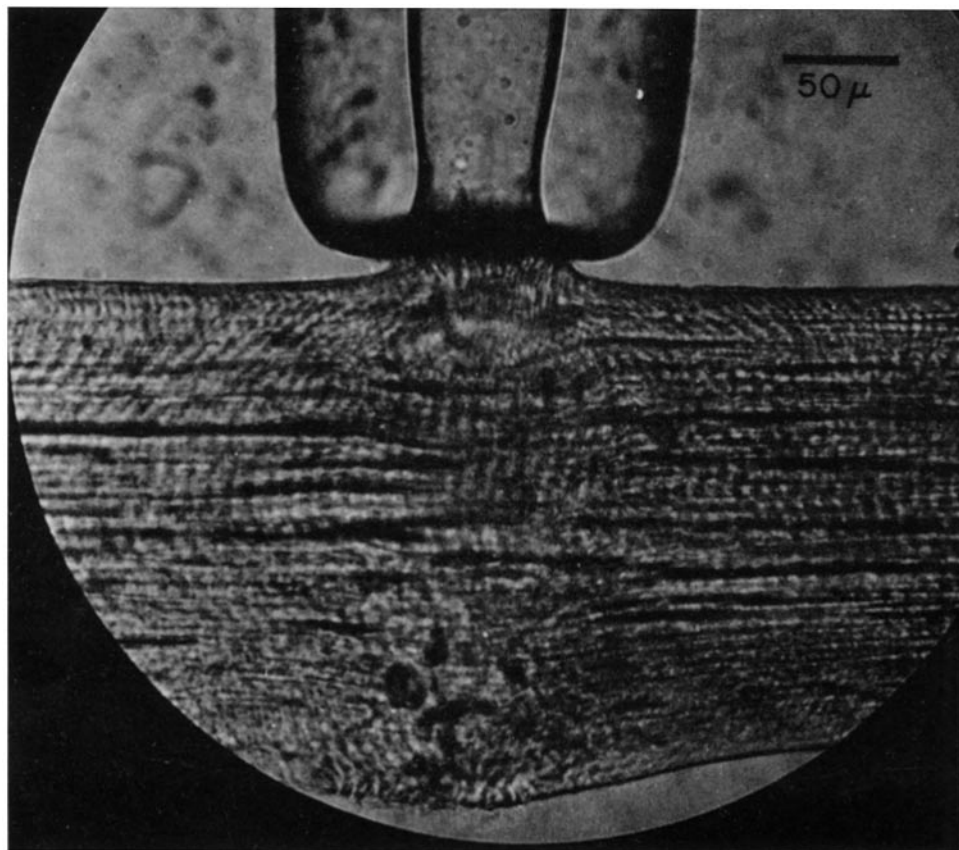

FIGURE 8.   Photomicrograph of the fiber during a local contraction initiated by a strong current of 0.8 msec duration producing a potential difference of 600 mv across the contact resistance of the pipette. Note that the sarcomere spacings at the center remain unchanged, while the contraction is produced at the upper and lower edges of the fiber.

curately the difference in the reaction time of two or three different parts across the fiber. A number of fine carbon particles were attached to the fiber so that the time sequence of contraction at each elementary part of the fiber was clearly recorded. The above is a modification of the "granule method" of Kamada and Kinosita (1943). An example of the records is shown in Fig. 10. The average velocity of the spread of contraction around the whole perimeter of the fiber was found to be of the order of 1 cm/sec (range 0.5–3 cm/sec in eight measurements on five different fibers) at room temperatures of 21–24°C.

The velocity of the spread was maximum in fresh preparations and gradually decreased with the lapse of time.

4. OTHER OBSERVATIONS   It was frequently observed that the contraction did not occur symmetrically with respect to the axis of the pipette, but the contracting part moved quickly in the longitudinal direction apart from the pipette and returned to its initial position when the response was over. It was noticed that the greater the difference in length between the two noncontracting parts at both sides of the contracting part, the more remarkable was the longitudinal displacement of the contracting part; the displacement was
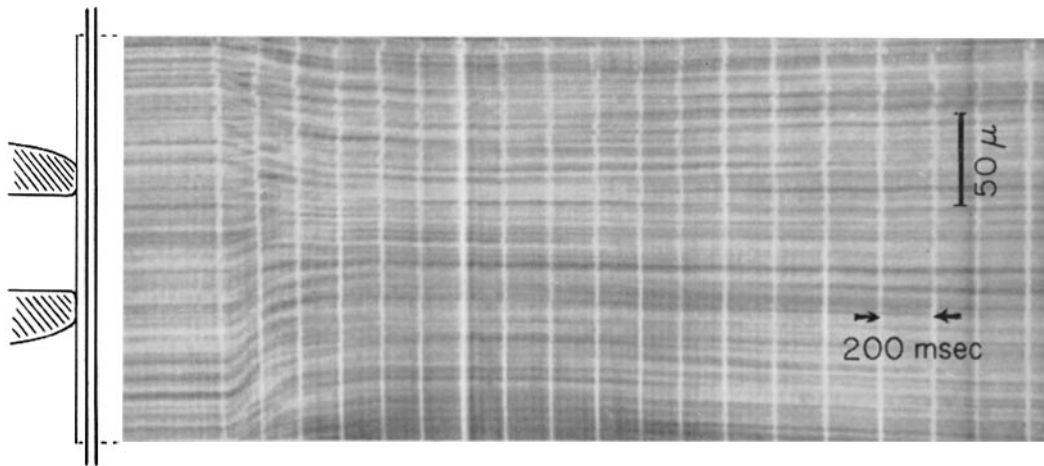

FIGURE 9.   Record showing time course of contraction. It was obtained from a cine-record shown in Fig. 7. The position of the slit with respect to the image of the fiber and that of the pipette (shaded area) are shown diagrammatically at the left-hand side of the figure.

minimized if the pipette was placed right at the middle of the fiber. Consequently, this phenomenon may be explained as being due to the difference in extensibility between the two noncontracting parts. As each noncontracting part is stretched towards the contracting part during the response, the contracting part may move longitudinally to one side where the resistance to stretch is higher than at the other side.

Another observation was that, if a strong negative current pulse of more than 100 msec was applied to the pipette, the resulting local contraction sometimes spread not only transversely but also longitudinally in each direction for a considerable distance with decrement. To make the cause of this longitudinal spread clear, the tension development during this response was recorded with the mechanoelectric transducer. It was found that the tension was produced not only during the application of the current, but also at the break of the current; the development of tension at the break of the current

was very rapid and much larger than that during the application of the current. Such a marked development of tension was always accompanied by the marked longitudinal spread of contraction. It may therefore be safe to conclude that the longitudinal spread of contraction occurs at the break of the current. Graded contractions occurring at the break of negative currents applied to a micropipette in contact with the surface of a frog muscle have
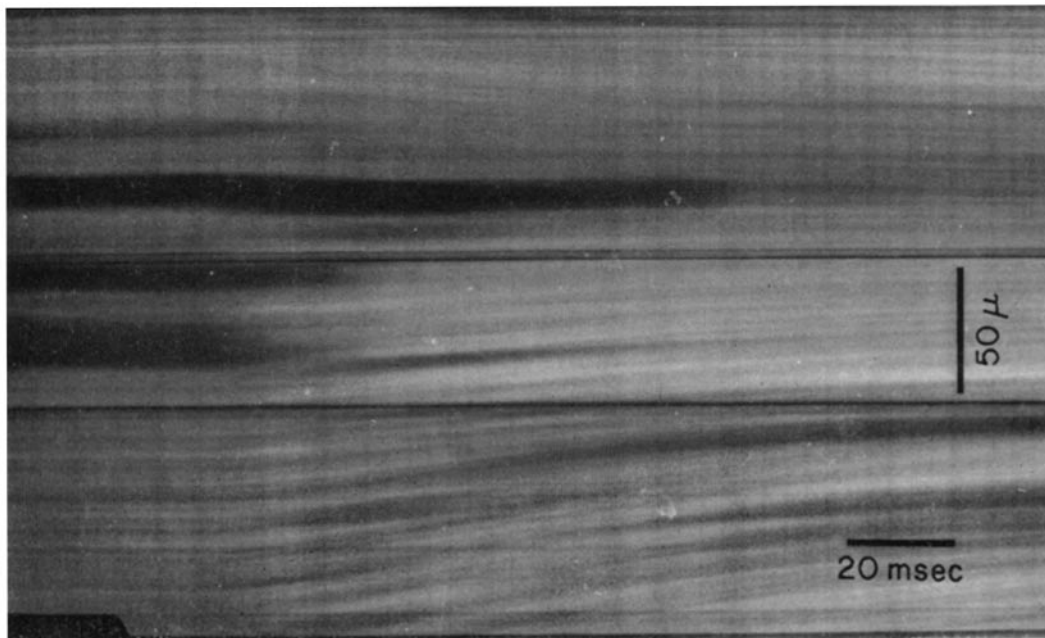

FIGURE 10. Record showing the early part of contraction. The onset of a current pulse is indicated by a downward deflection of an electromagnet, the shadow of which is projected at the bottom of the record.

been reported by Gelfan (1933). Contraction at the break of an intracellularly applied inward current has also been observed in crayfish muscle fibers (Garcia, personal communication). Since the contraction occurring at the break of the current was unfavorable for the observation of the transverse spread of contraction, brief current pulses of less than 10 msec duration were used which could elicit the response without producing contraction at the break of the current. Brief pulses were also effective for minimizing the above-mentioned longitudinal displacement of the contracting part.

DISCUSSION

The results obtained in the present experiments are summarized in Fig. 11. The contraction initiated by moderate depolarization spread inwards or

further transversely through the center of the fiber in a graded manner according to the amount and the area of depolarization (Fig. 11 A). The contraction initiated by strong negative current, on the other hand, was observed to spread around the whole perimeter but not through the center of the fiber, the response being elicited irrespective of the current duration provided the current strength was sufficient (Fig. 11 B). Both types of local contraction spread always along the striation pattern and not longitudinally. In other words, these local contractions were always confined to the sarcomeres in contact with the tip of the pipette for local activation. Therefore, it may be reasonable to suppose that the transverse tubular system is involved in these responses, and the present results will be discussed in connection with the properties of the transverse tubules within the muscle fiber.
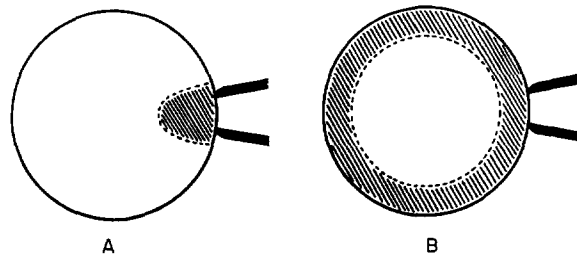

A   B

FIGURE 11. Diagram showing two different types of transverse spread of contraction initiated by local activation of crayfish muscle fibers. Each circle represents cross-section of the fiber. Pipette for local activation is shown at the right-hand side of each circle. Shaded area represents contracted region.

In agreement with the finding of Huxley and Taylor (1958), the contraction initiated by moderate depolarization was continuously graded according to the magnitude and duration of depolarization (Figs. 3–6). The greatest distance of the inward spread produced by them with a pipette of 2–10 μ diameter did not exceed some 10 μ. This has been taken as evidence that the inward conduction is a passive spread of an electrotonic potential; the influence of a local membrane depolarization is attenuated by spreading out over a broad front as it is conducted inwards along the transverse tubules. The above explanation accounts well for the result of the present experiments, namely that the increase of depolarized area increased the distance of transverse spread for a given amount of depolarization, since the larger the depolarized area is, the less readily the membrane depolarization is attenuated as it is conducted along the tubules. Thus, if a large part of the surface membrane was depolarized for a sufficiently long time the contraction could be made to spread transversely passing through the center of the fiber (Fig. 4), indicating that the depolarization effective for the initiation of contraction

may be conducted along the transverse tubular network not only in the inward direction, but also in the outward direction; i.e., from the center towards the surface of the fiber. This has already been suggested by Sugi and Kosaka (1964) who observed that a crayfish muscle fiber placed in a transverse DC field could be made to contract uniformly though the membrane depolarization was localized at one side of the fiber.

Successive brief depolarizations given at appropriate intervals could produce contraction more effectively for a given amount of total current applied to the pipette than did a prolonged depolarization (Fig. 5). This is also true with respect to the tension response of crayfish muscle fibers stimulated by transversely applied current; the summated tension produced by two successive brief pulses given at an appropriate interval was much larger than the tension attained by a single pulse the duration of which was equal to the sum of two successive pulses (Sugi and Kosaka, 1964; Fig. 9). It has been suggested that the next step leading to contraction, from the transverse tubular system to the myofibrils, is the release of calcium from some component of the sarcoplasmic reticulum (Huxley, 1959; Hodgkin and Horowicz, 1960; Costantin and Podolsky, 1965; Winegrad, 1965). If it is assumed that the degree of activation of myofibrils is proportional to the amount of calcium released from the sarcoplasmic reticulum over a wide range of the amount of calcium, this result may be taken to indicate that the rate of release of calcium may be reduced gradually during a maintained depolarization due to some inactivation process, and this process may be removed during a repolarization phase between successive depolarizations. Some inactivation of the contractile mechanism has been proposed to explain the spontaneous relaxation of potassium contracture in frog muscle fibers, though its supposed mechanism is quite different from that stated above (Hodgkin and Horowicz, 1960; Lüttgau, 1963).

The finding that the summation of local contraction is accompanied by an increase in the distance of transverse spread (Fig. 5) has supported the view (Sugi and Kosaka, 1964) that the gradation of contraction in crustacean muscle fibers depending on the frequency of motor nerve impulses (Hoyle, 1957) is achieved mainly by changing the number of activated myofibrils lying in parallel within the fiber. Each nerve impulse normally produces junction potentials localized around nerve terminals distributed along the fiber surface. Hence, it may not be unreasonable to suppose that the summation of nonpropagated contractions around each nerve terminal may occur in a manner similar to that observed in local activation experiments; the myofibrils near the fiber surface are activated by a single or a series of nerve impulses at low frequencies, while the myofibrils at the center of the fiber are only activated during a large summated contraction produced by a series of nerve impulses at high frequencies. As a matter of fact, it has been shown that

the graded nature of potassium contracture in frog muscle fibers results from the change in the number of activated myofibrils (Gonzales-Serratos, 1965). The effect of manganese on the tension response produced by intracellularly applied outward current and the local contraction initiated by local activation (Fig. 6) may be taken to indicate that manganese may change the relation between membrane potential and contraction by reducing the number of activated myofibrils for a given amount of depolarization.

The most remarkable result of the present experiments was that, in contrast to the contraction initiated by moderate depolarization, the contraction initiated by strong negative current was observed to spread around the whole perimeter but not through the center of the fiber, forming a ring-shaped region of contraction around the fiber (Figs. 7, 8, and 11 B). Another remarkable feature of this response was that it could be elicited even with a brief pulse of less than 1 msec duration provided that its strength was sufficient. As mentioned previously, the response was accompanied by the reduction of membrane resistance under the pipette, providing a low resistance path from the interior of the fiber to the interior of the pipette. It follows from this that the resulting considerable flow of current through the fiber may be an essential factor for the initiation of the response. Although the response may reflect the properties of the transverse tubular system to be investigated in the future, the interpretation of its underlying mechanism seems at present to be somewhat puzzling. It is difficult to explain the response by the hypotheses of previous authors; the outward flow of current across the surface membrane is localized under the pipette, while hyperpolarization of the surface membrane is produced over a large area due to the inward flow of current around the pipette. This type of local contraction can also be initiated in frog fast muscle fibers (Sugi and Ochi, 1965 *b*, *c*). Details of the results obtained on frog muscle fibers will be presented in the following paper (Sugi and Ochi, 1967) together with a discussion of the possible mechanism of the response.

REFERENCES

ANDERSSON-CEDERGREN, E. 1959. Ultrastructure of motor end plate and sarcoplasmic components of mouse skeletal muscle fiber as revealed by three dimensional reconstructions from serial sections. *J. Ultrastruct. Res. Suppl.* **1**:5.

ATWOOD, H. L. 1963. Difference in muscle fiber properties as a factor in "fast" and "slow" contraction in *Carcinus. Comp. Biochem. Physiol.* **10**:17.

ATWOOD, H. L., and B. S. DORAI RAJ. 1964. Tension development and membrane. responses in phasic and tonic muscle fibers of a crab. *J. Cell. Comp. Physiol.* **64**:55.

Brandt, P. W., J. P. Reuben, L. Girardier, and H. Grundfest. 1965. Correlated morphological and physiological studies on isolated single fibers. I. Fine structure of the crayfish muscle fiber. *J. Cell Biol.* 25:233.

Costantin, L. L., and R. J. Podolsky. 1965. Calcium localization and the activation of striated muscle fibers. *Fed. Proc.* 24:1141.

Dorai Raj, B. S. 1964. Diversity of crab muscle fibers innervated by a single motor axon. *J. Cell. Comp. Physiol.* 64:41.

Endo, M. 1964. Entry of a dye into a sarcotubular system of muscle. *Nature.* 202:1115.

Falk, G., and P. Fatt. 1964. Linear electrical properties of striated muscle fibres observed with intracellular electrodes. *Proc. Roy. Soc. (London), Ser. B.* 160:69.

Fatt, P., and B. L. Ginsborg. 1958. The ionic requirements for the production of action potentials in crustacean muscle fibres. *J. Physiol., (London).* 142:516.

Fatt, P., and B. Katz. 1953. The electrical properties of crustacean muscle fibres. *J. Physiol., (London).* 120:171.

Gelfan, S. 1933. The submaximal responses of the single muscle fibre. *J. Physiol., (London).* 80:285.

Girardier, L., J. P. Reuben, P. W. Brandt, and H. Grundfest. 1963. Evidence for anion-permselective membrane in crayfish muscle fibers and its possible role in excitation-contraction coupling. *J. Gen. Physiol.* 47:189.

Gonzales-Serratos, H. 1965. Differential shortening of myofibrils during contraction of single muscle fibres. *J. Physiol., (London).* 179:12P.

Hodgkin, A. L., and P. Horowicz. 1960. Potassium contractures in single muscle fibres. *J. Physiol., (London).* 153:386.

Hoyle, G. 1957. Comparative Physiology of the Nervous Control of Muscular Contraction. Cambridge, The University Press.

Huxley, A. F. 1957. Muscle structure and theories of contraction. *Progr. Biophys. Biophys. Chem.* 7:255.

Huxley, A. F. 1959. Local activation of muscle. *Ann. N.Y. Acad. Sci.* 81:466.

Huxley, A. F., and L. D. Peachey. 1964. Local activation of crab muscle. *J. Cell Biol.* 23:107A.

Huxley, A. F., and R. W. Straub. 1958. Local activation and interfibrillar structures in striated muscle. *J. Physiol., (London).* 143:40P.

Huxley, A. F., and R. E. Taylor. 1958. Local activation of striated muscle fibres. *J. Physiol., (London).* 144:426.

Huxley, H. E. 1964. Evidence for continuity between the central elements of the triads and extracellular space in frog sartorius muscle. *Nature.* 202:1067.

Kamada, T., and H. Kinosita. 1943. Disturbances initiated from naked surface of muscle protoplasm. *Japan. J. Zool.* 10:469.

Kuffler, S. W. 1946. The relation of electric potential changes to contracture in skeletal muscle. *J. Neurophysiol.* 9:367.

Lüttgau, H. C. 1963. The action of calcium ions on potassium contractures of single muscle fibres. *J. Physiol., (London).* 168:679.

Ogura, Y., and Y. Mori. 1963. Effect of crystalline tetrodotoxin on the crayfish nerve-muscle preparation. *Ann. Rept. Inst. Food Microbiol.*, 16:64.

Orkand, R. K. 1962 *a*. The relation between membrane potential and contraction in single crayfish muscle fibres. *J. Physiol., (London).* 161:143.

ORKAND, R. K. 1962 b. Chemical inhibition of contraction in directly stimulated crayfish muscle fibres. J. Physiol., (London). 164:103.

OZEKI, M., and H. GRUNDFEST. 1965. Different effects of tetrodotoxin in various electrogenic components. Fed. Proc. 25:209.

PEACHEY, L. D. 1965. The sarcoplasmic reticulum and transverse tubules of the frog's sartorius. J. Cell Biol. 25:209.

PEACHEY, L. D., and A. F. HUXLEY. 1964. Transverse tubules in crab muscle. J. Cell Biol. 23:70A.

PILGRIM, R. L. C., and C. A. G. WIERSMA. 1963. Observations on the skeleton and somatic musculature of the abdomen and thorax of Procambarus clarkii (Girard), with notes on the thorax of Panulirus interruptus (Randall) and Astacus. J. Morphol. 113:453.

PORTER, K. R., and G. E. PALADE. 1957. Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells. J. Biophys. Biochem. Cytol. 3:269.

STEN-KNUDSEN, O. 1954. The ineffectiveness of the "window field" in the initiation of muscle contraction. J. Physiol., (London). 125:396.

STEN-KNUDSEN, O. 1960. Is muscle contraction initiated by internal current flow? J. Physiol., (London). 151:363.

STRICKHOLM, A. 1962. Excitation currents and impedance of a small electrically isolated area of the muscle cell surface. J. Cell. Comp. Physiol. 60:149.

SUGI, H., and K. KOSAKA. 1964. Summation of contraction in single crayfish muscle fibres. Japan. J. Physiol. 14:450.

SUGI, H., and R. OCHI. 1965 a. The mode of transverse spread of contraction initiated by local membrane depolarization in crayfish muscle fibres. Proc. Japan Acad. 41:423.

SUGI, H., and R. OCHI. 1965 b. The mode of spread of local contraction in single frog muscle fibres. Dobutsugaku Zasshi. 74:207.

SUGI, H., and R. OCHI. 1965 c. The mode of transverse spread of contraction initiated by local membrane depolarization in frog muscle fibres. Proc. Japan Acad. 41:864.

SUGI, H., and R. OCHI. 1967. The mode of transverse spread of contraction initiated by local activation in single frog muscle fibers. J. Gen. Physiol. 50:2167.

VAN HARREVELD, A. 1936. A physiological solution for freshwater crustaceans. Proc. Soc. Exptl. Biol. 34:428.

WATANABE, A. 1958. Initiation of contraction by transverse and longitudinal current flow in single muscle fibers. Japan. J. Physiol. 8:123.

WERMAN, R., and H. GRUNDFEST. 1961. Graded and all-or-none electrogenesis in arthropod muscle. II. The effect of alkali-earth and onium ions on lobster muscle fibers. J. Gen. Physiol. 44:997.

WINEGRAD, S. Autoradiographic studies of intracellular calcium in frog skeletal muscle. J. Gen. Physiol. 48:455.