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LOCAL ACTIVATION OF STRIATED MUSCLE FIBRES

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The question how the excitation process in a muscle fibre reaches the myofibrils themselves has been discussed from time to time (Engelmann, 1873; Retzius, 1881; Tiegs, 1924), but was raised again in an acute form by Hill (1949). There was already at that time much evidence that the feature of excitation which is causally related to contraction was the reduction (or reversal) of the membrane potential (Kuffler, 1946; Katz, 1950); this in itself could not be expected to affect directly any structures more than a few Angstrom units away from the membrane, so that it was necessary to postulate some link by which myofibrils many microns away from the surface were brought into activity. Current flow in the substance of the muscle fibre appeared to be excluded as the causal agent by the experiments of Kuffler (1946; the conclusion has since been confirmed in other experiments by Sten-Knudsen (1954) and by Watanabe & Ayabe (1956); see also Taylor (1953)). Hill's calculations (1948) showed that the time for diffusion of a hypothetical activating substance, liberated at the surface during the action potential, would be too long to account for the rapidity (Hill, 1949) with which the whole of the contractile material was found to be brought into activity. It therefore seemed that there must be some specific mechanism for this inward spread of activation. The existence of connexions, at the level of the Z line, between adjacent myofibrils, and between superficial myofibrils and the sarcolemma (Enderlein, 1899; Heidenhain, 1911; von Boga, 1937; Draper & Hodge, 1949; Bennett & Porter, 1953), has suggested that this structure may be the route by which the fibrils are activated. The experiments to be described in this paper were begun as an attempt to test this possibility by finding whether contraction could be initiated by depolarizing a small area of membrane lying entirely between the attachments of two Z lines. This was found to be impossible in fibres from the frog (Huxley & Taylor, 1955*a*, *b*), in agreement with the

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hypothesis, but the interpretation was complicated by the different localization of sensitivity to depolarization that was found in crab muscle (Huxley, 1956). More recent experiments on lizard muscle (Huxley & Straub, 1958) suggest that a component of the sarcoplasmic reticulum (Porter & Palade, 1957) is the structure concerned, rather than the Z line as such.

METHODS

Principal of the method

The potential difference across the surface membrane of a muscle fibre was altered in a small area, without appreciably changing the membrane potential elsewhere, by applying current to a pipette whose tip, a few microns in diameter, was in contact with the surface of the fibre. The potential of the fluid within the pipette was thus changed, while the current was so small that the potential differences it created in the external fluid outside the pipette, and in the interior of the fibre, were negligible. The pipette was applied to different positions relative to the striation pattern, and the occurrence of contractions was observed microscopically. All experiments were carried out at room temperature (17–20° C).

Localization of potential changes

The order of magnitude of the potential changes elsewhere than within the pipette may be estimated as follows, for the typical case of a pipette of 2μ external diameter in frog Ringer's solution.

Potential differences in external fluid outside the pipette. The distribution of potential around the tip was investigated in a model in which the diameter of the pipette was 3·3 mm external, 2·2 mm internal. The pipette was filled with, and immersed in, frog Ringer's solution, specific resistance $78\,\Omega$.cm. The resistance between the interior of the pipette and a remote electrode was found to increase by $500\,\Omega$ when the tip was brought to a distance of 0·16 mm (about 1/20 external diameter) from a flat insulating surface, representing the surface of the fibre. Assuming that all dimensions change in simple proportion, this corresponds to a resistance of 0·83 MΩ with a pipette of 2μ external diameter (this is about the mean value found) and a conducting layer $0{\cdot}1\,\mu$ thick between pipette and surface membrane. The variation of potential found on the insulating plane in these conditions is shown in Text-fig. 1, falling to a quarter of the maximum at the outside edge of the tip, and to a tenth at a half radius further out.

The maximum value of the potential was roughly equal to

$$i\,(\Delta R + 0{\cdot}38rd),$$

where i = current flowing,

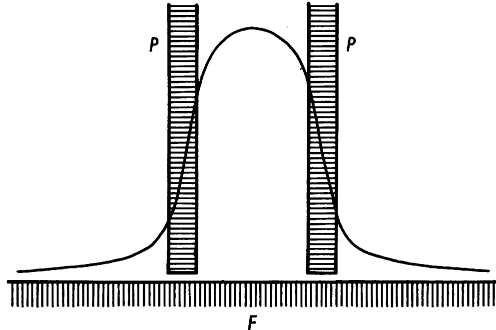
ΔR = increase of resistance of pipette as it is brought into position from a distant point,

r = resistance per unit length of the fluid in the pipette,

and d = external diameter.

Potential changes within the fibre. The area of surface membrane covered by the pipette is about 3×10^{-8} cm^2. Even if it is assumed that the resistance for unit area of membrane, when depolarized by the applied pulse, falls as low as $30\,\Omega.\text{cm}^2$, the total resistance of this patch of membrane is $10^9\,\Omega$, and the current flowing for 100 mV depolarization is 10^{-10} A. The total resistance presented by a fibre to current injected at a point is about 1 MΩ (Katz & Thesleff, 1957), so that the electrotonic potential produced within the fibre is of the order of 0·1 mV, which is altogether negligible.

If the area of membrane opposite the pipette undergoes an action potential, it is likely to pass an inward current of about 2 mA/cm^2 (Nastuk & Hodgkin, 1950), making a total current of $0{\cdot}6 \times 10^{-10}$ A, which is again negligible; the threshold strength of current needed to start a propagated action potential is of the order of 10^{-7} A (Fatt & Katz, 1951). The potential change created within the pipette by the action current will also be negligible, since the resistance of the seal formed by the tip of the pipette against the fibre was again of the order of 1 MΩ (p. 434). Similar considerations show that the corresponding disturbances were negligible also in the experiments on crab muscle.



Text-fig. 1. Distribution of potential on the surface of a muscle fibre when a pulse is applied to the pipette, as determined in model experiments (see text). The walls of the pipette, *PP*, and the gap between the tip of the pipette and the surface of the fibre, *F*, are drawn to scale.

Dissection

Frog. Single fibres were isolated from the semitendinosus muscles of frogs (*Rana temporaria*) by a procedure which did not differ in essentials from those of Asmussen (1932) and of Ramsey & Street (1938). Tubocurarine chloride (10^{-5} g/ml.) was added to the Ringer's solution, which was changed a few times during the dissection. Fibres of small or medium size were selected (diameter 40–80 μ approx.). The dissection was carried out at room temperature.

When an excitable fibre was obtained, it was transferred, either in a small scoop containing Ringer's solution or adhering to a thin glass rod, to the trough in which it was to be mounted on the microscope stage. It was held by gripping a piece of tendon at each end with screw-adjustable forceps attached to the mechanical stage.

Crab. Fibres of the extensor of the carpopodite were observed with their attachments intact. A walking leg of a shore crab (*Carcinus maenas*) was detached, and the shell of the meropodite cut away above and below to expose the muscle. The shell of the carpopodite was removed except for a small piece which was tied to the meropodite, so as to hold the muscle fibres at the greatest length they could naturally reach. Fibres were cut away from the muscle until an undamaged and fairly thin one was left at the edge in a satisfactory position for observation under the microscope.

Pipettes

Micropipettes of the type used for intracellular recording (Graham & Gerard, 1946) were drawn on a machine similar to that described by Alexander & Nastuk (1953). These were shortened to provide the required tip diameter by two methods. For the early experiments on frog fibres the tips were broken off in water by rubbing against a fine glass rod with a micromanipulator in the field of a phase-contrast microscope (objective n.a. 1·3). In the later experiments the tips were ground off at an angle of about 60° to the long axis by holding against a carefully cleaned Arkansas stone rotating slowly in a lathe, the pipette being removed frequently for examination under the phase microscope. The pipettes were filled either by boiling under reduced pressure or by means of suction.

Optical apparatus

Polarizing microscope. Many of the observations on frog fibres, and all those on crab muscle, were made with a polarizing microscope. The stand was of Swift's 'Survey' pattern, with Nicol prisms, equipped with a Zeiss D^* water immersion objective (focal length 4 mm, n.a. 0·75) and a Watson 'Holoscopic' condenser with the front lens removed to give a long working distance. The illuminating cone was limited by the polarizing prism to a square whose side (nearly parallel to the fibre axis) gave a n.a. of 0·41. A mica $\lambda/4$ plate was inserted above the polarizer, and the latter was rotated to provide compensation.

Interference microscope. The instrument described by Huxley (1954) was used. This has a water immersion objective of n.a. 0·88, and was used with an illuminating cone of n.a. 0·6–0·7.

With both microscopes the objective dipped directly into the saline surrounding the fibre that was under observation. To avoid short-circuits when current was applied, and to protect the fibre from metallic ions entering the saline from the lens mount, the metal surround of the front lens was coated before each experiment with a thin layer of paraffin and rubber lubricant (British Drug Houses Ltd.). As a further precaution, the objectives were insulated electrically from the microscope body.

Photographic arrangements. The contractions were recorded with a 16 mm cine-camera running at 16 frames/sec. The image was thrown directly on to the film by the microscope eyepiece, giving a magnification of 185 diameters with the polarizing microscope and 320 diameters with the interference microscope, determined by photographing a stage micrometer. A lightly silvered prism below the eyepiece deflected about 15% of the light into a visual eyepiece carrying a micrometer scale, adjusted so that an object focused on the graticule was also in focus on the film. The field was observed continuously during photography, at a magnification of about 800 diameters.

A ribbon filament lamp was used for visual observations, and a carbon arc was switched in for photography by moving a mirror. A heat-absorbing filter was used, but no colour filters.

Ilford 'Pan F' negative film was used.

Micromanipulators

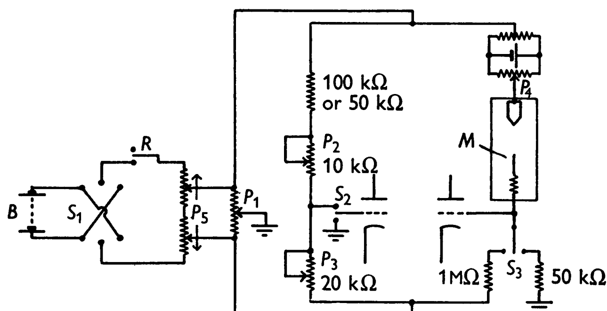
When the polarizing microscope was in use, the pipette was held in a Prior micromanipulator. It was brought into contact with the edge of the fibre, in focus under the microscope, by means of this instrument, and further movements were made by displacing the microscope stand, together with the fibre, by applying light finger pressure to the limb.

When the interference microscope was used, an additional fine movement, parallel to the muscle fibre, was incorporated in the Prior manipulator, and fine vertical movements were made by means of the stage fine adjustment on the microscope, which moved the fibre up and down, leaving the pipette stationary and in focus.

Electrical apparatus

The circuit used is shown in Text-fig. 2. The pulses (about 0·5 sec duration, at intervals of 1·5 sec) were made by closing the relay R, energized by an electronic square-wave generator. With switch S_2 up and S_3 to the left, the circuit became a Wheatstone bridge. This was used to measure the resistance of the pipette by setting P_2 to zero and adjusting P_3 to balance out the deflexion on the oscilloscope screen; the tip of the pipette was then brought into contact with the fibre surface and the increase of resistance determined from the setting of P_2 needed to restore balance. Both grids were kept near earth during the pulse by setting P_1 appropriately. Any d.c. component due to electrode potentials was balanced out by putting S_2 to its down position and adjusting P_4 until the oscilloscope trace was unaffected by changing the position of S_3.

When the effect of local depolarization was to be observed, S_2 was put to its down position and S_3 to the right. In this condition the oscilloscope deflexion was proportional to the current flowing in the pipette.



Text-fig. 2. Circuit diagram. B, 18 V dry battery. S_1, reversing switch. R, Carpenter high-speed relay. P_5, amplitude control. M, pipette. The two valves are the cathode-follower input of a differential direct-coupled amplifier and oscilloscope. For use of the other switches and potentiometers, see text.

Solutions

The composition of the solutions used with frog fibres is given in Table 1.

Solution A was used in the earlier, and solution B in the later experiments under the polarizing microscope. Solution C was used when a sodium-free medium was required.

Solution D was used in the experiments under the interference microscope. It contains enough dissolved protein to raise its refractive index to the mean value of the muscle fibres (Huxley & Niedergerke, 1954, 1958). In the experiments on frog fibres, the pipettes were filled with 120 mM-NaCl, except that when solution C was in use the pipettes also were filled with this solution. In the experiments on crab muscle the pipettes were filled with 570 mM-NaCl.

TABLE 1. Composition of solutions used in experiments on frog fibres; concentrations in m-mole/kg H_2O unless otherwise stated

Solution	Na^+	K^+	Ca^{2+}	Choline$^+$	Cl^-	HCO_3^-	PO_4 (pH 7)	Dextran* (g/100 ml.)	Protein† (g/100 ml.)
A‡	111·5	2·5	2·0	0	103	15	0	2·0	0
B	117·5	2·5	1·8	0	121	0	1·5	0	0
C	0	2·5	1·8	117·5	121	0	1·5	0	0
D‖	154	2·5	2·0	0	64·2	2·5	0	0	26·6

* 100 ml. of solution A contained 33·3 ml. of 'Intradex' (Glaxo Ltd).
† Crystallized bovine plasma albumin (Armour Laboratories Ltd).
‡ The pH of solution A was brought to 7·2 by adding a small amount of HCl.
‖ The NaOH used in making up this solution (0·2 m-equiv/g protein) brought its pH to 7·4.

RESULTS

Isolated fibres from the frog

Localization of sensitivity to depolarization

The first experiments were made on isolated frog muscle fibres under the polarizing microscope (Huxley & Taylor, 1955*a*, *b*). The fibre was usually stretched to a sarcomere length of about 3μ (some 20% beyond the maximum length in the body). The effect of applying negative electric pulses (duration about 0·5 sec, with intervals of about 1 sec between pulses) to a pipette of 2μ tip diameter in contact with the edge of the fibre is shown in Pl. 1, figs. 1–4.

If the pipette was applied opposite an A band no movement was produced (Pl. 1, figs. 1, 2) unless the strength was raised so high that irreversible changes occurred (see below, p.434). If, however, the pipette was opposite an I band it was often found that each pulse caused a local contraction, as shown in Pl. 1, fig. 4.

It appeared that a contraction was never produced unless the tip of the pipette overlapped the centre of the I band. This is the position of the Z line, but that structure was not visible in frog fibres under the polarizing microscope with the wide illuminating cone that we employed (it can be detected by reducing the cone, but it is then seen in virtue of its high refractive index, not birefringence). In order to check more precisely whether the sensitive point coincided with the Z line, similar experiments were carried out under the interference microscope. It was regularly found that contractions were caused only if the Z line was overlapped by the tip of the pipette.

Localization around the fibre perimeter. This phenomenon of local activation appeared at first to be somewhat capricious: even if the pipette was placed centrally over an I band, it was often found that no contraction occurred, and occasionally an I band became inactive after responding well for some time, or vice versa. The cause of this irregularity was found to be that, as the pipette was moved around the fibre along the line of contact of one Z membrane with the sarcolemma, responses were obtained at some positions but not at others. This was further investigated on the interference microscope, keeping the pipette stationary or moving it only in the horizontal plane while the fibre was moved up or down with the stage focusing movement, which operated with no detectable backlash. Some results of two experiments of this kind are shown in Text-fig. 3. Along any one Z line sensitive regions were found at intervals of about 5μ. These sensitive regions were not at exactly the same level on adjacent Z lines, but in some experiments (e.g. F, Text-fig. 3) an unmistakable correlation was found.

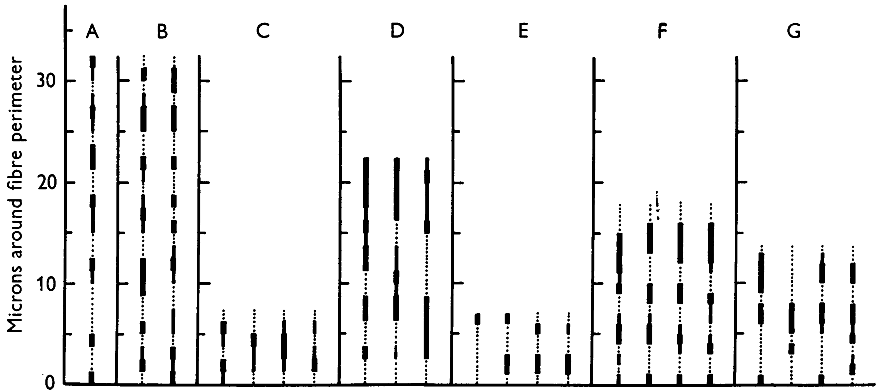
The extent of vertical movement within which responses were obtained consecutively was generally greater than the diameter of the tip of the pipette, suggesting that each sensitive region did not consist simply of a single sensitive spot on the fibre surface. In a few cases the range of movement within which responses were continuously obtained was several times the pipette diameter, showing clearly that the sensitive region was of considerable extent.

Nature, spread and gradation of the response

Nature of the contraction. Whenever a response was seen, it consisted in a movement towards one another of the A bands flanking the I band to which the pipette was applied. The A bands themselves appeared not to change in width during their movement, as is to be expected from earlier observations on frog fibres with the interference microscope (Huxley & Niedergerke, 1954,

1958) and on separated myofibrils under phase contrast (H. E. Huxley & Hanson, 1954; Harman, 1954). The nature of the contraction is well shown in Pl. 1, figs. 5–8.

Symmetry of the contraction. When the interference microscope was used, the Z line was visible, and it was invariably found that this structure remained central in the I band during a local contraction, i.e. the two halves of the I band shortened by equal amounts. This was true even when the edge of the pipette was placed over the Z line, and a weak contraction was produced (Pl. 2, figs. 1–3). In this case, if the two halves of the I band had been activated from different points on the surface, separated by only (say) $0{\cdot}1\mu$ along the fibre axis, the strength of depolarization they would have experienced would have



Text-fig. 3. Diagram showing distribution of sensitivity to depolarization at different points along line of contact between Z line and sarcolemma in frog fibres. Each vertical line corresponds to a single Z line; the lines grouped under each letter correspond to adjacent Z lines, tested one after another at each level. Thick full line, well-marked response; narrow full line, weak or doubtful response; dotted, no response. A–D on one fibre; E–G on another.

differed by some 30% (Text-fig. 1), and it would be expected that the amount of shortening in the two halves would have been detectably different.

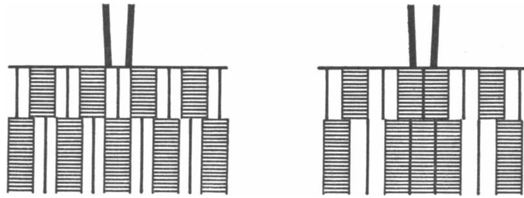
Asymmetrical contractions were also never seen when a sequence of points along the attachment of one Z membrane to the sarcolemma were tested.

On several occasions contractions were observed at places where a 'vernier formation' was visible a few microns from the edge of the fibre, so that the I band to which the pipette was applied terminated at the vernier opposite an A band. A single position was once found where the contraction of one I band near the edge of the fibre was continued across the vernier, into both the two nearest I bands (Text-fig. 4). Each of these I bands contracted completely, showing that the activation affected both sides of them.

These observations make it highly probable that the half-sarcomeres on either side of each Z line are activated by a single structure.

Movement of water and dissolved material. The shortening of a single I band

induced by local depolarization was not accompanied by any detectable expansion perpendicular to the fibre axis. This means that the volume enclosed between the mid planes of the adjacent A bands must have decreased to about half during the contractions, presumably by the expulsion of water. During the first 1 or 2 sec after it had relaxed, the I band was seen under the interference microscope (Pl. 2, figs. 4–6) to have a refractive index markedly lower than its neighbours', indicating that dissolved materials (presumably mostly proteins) had been squeezed out of the I band together with water during the contraction, and returned more slowly than the water after relaxation.



Text-fig. 4. Sketch showing spread of the local contraction across a 'vernier formation' observed on one occasion. Frog fibre; A bands shaded. When the pipette was applied over the adjacent Z lines at the same level, in one case no contraction was obtained, and in the other, a contraction which reached only as far as the vernier formation.

Contraction bands. When a powerful contraction of a single I band was observed under the interference microscope, a dense line was sometimes seen at the position where the two adjacent A bands came into contact. Under the polarizing microscope, however, a thin gap was always seen between the adjacent A bands (Pl. 1, figs. 6–8). These observations are in agreement with the classical descriptions of the formation of contraction bands (Engelmann, 1873; Rollett, 1891) and with recent observations on isolated fibrils (Hanson & H. E. Huxley, 1955, p. 245).

Absence of longitudinal spread. In no case was any shortening observed anywhere except in an I band to which the pipette was applied. This was so even when the contraction spread some 10μ inwards as shown in Pl. 1, figs. 6–8, although the adjacent I bands were only $1–2\mu$ away.

Gradation of inward spread. The speed and extent of the shortening, and usually also the distance to which the contraction spread inwards from the fibre surface, were graded according to the strength of the pulse applied. In our earliest reports (Huxley & Taylor, 1955*a*, *b*) this statement had to be qualified because none of the fibres was capable of giving an all-or-none response to ordinary stimulation at the end of the experiment, and it was possible that a fibre in good condition might have responded to local depolarization with, for example, an all-or-none response involving one I band across the whole cross-section of the fibre. Such possibilities were ruled out in later experiments in which the fibres were still able to conduct action potentials

past the point that was under observation, and to give all-or-none twitch responses to ordinary stimulation, after the local contraction had been observed. In all these cases the response to local depolarization was smoothly graded with stimulus strength, and there was no suggestion of all-or-none behaviour.

The greatest distance inwards to which the contraction was observed to spread was about 10μ; it appeared to be easier to obtain a contraction of this extent when using a pipette of 4μ diameter than with one of 2μ. No greater inward spread was obtained with a pipette of 10μ diameter, although several sarcomeres were contracting simultaneously in this case. On a few occasions the contraction appeared not to involve the most superficial layer of myofibrils (1–2μ) although it was well marked further from the surface.

Local contractions were observed on a few occasions with the oblique tip of the pipette in contact with the upper surface of the fibre. The shortening was then also seen to spread up to about 10μ in each direction around the periphery of the fibre.

Response in the absence of sodium

The effect was still present when the sodium of the Ringer's solution was replaced by choline, and the pipette was also filled with this solution.

Magnitude of membrane potential changes

The increase of resistance of the pipette when its tip was brought into contact with the surface of a muscle fibre was measured in most experiments by means of the bridge arrangement described on p. 429. The values obtained are somewhat arbitrary, since they increased as the pipette was pressed harder against the fibre. For a given size of pipette, the values varied over a range of about 2:1 on different fibres, the mean being a little under 1 MΩ for a pipette of 2μ external diameter, and varying inversely with diameter. No change in contact resistance could be detected when the pipette was moved from an I band to a nearby A band, or vice versa.

The potential change at the centre of the pipette was calculated by means of the formula given on p. 429, assuming the internal diameter of the pipette to be two-thirds of the external. Values obtained in this way ranged from 50 to 150 mV when a just-detectable contraction was being elicited, and from 50 to 300 mV when marked contractions, suitable for photographic recording, were produced. These values are likely to be overestimates of the membrane potential change opposite the sensitive spots themselves, since they were obtained, in most cases, without careful adjustment of the pipette in the vertical direction to a point of maximum sensitivity. In addition, there must be a large random error due to variation in the pressure with which the pipette was applied to the fibre.

Effect of overstimulation

The local contractions described so far were reversible, in that the I band extended again as soon as the current through the pipette stopped; they were also repeatable at will at the same point on the fibre. With stronger pulses in either direction, however, irreversible contractions were produced. These differed in the following respects from the reversible contractions:

(1) They persisted after the termination of the applied current.

(2) They involved many sarcomeres.

(3) In many cases when a contraction of this kind was induced in a fresh fibre, the whole fibre moved vigorously, suggesting that the whole or a large part of the fibre underwent a twitch.

(4) If the pipette was kept at exactly the position on the fibre at which it had been when the contraction was initiated, then subsequent application of a positive pulse to the pipette increased the contraction, whereas a negative pulse caused partial relaxation.

These observations are all consistent with the supposition that this type of contraction is produced when the resistance of the surface membrane is drastically reduced as a result of applying too large a potential difference across it, providing a low resistance path from the interior of the pipette to the interior of the fibre. The membrane potential difference (estimated as described in the previous section) that was required to produce a contraction of this kind was a few tenths of a volt; it appeared to be less when the interior of the pipette was made positive than when it was made negative.

Crab muscle

Observations on crab muscle were undertaken in the hope of defining more precisely the positions of the sensitive sites with respect to the striation pattern, since the sarcomere width in the muscle used is 3–4 times as great as in vertebrate muscle. Unexpectedly, the positions were found to be different from those in frog muscle. Observations were made with the polarizing microscope only, and the fibres were immersed in artificial sea water.

Positions of sites sensitive to depolarization. As with frog muscle, a positive pulse produced no result unless it was so strong as to cause irreversible damage, while a negative pulse evoked a strictly localized contraction if the pipette was appropriately placed. In the case of crab muscle, however, the positions of the pipette at which contractions could be initiated were not opposite the Z lines, but at points close to each boundary between A and I bands. There was a suggestion that the sensitive points were just within the A band, and not precisely at the A–I boundary. Pl. 3 shows the pipette at a series of positions where negative pulses were applied, with, in each case, an indication whether a contraction was obtained or not.

Nature of the response. These muscle fibres differed from those of the frog in that the Z line was clearly visible under the polarizing microscope even with the wide illuminating cone that was employed. It was found (Pl. 4) that when a local contraction was produced, the Z line was pulled over to the side of the I band to which the pipette was applied, until it came into contact with the A band. The other half of the same I band never took part in the contraction, and it was usually possible to see that it was actually stretched. No shortening was ever seen in other I bands. The strength of response and the extent of inward spread were graded continuously with the strength of the applied pulse, with no suggestion of all-or-none behaviour.

DISCUSSION

Relationship to normal contraction

There seems no reason to suppose that in these experiments the fibrils are activated by a different route from the one employed in normal contractions. The graded nature of the response is no difficulty, since it has long been recognized (Adrian, 1922; Gelfan, 1933) that the all-or-none character of the twitch of a frog muscle fibre is imposed by the all-or-none electrical response (action potential) of the membrane, and this was eliminated in our experiments as discussed on p. 427. Twitch-like contractions, graded according to the strength of the applied shock, have been recorded by Brown & Sichel (1936) and by ourselves (unpublished) in isolated fibres in which the action potential mechanisms had been lost or eliminated by immersion in a Na-free medium. The normal activity of crab muscle in any case consists largely of continuously graded responses (Katz, 1949). In one of the present series of experiments we applied pulses of a few milliseconds duration to the pipette; the local contraction was obtained and its duration was of the same order as that of a normal twitch.

Limitation of inward spread

Another respect in which these contractions differ from normal twitches or tetani of frog muscle is that they extended only some 10μ inwards from the tip of the pipette, in fibres which might be 70μ in diameter. A simple explanation of this difference is that the influence of local depolarization becomes spread out over a broad front as it progresses inwards and is not maintained in amplitude by any regenerative process. This factor would not arise in a normal contraction when the whole perimeter of the fibre is depolarized in each action potential.

Evidence that depolarization is the effective agent

The strict localization of the effect to the I band (or, in the crab, to the half-I-band) to which the pipette was applied shows that the immediate effect of the applied current on the fibre was at, or very close to, the surface membrane,

the inward spread taking place along some specialized structure. This consideration makes it extremely unlikely, for example, that the very small (p. 427) currents in the interior of the fibre could be the causative agent. It seems practically certain that this was the change in potential difference across the membrane itself, as appears to be the case when current is applied to a large area of muscle membrane (Kuffler, 1946; Sten-Knudsen, 1954; Watanabe & Ayabe, 1956).

Relation to Gelfan's observations

Gelfan (1933) recorded graded contractions of single fibres on the surface of a frog's sartorius muscle in response to electric pulses applied to a microcapillary in contact with the fibre surface. These resembled the irreversible contractions that we observed (p. 434) when too large a current was applied, in the following respects:

(1) They involved an appreciable length of the muscle fibre.
(2) Positive current fed to the pipette caused contraction, while negative current reduced the degree of shortening that was already present.
(3) Propagated activity was sometimes initiated at the start of a contraction which subsequently was localized to the neighbourhood of the capillary and was graded with current strength.

It therefore seems fairly certain that the same phenomenon was being observed in both cases. Gelfan explained the reversal of the usual polarity by supposing that the contraction was caused by 'induced cathodes' flanking the anodal capillary; our explanation (p. 435) is equivalent to this, with the added assumption of a drastically reduced membrane resistance opposite the tip of the pipette.

Gelfan did not describe contractions limited to a single striation; it is unlikely that they would have been visible under his conditions.

Mechanism of inward spread

It seems necessary to postulate that the influence of the membrane potential change is conducted inwards along a definite structure related to the striation pattern, in order to explain the localization of the shortening to a single I band (or half-I-band). The discontinuous distribution of sensitivity along the line of contact of a single Z line with the sarcolemma in the frog suggests that the structure in question is a network rather than a complete disk. As to the nature of the influence that is conducted inwards, the only direct evidence provided by our experiments is that the spread is not of an all-or-none kind. It is therefore likely to be a passive process.

The simplest hypothesis consistent with our results is that each fibre contains networks of tubules, electrically continuous with the external fluid, in

the transverse planes corresponding to the positions at which surface depolarization is effective (Z line in frog; near to boundaries of A band in crab), and that reduction of the potential difference across the walls of these tubules activates the neighbouring myofibrils. Tubular or vesicular structures forming networks in between the myofibrils have been seen in electron micrographs of muscle from many animals (Bennett & Porter, 1953; Edwards & Ruska, 1955; Robertson, 1956; Porter & Palade, 1957), and in each case the authors referred to have suggested that the structures might be concerned with the conduction of an activating process to the myofibrils. In amphibian (*Amblystoma*) muscle, Porter & Palade (1957) describe at the level of the Z line a characteristic grouping of such tubules or vesicles, which they refer to as a 'triad'. Its position makes it (or one of its components) an attractive candidate for being the inward conductor of the influence of membrane depolarization, but Porter & Palade state that it is not continuous across the fibre, whereas continuity exists between the tubular elements at the middle of the A band.

A further test of the possible role of the triads has been suggested by: (*a*) Porter & Palade's finding that in mammalian muscle there are two triads in each sarcomere, one near each boundary of the A band; and (*b*) Robertson's (1956) description of tubules resembling the centre element of a triad, at similar positions, in muscle from a lizard. If the triads are indeed the structures concerned, it might therefore be expected that lizard muscle would respond to local depolarization more nearly as has been described in this paper for crab muscle than for frog muscle. Huxley & Straub (1958) have carried out the experiment and found that the shortening obtained in this way was confined to one half of the I band when the pipette was placed over the adjacent A–I boundary, the Z line being pulled across into contact with the A band on that side just as we found in crab fibres. Further, one of us (A. F. H.), has confirmed with the electron microscope that structures closely resembling Porter & Palade's triads are present in lizard muscle at levels near each end of the A band. Hence, both in frog and lizard muscle, the region which responds as a unit to local depolarization is centred on the position where triad structures are found, although these positions are very different in the two animals. This suggests strongly that one of the components of the triads is the structure involved, but further study of the tubular elements with the electron microscope, both as regards their comparative anatomy and as regards their continuity and connexions with the surface membrane, is required before this possibility can be regarded as established.

There is little evidence concerning the final step, from these hypothetical transverse networks to the filaments themselves. Some possibilities are discussed elsewhere (Huxley, 1957).

SUMMARY

1. The membrane potential of small areas of the surface of surviving muscle fibres from frog and crab was changed by applying electric current to a pipette whose tip was in contact with the fibre.

2. Reversible contractions, whose strength and degree of spread towards the centre of the fibre varied with the applied current, were produced only if the membrane was depolarized at certain positions in relation to the striation pattern.

3. In frog muscle fibres these sensitive spots were distributed along the line of contact of each Z line with the sarcolemma. The contraction consisted in shortening of both halves of the I band which contained the Z line in question.

4. In crab muscle at maximum *in situ* length, the sensitive spots lay close to each boundary between A and I, probably just within the A band. The contraction consisted in an approximation of the adjacent Z line to the A–I boundary in question, the other half of the I band being unaffected.

5. The contraction never spread longitudinally, although in frog fibres it commonly spread inwards for a distance several times the striation spacing.

6. With stronger pulses of either polarity irreversible local shortening was produced. This agreed in all essential respects with the local contractions studied by Gelfan in 1933.

7. The results on frog muscle, together with those of Huxley & Straub (1958) on lizard muscle, suggest that some component of the 'triads' described by Porter & Palade (1957) in the sarcoplasmic reticulum may be the structure along which the influence of membrane depolarization is conducted inwards.

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REFERENCES

ADRIAN, E. D. (1922). The relation between the stimulus and the electric response in a single muscle fibre. *Arch. néerl. Physiol.* **7**, 330–332.

ALEXANDER, J. T. & NASTUK, W. L. (1953). An instrument for the production of microelectrodes used in electrophysiological studies. *Rev. sci. Instrum.* **24**, 528–531.

ASMUSSEN, E. (1932). Über die Reaktion isolierter Muskelfasern auf direkte Reize. *Pflüg. Arch. ges. Physiol.* **230**, 263–272.

BENNETT, H. S. & PORTER, K. R. (1953). An electron microscope study of sectioned breast muscle of the domestic fowl. *Amer. J. Anat.* **93**, 61–105.

BROWN, D. E. S. & SICHEL, F. J. M. (1936). The isometric contraction of isolated muscle fibres. *J. cell. comp. Physiol.* **8**, 315–328.

DRAPER, M. H. & HODGE, A. J. (1949). Studies on muscle with the electron microscope. I. The ultrastructure of toad striated muscle. *Aust. J. exp. Biol. med. Sci.* **27**, 465–503.

EDWARDS, G. A. & RUSKA, H. (1955). The function and metabolism of certain insect muscles in relation to their structure. *Quart. J. micr. Sci.* **96**, 151–159.

ENDERLEIN, G. (1899). Beitrag zur Kenntniss des Baues der quergestreiften Muskeln bei den Insekten. *Arch. mikr. Anat.* **55**, 144–150.

ENGELMANN, T. W. (1873). Mikroskopische Untersuchungen über die quergestreifte Muskelsubstanz. Zweiter Artikel. Die thätige Muskelsubstanz. *Pflüg. Arch. ges. Physiol.* **7**, 155–188.

FATT, P. & KATZ, B. (1951). An analysis of the end-plate potential recorded with an intra-cellular electrode. *J. Physiol.* **115**, 320–370.

GELFAN, S. (1933). The submaximal responses of the single muscle fibre. *J. Physiol.* **80**, 285–295.

GRAHAM, J. & GERARD, R. W. (1946). Membrane potentials and excitation of impaled single muscle fibers. *J. cell. comp. Physiol.* **28**, 99–117.

HANSON, J. & HUXLEY, H. E. (1955). The structural basis of contraction in striated muscle. *Symp. Soc. exp. Biol.* **9**, 228–264.

HARMAN, J. W. (1954). Contractions of skeletal muscle myofibrils by phase microscopy. *Fed. Proc.* **13**, 430.

HEIDENHAIN, M. (1911). *Plasma und Zelle.* Zweite Lieferung, p. 613. Jena: Fischer.

HILL, A. V. (1948). On the time required for diffusion and its relation to processes in muscle. *Proc. Roy. Soc.* B, **135**, 446–453.

HILL, A. V. (1949). The abrupt transition from rest to activity in muscle. *Proc. Roy. Soc.* B, **136**, 399–420.

HUXLEY, A. F. (1954). A high-power interference microscope. *J. Physiol.* **125**, 11–13*P*.

HUXLEY, A. F. (1956). Local activation of striated muscle from the frog and the crab. *J. Physiol.* **135**, 17–18*P*.

HUXLEY, A. F. (1957). Muscle structure and theories of contraction. *Progr. Biophys.* **7**, 255–318.

HUXLEY, A. F. & NIEDERGERKE, R. (1954). Interference microscopy of living muscle fibres. *Nature, Lond.*, **173**, 971–973.

HUXLEY, A. F. & NIEDERGERKE, R. (1958). Measurement of the striations of isolated muscle fibres with the interference microscope. *J. Physiol.* **144**, 403–425.

HUXLEY, A. F. & STRAUB, R. W. (1958). Local activation and interfibrillar structures in striated muscle. *J. Physiol.* **143**, 40–41*P*.

HUXLEY, A. F. & TAYLOR, R. E. (1955*a*). Activation of a single sarcomere. *J. Physiol.* **130**, 49–50*P*.

HUXLEY, A. F. & TAYLOR, R. E. (1955*b*). Function of Krause's membrane. *Nature, Lond.*, **176**, 1068.

HUXLEY, H. E. & HANSON, J. (1954). Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature, Lond.*, **173**, 973–976.

KATZ, B. (1949). Neuro-muscular transmission in invertebrates. *Biol. Rev.* **24**, 1–20.

KATZ, B. (1950). Quoted by HILL, A. V. in A discussion on muscular contraction and relaxation: their physical and chemical basis. *Proc. Roy. Soc.* B, **137**, 45–47.

KATZ, B. & THESLEFF, S. (1957). On the factors which determine the amplitude of the 'miniature end-plate potential'. *J. Physiol.* **137**, 267–278.

KUFFLER, S. W. (1946). The relation of electric potential changes to contracture in skeletal muscle. *J. Neurophysiol.* **9**, 367–377.

NASTUK, W. L. & HODGKIN, A. L. (1950). The electrical activity of single muscle fibers. *J. cell. comp. Physiol.* **35**, 39–73.

PORTER, K. R. & PALADE, G. E. (1957). Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells. *J. biophys. biochem. Cytol.* **3**, 269–300.

RAMSEY, R. W. & STREET, S. F. (1938). The alpha excitability of the local and propagated mechanical response in isolated single muscle fibers. *J. cell. comp. Physiol.* **12**, 361–378.

RETZIUS, G. (1881). Zur Kenntniss der quergestreiften Muskelfaser. *Biol. Untersuch.* Ser. 1, **1**, 1–26.

ROBERTSON, J. D. (1956). Some features of the ultrastructure of reptilian skeletal muscle. *J. biophys. biochem. Cytol.* **2**, 369–380.

ROLLETT, A. (1891). Untersuchungen über Contraction und Doppelbrechung der quergestreiften Muskelfasern. *Denkschr. Akad. Wiss. Wien*, **58**, 41–98.

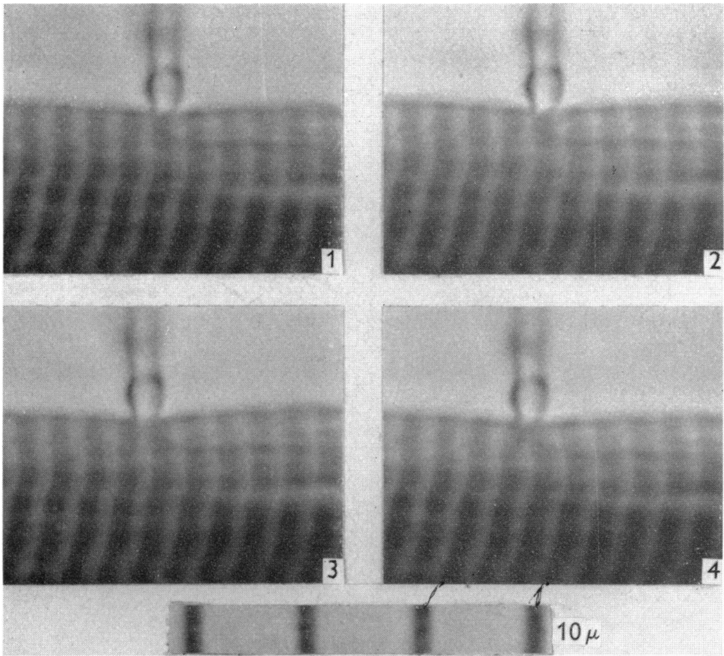
STNE-KNUDSEN, O. (1954). The ineffectiveness of the 'window field' in the initiation of muscle contraction. *J. Physiol.* **125**, 396–404.

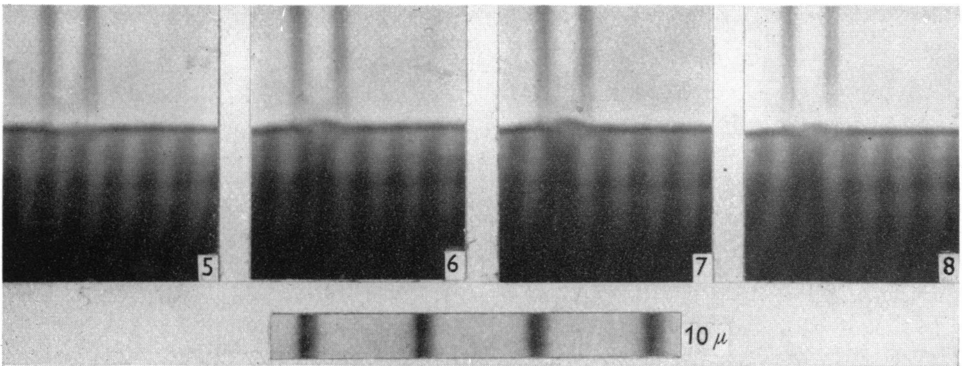
TAYLOR, R. E. (1953). The contractile process is not associated with potential changes. *J. cell. comp. Physiol.* **52**, 103–123.

TIEGS, O. W. (1924). On the mechanism of muscular action. *Aust. J. exp. Biol. med. Sci.* **1**, 11–29.

VON BOGA, L. (1937). Beiträge zur Kenntnis des Muskelgewebes von Trichopterenlarven. Eine mikroskopische Studie des feineren Baues des Insektenkörpers. *Z. Zellforsch.* **27**, 568–602.

WATANABE, A. & AYABE, R. (1956). Local contraction in a single muscle fiber elicited by electrical stimulation. *XIV Jap. med. Congr.* 17–19.





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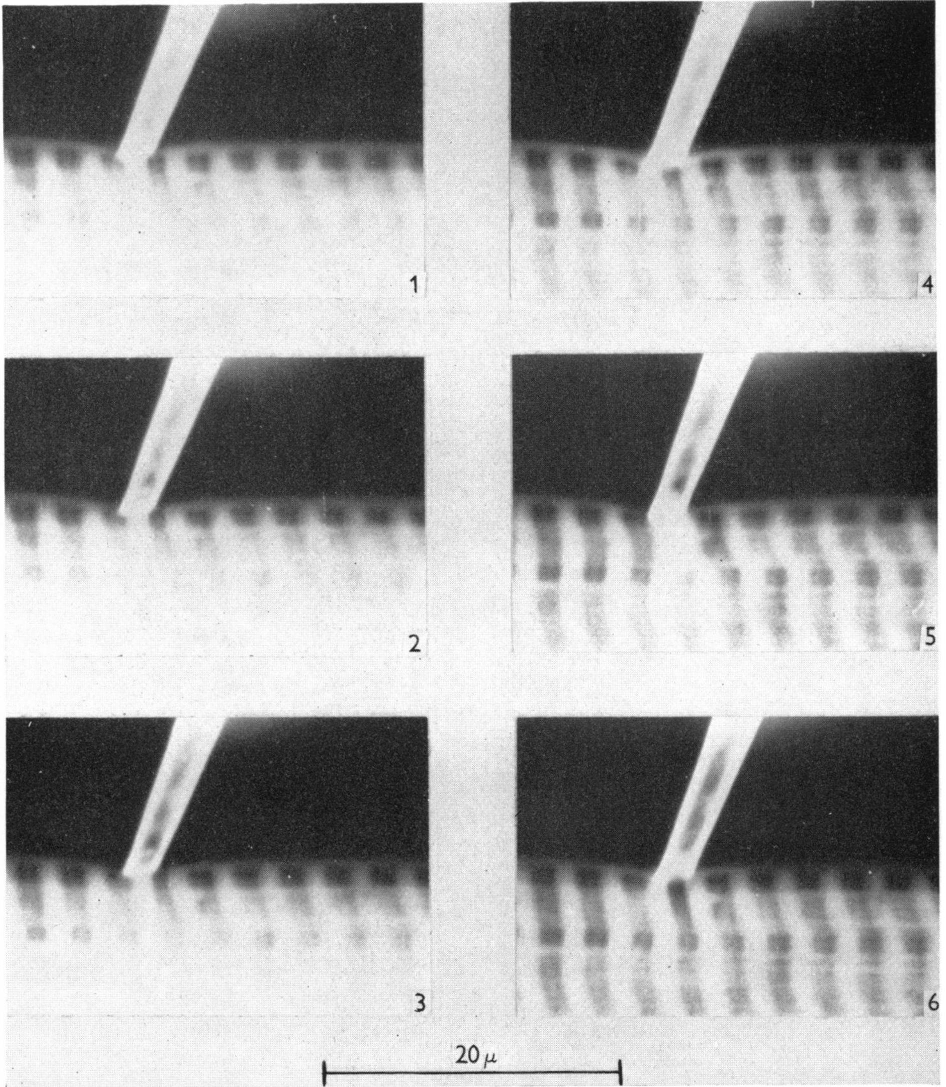
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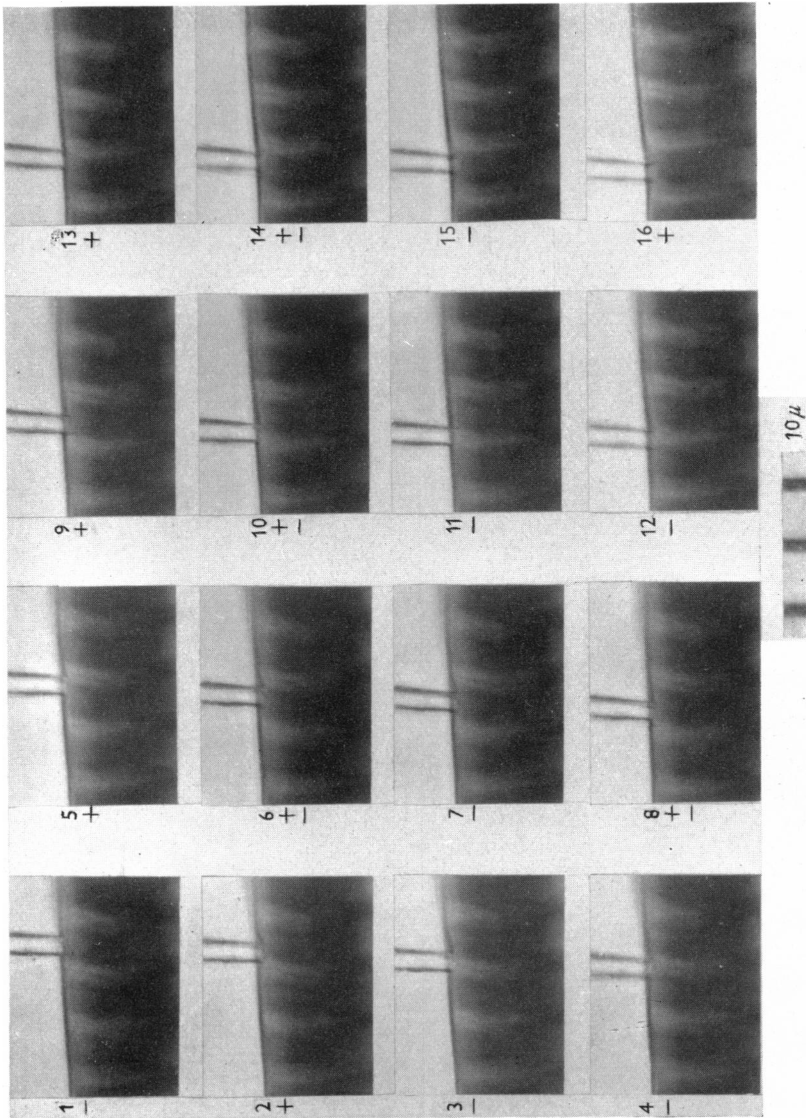
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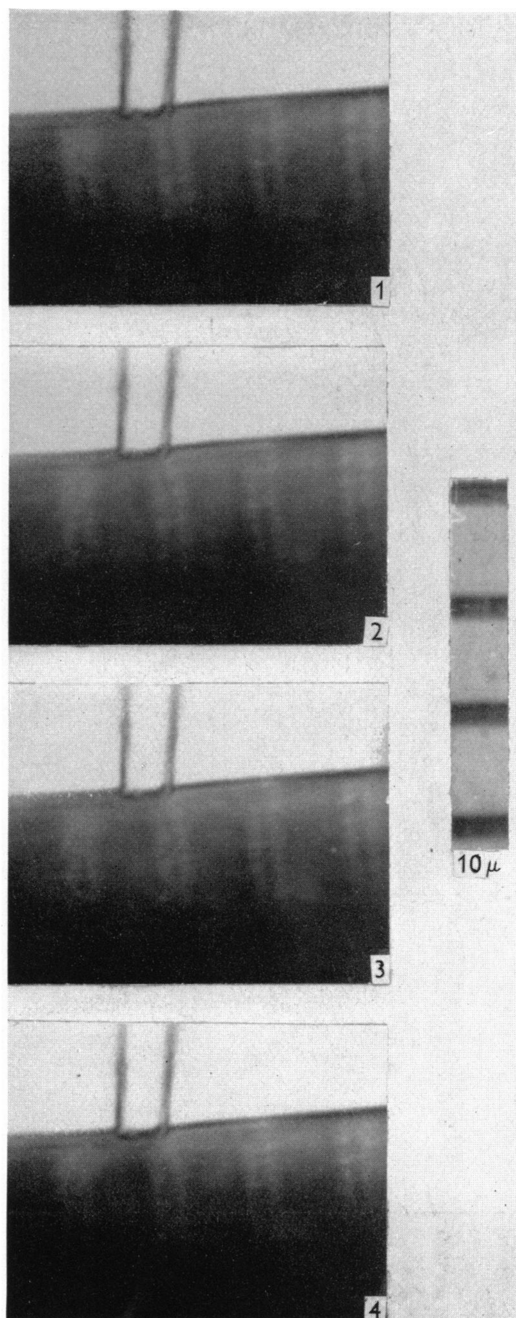
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EXPLANATION OF PLATES

Plate 1

Figs. 1–4. Edge of isolated muscle fibre from the frog, with pipette in contact. Polarized light, compensated so that A bands appear dark. Pipette applied in figs. 1 and 2 to an A band, and in figs. 3 and 4 to an I band. In each case, the left-hand picture is taken just before, and the right-hand picture during, a negative pulse applied to the pipette; a contraction is produced only if the pipette is opposite an I band (fig. 4).

Figs. 5–8. Successive frames from a cine film (16 frames/sec) showing nature of the shortening induced by local depolarization of a frog fibre; polarized light, compensated so that A bands appear dark. The onset of a negative pulse applied to the pipette occurs between figs. 5 and 6.

Plate 2

Figs. 1–3. Successive frames from a cine film showing that in a frog fibre the response is symmetrical about the Z line. Although the pipette is placed so that its right-hand edge is over the centre of the I band which shortens, the Z line remains central in the I band during the weak contraction which is produced. Interference microscope, compensated so that high refractive-index bands (A and Z) appear bright.

Figs. 4–6. Frog fibre before (fig. 4), during and immediately after (fig. 6) application of a negative pulse to the pipette. Interference microscope, compensated so that high refractive-index bands appear bright. By comparing fig. 6 with fig. 4 it is seen that the I band which has just relaxed is darker (lower refractive index) than before the contraction.

Plate 3

Crab muscle fibre. Polarizing microscope, compensated so that A (and Z) appear dark. Selected frames from a cine film, showing *positions* of the pipette at which contractions were produced (+) or were not (−); ± indicates a weak contraction. Each of these exposures was taken just before the beginning of the test pulse, so that the contractions themselves are not shown.

Plate 4

Nature of the contraction induced in a crab fibre by local depolarization. Polarized light, compensated so that A and Z appear dark. Consecutive frames from a cine film (16 frames/sec). A negative pulse begins between figs. 1 and 2.