IS MUSCLE CONTRACTION INITIATED BY INTERNAL CURRENT FLOW?

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It is generally agreed that of the series of events causing contraction in a muscle fibre the first is a reduction of the potential difference across the membrane ('depolarization'). Regarding the succeeding processes which lead eventually to activation of the contractile proteins little is known at present. These processes do not seem to involve inward diffusion of a substance liberated at the surface (Hill, 1948), and some other mechanism must be looked for by which the influence of a potential change across the membrane is conveyed inwards over a considerable distance (more than $50\,\mu$). Quite recently a clue has been provided by experiments of Huxley & Taylor (1958) which strongly suggest that in frogs the disturbance is conducted inwards along a structure adjacent to the Z membrane.

During the passage of an action potential a transient electric current of considerable magnitude flows longitudinally through the interior of the muscle fibre. To account for the rapid onset of contraction it has sometimes been suggested that this current itself, and not the change of the membrane potential, is the causative factor setting off contraction. An elaborate treatment of this hypothesis has been given by Bay, Goodall & Szent-Györgyi (1953), who gave the moving internal electric field the name 'window field'. In spite of its attractive simplicity, this theory has generally received little support. In the first place the theory is only applicable to a limited extent, since in many muscle fibres (slow fibres of frog and many arthropod muscles) a nerve stimulus does not give rise to a propagated action potential, but only to a depolarization which spreads out passively for a short distance from each terminal region. Nevertheless, a nerve impulse causes the fibre to contract because numerous nerve terminals are distributed along the fibre surface, which in this way is depolarized everywhere almost simultaneously. Secondly, in twitch muscle fibres where the theory should apply it has been shown that, in the absence of propagation, muscles exposed to a longitudinal homogeneous electric field contract only in the terminal portion of the cathodic region, where the membrane potential is reduced, while no contraction occurs in the mid region, where the current density inside the

fibre is at a maximum, or at the anodic region (Sten-Knudsen, 1954). Moreover, stimulation of a non-propagating muscle with a transverse a.c. field causes the muscle to contract along its whole length and to produce almost the whole tetanus tension (Katz & Lou, 1947; Sten-Knudsen, 1954). In this case the internal longitudinal current flow is negligible.

Recently Csapo & Suzuki (1958) have claimed that they have restored the experimental foundation for the 'window field' theory. Their evidence is based upon three types of observation on the sartorius muscle, which consists of fast striated fibres arranged in a longitudinal but not strictly parallel manner. (a) When a muscle is suddenly exposed to a strong KCl solution (20 mM-K or more), it goes into a permanently contracted state (contracture), but the tension produced is only a small fraction of the normal tetanus tension. (b) When the KCl concentration is reduced to 16 mM-K, i.e. slightly less than would produce contracture and considerably more than is necessary to block propagation of the action potential, a strong longitudinal a.c. field (6–20 V/cm) causes contraction along the whole length of the muscle. Csapo & Suzuki observed that the shortening was greater in the middle portion than at the ends, and at threshold strength (4 V/cm) only the middle portion shortened. (c) The high-frequency transverse field is only effective within a very narrow range of K concentrations above the minimum blocking concentration. From these observations Csapo & Suzuki concluded that the longitudinal internal current is more directly linked to contraction than depolarization, but the latter is an essential 'priming' step which must precede or coincide with an effective current flow.

This hypothesis presented an attempt to reinterpret part of the evidence brought forward against the original 'window field' theory. If true, it would, in spite of its complicated mechanism, have an important bearing upon the problem of excitation–contraction coupling, as is indicated in a recent review (Gelfan, 1958). For these reasons it was thought desirable to repeat Csapo & Suzuki's experiments. To avoid the complications introduced by using a whole muscle with the fibres not all running in the same direction, the present study was performed on single isolated fibres. It will be shown that there is a distinct discrepancy between Csapo & Suzuki's observations and my own, and the reasons for this have been elucidated. Neither the experimental findings nor the theoretical treatment lend any support to the 'window field' theory in its old or new form. A preliminary report of some of the results was made at the Second Conference on Muscular Contraction held by the New York Academy of Sciences (Buchthal & Sten-Knudsen, 1959).

METHODS

Preparation. Single fibres from the semitendinosus muscle of German brown frogs (*Rana temporaria*) were isolated at room temperature in a Ringer's solution of the following composition (mM): NaCl 116, KCl 2, CaCl$_2$ 1·8. A small bundle of 20–50 fibres, with free tendinous parts 2–3 mm long at either end, was transferred to the experimental Perspex trough in which the final isolation took place (Text-fig. 1). The two tendons were clamped by the two pairs of Perspex forceps (1) and (2) and rotated by them (3) and (4) so as to expose the most convenient places for the separation of the fibres. It is important for the survival of the fibres to cut rather than to tear the connective tissue which holds them together. When the fibres are held firmly together it is an advantage to 'cut' the connective tissue by means of a broken micro-electrode fixed to a preparation needle.
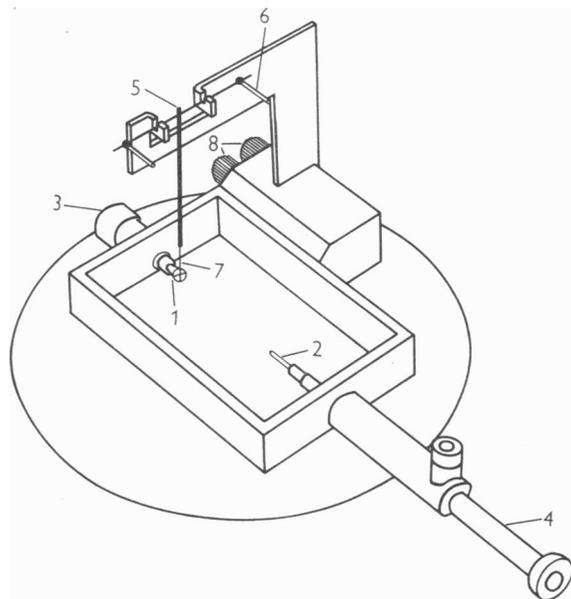
Text-fig. 1. Combined dissection and experimental Perspex trough for longitudinal and transverse a.c. stimulation of single muscle fibres. The tendon of a small fibre bundle is clamped to the pair of forceps (1) and (2) and rotated by them (3) and (4) to facilitate isolation of a fibre. For isotonic recording the lever axis (5) is placed in the groove of the gallows and one tendon end is pierced by the lever needle (7) and cut free from (1), the load on the fibre depending on the length of the counterweights (6). The final adjustments are made by the set screws (8) and the bar (4) of the pair of forceps (2). For isometric recording the lever is replaced by an electromechanical transducer. For longitudinal or transverse stimulation platinum plate electrodes are placed at either end or side walls.

Marking and photography. Small graphite particles, 20–30 in number, were placed upon the upper surface of the fibre so that length changes could be watched and photographed. At the terminal portions of the fibre marks were placed at intervals of 0·5–1 mm. Once these particles are in position they stick firmly to the surface and move with it. The whole length of the fibre was photographed with transmitted light on 9 × 12 cm plates (fine-grain, Isopan F) with an ordinary photographic objective (Sonnar 1:1·5, focal length 5 cm, exposure time 1/25–1/50 sec). The primary magnification was 4·5 times.

Mounting. The one tendon end of the fibre was fixed to the pair of forceps (2) while the other was fixed to the isotonic lever (5) of aluminium tube, 44 mm long and 0·6 mm in diameter. The torque produced by the counterweight (6) fixed on the lever axis was adjusted so that the fibre was loaded with about 12 dynes when fixed to the lever needle (7). The position of the lever was adjusted by screws (8) and the tendon was then pierced by the lever needle and cut free from the left pair of forceps (1). Finally, the fibre was adjusted perpendicularly to the end walls of the trough and the right pair of forceps (4) was pulled out to make a free distance of 3–4 mm between the needle and the left pair of forceps. For isometric recording the lever was replaced by an electromechanical transducer (R.C.A. 5734), one tendon end being tied to the elongated pin of the transducer.

Stimulation. The electrodes consisted of thin platinum plates affixed to Perspex plates 2 mm thick. For longitudinal stimulation plates covering the end walls of the trough were placed perpendicularly to the fibre axis, each plate being provided with a small slit for the forceps. Since the distance from each plate to the terminal portions of the fibre was more than five times the width of the slit, any non-homogeneity of the field around the fibre caused by the slit could be neglected. For transverse stimulation two platinum plates 6 cm long placed parallel to the fibre were used. The electrodes were connected to the output of an a.c. power amplifier with adjustable output impedance, providing an output of 15 W with a total distortion less than 0·1 %. In the bath this corresponded to a longitudinal a.c. field of 7·4 V/cm, r.m.s. The power amplifier was driven by a sine wave generator and the a.c. voltage across the electrodes was measured by recording the two-way rectified and filtered output from the power amplifier.

The fibre was tested for its ability to give propagated twitches and, if found satisfactory, left for more than an hour. Only fibres giving proper twitches after this time were used for the experiment. The muscle impulses were blocked by adding a sufficient amount of a 120 mM-KCl stock solution to raise the concentration in the Ringer's solution to 14–16 mM (i.e. 7–8 times the normal value). After the experiment the K concentration of the excess K Ringer's solution was determined by means of a flame photometer.

General procedure. When blocked, the fibre was photographed at rest and during longitudinal a.c. stimulation, about 2 sec after the beginning of the stimulation. The fibre was observed through a dissecting microscope throughout this procedure to be certain that the contracted state of the fibre was constant during the stimulation period. At intervals of 5 min, the field strength was altered and the fibre again photographed at rest and during a.c. stimulation. The temperature was raised by less than 0·5° C with maximum power delivered to the bath for 2 sec.

RESULTS

The localization of contraction in the longitudinal a.c. field

Contraction with reversible shortening and relaxation. An example is shown in Pl. 1. The fibre was 18 mm long; it was soaked in 14 mM-K Ringer's solution and the time required to produce the block was about 2 min. The whole length of the fibre marked with graphite particles is shown on the left-hand side of Pl. 1, both at rest (*r*) and during stimulation (*c*) with a longitudinal a.c. field (100 c/s) at about 5 V/cm, r.m.s. strength, the two frames being aligned with respect to a mark in the mid region of the fibre. The effect of the field is to cause an average shortening of about 8 % but with contraction entirely localized to the end regions. The main part of Pl. 1 shows enlargements of the end portions of the fibre with all frames aligned with respect to one mark placed at a distance of about

5 mm from the ends. The upper row of each set shows the resting fibre, and those below show the effect of the longitudinal field (100 c/s). With a field strength of 0·73 V/cm, r.m.s. (threshold) a shortening was just barely visible at the most terminal portions of the fibre, as estimated by making use of 'natural' marks on the fibre, and extended a distance of less than 0·2 mm from the tendon. With increasing field strengths shortening increased strongly and at 5·55 V/cm the portions nearest to the tendons had
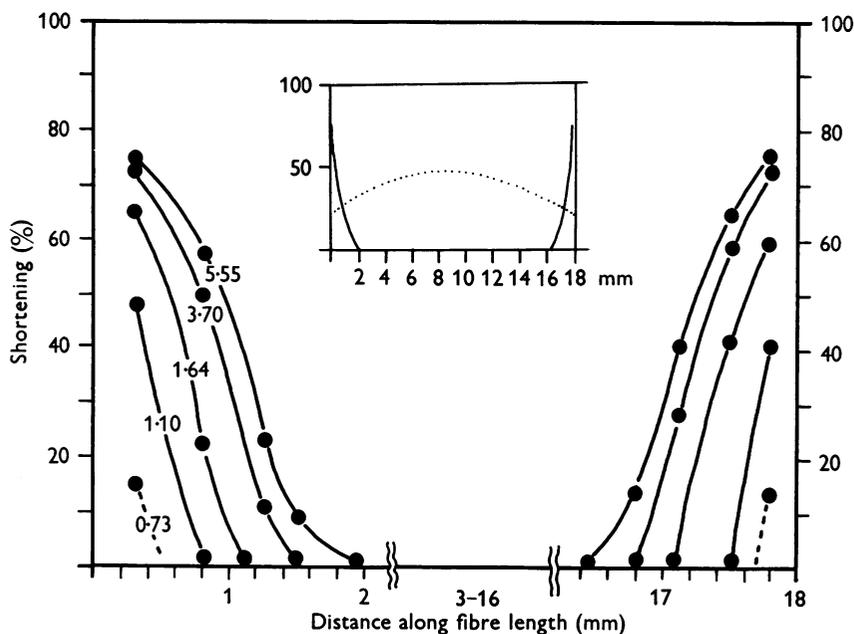
Text-fig. 2. To show the extent and degree of shortening along the fibre during longitudinal a.c. stimulation (100 c/s), the field strength (V/cm, r.m.s.) is indicated by the numbers on the curves. Ordinate, relative shortening of each length element; abscissa, distance along the length of the fibre, with the mid region strongly compressed. In the inset diagram is shown the distribution of shortening along the whole fibre (full line); dotted line, an example of Csapo & Suzuki's (1958, Fig. 3, 8 V/cm, 60 c/s) results.

shortened by almost 80 %. However, the total average shortening of the fibre was only 8 % (0·7–0·8 mm of each end region in a fibre of 18 mm length). The effect of the field on the extent of contraction and the degree of shortening along the fibre is shown in Text-fig. 2. The relative shortening of each length element, $(L_r - L_c)/L_r$, where L_r and L_c are the length of each element in the resting and stimulated state, is plotted against its position in the resting fibre; the numbers on the curves indicate the value of each field strength. With increasing field strength both the degree and the extent of contraction increased. Thus, at 3·7 V/cm maximum shortening was almost 80 %. However, this extreme degree of shortening was

confined to about the 0·2 mm next to the tendon, and the strength of the contraction decreased steeply with the distance from the end of the fibre; the whole extent of the contracted region comprising only 1·5 mm of each fibre end. An increase in the field strength to 5·55 V/cm resulted in an over-all increase in the extent of contraction by 0·2 mm, while the maximum shortening only increased very little. Thus, even at such high field strengths contraction only extended over less than 2 mm at the end regions, while the remaining 14 mm length of the fibre (80 %) showed no sign whatsoever of contraction. In the inset diagram of Text-fig. 2 is shown the extreme concave shape of the graph in contrast to the convex curves presented by Csapo & Suzuki (1958), one of which for the sake of comparison is indicated by the dotted line in the diagram.

With field strengths up to 5 V/cm (100 c/s) the fibre returned to its original length after each stimulation. Therefore, to present the data in the least complicated manner the photographs in the resting state taken before each stimulation are not shown in Pl. 1.

Irreversible effects of too high field strengths. With field strengths above 5 V/cm (100 c/s) the end portions of the fibre never completely returned to their initial relaxed state, indicating that the longitudinal field at these intensities also produced some permanent after-effects in the fibre. In addition to the extreme shortening of the end regions of the fibre, field strengths well above 5 V/cm also caused the fibre to shorten in its whole length. Both effects are illustrated in Pl. 2. The row *r* of the upper set of photographs shows the resting fibre. It was photographed 5 min after a 2 sec stimulation at 5·55 V/cm (100 c/s). A shortening of about 10 % was still present in the regions occupying the 0·2–0·3 mm next to the tendons (cf. Pl. 1). The second row shows the effect of a longitudinal field of 5·8 V/cm strength. In the first phase of stimulation contraction was as usual confined only to the ends of the fibre. After about 1 sec stimulation a new region of contraction, appearing as a local bulge (indicated by the arrow), was created next to the extremely contracted terminal portions. This locally shortened region moved along the fibre and vanished after travelling a few millimetres, to be followed by a new 'wave' originating from the same place. During this time the lever end showed small irregular movements and after about 3 sec of stimulation the fibre had shortened further by a few millimetres, the ends, however, remaining extremely shortened. When the field was increased well above this 'threshold', e.g. to 7·3 V/cm, the pattern of contraction changed. In addition to the extreme shortening at the ends the extent of contraction suddenly spread rapidly from both end regions towards the middle of the fibre, which ended up with an average total shortening of about 40 %. However, this type of contraction only slightly resembled a normal isotonic tetanus. It differed in so far as

'waves' of contraction travelled along the whole length of the fibre so that local contractions and relaxations occurred alternately down the length of the fibre, with the result that the lever end moved irregularly with an amplitude of several millimetres. Moreover, the strongest contraction was confined to the end regions, the maximum shortening here being almost 80 % in contrast to the average shortening of about 40 % of the whole fibre. The next two photographs (7·3 V/cm on Pl. 2) were taken after the over-all shortening had occurred and show the difference in thickness and relative shortening along the length of the fibre, especially at the terminal portions of both ends. To illustrate the after-effects of strong stimulation, the rows *b* and *b'* in the lower set in Pl. 2 show the fibre photographed 4 sec after the termination of stimulation with an a.c. field of 7·3 V/cm applied for 3 sec. For the sake of comparison the initial fibre length is shown in rows *a* and *a'*. A partial contracture of more than 50 % shortening remained at the end regions for a distance of about 1 mm measured from the tendons, and only a very slight change in this state was observed 5 min later. The field strength ('threshold') which was just strong enough to set off these wave-like contractions gradually increased with repeated stimulations. After about 10 stimulations the fibre gave no response, even at the highest field strength available. Thus, there seems to be no doubt that longitudinal a.c. stimulation at high field strengths produced irreversible changes in the fibre, presumably by damaging the fibre membrane at the end regions where the membrane is subjected to an excessive potential difference. It is then observed that a slight misalignment of the fibre with respect to the direction of the field will temporarily restore its responsiveness, presumably because the current can then enter a region where the membrane is still undamaged.

The response of the muscle fibre just described occurred not only with 14 mM-K Ringer's solution and at 100 c/s. It was also observed, for instance, in fibres blocked with 11 mM-K Ringer's solution, or with procaine or stimulated with 500 c/s. In these cases the threshold field strength for the appearance of the wave-like contractions was, however, somewhat higher.

The effectiveness of the transverse a.c. field

Katz & Lou (1947) have shown that a transversely applied a.c. field caused contraction in a muscle which had been rendered non-propagating by excess potassium. With an a.c. field of 2–4 V/cm (1000 c/s), muscles produced about 80 % of the tetanus tension, when blocked with 12 mM-K Ringer's solution (Sten-Knudsen, 1954; Csapo & Wilkie, 1956) and it was concluded that in these experiments the muscle is activated electrically without the intervention of a longitudinal current (Sten-Knudsen, 1954). Csapo & Suzuki (1958) have now suggested that these muscles were in

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some kind of 'transient' state between propagating and non-propagating conditions. If so, the experimental fact that a high-frequency transverse field produces contraction should be evaluated with caution. This suggestion is said to be substantiated by their claim that the high-frequency transverse field only causes contraction in a narrow range of K concentrations equal to or just above the minimum blocking concentration. And it is stated that a further depolarization of the membrane by only a few millivolts, e.g. by addition of 1–2 mM excess K to the Ringer's solution, abolished altogether the stimulating effect of the high-frequency transverse field.
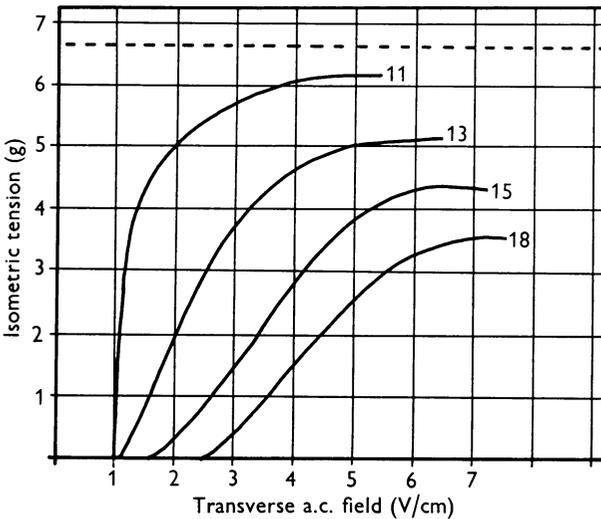
Text-fig. 3. Superimposed tracings of tension–field strength curves with m.ext. long.dig. IV soaked in excess K Ringer's solution, the numbers on the curves indicating the K concentrations (mM). Ordinate, isometric tension (g); abscissa, transverse a.c. field strength (V/cm, 600 c/s). Dotted line, maximum isometric tension. For further explanation see text.

It is difficult to imagine how a transverse a.c. field could restore impulse conduction in a muscle blocked with 12 mM-K Ringer's solution and in fact no action potentials are observed experimentally (Sten-Knudsen, unpublished). It was therefore considered desirable to see if Csapo & Suzuki's observations could be confirmed. In experiments of this type it is important that a reduced mechanical response, caused by excessive stimulation, should not be confused with effects of excess K concentrations on the force of contractions. Therefore, to reduce the total duration of stimulation at each K concentration, the muscle was stimulated for 2 sec, with the a.c. field continuously increasing from zero to maximum field strength. Direct recording of force as a function of field strength was made by connecting the a.c. voltage across the electrodes (two-way rectified and filtered)

to the X-plates of the oscilloscope, while tension was recorded on the Y-plates. In Text-fig. 3 are shown superimposed tracings of tension–field strength curves (at 600 c/s) with the m.ext.long.dig. IV soaked in excess K Ringer's solution, the numbers on the curves indicating the K concentrations in millimoles. Before each a.c. stimulation the muscle was soaked in the excess K Ringer's solution for more than 15 min to let it equilibrate, and before changing to a new K concentration the muscle was soaked in normal Ringer's solution for about half an hour and then checked for the tetanus tension, indicated by the dotted line in Text-fig. 3. The effect of the excess K Ringer's solution consists partly in shifting the curves to the right, along the field axis, and partly in reducing the maximum tension. Thus, with 11 mM-K (membrane potential, according to Adrian (1956), − 62 mV), threshold was about 1 V/cm and maximum tension 93 % of the normal tetanus tension, while with 18 mM-K (membrane potential: − 50 mV) threshold was 2·5 V/cm and maximum tension 53 % of the tetanus tension. Although the mechanical response of the non-propagating muscle decreased with increasing K concentrations, the muscle was nevertheless able to develop an appreciable amount of tension, even when soaked in 18 mM-K Ringer's solution. Hence, the present results do not confirm the observations of Csapo & Suzuki (1958), according to which high-frequency transverse stimulation was only effective at K concentrations equal to or less than 12 mM.

DISCUSSION

Recently Csapo (1957) and Csapo & Suzuki (1958) have attempted to revive the now obsolete 'window field' theory of Bay *et al.* (1953) by putting forward the hypothesis that it is the *combined action* of depolarization and internal current flow which causes contraction in a muscle fibre. The modified 'window field' theory is said to be consistent with the view that the fibrils within the fibre are stimulated to contract by the longitudinal current flowing through them, e.g. during the passage of an action potential along the fibre. In this hypothesis depolarization is considered necessary, but not sufficient; in addition, internal current flow (assumed to be ineffective with normal membrane potential) is required to cause the fibrils to contract. This concept has also been advocated at some length in a recent review (Gelfan, 1958). What Csapo & Suzuki (1958) appear to have done is to modify the 'window field' theory of Bay *et al.* (1953) so as to make it consistent with some of the evidence which was brought forward against it in its original form. However, their supporting evidence differs in essential aspects from the results of all the other investigations in the same field (Katz & Lou, 1947; Sten-Knudsen, 1954; Csapo & Wilkie, 1956; Hodgkin & Horowicz, 1956). The purpose of the present study is twofold, (i) to test whether Csapo & Suzuki's theory is compatible with

the available evidence and (ii) to find out whether Csapo & Suzuki's own results require a new theory or can readily be explained on the more commonly held view, namely, that sudden depolarization of the surface membrane of the fibre is responsible for eliciting contraction (see, for example, Katz, 1950).

Csapo & Suzuki assign to the longitudinal field of the action potential the role of moving ions over a distance of 2·5 μ, i.e. over the length of a sarcomere, and claim that the maximum gradient of the action potential (about 3 V/cm) can move Ca^{2+} over a distance of 2μ within a period of 30 msec. Now suppose that the mobilities of Ca^{2+} and K^+ within the cell are of the same order of magnitude, which for K^+ is about 5×10^{-4} cm.sec^{-1}/ V.cm^{-1} (Hodgkin & Keynes, 1953). A field of 3 V/cm will then move the ions over a distance of only $0·45\mu$ in 30 msec ($= 5 \times 10^{-4} \times 3 \times 3 \times 10^{-2}$ cm). But Csapo & Suzuki have ignored the fact that this maximum field of 3 V/cm is not maintained for 30 msec, but only for about 0·3 msec, after which it is immediately reversed in sign. Thus, the maximum displacement of a Ca^{2+} ion during the passage of an action potential is likely to be not 2μ but of the order of $0·0045\mu$. This does not rule out the possibility that some internal ionic displacement may be instrumental in activating the contractile material, though it cannot be anything like the value stated.

The original 'window field' theory proposed by Bay *et al.* (1953) was opposed on the grounds that, in the absence of propagation, internal current flow causes contraction only at points on the fibre where the membrane is being depolarized and not along its whole length (Kuffler, 1946; Sten-Knudsen, 1954; Huxley & Taylor, 1955, 1958; Watanabe & Ayabe, 1956). The modified 'window field' theory of Csapo & Suzuki (1958) is consistent with this set of observations, although it is difficult to reconcile this theory with the observation that a longitudinal d.c. field, when applied to a muscle in moderate contracture, causes relaxation in part of the anodic region, while contraction is enhanced only in the cathodic region (Kuffler, 1946; Sten-Knudsen, 1954). But, to make Csapo & Suzuki's theory applicable in general, it would follow that membrane depolarization never can cause contraction in a muscle fibre without the intervention of a strong internal current flow. Now, it is possible to depolarize a muscle fibre along its whole length and at the same time to prevent any internal current flow parallel to the longitudinal axis of the myofibrils. Nevertheless, in such cases full, or almost full, tetanus tension is obtained. This has been accomplished in two ways:

(1) When single fibres are made non-conducting by Na-free Ringer's solution, a rapid application of high K solution gives maximum tension (Hodgkin & Horowicz, 1956). Csapo & Suzuki (1958) reject this experi-

ment as inconclusive by suggesting that effective internal currents were generated during the longitudinal application of the K-rich solution. It is clearly open to them to repeat the Hodgkin–Horowicz experiment with transverse application of the high-K Ringer's solution.

(2) Nearly the full tetanus tension can be elicited in a non-propagating muscle by stimulating it transversely with an a.c. field (Katz & Lou, 1947; Sten-Knudsen, 1954; Csapo & Wilkie, 1956). In this case there is no longitudinal internal current, and the transverse current can also be made quite small. Csapo & Suzuki (1958) reject this evidence and suggest that fibres blocked with 12 mM-K Ringer's solution, are in a 'transient' state between propagating and non-propagating condition. Further they assert that an addition of 1–2 mM-K, i.e. a further depolarization by only a few millivolts, abolishes altogether the effectiveness of the transverse field. The results of the present investigation show that this statement is incorrect, since a muscle soaked in 18 mM-K Ringer's solution produced an appreciable amount of tension (50 % of tetanus tension) when stimulated with a transverse field (600 c/s). It is not possible to see how Csapo & Suzuki obtained their contrary results, for they did not state the conditions under which their observations were made. Perhaps they failed to recognize that increasing the K concentration shifts the tension–field-strength curves, as is shown in Text-fig. 3. Thus, with a field strength of 3 V/cm, tension was 87 % of the tetanus tension when the muscle was soaked in 11 mM-K Ringer's solution; in 18 mM-K Ringer's solution the response amounted only to 7 %, but a maximum tension of 50 % tetanus tension could still be developed at this concentration if adequate field strengths were used.

Csapo & Suzuki (1958) have been unable to invalidate the observations mentioned in (1) and (2), which are clearly at variance with their theory. That a non-propagating muscle contracts at the cathodic region in a longitudinal electric field has been observed by various investigators during the course of the past hundred years (Wundt, 1859; Kühne, 1860; du Bois-Reymond, 1875; Jendrássik, 1879; Hermann, 1886; Kuffler, 1946; Sten-Knudsen, 1954), Csapo & Suzuki (1958) representing the sole exception. Thus, except for their own results, Csapo & Suzuki's modified theory remains inconsistent with all the evidence which is at present available.

A result which Csapo & Suzuki cite in favour of their theory is the observation that only a small fraction of the tetanus tension was produced by soaking a whole muscle in excess-K Ringer's solution. But this fact has been well known to other workers and it has usually been ascribed to the fact that it takes time for potassium to diffuse into a muscle, during which the contractile response declines (see e.g. Hodgkin & Horowicz, 1956). Csapo & Suzuki's main evidence, obtained on a whole sartorius

muscle can be summarized as follows: (1) Longitudinal a.c. stimulation was only effective if the non-propagating muscle was deeply depolarized by the excess-K Ringer's solution. (2) At threshold field strength (4 V/cm, 60 c/s) only the middle portion shortened. (3) With strong a.c. fields (6–20 V/cm) the middle portions shortened more than the rest. And (4) during propagated activity shortening was strongest in the middle portion (some 30 %). The experimental conditions of the experiments reported in this paper were quite similar to those of Csapo & Suzuki (1958), except that single fibres were used. This makes it possible to align the fibre in the direction of the field and provides a better discrimination of changes in different regions along the fibre. The experiments showed that *contraction was always strongest at the end regions* and, with field strengths in the range of 0·7–5·5 V/cm (100 c/s), localized solely to these parts of the fibre. The results are clearly at variance with the Csapo–Suzuki theory. However, it was also found in the present work that large fields (e.g. higher than 5·5 V/cm, 100 c/s) caused the fibre to shorten along its whole length. This is the only observation which resembles those of Csapo & Suzuki, but the contraction is only slightly similar to a normal tetanus and its origin can be adequately explained in terms of changes at the membrane. It thus remains an open question whether this observation alone necessitates the introduction of a radically different theory of activation.

When a parallel-fibred muscle is placed longitudinally in a homogeneous d.c. field, simple application of the core conductor equations (Hodgkin & Rushton, 1946) shows that almost the total current which flows in the fibre must enter and leave at its terminal region. These results were used to describe qualitatively the way a non-propagating muscle was activated when placed longitudinally in a homogeneous d.c. field (Sten-Knudsen, 1954). Csapo & Suzuki (1958) assumed the same results for the somewhat different situation where the field was alternating. The solution of the core conductor equations for this case (see Appendix 1) shows that the amplitude of the voltage oscillations across the membrane diminishes towards the fibre mid point according to the same general law as in the d.c. case, with the exception that (a) the parameter for the attenuation (the a.c. space constant) now is the square root of twice the ratio between the membrane a.c. resistance of one unit length and the longitudinal internal resistance per unit length, (b) there is a progressive phase shift of the voltage fluctuations along the length of the fibre. In Text-fig. 4 (curve a) is shown the amplitude–distance relationship of the membrane polarization (disregarding phase relations) during the peak of a longitudinal a.c. field (100 c/s), computed from the numerical data of Katz (1948) and Nicholls (1956). During each half cycle, activation will occur only in the region which is sufficiently depolarized, causing a contraction which will

last as long as a normal twitch. This contraction, once started, will not be altered by the following hyperpolarizing phase. In agreement with the findings in the present investigation it is therefore to be expected that the fibre will shorten only at its ends as long as the field strengths are moderate. If the fibre is depolarized close to its contracture threshold with excess-K Ringer's solution, only a small additional depolarization, say 5–10 mV, is needed to cause activation. It was to a muscle in this condition that Csapo & Suzuki (1958) applied strong fields (6–20 V/cm), the implications of which were apparently not fully realized by them. It appears from curve *a* in Text-fig. 4 that a field of 10 V/cm is likely to produce activation in
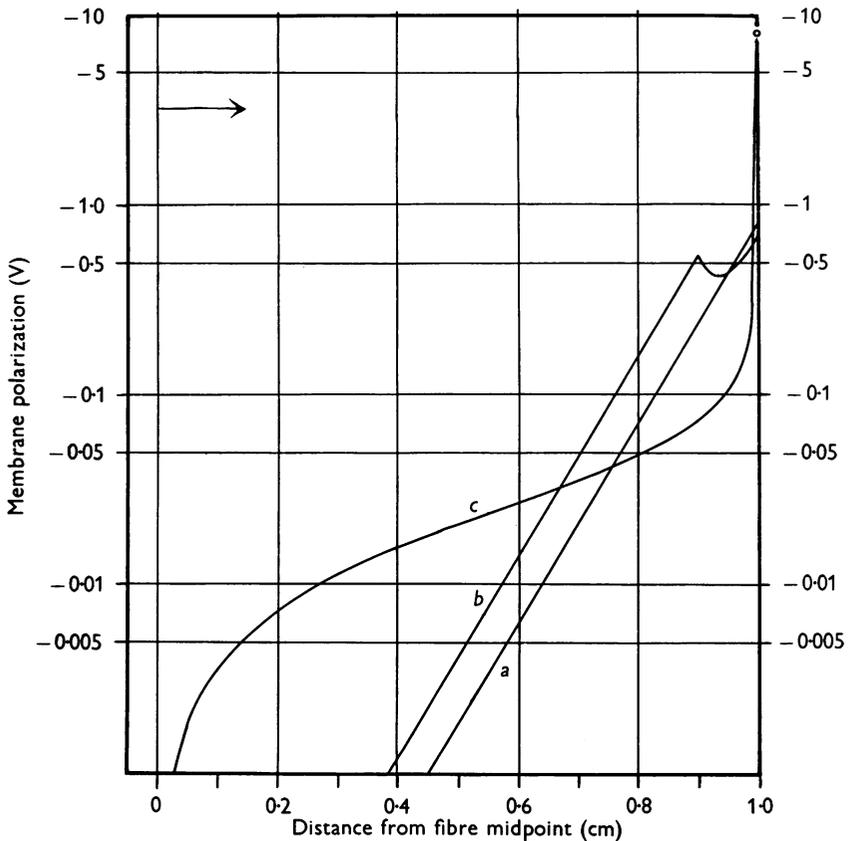
Text-fig. 4. Calculated amplitude–distance relationship of membrane polarization on a fibre 2 cm long during the peak of a longitudinal a.c. field (10 V/cm, r.m.s., 100 c/s). Ordinate, membrane polarization, log scale; abscissa, distance from fibre mid point. *a*, application of the core-conductor equations assuming constant parameters along the fibre length; *b*, the effect of a reduced space constant in the terminal 1 mm portion of the fibre; *c*, the fibre tapering in the shape of a long prolate ellipsoid. For further explanation, see text and appendix.

about one-fifth of the fibre at each end, but none in the middle. As was mentioned earlier, any observed shortening in the middle can be adequately accounted for by such complicating geometrical factors as tapering of the fibre towards its ends, sudden irregularities in fibre diameter and misalignment of the fibre in the direction of the field. It is possible that Csapo & Suzuki (1958) were led to their conclusions because they assumed (without explicitly stating so) that these second-order effects could be neglected; but they can, in fact, be shown to be far from negligible. In the first place, tapering of the fibre towards its ends results both in an increased membrane polarization at the end regions and in a less steep decline of polarization towards the mid point of the fibre. This is illustrated in curve b, Text-fig. 4, which shows the effect of a reduced space constant in the terminal 1 mm portion of the fibre corresponding, e.g. to a reduction in fibre diameter by half (see Appendix 2), and in curve c, Text-fig. 4, which shows the membrane polarization in a fibre gradually tapering in the shape of a long prolate ellipsoid (see Appendix 4). In the latter case the area of membrane polarization has increased so that a field of 10 V/cm is likely to activate about 80 % of the whole muscle fibre. In the second place it is scarcely possible to place all the fibres of a whole sartorius muscle parallel to the direction of the field. With an angle of misalignment of 5°, which is easily possible with this muscle, and a field of 10 V/cm, the transverse component of the field is $E_t = 14.14 \times \sin 5° = 1.233$ V/cm. This component of the field will cause an a.c. membrane polarization along the whole length of the fibre, the peak value of which is 12 mV (see Appendix 3). This additional depolarization will be sufficient to cause activation also in the mid region of the fibre, where a properly aligned fibre will not show any membrane polarization.

The effect which a strong longitudinal field (10 V/cm, or more) produces on the membrane, especially at the fibre ends, was also neglected by Csapo & Suzuki. A simple consideration shows that with a field of 10 V/cm, r.m.s. (100 c/s) and a muscle 3 cm long a potential difference of about 10 V will be produced across the extreme terminal part of the fibre membrane, provided that its dielectric strength is not exceeded. In view of this fact it was hardly surprising to find in the present investigation that the fibre gradually deteriorated at its ends. The irregular shortening which occurred at high field strengths displayed a similarity with the 'galvanic waves' of the type described by Hermann (1886). They are probably due to the local increase in thickness of the extremely shortened fibre ends, causing more current to enter this region as a result of its decreased longitudinal internal resistance. Under certain conditions, depending upon the degree of change in the electric constants in the region of bulge, part of this current will be forced to leave the fibre again in the

region next to the bulge and thus to create a new cathodic region which causes the local contraction to move along the fibre (see Appendix 2).

Thus it is most likely that Csapo & Suzuki's (1958) findings are due to the several complicating factors in their experimental situation. The sartorius muscle was depolarized close to its contracture threshold by the 16 mM-K Ringer's solution. The combination of great field strengths, misalignment of the fibres in the direction of the field, which inevitably occurs with a sartorius muscle, and irregularities of the fibre diameters which are unavoidable at their ends, provided sufficient depolarization along the whole length of the fibre to produce a contraction at all points of the fibre. It may be that part of the response which Csapo & Suzuki (1958) observed was of the 'galvanic wave' type, since the irregularity of the movements of the individual fibres would pass unnoticed. In their sartorius muscle of 3 cm length they observed a threshold of 4 V/cm for an over-all contraction. To produce the same potential drop along a fibre 2 cm long (as in the present case) the longitudinal field must be raised to about $4 \times 3/2 = 6$ V/cm. This is close to the threshold for the 'galvanic wave' contraction in the present experiments, while the threshold for the contraction at the fibre end was only 0·73 V/cm (equivalent to 0·5 V/cm in the 3 cm long sartorius muscle). Furthermore, excessive potential differences which must have existed across the membrane at the endregions of the fibre in Csapo & Suzuki's experiments (1958) may partly explain their finding that contraction was strongest in the middle of the muscle. Even if the ends were not responding optimally it would still be possible to achieve adequate activation of the rest of the fibre. Therefore, with proper regard to the various complexities in Csapo & Suzuki's experimental conditions, their results cannot be considered to contradict, but rather to substantiate, the view that it is membrane depolarization, regardless of internal longitudinal current flow, which sets off contraction. And their results cannot be said to necessitate a new theory of activation.

Szent-Györgyi (1956) considers the stimulating effect of transverse a.c. stimulation to be due to some internal disturbance of electron distribution of the contractile proteins caused by the transverse current, rather than to the effect of successive depolarizing half cycles. The Watanabe–Ayabe (1956) experiment (current flow between two micro-electrodes in the interior of the fibre gives no contraction) casts doubt on this concept, because in this experiment there must have been a considerable transverse component of the current around each electrode. Pointing in the same direction is the observation that the threshold of transverse stimulation increases with the frequency; a rough calculation shows that the internal transverse field in the range of frequencies used must have increased by almost 170 times (C. Guld & O. Sten-Knudsen, unpublished).

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There is strong evidence (Huxley & Taylor, 1955, 1958) that the disturbance of the surface membrane is conducted inwards along some structures in the middle of the I bands. Huxley (1957) has tentatively suggested that (*a*) the mechanism of conduction is an electrical one taking place in a cable-like structure and (*b*) activation is in some way conveyed from the channels to the action filaments as a result of a change in the potential difference across the walls. According to this scheme the internal current flow which elicits contraction is that which traverses the walls of the channels, possibly by moving some particular ion from one compartment to the other. If this scheme is correct it also explains why Szent-Györgyi's 'window field' has been found ineffective in causing contraction. It is natural to assume that the threshold for the change in potential difference across the channel walls is equal to that across the whole fibre membrane, say 10 mV in a K-blocked fibre. To produce this amount of depolarization on a channel wall of 100 Å diameter requires a 'window-field' strength which is, approximately at least, $E = 0.5 \times 10^{-2}/10^{-6} = 5000$ V/cm (see Appendix 3, eqn. 3.11), which is far above anything which can be obtained experimentally without damaging the fibre.

SUMMARY

1. Csapo & Suzuki have put forward the theory that it is the combined action of depolarization *and* internal current flow which elicits contraction. This is a modification of the now discarded 'window field' theory of Bay *et al.* (1953). Membrane depolarization is considered a necessary 'priming' step which by itself is ineffective in setting off contraction. This theory is said to be consistent with the experimental facts. But to support the theory Csapo & Suzuki have presented observations which differ in essential aspects from those of other investigators.

2. The experiments of Csapo & Suzuki have been repeated on isolated single fibres, by means of a photographic technique which provided good resolution of events taking place at different points along the length of the fibre. Csapo & Suzuki's main observation could not be reproduced, namely that a longitudinal a.c. field, when applied to a muscle placed in a high-K Ringer's solution, causes the greatest amount of shortening in the middle portion. Neither could their claim be confirmed that transverse a.c. stimulation is ineffective if the external K concentration is higher than 12 mM.

3. Csapo & Suzuki's theory is shown to be inconsistent with the evidence at present available. It is further shown that their results do not require a new theory of activation, but can be explained on the current view, that it is membrane depolarization which initiates contraction.

4. In a theoretical Appendix is derived the form of the core-conductor

equation appropriate for alternating fields. It is also shown how the distribution of membrane polarization varies according to the shape of the fibre.

APPENDIX
Calculation of the membrane polarization of a muscle fibre placed in a homogeneous a.c. field

Let

E $= \mathscr{E}\cos\omega t =$ undisturbed parallel a.c. field (V/cm); $\omega = 2\pi\nu$, where ν is the frequency (c/s);

x, y, z = Cartesian reference system placed with the z-axis coinciding with the direction of the fibre axis and the origin being placed at the mid point of the fibre;

r, ϕ, z = cylindrical co-ordinate reference system defined by: $x = r\cos\phi$, $y = r\sin\phi$, $z = z$, where ϕ is the angle between the x-axis and r.

η, θ, ϕ = prolate spheroidal co-ordinate reference system (cf. Hobson, 1931) defined by: $z = f\cosh\eta\cos\theta$, $x = f\sinh\eta\sin\theta\cos\phi$, $y = f\sinh\eta\sin\theta\sin\phi$, where f is the focal distance. η is the parameter for a family of confocal prolate ellipsoids: $(z/f\cosh\eta)^2 + (x^2+y^2)/(f\sinh\eta)^2 = 1$, $1 \leqslant \eta < \infty$, and θ is the parameter for the orthogonal system of confocal hyperboloids:

$$(z/f\cos\theta)^2 - (x^2+y^2)/(f\sin\theta)^2 = 1, \quad 0 \leqslant \theta \leqslant \pi.$$

A point in space is specified as the intersection between these surfaces with a plane passing through the z-axis and making an angle ϕ with the x-axis;

$2L$ = length of the fibre (cm);

a = radius of the fibre (cm);

ψ_i = potential of myoplasm caused by E (V);

ψ_e = potential in Ringer's fluid (V);

V_m = potential difference across the membrane in excess of the normal resting membrane potential $= \psi_e(a) - \psi_i(a)$, (V);

I = longitudinal current in myoplasm, positive in the direction of increasing z (A);

I_m = current entering the myoplasm per unit length (A/cm);

R_i = longitudinal resistance of myoplasm per unit length (Ω/cm);

R_m = membrane resistance of one unit length (Ω.cm);

C_m = membrane capacitance per unit length (F/cm);

ρ_i = specific resistivity of myoplasm (Ω.cm);

ρ_m = specific surface resistivity of membrane (Ω.cm²);

c_m = capacitance per unit area of membrane (F/cm²);

ρ_e = specific resistivity of Ringer's fluid = 100 Ω.cm at 20° C;

t = time (sec);

i = $\sqrt{-1}$.

The following average values are used (Katz, 1948; Nicholls, 1956):

$$\rho_m = 4\,\mathrm{k}\Omega.\mathrm{cm}^2, \quad \rho_i = 300\,\Omega.\mathrm{cm}, \quad c_m = 4\,\mu\mathrm{F/cm}^2.$$

1. *Application of the core conductor equations to calculate membrane polarization caused by a longitudinal a.c. field, assuming constant parameters*

We have from the fundamental core conductor equations (Hodgkin & Rushton, 1946)

$$I_m = \frac{V_m}{R_m} + C_m\frac{\partial V_m}{\partial t} = \frac{\partial I}{\partial z} = -\frac{1}{R_i}\frac{\partial^2\psi_i}{\partial z^2},$$

Since the diameter of the fibre is small as compared to its length, the disturbing effect of the fibre upon the parallel field is small. As a first order of approximation we have therefore: $\psi_e(z, a) = -Ez$ and $\partial^2 \psi_i/\partial z^2 = \partial^2(-Ez - V_m)/\partial z^2 = -\partial^2 V_m/\partial z^2$. The equation to be solved for V_m is therefore

$$\frac{\partial^2 V_m}{\partial z^2} = R_i C_m \frac{\partial V_m}{\partial t} + \frac{R_i}{R_m} V_m, \tag{1.1}$$

together with the conditions: (i) $V_m(0) = 0$, (ii) $I(L) = I(-L) = 0$, or $\partial V_m/\partial z = -E$, for $z = \pm L$, and (iii) $E = \mathscr{E} \cos \omega t$. Condition (ii) is equivalent to the assumption that no current enters at the end surfaces of the fibre. To find the stationary solution we seek, as originally suggested by Stokes (1856), a solution of eqn. (1.1) of type

$$V_m = Z(z) \exp(i\omega t), \tag{1.2}$$

where $Z(z)$ is a function of z alone. Substituting eqn. (1.2) into eqn. (1.1) it follows that Z must satisfy

$$\frac{d^2 Z}{dz^2} - \frac{Z}{\lambda_c^2} = 0, \tag{1.3}$$

where λ_c is the complex space constant

$$\lambda_c^{-1} = \sqrt{\{\omega R_i C_m (i + 1/\omega C_m R_m)\}}. \tag{1.4}$$

At 100 c/s, $\omega C_m R_m = \omega c_m \rho_m = 10$, hence

$$\lambda_c^{-1} \approx \sqrt{\{i\omega C_m R_i\}} = (1+i)\sqrt{\{\omega R_i C_m/2\}} = (1+i)m.$$

The solution of eqn. (1.3) which satisfies (i), (ii) and (iii) is

$$V_m = -\mathscr{E} \frac{\sinh[z(1+i)m]}{m(1+i) \cosh[L(1+i)m]} \exp(i\omega t). \tag{1.5}$$

The real part of eqn. (1.5) may be reduced to the form

$$V_m = -\frac{1}{\sqrt{2}\,m} \mathscr{E} \sqrt{\left/ \left\{ \frac{\sinh^2(mz) + \sin^2(mz)}{\cosh^2(mL) - \sin^2(mL)} \right\} \right.} \cos(\omega t + \gamma(z) - \gamma_1 - \pi/4), \tag{1.6}$$

where $\tan \gamma(z) = \tan(mz) \coth(mz)$ and $\tan \gamma_1 = \tan(mL) \tanh(mL)$. At 100 c/s, $m = \sqrt{(\omega C_m R_i/2)} = 12{\cdot}2$. For $L = 1$, $\cosh(mL) \sim 10^5 \gg \sin(mL)$ and $\sinh^2(mz) \gg \sin^2(m)$ for $z > 2$ mm. Eqn. (1.6) is therefore equivalent to

$$V_m = -\frac{1}{\sqrt{2}} \mathscr{E} \frac{\sinh(mz)}{m \cosh(mL)} \cos(\omega t + \gamma(z) - \gamma_1 - \pi/4), \tag{1.7}$$

which shows that the amplitude of the oscillations of V_m diminishes like $\sinh(mz)$, which in principle is equivalent to the steady d.c. case with the exception that the space constant which governs the attenuation is $1/m = \sqrt{(2/\omega C_m R_i)}$ instead of $\lambda = \sqrt{(R_m/R_i)}$. On the other hand there is a progressive shift in the phase of V_m along the length of the fibre. However, if only maximal values of the voltage fluctuations across the membrane are considered they can satisfactorily be described by the equation of the well-known form

$$\frac{d^2 V_m}{dz^2} - m^2 V_m = 0, \tag{1.8}$$

where m^{-2} is twice the ratio between the membrane a.c. resistance and the longitudinal internal resistance per unit length.

2. The effect of irregularities in the fibre diameter

Since $R_i = \rho_i/\pi a^2$, $R_m = \rho_m/2\pi a$, $C_m = c_m 2\pi a$, any local change in a will change the pattern of current entry into the fibre. Let it be assumed, for the sake of simplicity, that the irregularity is symmetric with respect to the mid point of the fibre such that in the region $|l| \leqslant |z| \leqslant |L|$ we have $V_m = F(z)$, $R_i = R_0$, $m = m_0$; while in the region $-l \leqslant z \leqslant l$ we have $V_m = G(z)$, $R_i = R_1$, $m = m_1$.

The proper solution of eqn. (1.8) for F is

$$F(z) = A \exp(m_0 z) + B \exp(-m_0 z), \tag{2.1}$$

while that for G is
$$G(z) = C \sinh(m_1 z), \tag{2.2}$$

where A, B and C are constants to be determined from the boundary conditions: (i) $dF/dz = -E$, for $z = L$; (ii) $F(l) = G(l)$, and (iii) $(E + dF/dz)/R_0 = (E + dG/dz)/R_1$ for $z = l$. (ii) and (iii) are equivalent to the conditions that both V_m and I must be continuous at $z = l$. Insertion of eqns. (2.1) and (2.2) into (i), (ii) and (iii) gives three simultaneous equations for the determination of A, B and C from the values of which the final solution may be given as

$$F(z) = -\frac{1}{\sqrt{2}} \mathscr{E} \frac{\alpha \cosh(m_1 l) \sinh m_0(z-l) + [\cosh m_0(z-l) - \beta \cosh m_0(L-z)] \sinh(m_1 l)}{m_0 [\sinh(m_1 l) \sinh m_0(L-l) + \cosh(m_1 l) \cosh m_0(L-l)]},$$

$$G(z) = -\frac{1}{\sqrt{2}} \mathscr{E} \frac{[1 - \beta \cosh m_0(L-l)] \sinh(m_1 z)}{m_0 [\sinh(m_1 l) \sinh m_0(L-l) + \cosh(m_1 l) \cosh m_0(L-l)]},$$

where $\alpha = R_0 m_1 / R_1 m_0$; $\beta = 1 - R_0/R_1$. If $\beta \cosh m_0(L-l) > 1$, part of the current which has entered the fibre will leave again already in the transition region near $z = l$.

3. Transverse field

The fibre is regarded as an infinite circular cylinder of radius a and internal conductivity $1/\rho_i = g_i$ (mho/cm) covered with an infinitely thin layer of surface resistivity ρ_m. The x-axis is placed in the direction of the undisturbed field, E, the potential of which is $\psi_0 = -Ex$. The current density has nowhere a z-component and the potential function ψ of the current field must in all space satisfy the two-dimensional Laplace equation

$$\nabla^2 \psi = \frac{\partial}{\partial r} \left(r \frac{\partial \psi}{\partial r} \right) + \frac{1}{r} \frac{\partial^2 \psi}{\partial \phi^2} = 0, \tag{3.1}$$

together with the conditions: (i) the disturbing effect of the cylinder on the original parallel field vanishes at great distances from the origin, so: $\psi \to -Ex = -Er \cos\phi$, for $r \to \infty$. (ii) $\psi(r, \phi) = \psi(r, -\phi)$, since ψ must be symmetrical with respect to the $x-z$ plane. (iii) ψ remains finite at the origin and throughout the whole space the $y-z$ plane is an equipotential which arbitrarily may be assigned the value zero, so $\psi = 0$ for $\phi = \pm\frac{1}{2}\pi$ and all r. From the general solution of eqn. (3.1)

$$\psi = (A_0 \phi + B_0)(C_0 \ln r + D_0) + \Sigma(A_n r^n + B_n r^{-n})(C_n \cos n\phi + D_n \sin n\phi), \tag{3.2}$$

(cf. Smythe, 1950). We seek solutions for ψ_e and ψ_i which satisfy conditions (i), (ii) and (iii). These are

$$\psi_e = -Er \cos\phi + \Sigma B_n r^{-n} \cos n\phi, \tag{3.3}$$

$$\psi_i = \Sigma A_n r^n \cos n\phi. \tag{3.4}$$

Since the membrane conductivity $\delta a / \rho_m$ (where δa is the membrane thickness) is much smaller than g_e and g_i, practically no current will flow tangentially within the membrane. Therefore, the normal component of the current density flowing across the interface between the outer side of the membrane and the exterior region, $-g_e(\partial \psi_e/\partial n)_a$, must be equal to that, $-g_i(\partial \psi_i/\partial n)_a$, flowing across the inner membrane into the fibre. The boundary conditions to be satisfied for ψ_e and ψ_i are thus

$$g_e \left(\frac{\partial \psi_e}{\partial r} \right)_a = g_i \left(\frac{\partial \psi_i}{\partial r} \right)_a, \tag{3.5}$$

since $\partial/\partial n = \partial/\partial r$, and
$$\psi_i(a, \phi) - \psi_e(a, \phi) = -g_i \rho_m \left(\frac{\partial \psi_i}{\partial r} \right)_a. \tag{3.6}$$

To determine the constants A_n and B_n, eqns. (3.3) and (3.4) are inserted into (3.5) and (3.6).

It can be shown that all the coefficients vanish identically to zero except those for $n = 1$, the determination of which is given by the two simultaneous equations

$$g_i A_1 + a^{-2} g_e B_1 = -g_e E, \tag{3.7}$$

$$(a + g_i) A_1 - a^{-1} B_1 = -aE. \tag{3.8}$$

Solving these equations for A_1 and B_1 and inserting into eqn. (3.3) and (3.4) gives

$$\psi_e = -Er \cos \phi - \frac{a^2}{r} \frac{1 + \rho_m g_i/a - g_i/g_e}{1 + g_i/g_e + \rho_m g_i/a} E \cos \phi, \tag{3.9}$$

$$\psi_i = -\frac{2}{1 + g_i/g_e + \rho_m g_i/a} Er \cos \phi, \tag{3.10}$$

from which it follows that the membrane polarization, $V_m = \psi_e(a, \phi) - \psi_i(a, \phi)$, is

$$V_m = -2 \frac{\rho_m g_i/a}{1 + g_i/g_e + \rho_m g_i/a} (aE) \cos \phi \tag{3.11}$$

At 100 c/s, ρ_m, can be replaced solely by the a.c. surface resistivity of the membrane ($= 400 \ \Omega.cm^2$), and for a fibre of 100 μ in diameter eqn. (3.11) becomes

$$V_m = -0.99 \times 10^{-2} E \cos \phi. \tag{3.12}$$

4. *The effect of tapering along the fibre*

To solve this case by means of the core conductor equations, R_i, R_m and C_m must be considered functions of the distance z along the fibre. The fundamental equation takes then the modified form

$$\frac{\partial^2 V_m}{\partial z^2} - \frac{1}{R_i} \frac{dR_i}{dz} \frac{\partial V_m}{\partial z} = \frac{R_i}{R_m} V_m + R_i C_m \frac{\partial V_m}{\partial t} + \frac{1}{R_i} \frac{dR_i}{dz} E, \tag{4.1}$$

However, even in the case of a steady d.c. field, eqn. (4.1) can only be solved in terms of known functions by assuming a special regular geometry of the fibre. In the simplest case where the fibre tapers linearly towards the ends at an angle α, the general solution of eqn. (4.1) takes the form (the independent variable z has been replaced by the fibre radius r)

$$V_m(r) = A I_1(k\sqrt{r})/\sqrt{r} + B K_1(k\sqrt{r})/\sqrt{r},$$

where $k^2 = 4R_m/R_i \alpha^2$ and I_1 and K_1 are the modified Bessel functions of the first order, but this expression is not very suited for practical computations.

If the fibre is considered as a long prolate ellipsoid it is possible to obtain a first-order approximation of the membrane polarization by a straightforward solution of the volume conduction equations as in 3. A short outline of the procedure is as follows: The potential ψ is calculated in prolate spheroidal co-ordinates. Since ψ is independent of ϕ (rotational symmetry with respect to the fibre axis) the Laplace equation takes the form

$$\nabla^2 \psi = \frac{\partial^2 \psi}{\partial \eta^2} + \coth \eta \frac{\partial \psi}{\partial \eta} + \frac{\partial^2 \psi}{\partial \theta^2} + \tan \theta \frac{\partial \psi}{\partial \theta} = 0. \tag{4.2}$$

A solution of eqn. (4.2) of the form $\psi = V_1(\eta) V_2(\theta)$ makes 4.2 separate into two ordinary differential equations of Legendre's type. The proper solutions for a conducting homogeneous ellipsoid are selected and the same type of boundary conditions as (3.5) and (3.6) are applied. Since most of the current enters at the poles, the boundary conditions are adjusted to fit the present physical model correctly at the fibre axis. From these conditions ψ_e can be determined. Since the membrane polarization is $V_m = -i_m \rho_m$, where i_m is the normal component of the membrane current density, we have

$$V_m = \rho_m g_e \frac{\partial \psi_e}{\partial n} = g_e \rho_m \frac{\partial \psi_e}{\partial \eta} \frac{\partial \eta}{\partial n} \quad \text{for} \quad \eta = \eta_0, \tag{4.3}$$

where $\eta_0 = \text{artanh} \ (a/L)$ is the ellipsoidal co-ordinate defining the fibre surface and n is

the distance measured along the normal to the ellipsoidal surface. This leads to the final result

$$-V_m = \frac{\rho_m g_i [1 - Q_1 (\cosh \eta_0)/\cosh \eta_0 Q_1' (\cosh \eta_0)]}{1 + \rho_m g_i/f \cosh \eta_0 - g_i Q_1 (\cosh \eta_0)/g_e \cosh \eta_0 Q_1' (\cosh \eta_0)} \left\{ \frac{\cosh^2 \eta_0 - 1}{\cosh^2 \eta_0 - \cos^2 \theta} \right\}^{\frac{1}{2}} E \cos \theta,$$

where Q_1 is the Legendre function of the second kind and $\cos \theta = z/L$. With $a/L = 10^{-2}$, $\cosh \eta_0$ is very nearly equal to unity, which makes $Q_1 (\cosh \eta_0)/Q_1' (\cosh \eta_0) \ll 1$. Disregarding this term in the above equation and substituting $f \cosh \eta_0 = L$ it simplifies to

$$-V_m = \frac{\rho_m g_i/L}{1 + \rho_m g_i/L} \left\{ \frac{\cosh^2 \eta_0 - 1}{\cosh^2 \eta_0 - \cos^2 \theta} \right\}^{\frac{1}{2}} \cos \theta \ (LE). \tag{4.4}$$

For a fibre 2 cm long and 100μ in maximum diameter $\eta_0 = \text{artanh} (0.005) = 0.005$ and $\cosh^2 \eta_0 = 1.00002$. Using the average values of g_i and c_m and calculating ρ_m at 100 c/s ($= 400 \ \Omega.\text{cm}^2$) the maximum polarization which occurs at the poles is

$$V_m^0 = 0.57 \times EL. \tag{4.5}$$

For all points on the surface except the most terminal portions ($z \leqslant 0.9996L$) eqn. (4.4) is given with an error less than 1% by

$$V_m = -2.55 \times 10^{-3} \cot \theta.EL, \tag{4.6}$$

where $\theta = \text{arc} \cos (z/L)$.

REFERENCES

ADRIAN, R. H. (1956). The effect of internal and external potassium concentration on the membrane potential of frog muscle. *J. Physiol.* 133, 631–658.

BAY, Z., GOODALL, M. C. & SZENT-GYÖRGYI, A. (1953). The transmission of excitation from the membrane to actomyosin. *Bull. math. Biophys.* 15, 1–13.

BUCHTHAL, F. & STEN-KNUDSEN, O. (1959). Impulse propagation in striated muscle fibres and the role of the internal currents in activation. *Ann. N.Y. Acad. Sci.* 81, Art. 2, 422–445.

CSAPO, A. (1957). Depolarized muscle, a missing link. *Science*, 126, 1230.

CSAPO, A. & SUZUKI, T. (1957). A preliminary note on excitation-contraction coupling. *Proc. nat. Acad. Sci., Wash.*, 43, 278–281.

CSAPO, A. & SUZUKI, T. (1958). The effectiveness of the longitudinal field, coupled with depolarization in activating frog twitch muscles. *J. gen. Physiol.* 41, 1083–1098.

CSAPO, A. & WILKIE, D. R. (1956). The dynamics of the effect of potassium on frog's muscle. *J. Physiol.* 134, 497–514.

DU BOIS-REYMOND, E. (1875). Ueber Elektrotransfusion am erregbaren Muskel. In *Gesammelte Abhandlungen zur allgemeinen Muskel- und Nervenphysik*, pp. 126–130. Leipzig: Veit.

GELFAN, S. (1958). Muscle. *Annu. Rev. Physiol.* 20, 67–96.

HERMANN, L. (1886). Ueber das galvanischen Wogen des Muskels. *Pflüg. Arch. ges. Physiol.* 39, 597–623.

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

HOBSON, E. W. (1931). *The Theory of Spherical and Ellipsoidal Harmonics*, p. 412. Cambridge University Press.

HODGKIN, A. L. & HOROWICZ, P. (1956). The effect of sudden changes in the external medium on the tension and membrane potential of single muscle fibres. *Abstr. XX int. physiol. Congr.*, 442.

HODGKIN, A. L. & KEYNES, R. D. (1953). The mobility and diffusion coefficient of potassium in giant axons from *Sepia. J. Physiol.* 119, 513–528.

HODGKIN, A. L. & RUSHTON, W. A. H. (1946). The electrical constants of a crustacean nerve fibre. *Proc. Roy. Soc. B*, 133, 444–479.

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

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

HUXLEY, A. F. & TAYLOR, R. E. (1958). Local activation of striated muscle fibres. *J. Physiol.* **144**, 426–441.

JENDRÁSSIK, A. E. (1879). Ueber die Ursache der in den querstreiften Muskeln unter der Einwirkung constanter Strömen auftretenden Strömungserscheinungen. *Arch. Anat. Physiol., Lpz. (Physiol. Abt.)*, 300–341.

KATZ, B. (1948). The electrical properties of the muscle fibre membrane. *Proc. Roy. Soc. B*, **135**, 506–534.

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. & LOU, H. C. (1947). Transverse stimulation of muscle with alternating current. *J. Physiol.* **106**, 30P.

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

KÜHNE, W. ((1860). Ueber das Porret'schen Phänomen am Muskel. *Arch. Anat. Physiol., Lpz. (Physiol. Abt.)*, 542.

NICHOLLS, J. G. (1956). The electrical properties of denervated skeletal muscle. *J. Physiol.* **131**, 1–12.

SMYTHE, W. R. (1950). *Static and Dynamic Electricity*, 2nd ed., p. 64. New-York: McGraw-Hill.

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

STOKES, G. G. (1856). On the effect of the internal friction of fluids on the motion of pendulums. *Trans. Camb. Phil. Soc.* **9**, 8–106.

SZENT-GYÖRYGI, A. (1956). General views on the chemistry of muscle contraction. *Adv. Cardiol.* **1**, 6–51. Basel; New-York: S. Karger.

WATANABE, A. & AYABE, R. (1956). Local contraction in single muscle fibres elicited by electrical stimulation. *XIV Japan Med. Congr.* pt. 2, 17–19.

WUNDT, W. (1859). Ueber den Verlauf der Muskelzusammenziehung bei direkter Muskelreizung. *Arch. Anat. Physiol., Lpz. (Physiol. Abt.)*, 549–552.

EXPLANATION OF PLATES

PLATE 1

Photographs of reversible isotonic a.c. contractures in a non-propagating isolated single fibre from the semitendinosus muscle from frog. Left-hand side: photographs of the whole length of the fibre marked with graphite particles. (*r*), resting fibre; (*c*) longitudinal a.c. field at about 5 V/cm (100 c/s). Right-hand side: enlargements of the end portions of the fibre with all the frames aligned with respect to one mark placed at a distance of about 5 mm from the ends. Upper row of each set shows the resting fibre (*r*), and those below the effect of the a.c. field, the numbers indicating the value of each field strength. The displacement of the graphite granules is shown by the black lines. For further description, see text.

PLATE 2

To show the irreversible effect of too high field strength. For explanation, see text and legend to Pl. 1. Note the difference in thickness and degree of shortening along the length of the fibre, which characterizes the 'wave-like' contractions. *b* and *b'* show the fibre ends 4 sec after stimulation with 7·3 V/cm as compared with the initial fibre length *a* and *a'*.

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