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SLOW FIBRES IN THE EXTRAOCULAR MUSCLES OF THE CAT

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Two distinct types of striated muscle fibres, twitch and slow fibres, have been demonstrated by functional studies in frogs (Kuffler & Vaughan Williams, 1953*a*, *b*) and in chickens (Ginsborg, 1960*a*, *b*). During electrical stimulation the former muscle fibres show propagated impulse activity and non-graded contractile activation; the latter usually do not display impulse activity and undergo a graded contraction dependent upon the extent of membrane depolarization by multiple junctional potentials. Morphological studies have also shown the presence of the two fibre types, differing in their fibril structure and innervation (Krüger, 1949; Günther, 1949; Krüger & Günther, 1958; Hess, 1960). One type of fibre has large, irregular, poorly defined fibrils (Felderstruktur) and multiple motor endings of the 'en grappe' type; the other type of fibre shows a fibrillar pattern in which the fibrils are regular, distinct and punctate (Fibrillenstruktur) and receives a single ending of the end-plate or 'en plaque' type. Hess (1961*a*) has shown that the anterior latissimus dorsi muscle of the chick, which contains only slow fibres (Ginsborg, 1960*a*, *b*), is composed exclusively of fibres of the Felderstruktur type; and the posterior latissimus dorsi, from which only twitch-type electrical activity was recorded (Ginsborg, 1960*a*, *b*), is composed almost entirely of Fibrillenstruktur type fibres. Further confirmation of the relation between twitch type behaviour and Fibrillenstruktur fibres, and between slow type behaviour and Felderstruktur fibres was provided by the recent study of Peachey & Huxley (1962) in frog muscle fibres.

In the mammal the situation is less clear. Krüger (1957) described Felderstruktur fibres in several muscles but no functionally slow fibres (as defined above) have thus far been demonstrated in these sites. Hess (1961*b*) found fibres of the Felderstruktur type in the extraocular muscles of the guinea-pig and found that they were innervated by multiple endings of the 'en grappe' type. There has been no definitive evidence that these

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fibres are of the physiologically slow type, but some previous observations suggest that this might be so. Thus, it has been shown that acetylcholine produces a contracture in mammalian extraocular muscles, i.e. shortening unaccompanied by muscle action potentials (Duke-Elder & Duke-Elder, 1930; Brown & Harvey, 1941; Luco, Eyzaguirre & Perez, 1947). Such behaviour is characteristic of slow muscle fibres in the frog (Kuffler & Vaughan Williams, 1953*b*).

In the present study, it is shown that morphologically the extraocular muscles of the cat have two distinct types of muscle fibre, similar to the twitch and slow fibres of frog and chicken in the arrangement of their fibrils and in their innervation. Physiological evidence is also presented showing that there are two functionally distinct fibre types: slow fibres exhibiting only local junctional potentials and graded contractions, and twitch fibres showing all-or-none impulse activity and twitch-type contractions. From the distribution of the two fibre types, the relation between functional and morphological characteristics of the twitch and slow fibres is shown to be similar to that in frog and chicken. The muscle fibres under discussion here are all extrafusal; the cat eye muscles have no spindles (Cooper & Fillenz, 1955). A partial account of this work has already been presented (Pilar & Hess, 1962).

METHODS

Morphology

The eye muscles (superior rectus, medial rectus, lateral rectus, inferior rectus, superior oblique, inferior oblique) of the cat were used. For study of muscle structure whole muscles or pieces of muscle were fixed outstretched in either Dalton's or Susa's fluid. The tissues were dehydrated, embedded in epon, and thin (200–400 Å) and thick (1–2 μ) sections were cut. The thick and thin sections cut from Dalton-fixed material were examined in the phase contrast and electron microscopes after treating the sections with lead salts (lead tartrate or lead hydroxide) at a high pH to increase contrast. The thick sections of Susa-fixed material were stained with an azure II–methylene-blue mixture and studied in the light microscope.

Nerve endings were stained with a modified Koelle cholinesterase method. Muscles were fixed outstretched in 10% glyoxal treated with calcium carbonate, teased, incubated in acetylthiocholine or butyrylthiocholine iodide, placed in dilute ammonium sulphide, and mounted in glycerine. Some tissues were treated with di-isopropyl phosphofluoridate before being placed in the incubation medium.

To correlate the type of nerve ending and type of muscle fibre, muscle fibres after cholinesterase staining and mounting in glycerine were rinsed in water, refixed in Dalton's fluid, dehydrated, embedded in epon, sectioned and examined with the electron microscope.

Physiology

Isolated neuromuscular preparations of the superior oblique muscle of the cat were used. Animals were anaesthetized with sodium pentobarbitone (Nembutal, Abbott Laboratories, 40 mg/kg), and after exposure of the trochlear nerve were killed, either with Nembutal or by destruction of the medulla oblongata. Oxygenated Krebs's solution (mM): (NaCl 115, KCl 4·6, KH_2PO_4 1·15, $CaCl_2$ 2·5, $NaHCO_3$ 4·1, $MgSO_4$ 1·15, glucose 8·85), bubbled with

a gas mixture containing 95% O_2, 5% CO_2 (Boyd & Martin, 1956*a*), was allowed to flow over the tissues until removal of the nerve and muscle was complete. The entire muscle was removed from its origin around the optic foramen to the round tendon passing through the trochlea.

All experiments were done at room temperature (20–25° C). Two sets of experiments were performed: in one, intracellular recording techniques were used to obtain records of electrical changes in individual muscle fibres; in the other, tension changes in the whole muscle were recorded.

For intracellular recording the preparation was mounted in a lucite chamber through which oxygenated Krebs's solution flowed at a rate of 2·5 ml./min. External stimulation to the nerve was applied through a pair of platinum electrodes. The recording arrangement was identical with that described by Martin & Pilar (1963). Glass micropipettes, filled with 3M-KCl and having resistances of 40–80 MΩ, were used. These were connected to a unity-gain negative-capacity pre-amplifier for recording and a Wheatstone bridge arrangement was used to pass polarizing current through the same electrode.

In order to avoid movement artifacts during nerve stimulation, the muscle was stretched in the chamber. In addition, neuromuscular transmission was partially blocked by increasing the concentrations of Mg and Ca in the bathing solution while maintaining its molarity by adjusting the Na concentration. Usually, the Mg and Ca concentrations required to maintain the muscle without visible movement during nerve stimulation were about 20 and 8 mM respectively. Slight adjustments were made in the Ca concentration so that spikes could be recorded from a few fibres. The increased Mg concentration presumably decreased the amplitude of the end-plate potential (e.p.p.) and increased the threshold for initiation of a propagated spike (del Castillo & Engbaek, 1954), while the increased Ca concentration tended to restore the e.p.p. amplitude and raise the threshold still further (del Castillo & Stark, 1952; Boyd & Martin, 1956*b*).

For recording tension changes the muscle was mounted vertically in a lucite chamber with the proximal end attached firmly to a fixed clamp. The tendon end was tied with a linen thread to a vacuum tube transducer (RCA 5734) for isometric tension recording. The nerve was mounted on a pair of silver electrodes, and stimulating pulses of 0·1 msec duration applied. A system of communicating tubes allowed solutions to be changed. The chamber was drained for recording, except during the acetylcholine (ACh) experiments.

RESULTS

Morphology

Muscle fibres. Cross-sections of the superior oblique muscle, examined by light microscopy, reveal two different kinds of muscle fibre (Pl. 2*C*). In one, usually the larger fibre (about 30–45 μ in diameter), the fibrils have a punctate appearance and are regularly spaced; in the other, usually the smaller fibre (about 10–15 μ in diameter), the fibrils appear larger, of more variable size, and are not regularly separated from each other. Cross-sections examined in the electron microscope show that in the fibres in which the fibrils have a punctate appearance, the sarcoplasmic reticulum generally surrounds and delineates clearly the individual fibril (Pl. 1*A*). In contrast, the other type of muscle fibre shows fibrils only partly surrounded by sarcoplasmic reticulum, so that at some points adjacent fibrils appear confluent and irregular in size and shape (Pl. 1*B*). Longitudinal sections also reveal conspicuous differences

between the two fibre types. In one type (Pl. 2*D*) fibrils may be seen with sarcoplasmic reticulum extending along both sides of the fibrils, separating the latter distinctly. In the other (Pl. 2*E*) the sarcoplasmic reticulum is less extensive, does not extend unbroken along the length of each fibril and hence only partly separates them.

The two types of fibres described above correspond almost exactly to the twitch and slow type extrafusal muscle fibres described morphologically in the frog and chicken, and in the extraocular muscles of the guinea-pig. Those with punctate fibrils surrounded by sarcoplasmic reticulum, with the appearance of Fibrillenstruktur, are characteristic of twitch fibres, while those with larger, more irregular fibrils (Felderstruktur) only partly surrounded by sarcoplasmic reticulum are typical of the slow-fibre type.

Nerve endings. The nerve endings seen on teased fibres after cholinesterase staining are of two types. One shows relatively large circular or elliptical areas (Pl. 3*A*, *B*, *C*, *D*), consisting of finger-like projections with intensely staining borders disposed in various complex configurations (Pl. 4*A*, *B*, *C*, *D*): these resemble the motor end-plates in other mammalian muscles. The other type of ending is generally smaller, less densely stained, simpler, but more variable in size and shape. At low magnification these usually appear as thin, elongated profiles of cholinesterase-staining material, usually multiple along the fibre length (Pl. 3*E*, *F*, *G*). Under higher magnification these profiles can be seen to consist of oval or more extended elements with most of the density in their margins (Pl. 4*E*, *F*, *G*, *H*). Although this type of ending in the cat extraocular muscles does not have a grape-like appearance, it is probably analogous to the 'en grappe' terminal in other species.

Both the above types of ending have true and pseudocholinesterase. Thus, both show staining with incubation in acetylthiocholine after treatment with di-isopropyl phosphofluoridate (revealing the presence of true cholinesterase), or after incubation with butyrylthiocholine (revealing pseudocholinesterase). Muscle fibres from skeletal muscles treated with di-isopropylfluorophosphate and incubated in butyrylthiocholine do not stain.

The distribution of the two types of endings is strikingly different. Only one ending of the end-plate type can be found on a single muscle fibre (Pl. 3*A*, *B*, *C*, *D*). Since the fibres are quite thin, they cannot be teased out for a great length. Thus, it cannot be concluded that a single muscle fibre receives only one end-plate. If, however, there are multiple endings, they must be far apart. This is in marked contrast to the other type of ending, where many endings can be seen to terminate at multiple points along the fibre length (Pl. 3*E*, *F*, *G*). They are densely distributed, in many

cases with only short distances (10–30 μ) separating them (Pl. 4*F*, *G*, *H*). The two different types of nerve ending are never seen on the same muscle fibre.

Both end-plate and distributed type endings are found in all the six extraocular muscles examined (the four recti and two obliques). Since the superior oblique was used for physiological studies, the distribution of its endings will be considered in detail. In this muscle the multiply-innervated fibres appear to be relatively more numerous. The two types of ending also have quite different distributions. The central portion of the muscle near the tendon consists essentially of muscle fibres with multiply-distributed endings; end-plates are virtually absent from this region. In the proximal belly of the muscle fibres with the two types of endings are intermingled. As the tendon is approached the muscle fibres with end-plate endings come to occupy the more lateral portions of the muscle and end more laterally and slightly more proximally than the muscle fibres with distributed endings. The latter fibres are inserted more distally, in the more medial portion of the tendon. Although the fibres with end-plates occupy the major portion of the muscle length, their motor endings tend to be concentrated toward the origin end.

Correlation of muscle-fibre and nerve-ending types. Muscle fibres first examined for the type of nerve ending by cholinesterase staining were subsequently examined by electron microscopy (see Methods). Those fibres with end-plate type endings show relatively small, separate and punctate fibrils (Pl. 2*A*). In spite of their harsh treatment, they can be seen to be similar to the twitch-type fibres described above. Muscle fibres with demonstrated distributed endings, on the other hand, show large fibrils with ill-defined borders and poor separation (Pl. 2*B*), similar to the slow-fibre type described above.

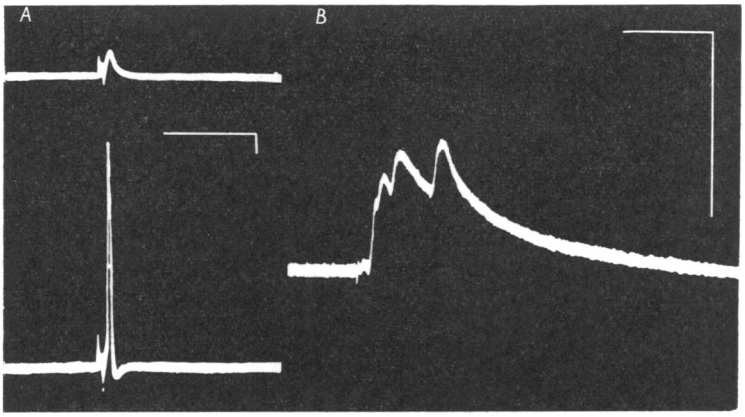
Physiology

Intracellular recording

Resting potentials and responses to nerve stimulation. Two distinct types of muscle fibre could be distinguished on the basis of their response to nerve stimulation. In one group of fibres motor nerve stimulation produced all-or-none action potentials, which had an average amplitude of 80 mV (Text-fig. 1*A*, lower record). If the recording electrode was inserted near the proximal end of the muscle, end-plate potentials (e.p.p.s) could be detected, as in the example of Text-fig. 1*A*, upper record, where after high-frequency stimulation the spike potential failed to be initiated and the e.p.p. alone could be seen. When insertions were made near the tendinous end of these fibres, no e.p.p.s could be detected. The resting potentials of 35 fibres showing propagated impulses ranged from 50 to

80 mV, with an average of 65 mV. When recorded at a point presumably near the end-plate focus, the e.p.p. had a rise time of between 2 and 3 msec and decayed to half amplitude in 2–4 msec. Fibres with the above characteristics were considered to be twitch or 'fast' fibres.

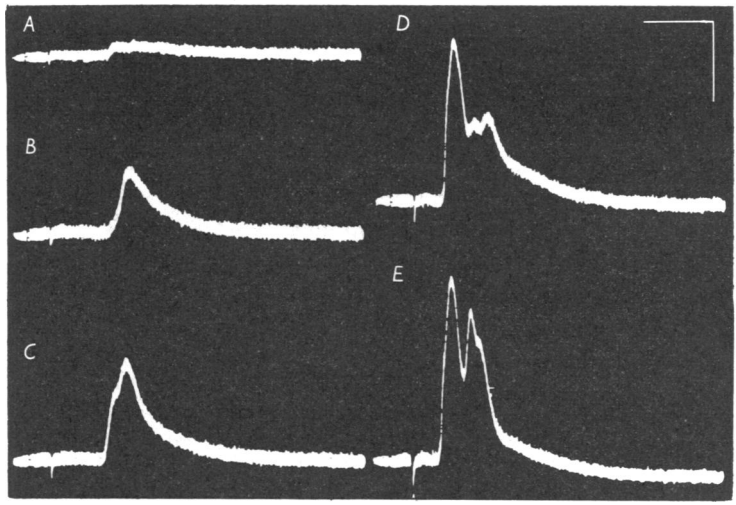
Response of a second type of muscle fibre is shown in Fig. 1*B*. Supramaximal nerve stimulation in such fibres yielded a complex junctional potential reaching a maximal depolarization of 12–14 mV. The various components of the potential had rise times ranging from 3 to 15 msec and the time to half decay of the potential was about 30 msec. Responses of



Text-fig. 1. Response to nerve stimulation of two types of extrafusal fibres in the superior oblique muscle. Intracellular recording. *A*. Typical action potential (bottom) and end-plate potential (top) recorded from the same twitch fibre after stimulation at 20/sec. Resting potential, 70 mV. *B*. Typical complex response of slow muscle fibre during maximal stimulation of the nerve. No action potential is seen. Each peak corresponds to a small junctional potential elicited in neuromuscular junctions near the point of insertion of the pipette. Resting potential, 45 mV. Calibration: 10 mV; 40 msec.

this type of fibre could be recorded anywhere along the length of the muscle. Complex junctional potentials, such as that in Text-fig. 1*B*, could be graded into a number of individual components by varying the strength of stimulation to the motor nerve. An example is shown in Text-fig. 2, in which records were taken in sequence as the stimulus strength was gradually increased from just above threshold (*A*) to maximal (*E*). The complex response to maximal stimulation can be seen to have resulted from the summation of numerous components of varying latency, amplitude and time course. Such responses are similar to the 'small junctional potentials' (s.j.p.s) described in slow muscle fibres of the frog (Kuffler & Vaughan Williams, 1953*a*), and chicken (Ginsborg, 1960*b*). The variation in amplitude and time course of the individual components

suggests that they arose from junctions scattered along the muscle fibre at varying distances from the recording pipette. The presence of numerous components suggests, in addition, that there were many points of innervation, closely spaced with respect to the space constant of the muscle fibre. Finally, the variations in threshold and latency indicate that the multiple innervation was polyneuronal. It may be noted in Text-fig. 2*E* that the decay of the s.j.p. was followed by a phase of hyperpolarization; this usually occurred when the s.j.p. exceeded 10 mV in amplitude. Similar



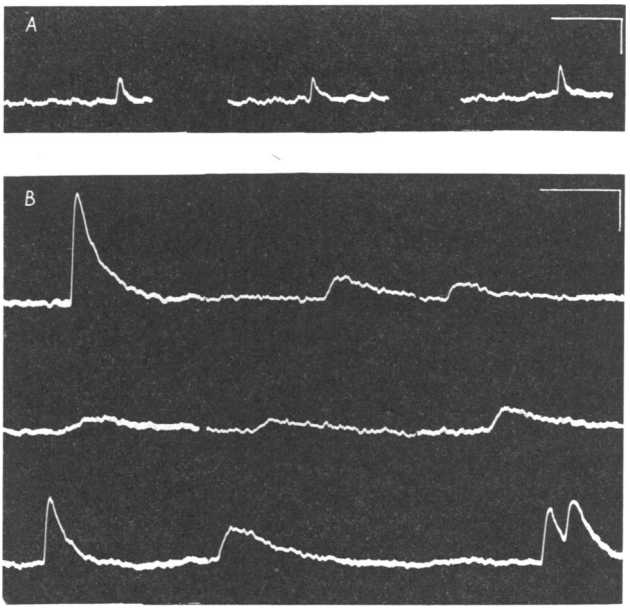
Text-fig. 2. Intracellular response obtained from a slow muscle fibre with varying strength of stimulus applied to motor nerve. Stimulus gradually increased from *A* to *E*. In *E* a late phase of hyperpolarization follows the decay of the potential. The observed decrease in latency of response was probably due to spread of the stimulating current as the stimulus strength was increased. Calibration: 1 mV; 20 msec.

hyperpolarization following s.j.p.s in slow muscle fibres of the frog and chicken has been ascribed to 'delayed rectification' of the fibre membrane (Burke & Ginsborg, 1956; Ginsborg, 1960*b*). The resting potentials of 20 fibres of the slow type studied ranged from 30 to 70 mV with an average of 45 mV. Magnesium- and calcium-enriched Krebs's solution was used with 12 of these muscle fibres and with the rest normal Krebs's solution was employed. In only one fibre in the latter group was it possible to evoke a spike potential by nerve stimulation.

Spontaneous activity. In both twitch and slow fibres miniature junctional potentials were seen to occur spontaneously. An example from a twitch fibre is shown in Text-fig. 3*A*. These were similar to spontaneous miniature e.p.p.s seen in other muscles (Fatt & Katz, 1952; Boyd & Martin, 1956*a*;

Liley, 1956). They had maximal amplitudes of 0·25–2·0 mV, rise times between 0·5 and 1·5 msec, and time to half decay ranged from 0·8 to 3·5 msec. As with the e.p.p.s evoked by nerve stimulation, they could only be detected in the proximal part of the muscle.

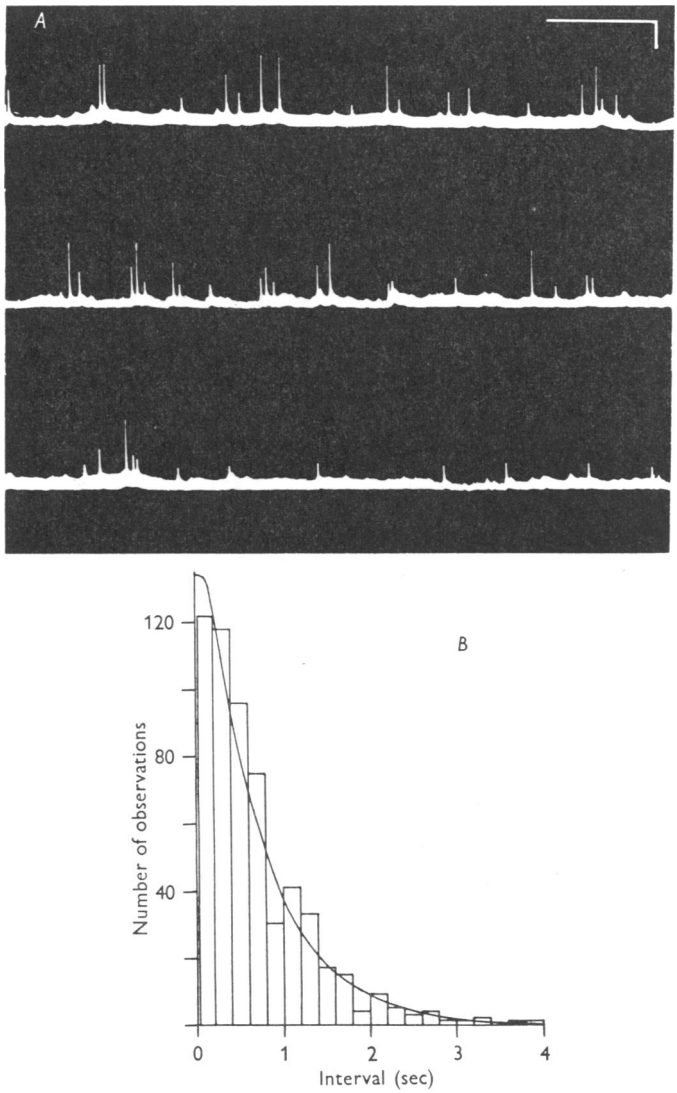
An example of the spontaneous miniature junctional potentials from slow fibres (miniature s.j.p.s) is shown in Text-fig. 3*B*. These varied in amplitude from base-line noise level to a maximum of about 6 mV and



Text-fig. 3. Spontaneous miniature potentials: *A*, twitch muscle fibre; *B*, slow muscle fibre. Note the longer and more variable decay of the spontaneous miniature potentials of the slow fibres in comparison with the fairly constant decay of the fast fibres. Normal Krebs's solution. Calibration: 1 mV; 10 msec.

had rise times from 2 to 24 msec. Since they occurred at low frequency (1–3/sec) with an apparent random distribution of intervals (see below), it is unlikely that the larger potentials were due to summation of two or more unit potentials. Rather, the large maximal amplitudes were probably related to the high effective impedance of the slow muscle fibres, which was in the order of 7 MΩ, as compared to about 0·9 MΩ for the twitch fibres (see Katz & Thesleff, 1957).

In contrast to the miniature e.p.p.s, the miniature s.j.p.s could be recorded from any part of the muscle. Both types of miniature potential were abolished when D-tubocurarine 10 μg/ml. was added to the bathing solution. The average frequency of the miniature s.j.p.s varied from one



Text-fig. 4. *A*. Continuous recording of the spontaneous miniature potentials from a slow muscle fibre. Calibration: 2 mV; 2 msec. Normal Krebs's solution. *B*. Histogram of 578 spontaneous miniature potentials from one slow muscle fibre, showing frequency of occurrence of intervals between times t and $t+\Delta t$. Mean interval (T) 675 msec, class width (Δt) 200 msec. Curve calculated from equation

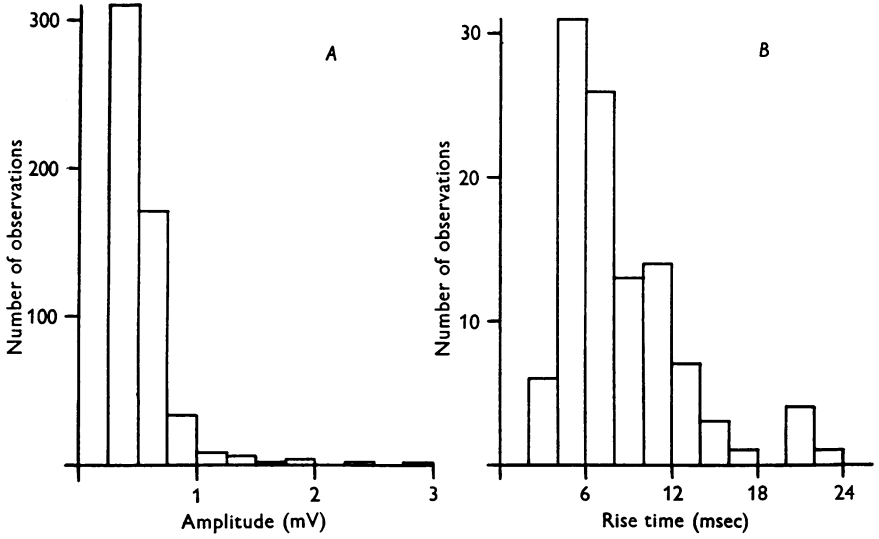
$$n = \frac{N\Delta t}{T} e^{-t/T},$$

where n is expected number of observations in each class and N the total number of observations. Note that at the beginning the curve has a different slope. Since intervals of less than 20 msec could not be resolved, the first class width is reduced to 180 msec.

fibre to another but was generally constant in any given fibre. An example of the discharge pattern of a miniature s.j.p., recorded on a slow time base, is shown in Text-fig. 4*A*. A histogram of the distribution of intervals between individual discharges (Text-fig. 4*B*) fits closely with the expected distribution for random events.

TABLE 1. Characteristics of spontaneous miniature junctional potentials in twitch and slow fibres

Temp. 20–25° C	Maximum amplitude (mV)	Rise time (msec)	Half-decay (msec)	Frequency (sec^{-1})
Twitch fibre	2	0·5–4·0	0·8–3·5	1·0–3·4
Slow fibre	6	2·0–24	5·0–35	1·2–2·5



Text-fig. 5. Histograms of amplitude distribution (*A*) and distribution of rise time (*B*) of spontaneous miniature s.j.p.s. Note that both distributions are skewed.

The characteristics of the miniature s.j.p.s and e.p.p.s are summarized in Table 1. The principal difference between the two types of spontaneous potential was in time course. The longer average duration of the miniature s.j.p.s may be attributed in part to the longer time constant of the slow muscle fibre membrane and in part to the presence of multiply-distributed endings, many of the potentials presumably being recorded at some distance from their point of initiation. Further evidence consistent with this view is presented in the histograms of Text-fig. 5. Text-figure 5*A* show the amplitude distribution of 578 miniature s.j.p.s recorded during one insertion in a slow fibre. The distribution is skewed, unlike that of

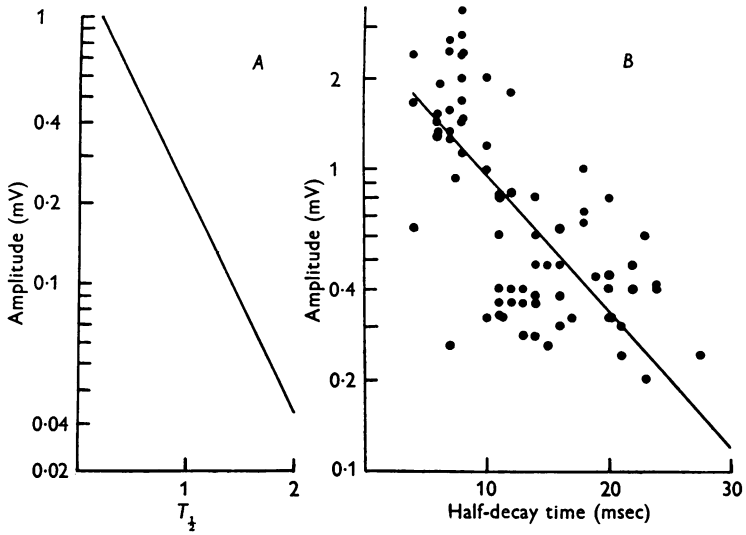
miniature e.p.p.s (Fatt & Katz, 1952). This type of distribution could result if the potentials originated at numerous points along the muscle fibre, located at varying distances from the recording pipette. The distribution of rise times of the same population of miniature s.j.p.s is shown in Text-fig. 5*B*. This distribution is also skewed with a large variance, in contrast to the rise times of miniature e.p.p.s which show only slight variations. It should be noted that the rise time distribution differs from that which might be expected from the amplitude distribution; since small potential amplitudes are most frequent, one might expect that the longer rise times would occur most often. Since this is not the case, it may be concluded that the smaller potentials are not necessarily those arising at the greater distances from the recording electrode. This suggests that the amplitude of the miniature s.j.p.s at individual endings along the fibre may vary considerably.

The suggestion that the size of the unit potentials is not the same at all the junctions on a slow fibre may be tested by comparing the recorded data with the relation between amplitude and half decay predicted if potentials of uniform amplitude originated at point sources at varying distances from the recording electrode along a passive muscle membrane. From the equations of Hodgkin & Rushton (1946), a relation between amplitude and half decay may be derived, as shown in Text-fig. 6*A*. The maximal potential, of unity amplitude, should decay to half its value in 0·22 time constants. The amplitude of potentials recorded from points farther away should decrease exponentially as the half-decay time increases, the relation being dependent only on membrane time constant. In the graph of Text-fig. 6*B* the amplitudes of miniature s.j.p.s recorded from a single slow fibre are plotted as a function of their half-decay times. The minimal half decay time was about 4 msec, suggesting a membrane time constant of approximately 18 msec. This was in agreement with directly measured time constants in other fibres which ranged from 18 to 45 msec. The figure of 18 msec was used to plot the theoretical relation between amplitude and half-decay time. The experimental points deviate considerably from the theoretical relation, again suggesting that the amplitudes of the miniature s.j.p.s may vary considerably at individual junctions. It may also be noted that the points do not fall into distinct groups on the graph, as might be expected if the endings were widely separated. This again is consistent with the morphological observation that the individual endings are separated by intervals of less than 30 μ.

Tension recording

Because of the particular distribution of motor nerve endings on twitch and slow fibres of the superior oblique, it was possible to curarize the

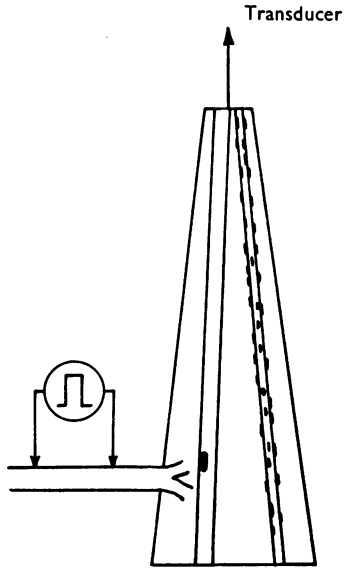
neuromuscular junctions of almost all the twitch fibres and leave unblocked transmission at many of the endings on the slow fibres. In this way the tension responses of the slow fibres could be studied in isolation. Text-figure 7 shows diagrammatically the distribution of nerve endings on the twitch and slow fibres in this muscle and a schematic view of the recording and stimulating arrangement. It may be recalled that end-plate potentials were found at the proximal end of the muscle and never near the tendon, while s.j.p.s of slow fibres could be found all along the muscle. With the muscle mounted vertically and the tendon uppermost, a solution



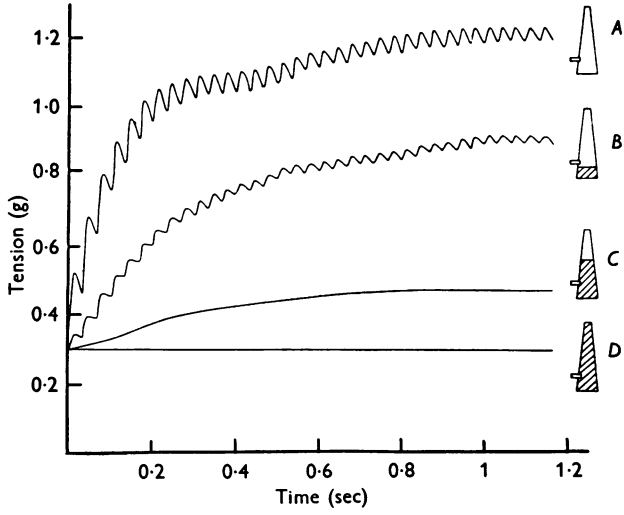
Text-fig. 6. *A*. Theoretical relation between amplitude and half-decay time of potentials of uniform size distributed along a fibre membrane as seen by micropipette inserted at one point (see text). Ordinate, amplitude expressed as fraction of maximal amplitude at point of insertion. Abscissa, half-decay time as fraction of membrane time constant. *B*. Amplitudes of the spontaneous miniature potentials of the slow muscle fibre plotted against half-decay time. The solid line is the predicted relation from *A*, assuming a membrane time constant of 18 msec.

containing D-tubocurarine could be allowed to fill the chamber to varying levels, thus exposing progressively increasing portions of the muscle to its blocking action.

Text-figure 8 shows the tension curves recorded following nerve stimulation at 30/sec when the muscle was exposed to varying extents in a solution containing D-tubocurarine 10 μg/ml. The stimulating frequency was well below that necessary to obtain fusion frequency (400 impulses/sec). The upper record (*A*) was obtained before curarization, and the



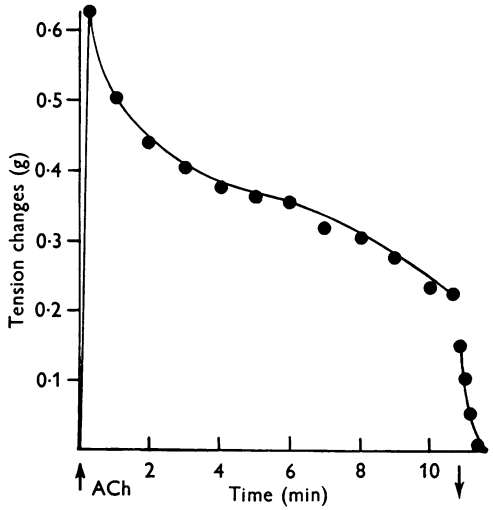
Text-fig. 7. Diagram of the muscle showing the distribution of multiple nerve endings on a slow muscle fibre on the right and the location of a single end-plate on a twitch muscle fibre on the left. The transducer at the top is attached to the tendon end of the muscle. The proximal end of the muscle is attached to a clamp in a moist chamber.



Text-fig. 8. Mechanical recording. Tension changes produced by maximal stimulation of the nerve at 30/sec. All the curves are plotted to the same scale from original films. In the original film of curves *C* and *D* the sensitivity of the oscilloscope was three times greater than that of curves *A* and *B*. Diagrams on the right show the different levels at which curarization was applied for each particular curve. D-tubocurarine 10 μg/ml. was applied for 20 min at each level.

individual tension increments produced by the nerve stimuli may be clearly seen. In the curve just below (*B*), approximately 1/5 of the muscle was exposed to D-tubocurarine; the tension developed during the tetanus is considerably reduced, but the tension increments following each stimulus are quite evident. When the proximal 2/3 of the muscle was exposed to curare (curve *C*), a smooth tension response was obtained with no sign of contractile increments at the stimulus frequency. When the entire muscle was exposed to the curare solution (*D*), no tension response was elicited by nerve stimulation.

The tension responses shown in Text-fig. 8 indicate that there are two contractile systems activated by nerve stimulation, with quite different



Text-fig. 9. Acetylcholine 'contracture'. Isometric tension recording from the superior oblique muscle soaked in Krebs's solution with ACh 10 μg/ml. Arrows indicate time of application and removal of ACh solution.

frequency characteristics. The system giving distinct tension increments at a stimulation frequency of 30/sec has its neuromuscular junctions at the proximal end of the muscle and may be identified with twitch fibres. The other system, producing a fused tension response at 30/sec, has at least some of its endings near the tendon end of the muscle and must be associated with slow fibres. The tension developed by the slow fibres when all but the distal 1/3 of the muscle has been exposed to curare was an appreciable fraction of the control tetanic tension (see Text-fig. 8). Since the contraction in the slow fibres is local and would be confined to the non-curarized region, the tension developed by the slow fibres in the normal

muscle should be several times greater. This suggests that a large number of the fibres in this muscle are 'slow' and that they may play an important role physiologically.

As noted above, several investigators have found that mammalian extraocular muscles can respond by a contracture to ACh (Duke-Elder & Duke-Elder, 1930; Brown & Harvey, 1941; Luco *et al.* 1947). Kuffler & Vaughan Williams (1953*b*) have shown that in the frog ACh contractures occur in fibres that are of the multiply-innervated slow type. Experiments on the effect of ACh on the superior oblique muscle confirmed these findings. An example is shown in Text-fig. 9. When the muscle was exposed to ACh 10 μg/ml. it abruptly developed a tension in excess of 0·6 g. This fell within the first few minutes to about 0·4 g and then declined much more gradually. A considerable tension remained until the ACh was washed out some 11 min after its application. Some of the initial tension rise when the ACh was first applied may have been due to activation of twitch fibres; the tension persisting after the first few minutes can be attributed to contracture in the multiply-innervated slow fibres. The magnitude of the tension developed during this contracture (0·3–0·4 g) is large relative to the tension produced following tetanic stimulation of the nerve to this muscle (see Text-fig. 8). This again indicates that there must be a relatively large number of slow fibres in this muscle.

DISCUSSION

The present experiments have shown that there are two distinct types of muscle fibre in the superior oblique muscle of the cat. Twitch-type fibres display all-or-none impulse activity, end-plate potentials and spontaneous miniature e.p.p.s; morphologically, they show punctate distribution of fibrils, clearly defined by sarcoplasmic reticulum, and are innervated by one, or certainly few, motor ending of the end-plate type. While these fibres were not studied extensively, they display all the usual attributes of twitch-type fibres. It is well known that these fibres in the extraocular muscles are unusual in the rapidity of their twitch contraction and the very high frequencies of stimulation required to attain a fused tetanic contraction.

The fibres herein identified as slow satisfy all the generally accepted physiological criteria for this fibre type and display the morphological characteristics and innervation pattern that is typical. These fibres show multiple junctional potentials which can be graded by varying the number of motor nerve fibres stimulated. Such s.j.p.s can be recorded anywhere along the muscle length, as can spontaneous miniature s.j.p.s. Tension records show that the time course of contraction in these fibres is much

slower than in the twitch fibres and that they produce a contracture on the application of acetylcholine. All these features are typical of slow-fibre systems. In all but one of the slow fibres examined no spike potential could be elicited by nerve stimulation. However, in view of the high calcium and magnesium concentrations used in this study, as well as the possibility of damage following impalement of these small fibres, it cannot be stated with certainty that these fibres usually do not produce propagated action potentials. The slow fibres show fibrils which are large, irregular in shape, and poorly defined in that they are only partly surrounded by sarcoplasmic reticulum. They are multiply innervated by endings densely distributed along their length. These relatively simple endings, analogous to 'en grappe' terminations in other muscles, are so numerous along the fibre length that only very short stretches of the muscle fibre lack nerve endings.

The presence of a slow-fibre system, involving a considerable number of the muscle fibres in an extraocular muscle, must be of real significance physiologically. It is of interest that this system occurs together with a system of twitch fibres of unusual rapidity. Extraocular muscles are required to make extremely rapid movements, as when the gaze is suddenly shifted or during scanning movements. They also must produce sustained contractions for long periods, as in maintaining the positions of the eyes for binocular fusion. The development, or perhaps persistence, of two muscle fibre systems may be related to the need for these two quite different types of contraction. While in other mammalian muscles 'tonic' contraction appears to be due to twitch type fibres which have rather slow contraction times, the twitch fibres of the extraocular muscles are so exceedingly fast that they may be unable to produce sustained contraction of sufficient smoothness. If this dual motor system in extraocular muscles did function in this manner, it would be of great interest to determine the manner in which the twitch and the slow systems are activated reflexly.

The presence of slow fibres in other mammalian muscles, such as the soleus, diaphragm and others, in which Felderstruktur fibres have been described (Günther, 1952; Krüger & Günther, 1955), seems doubtful from the physiological evidence now available. It is of significance that multiply-distributed nerve endings are not found on muscle fibres in these muscles (Hess, 1962). In these situations, the arrangement of fibrils may be either produced by artifact or may not be a sufficient criterion for identification of slow fibres. However, it is clear from the present study and from other investigations on striated muscle of frog, chicken and mammalian extraocular muscle, that muscle fibres showing both the diffuse, poorly delineated fibrils incompletely surrounded by sarcoplasmic reticulum and multiply-

distributed nerve endings are, thus far, invariably of the slow-fibre type physiologically.

SUMMARY

1. In the light and electron microscopes, Felderstruktur muscle fibres with multiple nerve endings have been found in all the extraocular muscles of the cat, in addition to Fibrillenstruktur muscle fibres with a single end-plate ending.

2. Intracellular recording in the superior oblique muscle allows the distinction of two kinds of muscle fibres: one with the characteristics of twitch muscle fibres and the other with the characteristics of slow muscle fibres similar to those described in the frog and chick.

3. Mechanical recording of tension change during nerve stimulation and after block of virtually all twitch fibres shows that the remaining slow fibres respond with a 'contracture'.

4. Correlation of the morphological and physiological data provides further evidence that the fibres with diffuse, poorly delineated fibrils incompletely surrounded by sarcoplasmic reticulum (Felderstruktur) and with multiply-distributed nerve endings are physiologically of the slow-fibre type.

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EXPLANATION OF PLATES

All photo-micrographs are from the cat superior oblique muscle.

PLATE 1

Electron micrographs, cross-section. The scale line represents 1 μ, $\times$ 16000. *A*, twitch type; *B*, slow type.

PLATE 2

A and *B*. Electron micrographs of cross-sections of muscle fibres with known type of nerve terminal, fixed in glyoxal, teased, stained for cholinesterase, mounted in glycerine, refixed in Dalton's fluid, embedded in epon. The scale line represents 1μ, $\times$ 32000. *A*, twitch type; these muscle fibres have individual end-plate endings. *B*, slow type; these muscle fibres have multiply distributed endings. *C*. Light micrograph, Susa-fixed, embedded in epon, stained cross-section. The scale line represents 10 μ, $\times$ 1500. The large fibres with punctate fibrils

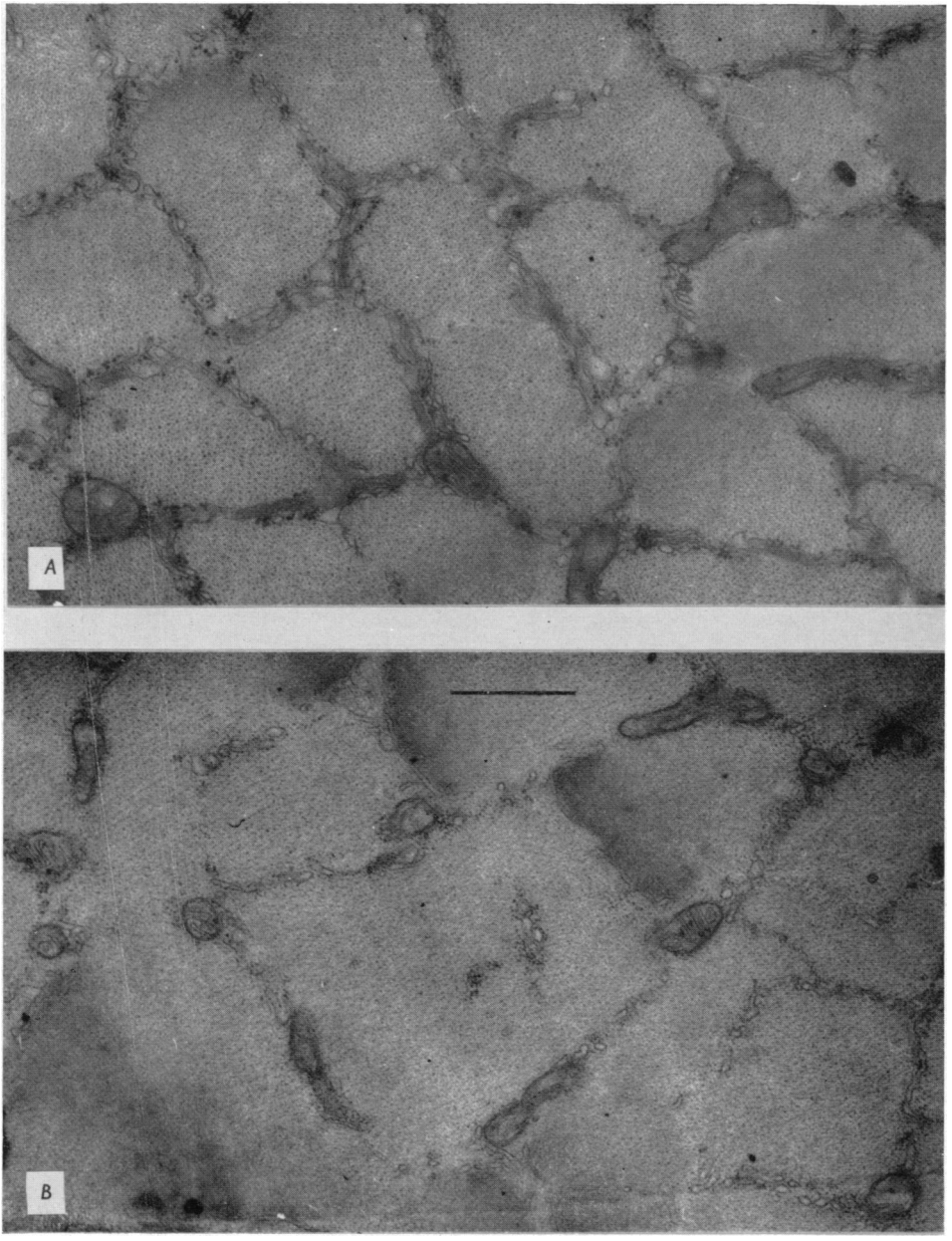
are of the twitch type; the small fibres with large, irregularly distributed fibrils are of the slow type. *D*, *E*. Electron micrographs, longitudinal section. The scale line represents 1 μ, $\times$ 16000. *D*, twitch type; *E*, slow type.

PLATE 3

Light micrographs, teased muscle fibres, cholinesterase stain. The scale line represents 10 μ, $\times$ 150. *A*, *B*, *C*, *D*, end-plate endings; *E*, *F*, *G*, multiply distributed endings.

PLATE 4

Light micrographs, teased muscle fibres, cholinesterase stain.
The scale line represents 10 μ, $\times$ 640. *A*, *B*, *C*, *D*, end-plate endings: *E*, *F*, *G*, *H*, multiply-distributed endings.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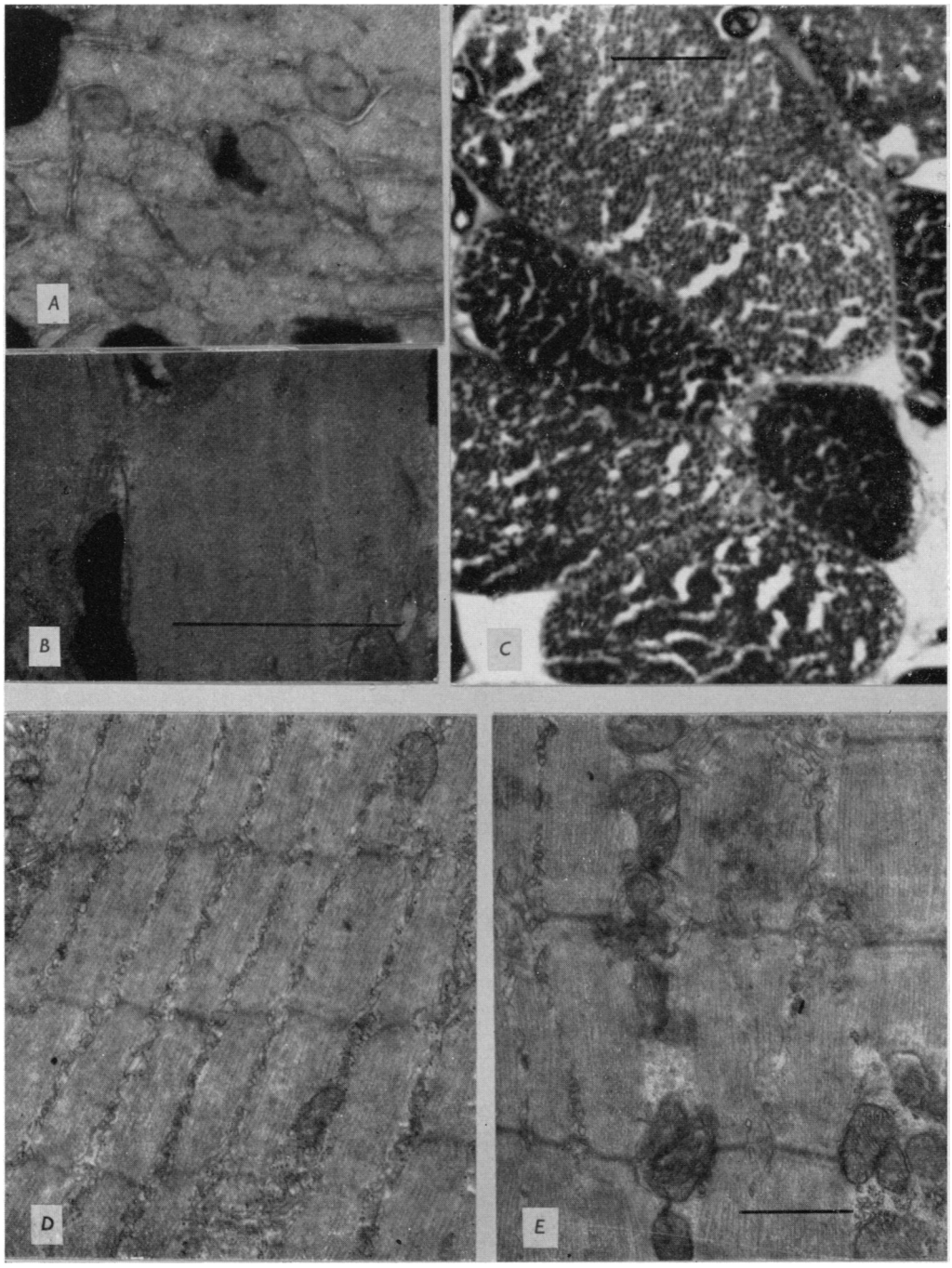
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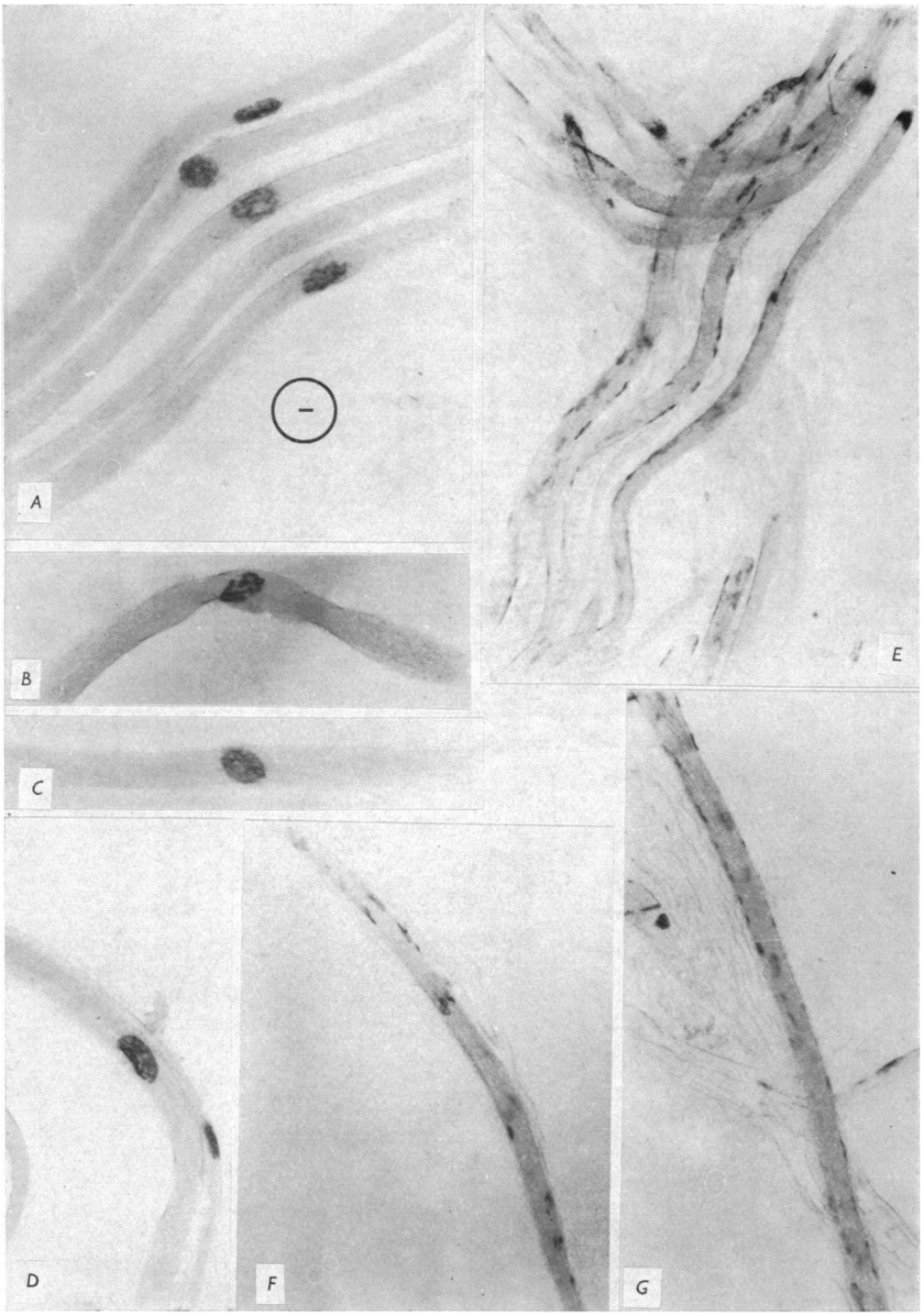
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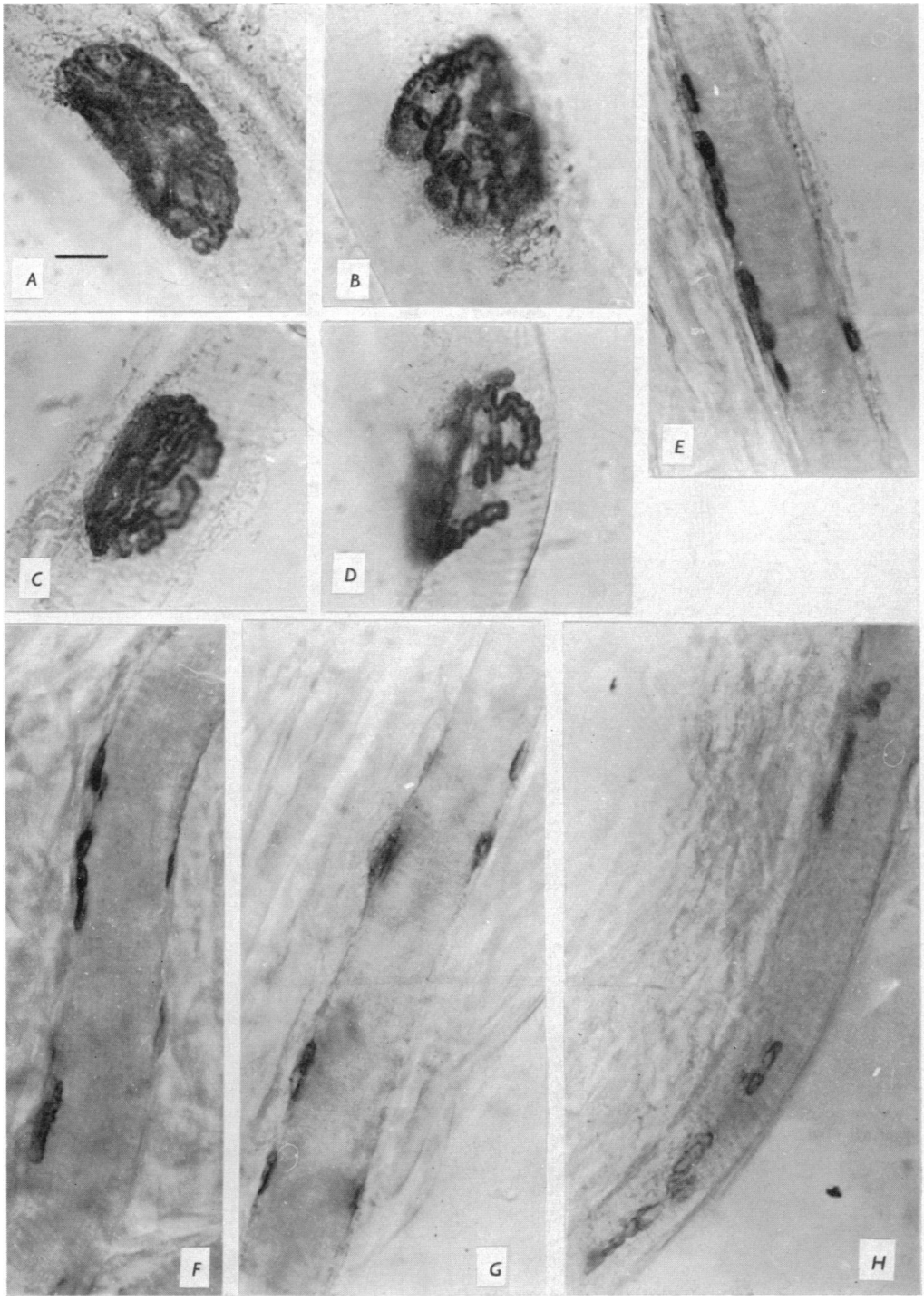
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