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A FURTHER STUDY OF ELECTRICAL RESPONSES IN SLOW AND TWITCH MUSCLE FIBRES OF THE FROG

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A number of muscles in the frog are known to contain two distinct nerve-muscle systems, a large-nerve (twitch) system and a small-nerve (slow) system (Kuffler, 1955). In twitch muscle fibres an all-or-none action potential is initiated at an end-plate region as a result of the action of the nerve transmitter (Eccles, Katz & Kuffler, 1941). The action potential propagates along the muscle fibre and brings about the chain of events leading to contraction. By contrast, the studies of Kuffler & Vaughan Williams, 1953*a*, *b*) and of Burke & Ginsborg (1956*b*) indicate that nerve stimulation does not produce action potentials in slow muscle fibres, and that contraction results from the depolarization produced by summation and electrotonic spread of numerous junctional potentials. Recently, Shamarina (1962) has claimed that nerve stimulation will produce all-or-none action potentials in a large percentage of 'tonic' muscle fibres in the tonus bundle of the frog's iliofibularis muscle. However, Shamarina's report does not provide evidence that the action potentials were recorded from muscle fibres innervated by small nerves. The aim of the present study was to determine whether muscle fibres in the tonus bundle which respond to nerve stimulation with propagated action potentials belong to the small-nerve system or large-nerve system, or constitute an intermediate type of muscle fibre.

METHODS

In the frog no single muscle or portion of a muscle is known to contain only 'slow' muscle fibres (Krüger, 1952; Kuffler & Vaughan Williams, 1953*b*). In order to prevent the whole muscle from twitching while studying the responses of the slow muscle fibres it has usually been necessary to stimulate the small-nerve fibres selectively (Kuffler & Vaughan Williams, 1953*a*). I-min (1960) has reported that following acute partial denervation of the iliofibularis one obtains a preparation which does not twitch in response to a single nerve stimulus. A partially denervated preparation similar to that described by I-min was used in these experiments.

Experiments were performed on the iliofibularis muscle of frogs (*Rana temporaria*) kept in the laboratory for 2–5 months. In about half the preparations studied the nerve to the iliofibularis was found to divide into two branches before entering the muscle, as indicated by I-min (1960). The thinner branch entered the muscle in the region of the tonus bundle (Somerkamp, 1928); the thicker branch coursed towards the belly of the muscle. The entrance of the thinner branch was proximal to the entrance of the thicker branch. The

ratio of the diameters of the two branches varied from about 2 : 1 to 10 : 1. In the remaining preparations the nerve was found to divide into 3–6 branches before entering the muscle. The pattern of innervation varied between one leg and the other in the same frog. Partial denervation was accomplished under a binocular microscope (× 30) by cutting all but the most proximal nerve branch.

This procedure did not always denervate all the muscle fibres outside the tonus bundle; that is, nerve stimulation occasionally produced twitch contractions in the 'twitch' portion of the muscle. In a few preparations evidence was obtained that neuromuscular junctions within the tonus bundle had been denervated: fibres in the tonus bundle were impaled in which spontaneous miniature end-plate potentials were recorded, but no response was produced in these fibres by nerve stimulation.

The sciatic iliofibularis preparation was bathed in Ringer's solution (mM: NaCl 115, KCl 2·0, $CaCl_2$ 1·8) at room temperature (18–25° C). Conventional micro-electrode techniques were employed for intracellular recording (Fatt & Katz, 1951). Movement during contraction was decreased by stretching the muscle to about 120 % of its *in situ* length. Extracellular recording was obtained by mounting the iliofibularis vertically, pelvic end up, at its *in situ* length, in a chamber similar to one used by Fatt (1950). One Ag–AgCl electrode was connected via a Ringer-agar-filled capillary to the pelvic tendon of the muscle. A second electrode was immersed in the Ringer's solution, the level of the fluid forming a movable electrode. Usually the level of the fluid was kept at that of the middle of the tonus bundle. The nerve was placed across two platinum stimulating electrodes in the moist air above the muscle. In some experiments the small-nerve fibres were selectively stimulated by the method described by Burke & Ginsborg (1956*a*). The length of nerve from the stimulating electrode to the muscle was between 20 and 25 mm. Isometric tension responses were recorded with the aid of a transducer valve (RCA 5734).

RESULTS

Electrical responses following acute partial denervation

Partial denervation by cutting all but the thin nerve branch leading to the tonus bundle was performed in 32 muscles. This procedure reduced the visible twitch response of the iliofibularis to a single stimulus applied to the sciatic nerve in 25 preparations and abolished the twitch response in 7 preparations (viewed at 30 ×). In 20 of these preparations, including 5 which did not twitch, the region of the tonus bundle was explored with an intracellular electrode. Three types of electrical responses could usually be recorded from different muscle fibres following a single nerve volley: (1) a muscle spike of 95–130 mV sometimes seen to be preceded by an end-plate potential (e.p.p.) step (cf. Fatt & Katz, 1951); (2) a subthreshold potential (5–28 mV) of short latency (1·6–3·0 msec) with the properties of the e.p.p. found normally in the frog's submentalis muscle (Kuffler, 1952) and occasionally in the uncurarized sartorius muscle (Fatt & Katz, 1951); (3) a long-latency (5–9 msec) end-plate potential (3–15 mV) with the properties of the slow junctional potential (s.j.p.) recorded from 'slow' muscle fibres (Kuffler & Vaughan Williams, 1953*a*; Burke & Ginsborg, 1956*a*, *b*). There was no difference in latency between the e.p.p. in fibres of type 1 in which the e.p.p. led to an all-or-none action potential and

fibres of type 2 in which it failed to do so. Also, there was no difference in the strength of the nerve stimulus required to elicit an action potential or a short-latency e.p.p. However, stronger nerve stimuli were needed to elicit long-latency junctional potentials.

The proportion of fibres giving a particular type of response varied greatly from one muscle to the next. In many preparations a strong twitch response followed a single nerve impulse, suggesting that a large number of fibres responded with a muscle spike; these preparations were difficult to study with intracellular electrodes and were not thoroughly examined. In others the twitch response was weaker and all three types of responses were recorded from different fibres. In each of the five preparations which failed to twitch to a single nerve stimulus, a few fibres were impaled which responded with a short-latency e.p.p. Careful surface exploration failed in some muscles to reveal any fibres with long-latency response, although repetitive small-nerve stimulation did produce a slow contraction. In other muscles as many as 12 fibres with long-latency responses were found on the surface of the muscle.

An important distinction between slow and twitch muscle fibres of the frog is that the former are multiply innervated by axons of small diameter, high threshold and slow conduction; the latter by axons of large diameter, low threshold, and fast conduction (Kuffler, 1955). It appears, therefore, that in the present experiments those fibres which responded to a nerve impulse with an e.p.p. of short latency (less than 3 msec), whether immediately followed by a spike or not, belonged to the twitch system, and that fibres which responded with the long-latency (greater than 5 msec) junctional potential belong to the slow system.

That the above classification is correct was supported by several other observations which may be compared with those of Kuffler & Vaughan Williams (1953*a*). The short-latency response (e.p.p.) was recorded from 105 fibres whose resting potential ranged between 80 and 100 mV, the long-latency response (s.j.p.) from 46 fibres with resting potentials between 40 and 85 mV (see Kiessling, 1960). Figure 1 shows that in successive recordings with a given stimulus intensity the amplitudes of both types of responses fluctuated. However, while the e.p.p. occurred usually in all-or-none fashion at a critical stimulus strength and did not increase with higher stimulus intensities, the s.j.p. showed several increments as the stimulus strength was raised. The abrupt changes in amplitude of the s.j.p. indicate that these muscle fibres are innervated by several axons. The different rates of rise of the several components of the s.j.p. result from the spatial distribution of the axon terminations along the muscle fibre. By contrast the e.p.p. had a more simple rising phase, as would be expected if only one region in the neighbourhood of the recording electrode was

innervated. Additional differences between the two types of response were that the long-latency response decayed more slowly and usually exhibited an after-hyperpolarization. All these distinguishing features are in accord with the characteristics described by Kuffler & Vaughan Williams (1953*a*) for the 'twitch' and 'slow' systems.

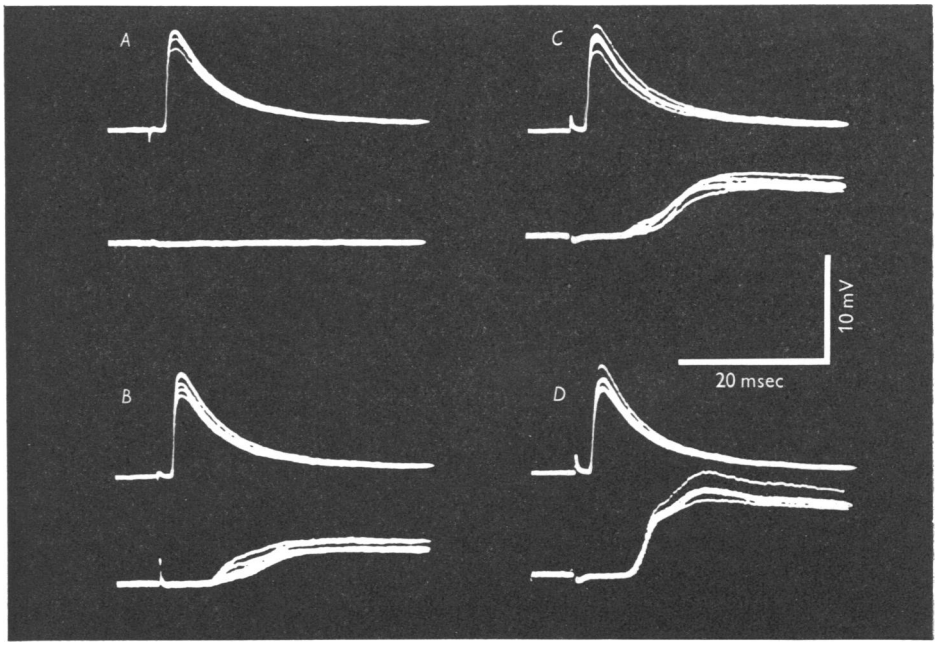

Fig. 1. Simultaneous intracellular recording from two muscle fibres within the tonus bundle. Stimulus intensity to the nerve increased in three steps from *A* to *D*. Five superimposed sweeps at each stimulus intensity. Top record from fibre with resting potential of 88 mV; bottom record from fibre with resting potential of 63 mV. Conduction distance was 23 mm.

On two occasions the amplitude of the short-latency response was found to vary abruptly with change in the stimulus intensity, indicating that occasionally the same region of a 'twitch' muscle fibre was innervated by more than one axon (cf. Kuffler, 1942). In 37 fibres, however, it was not possible to fractionate the short-latency response by finely grading the intensity of the nerve stimulus. Hyperpolarization did usually but not always follow the long-latency s.j.p. (cf. Oomura & Tomita, 1960), and in a few fibres hyperpolarization was seen during the decline of a short-latency action potential. In the latter cases hyperpolarization increased if there was a fall in membrane potential during repeated responses, resulting probably from mechanical damage during contraction. It is clear that multiple innervation of a fibre and hyperpolarization during the decline of the response are not, by themselves, unfailing criteria for differentiating between twitch and slow fibres.

Occasionally a fibre was impaled which responded to a single nerve impulse at times with a spike and at other times with a subthreshold e.p.p. In one preparation which initially failed to twitch to a single stimulus the

facilitation of neuromuscular transmission produced by stretching the muscle (Hutter & Trautwein, 1956) resulted in the muscle giving a visible twitch response. These observations further suggest that there is no clear distinction between fibres of response type (1) and (2), either in their innervation or in their ability to generate propagated action potentials. On the other hand, no fibre of type (3) was ever found to produce a spike potential. Over 200 innervated muscle fibres were examined during the course of the present study, and none was found to exhibit both short- and long-latency responses. Shamarina's suggestion (1962) that some of the muscle fibres in the tonus bundle are innervated by large as well as small axons is not supported by these experiments.

Quantum content of subthreshold e.p.p.

The amplitude of focally recorded e.p.p.s did not exceed 30 mV and was, therefore, below the threshold for the initiation of the muscle action potential (Fatt & Katz, 1951). A possible explanation for the small size of the e.p.p. is that there is a low output of transmitter from the nerve (Kuffler, 1952; Martin, 1955). The e.p.p. results from the synchronous release of a number of small 'packets' (quanta) of transmitter (del Castillo & Katz, 1954). In the absence of stimulation quanta are spontaneously released and give rise to miniature end-plate potentials (min. e.p.p.s). By measuring the amplitude of the min. e.p.p.s and of the e.p.p. one can determine the average number of quanta of transmitter released by the nerve impulse. Table 1 indicates how the quantum content of the e.p.p.

TABLE 1. Quantum content of e.p.p.

v (mV)	v_1 (mV)	v/v_1	m
6·1	0·52	11·7	12·8
8·6	0·33	26·0	29·2
26·6	0·44	60·5	85·9

v, mean e.p p. amplitude; v_1, mean amplitude of spontaneous miniature e.p.p.; v/v_1, uncorrected mean quantum content of e.p.p.; m, corrected mean quantum content.

was determined in three fibres. The nerve was stimulated 25 times at 5 sec intervals to obtain the mean amplitude of the e.p.p. The values are corrected for the non-linear relation between conductance and potential change which occurs when the e.p.p. amplitude is greater than about 5 mV (Martin, 1955). However, they do not take into account the slight depression of e.p.p. amplitude during stimulation at 5 sec intervals (Takeuchi, 1958) or the increase in transmitter output as a result of stretching the muscle to 120 % of its rest length (Hutter & Trautwein, 1956). Focally recorded min. e.p.p.s from different twitch fibres had an amplitude from 0·2 to 1 mV, and were within the range of min. e.p.p. amplitudes found in twitch fibres from other muscles (Katz & Thesleff, 1957).

Repetitive nerve stimulation

Shamarina (1962) reported that action potentials could be readily produced in 70% of the 'tonic' muscle fibres if repetitive nerve stimulation was used. In addition, Peachey & Huxley (1962) suggested that strong electrical stimulation may have elicited propagated action potentials from some of the slow fibres which they studied (cf. Burke & Ginsborg, 1956*a*).

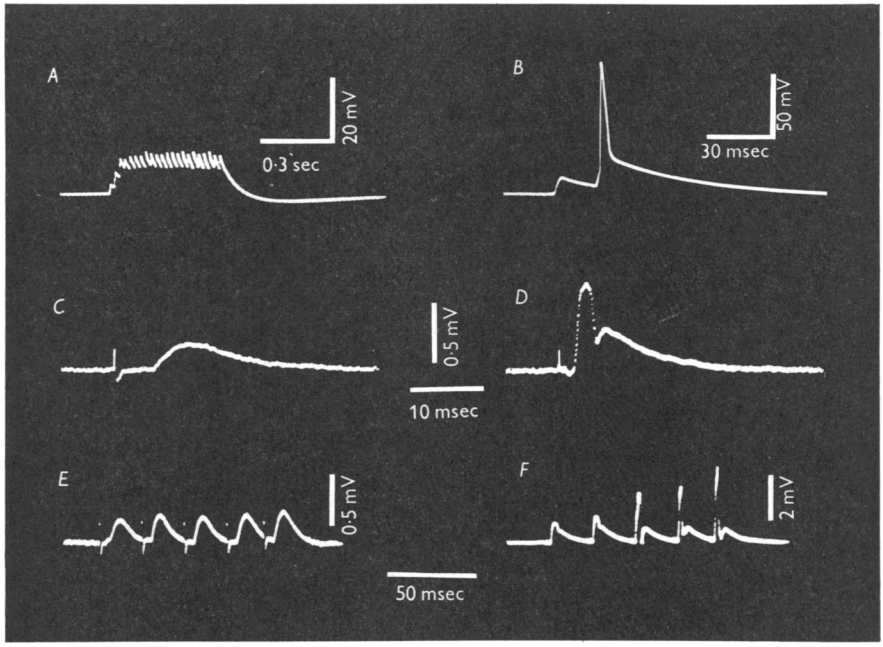

Fig. 2. *A*, Intracellular recording from slow muscle fibre during 22 stimuli to sciatic nerve at 50/sec: resting potential was 50 mV and latency of s.j.p. 6 msec. *B*, Intracellular recording from twitch muscle fibre during two nerve stimuli at 18 msec interval: resting potential was 80 mV and latency of e.p.p. 1·8 msec. *C–F*, Extracellular recording from partially denervated muscle. *C*, Selective stimulation of small-nerve fibres. *D*, Stimulation of large-nerve fibres. *E*, Stimulation of small-nerve fibres at 40/sec. *F*, Stimulation of large-nerve fibres at 40/sec. Vertical line on trace indicates time of stimulus in *C* and *D*. Note difference in latency for response.

The possibility that repetitive nerve stimulation will produce action potentials in slow fibres was, therefore, reinvestigated. When the microelectrode was inserted into a slow muscle fibre (latency for s.j.p. greater than 5 msec) repetitive nerve stimulation (20–60/sec) produced a summation of the s.j.p. until a 'plateau' depolarization was reached. The depolarization did not exceed one half the resting potential and never led to the appearance of an all-or-none action potential. Figure 2*A* illustrates the

response of one of the 23 fibres studied in this way. On the other hand, as shown in Fig. 2*B*, when the micro-electrode was in a twitch fibre (latency for e.p.p. less than 3 msec), repetitive stimulation of the nerve led to the appearance of an all-or-none spike.

Extracellular recording from partially denervated muscles confirmed these results. Figure 2*C* illustrates the response obtained when the small nerves were selectively stimulated. When the small nerves were repetitively stimulated at 40/sec (Fig. 2*E*) the amplitude of the recorded potential increased, but the slow time course was not changed. The response of the same preparation to stimulation of large-nerve fibres (at 1·2 times threshold) is illustrated in Fig. 2*D*. The latency for the response was short, indicating that the fast-conducting nerves had been stimulated. With this stimulus strength repetitive stimulation at 40/sec produced a marked change in both the amplitude and time course of the response. The 'spike' component increased during the tetanus, suggesting that the number of fibres producing full-size action potentials increased with successive stimuli. In 7 of 12 preparations studied with extracellular recording repetitive large-nerve stimulation did not result in an increase in the amplitude of successive action potentials. This would suggest that in these 7 preparations a single nerve impulse was sufficient to produce an action potential in almost all the innervated twitch fibres (cf. Wakabayashi & Iwasaki, 1962).

Mechanical responses

Isometric tension responses and extracellular electrical responses were simultaneously recorded during selective stimulation of large- or small-nerve fibres. Figure 3*A* indicates that a single nerve impulse in small-nerve fibres produced a slow potential change but no significant tension response. Repetitive small-nerve stimulation produced a measurable tension response without the appearance of a muscle 'spike' (Fig. 3*B*). A single impulse in large-nerve fibres produced a muscle spike accompanied by a twitch contraction (Fig. 3*C*). With repetitive large-nerve stimulation the muscle action potential amplitude slightly increased and a tetanic contraction was produced (Fig. 3*D*). This experiment confirms the previous conclusion (Kuffler & Vaughan Williams, 1953*a*, *b*) that the muscle twitch is produced by action potentials in muscle fibres innervated by large-nerve fibres and that repetitive small-nerve stimulation will produce tension responses from slow muscle fibres without a muscle spike. In some preparations a single impulse in small-nerve fibres did produce a visible 'slow' tension response (cf. Burke & Ginsborg, 1956*a*). This slow tension response was never found to be preceded by a muscle 'spike'.

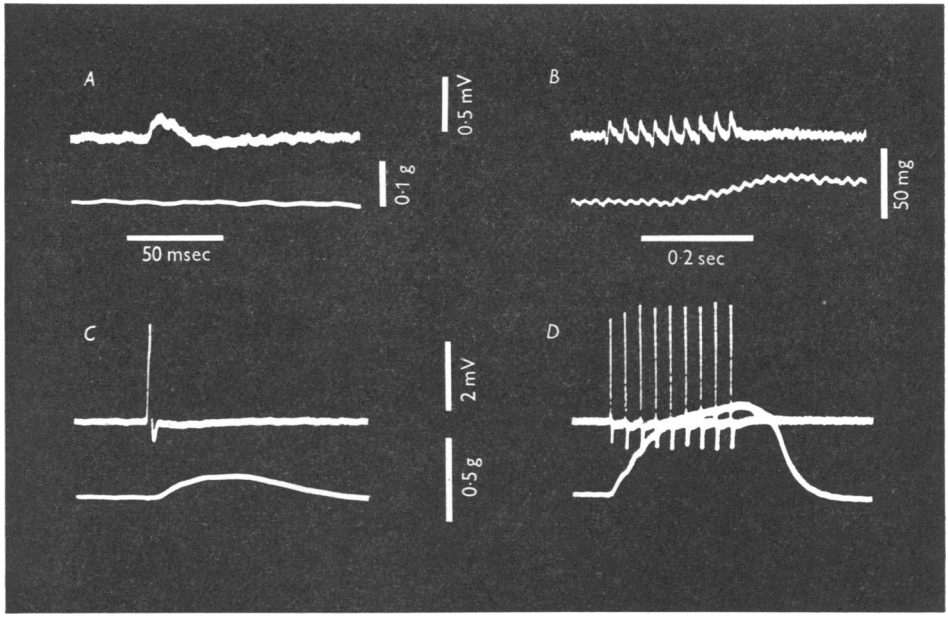

Fig. 3. Above, extracellular electrical responses and (below) isometric tension responses from a partially denervated muscle. *A*, Selective single stimulation of small-nerve fibres. *B*, Repetitive stimulation of small-nerve fibres at 42/sec. 'Noise' on tension trace results from background vibration. Note increased amplification of tension recording. *C*, Single stimulus applied to large nerve fibres (at twice threshold). *D*, Repetitive stimulation of large-nerve fibres at 42/sec.

DISCUSSION

Muscle fibres in the tonus bundle of the iliofibularis muscle in which propagated action potentials were recorded after single or repetitive nerve stimulation belong to the large-nerve (twitch) system, as they were found to be innervated by low-threshold rapidly conducting axons. Propagated action potentials were not recorded from 'slow' muscle fibres (innervated by high-threshold, slowly conducting axons) even after prolonged repetitive nerve stimulation. These results, therefore, confirm the observations of Kuffler & Vaughan Williams (1953*a*), and are opposed to the view put forward by Shamarina (1962) that nerve stimulation will produce action potentials in many 'slow' muscle fibres. The failure of the present study to find even a single fibre with both a short- and a long-latency response supports the previous physiological (Kuffler & Vaughan Williams, 1953*a*) and anatomical (Gray, 1957; Hess, 1960) evidence, that the small-nerve and large-nerve systems are separate.

The statistical distribution of the apparent conduction velocities

((latency of response)/(length of nerve from point of stimulation to muscle)) of the responses was bimodal and without a region of overlap. Apparent conduction velocities were either between 2·2 and 4·5 m/sec or between 7 and 14 m/sec. The studies of Kuffler & Gerard (1947) and Kuffler & Vaughan Williams (1953*a*) indicate that some large-nerve and some small-nerve fibres have conduction velocities of about 8 m/sec. However, their measurements were made along a length of nerve outside the muscle. The clear separation between the two systems observed in the present work is probably the result of the difference in myelination and branching of the two types of nerve fibres during their intramuscular course. The small-nerve fibres break up into trusses of fine non-myelinated axons which ramify for a considerable distance before reaching the slow fibres. The large-nerve fibres lose their myelin only when reaching an end-plate (Gray, 1957).

The present experiments do not support the conclusion of I-min (1960) that partial denervation is effective in completely separating the small-nerve system from the large-nerve system. I-min's conclusion is based on his observation that after cutting the thick nerve branch leading to the belly of the muscle the preparation did not 'twitch' after a single nerve stimulus, but did contract with repetitive nerve stimulation. In the present study muscle fibres were still found to be innervated by large axons even when partial denervation had abolished the twitch response to a single nerve impulse.

It has been estimated that an e.p.p. usually must result from the action of more than 100 quanta of transmitter to depolarize the end-plate region to the threshold for the propagated action potential (Martin, 1955). The relatively low quantum content of the e.p.p. found in fibres in which a single e.p.p. failed to initiate an action potential can account for the low safety factor for neuromuscular transmission. A large variation in the safety factor for transmission in other frog muscles has been suggested by results from the sartorius muscle (Fatt & Katz, 1951; Ralston & Libet, 1953; Wakabayashi & Iwasaki, 1962), submentalis muscle (Kuffler, 1952) and the ext. l. dig. IV (Martin, 1955). The physiological significance of this low safety factor is not known, and the possibility need be borne in mind that the incidence of transmission failure may be increased in isolated nerve-muscle preparations. A possible explanation for the relatively low quantum content of the e.p.p. observed at some end-plates is that the area of synaptic contact may be smaller at these junctions. In this connexion it is interesting to note that Hess (1960) finds that many 'twitch' endings in the tonus bundle are relatively shorter than the typical 'end-bushes' found in the twitch portion of the muscle.

SUMMARY

1. Neuromuscular transmission in the tonus bundle of the frog's iliofibularis muscle has been studied with the aid of partial denervation and by intracellular and extracellular electrical recording.

2. Individual muscle fibres were found to be innervated either by 'small' motor axons (high threshold, long latency for junctional potential) or 'large' motor axons (low threshold, short latency for junctional potentials).

3. In muscle fibres innervated by large axons all-or-none action potentials could be evoked by single or repetitive nerve stimulation. In muscle fibres innervated by small axons repetitive nerve stimulation produced summation of junctional potentials but never elicited an all-or-none action potential.

4. In some of the partially denervated muscles a single impulse in large nerve fibres failed to elicit a muscle spike and produced only a short latency end-plate potential in some of the muscle fibres.

5. The failure of a single nerve stimulus to lead to an action potential in some twitch fibres can be attributed to a low 'quantum content' of the end-plate potential.

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