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SOME PROPERTIES OF AVIAN SKELETAL MUSCLE FIBRES WITH MULTIPLE NEUROMUSCULAR JUNCTIONS

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It has recently been found that certain skeletal muscles of the bird contain fibres which are innervated at many points (Ginsborg, 1960; Ginsborg & Mackay, 1961). The purpose of this paper is to describe some of the characteristics of these fibres. In particular, it will be shown that they are innervated by several axons, that they are able to produce propagated action potentials and that they respond to prolonged depolarization with a prolonged contracture. The experiments have been mainly carried out on the chick, either on single multiply-innervated fibres of the isolated biventer cervicis, or on the isolated anterior latissimus dorsi nerve-muscle preparation. The advantage of the latter preparation is that the anterior latissimus dorsi is composed largely, or perhaps entirely, of fibres with multiple neuromuscular junctions (Ginsborg & Mackay, 1961). The properties of such fibres may therefore be inferred from the properties of the muscle as a whole. Some of the results have been briefly reported (Ginsborg, 1959; Ginsborg & Mackay, 1960).

METHODS

The preparations were removed from chicks (Brown Leghorns) about 3 weeks old. In two experiments latissimi dorsi were taken from a pigeon and a rook, both apparently adult, but of unknown age. The birds were previously anaesthetized with I.V. Na phenobarbitone solution 9 g/100 ml., 2 ml./kg, or decapitated. The biventer cervicis preparation has already been described (Ginsborg, 1960; Ginsborg & Warriner, 1960). The latissimi dorsi of the chick (Fig. 1) and the rook are in two separate parts, an anterior and a posterior, lying immediately beneath the skin and extending between the mid line and the humerus. In the pigeon the posterior part is not present. The anterior latissimi dorsi were removed together with part of the humerus to which the peripheral end is attached. The nerve supply to the muscle is contained in three separate trunks which lie side by side for some way. For electrical recording, one branch (*a*), which appears to innervate part of the 'central' end of the muscle, was generally cut in the course of removing the connective tissue lying over the surface of the muscle, in order to avoid artifacts.

Intracellular recording. The biventer cervicis was mounted in Krebs–Henseleit solution (1932) as has been described previously (Ginsborg, 1960). The methods for recording from and polarizing single fibres with micro-electrodes were conventional (Fatt & Katz, 1951). The nerve was freed from the cranial tendon which encapsulates it (Ginsborg & Warriner,

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1960) and the cut end was sucked into a capillary electrode (Furshpan & Potter, 1959). Stimuli were applied via an RF isolating unit (Schmitt, 1948) to minimize stimulus artifacts.

To avoid breaking an excessive number of electrodes it was necessary to reduce the muscle twitch which occurred on nerve stimulation. The experiments were therefore made at room temperature, and parts of the muscle were often made unresponsive to nerve stimulation either by cutting intramuscular branches of the nerve or by replacing part (up to 80 %) of the Na content of the bathing solution with tris-(hydroxymethyl)-amino-methane chloride (Lüttgau & Niedergerke, 1958).

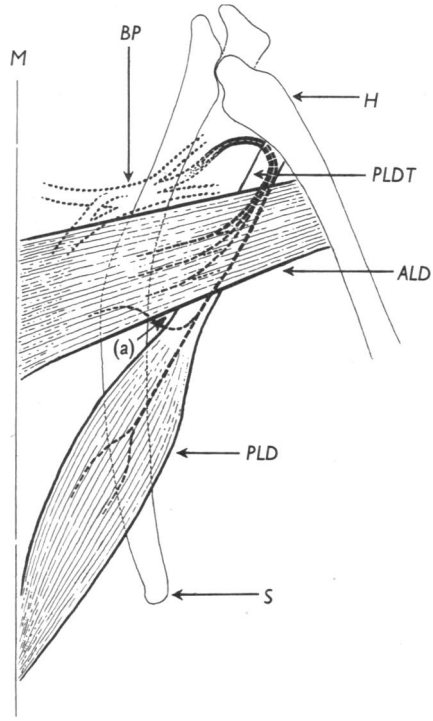

Fig. 1. Diagram of dorsal view of right latissimus dorsi of chick. The nerve supply is exposed by detaching the 'central' end of the muscles from the supraspinous ligament on the mid line and reflecting them about the humerus. Part of the scapula overlying the brachial plexus is then removed and the branches of the median nerve which supply the muscles may then be freed from the surrounding connective tissue. *ALD*, anterior latissimus dorsi; *BP*, brachial plexus; *H*, humerus, *M*, mid line; *PLD*, posterior latissimus dorsi; *PLDT*, tendon of *PLD*; *S*, scapula; *a*, see text.

Extracellular recordings were made from latissimus dorsi muscles with either platinum electrodes connected to the input of an a.c. amplifier, or with Ag–AgCl–agar wick electrodes connected to the input of a d.c. amplifier. For a.c. recording the muscles were usually mounted vertically on a multi-electrode assembly consisting of a rectangular Perspex frame into which ten horizontal platinum wires 4 mm apart were sealed. The nerve was threaded through a hole in the frame into which two concentric platinum annuli, which served as stimulating electrodes, were sealed. The preparations were mounted in a closed chamber containing Krebs–Henseleit solution, which was drained only for the short periods

required for recording. For d.c. recording one wick electrode was usually kept fixed at one end of the muscle, and the bathing fluid surface, below which dipped the second wick, served as the other recording electrode (Fatt, 1950). The preparations did not appear to deteriorate over periods of several hours.

Isometric tension changes were recorded conventionally with an R.C.A. 5734 mechano-electric transducer coupled either to an oscilloscope or to a pen recorder.

RESULTS

Intracellular recording from fibres with multiple end-plates

Multiple innervation. Figure 2 illustrates the response to nerve stimulation of three fibres of the biventer cervicis in which the miniature end-plate potentials were characteristic of muscle fibres with many neuro-

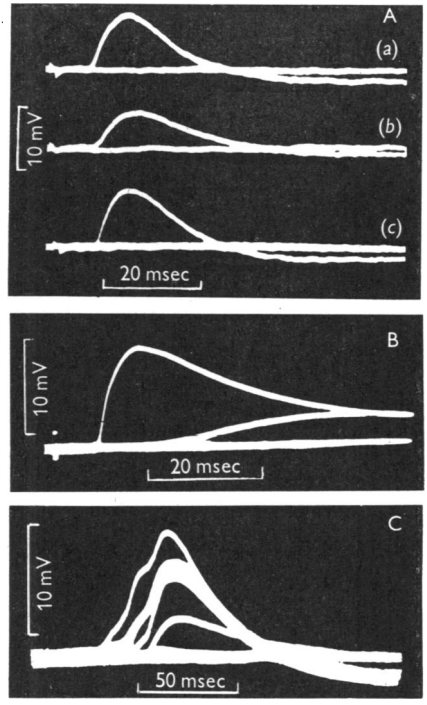

Fig. 2. Intracellular records of responses of three multiply-innervated fibres of biventer cervicis muscles *A*, *B* and *C*, to graded stimuli applied to the nerve. Temperature, 19° C. Movement of muscles reduced in *A* by cutting several intramuscular nerve branches, in *B* by replacing 80 % NaCl in bathing solution by tris-(hydroxymethyl)-amino-methane Cl. In *A* responses (*a*) and (*c*) were evoked by stimuli of equal strength.

muscular junctions along the fibre (see Ginsborg, 1960). It was evident that such fibres were innervated by several axons.

In several fibres the smaller responses, i.e. those to smaller stimuli, which therefore resulted from the stimulation of larger-diameter and more

rapidly conducting motor axons, occurred with a longer latency than those to the larger stimuli (Fig. 2*B* and *C*). This result suggests that very extensive branching of these axons occurs or that different axons supplying the same muscle fibre have very different lengths.

Action potentials in response to nerve stimulation. Of the seventy-one multiply-innervated fibres of the biventer cervicis muscles which were

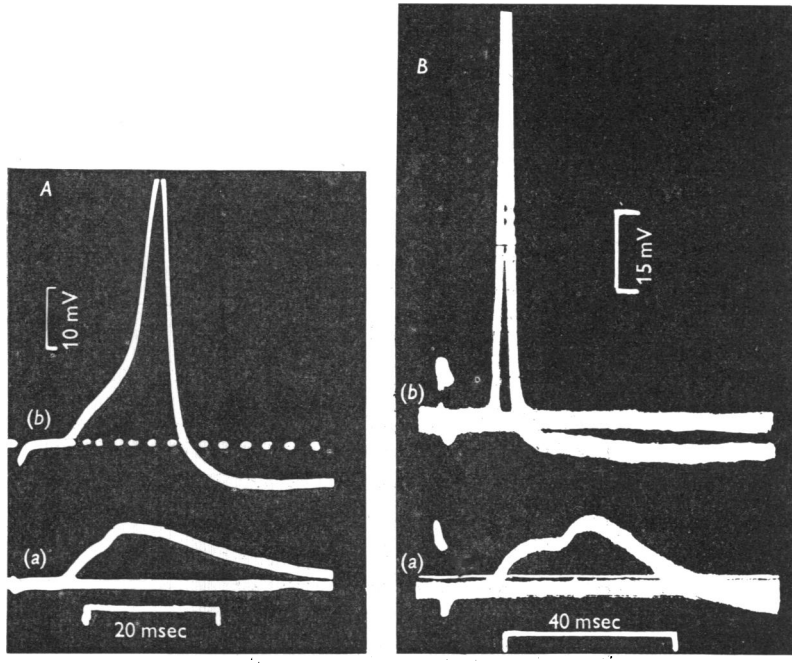

Fig. 3. Intracellular records of responses to submaximal (*a*) and maximal (*b*) nerve stimulation of two fibres, *A* and *B*, of biventer cervicis muscles. Temperature, 19° C. Muscle bathed in Krebs–Henseleit solution with 80 % NaCl replaced by tris-(hydroxymethyl)-amino-methane Cl.

tested only seven were observed to respond to maximal nerve stimulation with action potentials. Two examples are shown in Fig. 3. From the results to be described below it seems likely that the absence of action potentials in the remainder was due, not to a genuine inability to produce action potentials, but to the measures taken to reduce the muscle twitch (see Methods) and to the reduction in resting potential caused by the insertion of the micro-electrode.

Response to direct stimulation. Whenever it was possible to insert a second, polarizing, micro-electrode into a multiply-innervated fibre, it was possible also by some means to evoke an action potential. In three of nineteen fibres an electrotonic depolarization was effective (Fig. 4*A*).

Although measurements of resting potential were unreliable (see Methods in Ginsborg, 1960) in these three fibres the resting potentials appeared to be above 56 mV. The remaining fibres appeared to be in a state of 'cathodal depression' (see for example, Lorente de Nó, 1947), since they could only be stimulated either by 'anode-break' excitation (Fig. 4 B ii) or by applying an electrotonic depolarization after raising the resting potential at the site of the micro-electrodes by means of a steady hyperpolarizing current.

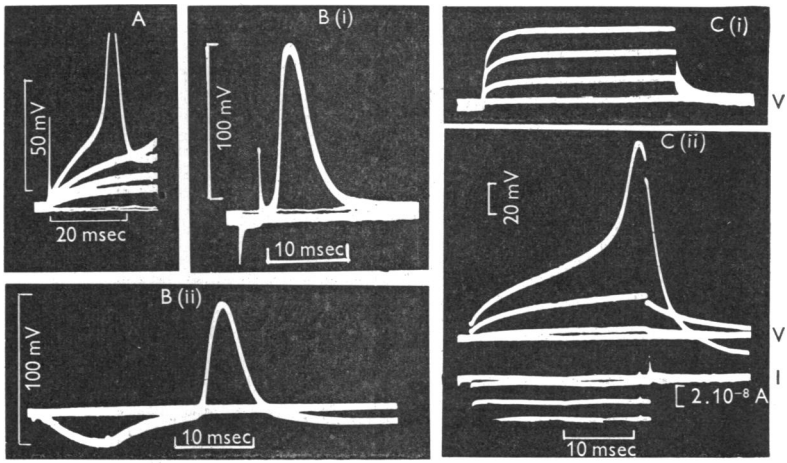

Fig. 4. Intracellular records of action potentials from three multiply-innervated fibres, *A*, *B*, *C*, of the biventer cervicis. Temperature, 18° C. *A*: resting potential, 66 mV; fibre stimulated with internal polarizing electrode close to recording electrode; three subthreshold responses also shown. *B*: (i) resting potential, 83 mV; stimulation by second micro-electrode external to fibre and 1 mm away from recording electrode; (ii) resting potential, 52 mV after insertion of polarizing electrode into the fibre; it could no longer be stimulated by depolarization, but responded to anode break excitation. Identical voltage and time scales in (i) and (ii). *C*: V, voltage; I, current; (i) resting potential, 58 mV; no action potential in response to depolarization; (ii) resting potential set to 100 mV by steady current (not shown) passed inwards across membrane; a sufficiently large outward current pulse (I) stimulated the fibre. Identical voltage and time scales in (i) and (ii).

Many of the responses, both end-plate and action potentials terminated with a phase of hyperpolarization (Figs. 2–4). It is very likely that this feature although involving 'delayed rectification' (Hodgkin, 1951; cf. Burke & Ginsborg, 1956), was associated, like the inability to produce action potentials, with the depression of the resting potential caused by inserting micro-electrodes into fibres of small diameter and therefore of high resistance (cf. Katz & Thesleff, 1957; Koketsu & Nishi, 1957*b*). This is strongly suggested by the experiment illustrated in Figs. 4 B (i) and (ii). In this fibre with a high resting potential, 83 mV, an action potential was evoked by external stimulation (i.e. by an external micro-electrode closely applied to the fibre and made negative with respect to the bath). This action potential was not followed by a phase of hyperpolarization (Fig. 4 B i). When the polarizing electrode was inserted into the fibre (Fig. 4 B ii) the resting potential fell by 31 mV and the fibre failed to respond to direct depolarization. It was, however, stimulated by the break of an anodic pulse, and the action potential was now followed by a phase of hyperpolarization.

Extracellular recording

The preceding results suggest that the multiply-innervated fibres in the muscles of the chick can produce propagated action potentials and that at least some of them do so on maximal nerve stimulation. More convincing evidence is provided by the results of experiments made on the anterior

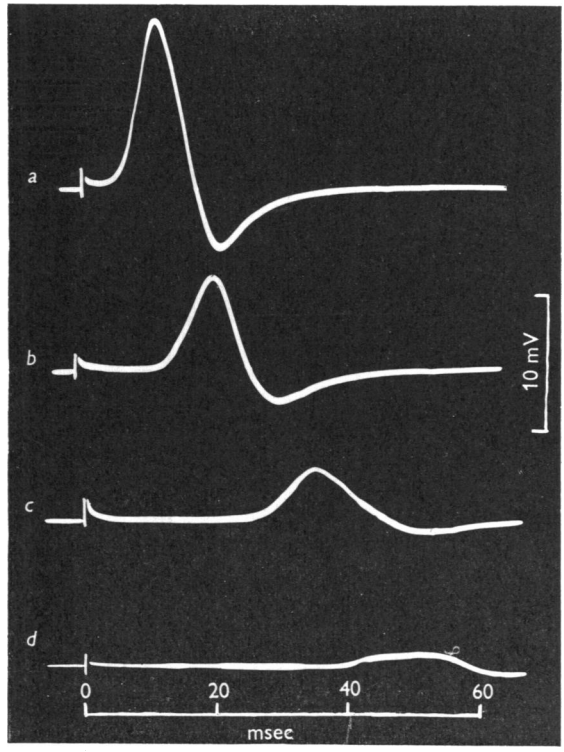

Fig. 5. Action potentials recorded externally from an anterior latissimus dorsi muscle in response to direct stimulation (neuromuscular transmission blocked by tubocurarine Cl 8×10^{-5} M). 21-day-old chick, length of muscle 23 mm. Temperature, 28° C. Stimulating electrodes 2 mm apart at peripheral end of muscle. Recording electrodes 3 mm apart; distance between inner recording and stimulating electrodes: *a*, 2 mm; *b*, 6 mm; *c*, 12 mm; *d*, 18 mm. Stimulus artifacts reinforced with short vertical lines.

latissimus dorsi, which has been shown to consist largely, or perhaps exclusively, of fibres with multiple neuromuscular junctions (Ginsborg & Mackay, 1961).

Response to direct stimulation. Figure 5 shows unequivocally that the fibres of the anterior latissimus dorsi are able to conduct action potentials and that at least some fibres extend the length of the muscle. The ampli-

tude, 14 mV, of the largest externally recorded action potential from the muscle was small, as compared, for example, to the amplitude of the action potential which can be recorded under favourable conditions from the frog's sartorius muscle (50–60 mV, Katz & Kuffler, 1941), but the fibres of the anterior latissimus dorsi of the 21-day-old chick are less than 20 μ in diameter, compared with 73 μ in the frog's sartorius (Katz, 1948, p. 523); and since the muscle was kept moist by vigorous bubbling with gas of the bathing fluid at the bottom of the chamber containing the muscle its extracellular resistance was probably low: a relatively small amplitude of the externally recorded action potential would therefore be expected (Hodgkin & Rushton, 1946).

The speed of conduction along the muscle was low; in six muscles, at temperatures between 28 and 34° C, the average speed of conduction varied between 0·41 and 0·70 m/sec. The slow speed of conduction cannot be entirely due to the small diameter of the fibres (Hodgkin, 1954) since the posterior latissimus dorsi, which is composed of fibres of somewhat smaller diameter, conducts at the rate of 2·3–2·8 m/sec (four muscles, 31–36° C).

Response to nerve stimulation. In Fig. 6 the responses to submaximal and maximal nerve stimulation of an anterior latissimus dorsi muscle are compared with the response of the same muscle to direct stimulation. The response to the submaximal stimulus was consistent with the fact that neuromuscular junctions are present along the whole length of the muscle. Action potentials initiated at the end of the muscle distant from the recording electrodes would be expected to take more than 30 msec to arrive at the electrodes (distance 22 mm, average speed of conduction 0·70 m/sec) and such action potentials may be seen to occur (Fig. 6*a*). On maximal nerve stimulation (Fig. 6*b*), however, the response was not longer in duration than the response to direct stimulation (Fig. 6*c*). Furthermore, if the electrodes were suitably placed, the peak amplitude of the response to maximal nerve stimulation was little smaller than the amplitude of the action potential evoked by direct stimulation. If one electrode was kept fixed and the other moved along the surface of the muscle there was only a small variation in the latency and time course of the response, the latency being least in the central part of the muscle, in which the nerves were seen to enter, and increasing towards each end of the muscle (Fig. 7). A simple way of explaining these results (cf. Katz & Kuffler, 1941) is to suppose that action potentials arise almost but not quite simultaneously at many neuromuscular junctions along each fibre of the muscle, the variation in latency being due to different conduction times along the motor axons to the neuromuscular junctions at different points along the muscle. In each fibre, then, after propagating through only a short distance, the action

potential collides with and abolishes the action potential from a neighbouring junction.

In some preparations isolated single maximal stimuli applied to the nerve gave rise to action potentials of irregular amplitude and duration. If, however, the stimuli followed a period of repetitive stimulation (for example, 100/sec for 30 sec), after a short interval the response became regular in amplitude and shorter in duration. In these preparations facilitation was apparently required at some of the junctions before action potentials were initiated at them (cf. Brown & Harvey, 1938; Hutter, 1952; Liley & North, 1953).

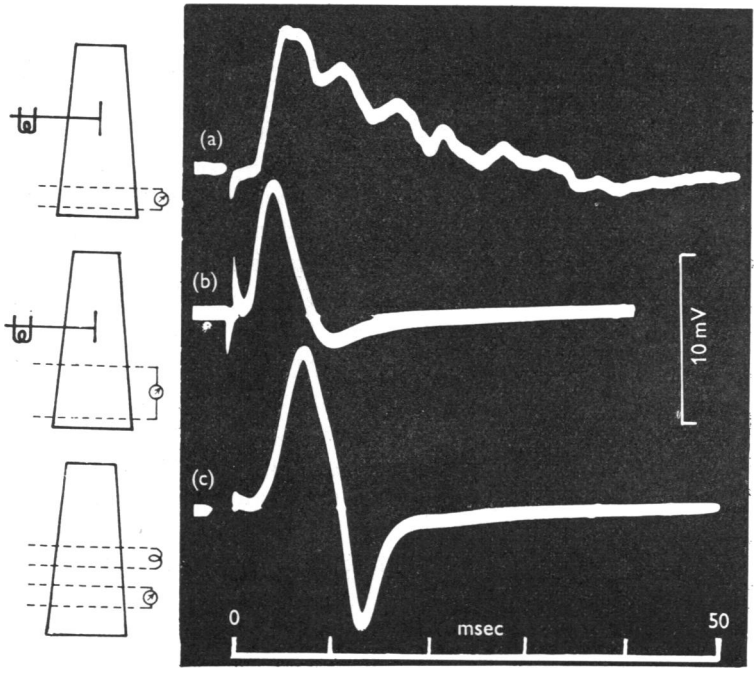

Fig. 6. Action potentials recorded externally from an anterior latissimus dorsi muscle 20-day-old chick. Muscle length, 28 mm. Temperature, 36° C. (*a*) Response to submaximal stimulation of nerve; recording electrodes 2 and 6 mm from central end of muscle; voltage calibration 1 mV. (*b*) Response to maximal nerve stimulation; recording electrodes 2 to 10 mm from 'central' end of muscle. (*c*) Response to direct stimulation (neuromuscular transmission blocked with 8×10^{-5} M tubocurarine Cl); recording electrodes 6 and 10 mm, stimulating 14 and 18 mm from 'central' end of the muscle. Voltage calibration (*b*) and (*c*) 10 mV.

Two tests of this explanation have been made, (*a*) by curarizing part of the muscle and (*b*) by cutting the nerve supply to part of the muscle.

Effect of curarizing part of the muscle. Figure 8 illustrates the effect on the action potential of blocking neuromuscular transmission in part of the muscle. In the 'curarized' region the latency of the action potential increases by the time necessary to propagate from neuromuscular junctions in the 'uncurarized' part of the muscle. In the 'uncurarized' part of the

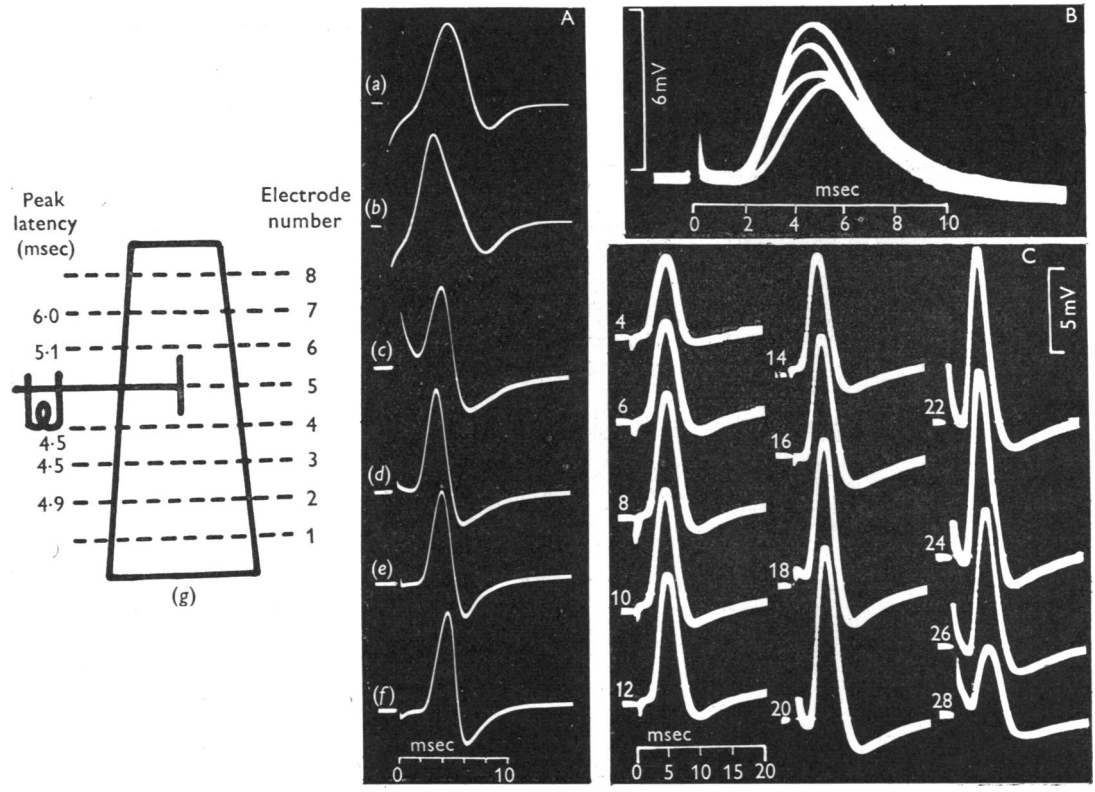

Fig. 7. Action potentials recorded externally from three anterior latissimi dorsi, *A*, *B*, *C*, in response to maximal nerve stimulation. *A*: 22-day-old chick; muscle length, 30 mm; temperature 33° C. Muscle mounted on multi-electrode assembly (*g*), electrodes 4 mm apart. Action potentials recorded between electrodes **7**, 8(*a*); **6**, 8(*b*); **5**, 1(*c*); **4**, 1(*d*); **3**, 1(*e*); **2**, 1(*f*); upward deflexion denotes negativity of electrode with bold number. Interval between first peak of response and stimulus artifact at different points along muscle indicated below in (*g*). *B*: 22-day-old chick; muscle length, 24 mm; temperature, 36° C; superimposed sweeps; indifferent electrode 2 mm from 'central' end of muscle which was treated with 132 mM-$MgCl_2$ to make responses monophasic (Niedergerke, 1956). Distance between electrodes in order of increasing amplitude of responses: 6, 8, 10, 12 mm; average speed of conduction subsequently determined after curarization, 0·5 m/sec. *C*: 28-day-old chick; muscle length, 34 mm; temperature, 35° C, d.c. recording. Indifferent agar wick electrode at 'central' end of muscle; second electrode, fluid surface. Distances in mm between fluid surface and indifferent electrode indicated by number at the side of each response.

muscle only minor changes occur in the action potential in spite of the curarization of the remaining two thirds of the muscle. These changes are probably accounted for by the diffusion of the tubocurarine applied to the lower part of the muscle into the 'uncurarized' region and the resulting blockade of the more susceptible junctions.

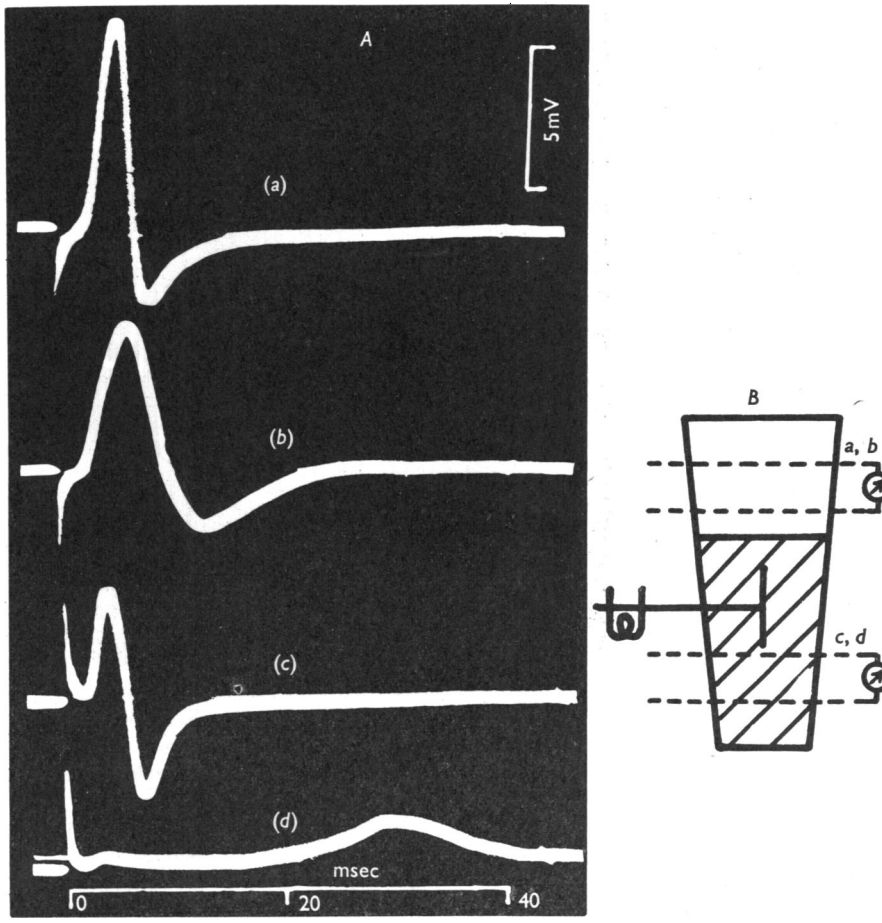

Fig. 8. Effect of curarization of part of muscle on externally recorded action potential from an anterior latissimus dorsi muscle: same preparation as Fig. 7*C*. Agar wick electrodes, d.c. recordings. (*a*) and (*c*) recorded (with electrodes in position shown in *B*) before curarization. (*b*) and (*d*) recorded from same positions after the part of the muscle shown shaded in *B* had been bathed in 5×10^{-5} M tubocurarine Cl for 7 min. Upward deflexion denotes negativity of electrode nearer the centre of the muscle.

Effect of cutting part of nerve supply. The nerve to the anterior latissimus dorsi branches extensively before entering the muscle; it is therefore possible by cutting the appropriate branches to denervate acutely part of

the muscle. The effect of this procedure on the indirectly evoked action potential is illustrated in Fig. 9. In the part of the muscle to which the nerve supply was 'intact' (Fig. 9*A a, d*) there were only minor changes in the time course and amplitude of the response. It is likely that these changes occurred because some muscle fibres had been totally denervated. In the 'denervated' region, the latency of the response was increased by the time taken for action potentials to propagate along the muscle from the most proximal junctions which remained innervated. The small reduction in the amplitude of the action potential in the 'intact' region, after cutting more than half the branches of the nerve to the muscle, shows that at least a majority of fibres in the muscle must be innervated by several axons. This result is also clear from the experiment illustrated in Fig. 9*B*. The action potential in the 'denervated' region evoked by nerve stimulation, again after cutting more than half the branches of the nerve, is very little smaller than the action potential evoked by direct stimulation of the whole muscle. The response of most of those fibres which extended between the position of the stimulating and recording electrodes must therefore have survived 'denervation' at least at one neuromuscular junction.

Mechanical recording

Response to nerve stimulation. Figure 10*A* illustrates the responses of three varieties of anterior latissimus dorsi muscles and shows that these muscles are capable of producing twitches to single maximal stimuli applied to the nerve. The anterior latissimus dorsi of the chick and the latissimus dorsi of the pigeon have both been shown directly to be made up largely or exclusively of multiply-innervated fibres and it may be inferred that this is also the case for the anterior latissimus dorsi of the rook (see Ginsborg & Mackay, 1961). It does not seem likely that the twitch was due to a small number of focally innervated fibres which have resisted detection histologically; the twitch in such fibres, as exemplified by the twitch of the posterior latissimus dorsi (a muscle which usually contains few, if any, multiply-innervated fibres (Ginsborg & Mackay, 1961) has a much more rapid time course (Fig. 10*B*). This is not conclusive evidence, however, since there may be differences in the speed of the twitch in different focally innervated fibres of the bird equivalent to the differences in speed between 'fast' and 'slow' mammalian muscle fibres (see, for example, Buller, Eccles & Eccles, 1960).

On repetitive stimulation of the nerve at minimum frequencies, varying from preparation to preparation between 15 and 35/sec, the anterior latissimus dorsi muscle responded with maximal sustained tension throughout the period of stimulation (up to 3 min). This behaviour was in contrast to that of the posterior latissimus dorsi. The tetanic tension (produced by

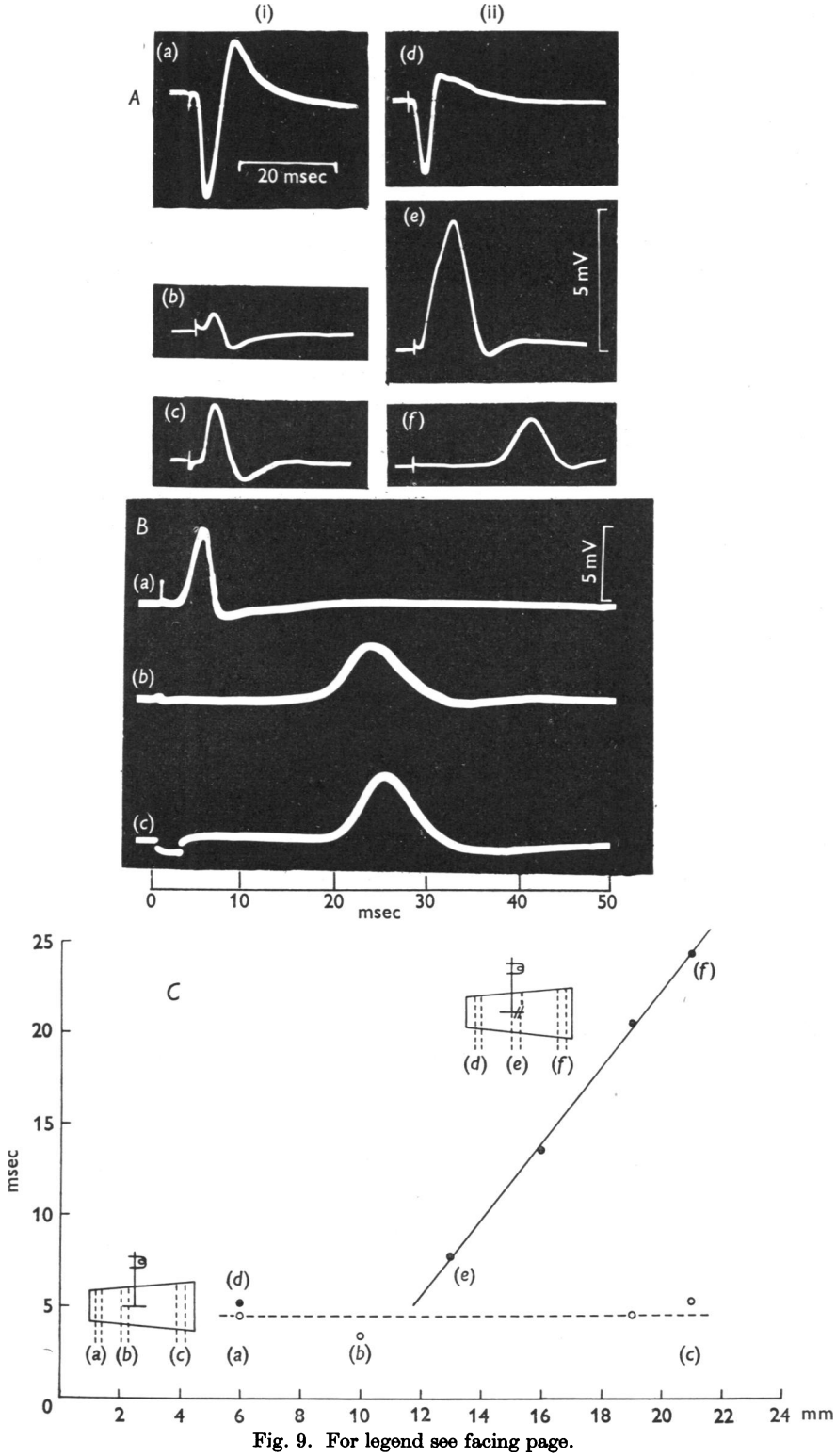

Fig. 9. For legend see facing page.

repetitive nerve stimulation at a minimum frequency in excess of 100/sec) in this muscle declined to less than half its maximum value in about 25 sec.

Response to depolarizing agents. When drugs known to cause depolarization at the motor end-plate (decamethonium, succinylcholine and carbaminoylcholine) were added to the fluid bathing the anterior latissimi dorsi, a prolonged increase in tension of the muscle resulted (Figs. 11 and

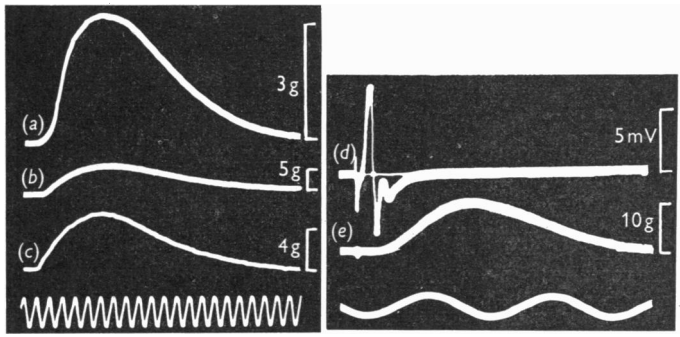

Fig. 10. 'Isometric twitches' in response to nerve stimulation. (*a*), anterior latissimus dorsi of chick; (*b*), anterior latissimus dorsi of rook; (*c*) latissimus dorsi of pigeon; (*e*), posterior latissimus dorsi of chick. The action potential (*d*) and twitch (*e*) were recorded simultaneously. Time marker, 20 msec. 37° C.

12*a*). The amplitude of the maximal isometric tension (a maximal effect was caused by decamethonium iodide (C_{10}), for example, at a concentration of $1{\cdot}7 \times 10^{-6}$ M in all three varieties of muscle tested) was almost identical with that produced by tetanic stimulation of the nerve; this result suggests that all the fibres in these muscles were taking part in the response. Contractures of similar amplitude but of longer duration were produced by bathing the muscle in a solution enriched in potassium. In

Fig. 9. Effect of 'denervating' part of muscle on externally recorded action potential. *A*: 23-day-old chick; muscle length 24 mm; temperature 29° C. (i) Nerve intact, (ii) branches of nerve to 'central' half of muscle cut. Recording electrodes 2 mm apart. Distance of the inner recording electrode from peripheral end of muscle indicated in *C* by letters affixed to points on graph which correspond to lettering of records. Upward deflexion in (*b*), (*c*), (*e*) and (*f*) and downward deflexion in (*a*) and (*d*) denotes negativity of inner electrode. Maximal nerve stimulation. *B*: 21-day-old chick; muscle length 26 mm; temperature 36° C. Recording electrodes 2 and 6 mm from 'central' end of muscle. Upward deflexion, negativity of inner electrode. (*a*) Nerve intact, maximal nerve stimulation; (*b*) branches to 'central' half of muscle cut, maximal nerve stimulation; (*c*) direct stimulation of curarized muscle: stimulating cathode 12 mm from inner recording electrode. *C*: variation in 'peak' latency at different points along normal (○) and partly denervated (●) muscle. Preparation of Fig. 9*A*. Abscissa, distance between inner recording electrode and peripheral end of muscle (mm). Ordinate, interval between first peak of action potential and stimulus artifact (msec).

seven experiments on anterior latissimi dorsi of the chick, the time taken for the tension to decay from the maximum to half the maximum value varied between 3 and 8 min in the presence of $1{\cdot}7 \times 10^{-6}$ M C_{10}, whereas this time varied between 9 and 15 min in the presence of 100 mM-KCl (replacing NaCl). In the rook (Fig. 12) the difference in duration was even

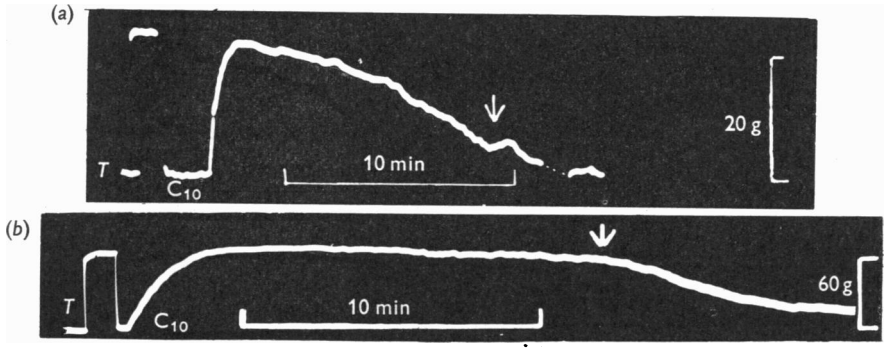

Fig. 11. Comparison between isometric tension produced by repetitive nerve stimulation at 40/sec, *T*, and by the addition of decamethonium iodide to the bath to give a final concentration of $1{\cdot}7 \times 10^{-6}$ M. (*a*) anterior latissimus dorsi of chick, (*b*) latissimus dorsi of pigeon. At arrows, bathing solution drained and replaced with Krebs–Henseleit solution. 37° C.

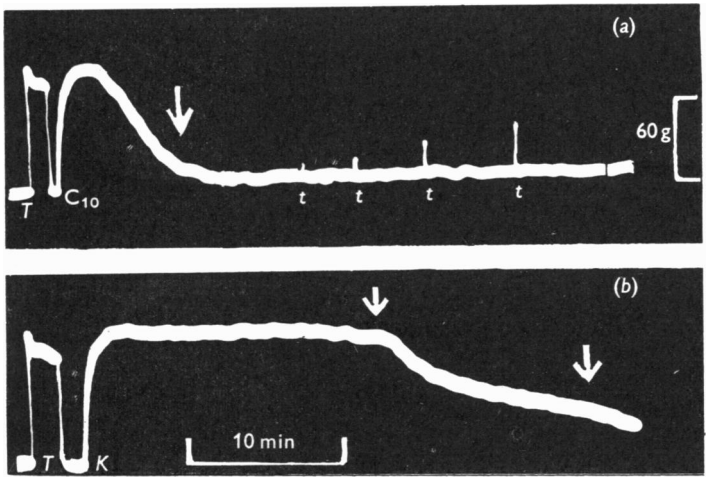

Fig. 12. Anterior latissimus dorsi of rook. Comparison between isometric tension produced (*a*) by decamethonium iodide $1{\cdot}7 \times 10^{-6}$ M, and that produced (*b*) by replacing 100 mM-NaCl with 100 mM-KCl. Common time and tension scales in (*a*) and (*b*). *T*, tetanic tension produced by nerve stimulation at 40/sec. At arrows, bath drained and refilled with Krebs–Henseleit solution. Recovery of neuromuscular transmission delayed after the contracture had subsided, as may be seen from the responses to the 5 sec test tetani, *t*, applied to the nerve. 37° C.

more pronounced. The shorter duration of the 'decamethonium contracture' probably reflects the relatively rapid decline of the depolarization of the membrane due to 'desensitization' (see for example, Thesleff, 1958; Fatt, 1950).

As a rule prolonged contractures could not be elicited from the posterior latissimus dorsi. In one out of sixteen experiments, however, a contracture of similar time course to that obtained in the anterior latissimus dorsi did occur; the maximum tension produced, 2 g, was, however, only about one tenth of the tetanic tension the muscle was able to develop. Since on histochemical staining of posterior latissimi dorsi a small band of fibres with multiple neuromuscular junctions was, on one occasion, observed (Ginsborg & Mackay, 1961), it seems possible that the anomalous response was due to the presence of such fibres in the muscle in question. Two other muscles which failed to give prolonged contracture in two experiments were the extensor carpi radialis profundus and the extensor digiti III and IV. No multiply-innervated fibres have been detected in these two muscles (Ginsborg, 1960; Ginsborg & Mackay, 1961).

DISCUSSION

It is evident that avian muscle fibres with multiple neuromuscular junctions are similar in certain ways to the slow fibres of the frog (Kuffler & Vaughan Williams, 1953*a*, *b*). Both types of fibre are innervated at numerous points by several axons and it seems probable, also, that both types of fibre are distinguished histologically from focally innervated fibres in another way, namely in having a 'Felderstruktur' as compared to a 'Fibrillenstruktur' (see Krüger, for example, 1952; Krüger & Günther, 1956). (For evidence relating to the frog, see Kuffler & Vaughan Williams, 1953*b*; for the bird, Ginsborg & Mackay, 1961). Both types of fibre respond to prolonged depolarization with prolonged contracture, a property which is not shared with focally innervated fibres of either the bird or frog (see, for example, Taylor, 1953; Kuffler & Vaughan Williams, 1953*b*; Horowicz & Hodgkin, 1956). On the other hand, one clear difference between the multiply-innervated fibres of bird and the slow fibres of the frog is the ability of the fibres of the bird to produce propagated action potentials; in this respect the fibres of the bird resemble certain multiply-innervated intrafusal fibres in the frog (Koketsu & Nishi, 1957*a*) and some crustacean muscles (Fatt & Katz, 1953).

Although it seems probable that in the bird and the frog multiple innervation, 'Felderstruktur', and the ability to produce prolonged contractures are all properties of the same variety of muscle fibre, the connexion between these properties is obscure. There is clearly no immediate connexion between type of innervation and ability to produce prolonged contracture;

however, evidence is accumulating which suggests that the innervation of a muscle fibre does in some unknown way affect both structural (Krüger & Günther, 1957) and functional (Buller *et al.* 1960) characteristics of the myoplasm. Nor can it be supposed that a 'Felderstruktur' as such confers the ability to produce prolonged contractures. Thus although 'Felderstruktur' fibres exist in the mammal (Krüger, 1952), Rückert (1930, 1930/1) found that the application of constant currents, which evoke prolonged contractures both in slow fibres of the frog and in multiply-innervated fibres of the bird, failed to do so in mammalian skeletal muscles, with the exception of certain foetal muscles and the diaphragm of the white mouse. The particular type of fine structure of the myoplasm which gives rise to the characteristic 'Felderstruktur' appearance of the cross-section of the fibre may be necessary, but it is apparently not sufficient to allow a fibre to undergo a prolonged contracture.

SUMMARY

1. Single skeletal muscle fibres, with multiple neuromuscular junctions, of the biventer cervicis of the chick have been investigated with microelectrodes. The results obtained were confirmed by those resulting from external recording from the anterior latissimus dorsi, which is made up largely or exclusively of fibres with multiple neuromuscular junctions.

2. These fibres are innervated by several motor axons, and respond to direct and to maximal indirect stimulation with propagated action potentials. In the fibres of the anterior latissimus dorsi action potentials on maximal indirect stimulation were initiated at many of the junctions on each fibre.

3. The mechanical response to 'depolarizing agents' of the anterior latissimus dorsi of the chick and the rook and of the latissimus dorsi of the pigeon was a prolonged contracture: the tension of the maximal contracture was approximately equal to the maximal isometric tetanic tension produced by nerve stimulation. As a rule the posterior latissimus dorsi of the chick did not respond with prolonged contractures; this muscle usually contains few if any fibres with a dense innervation.

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