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MECHANICAL AND ELECTRICAL RESPONSES OF SINGLE INNERVATED CRAB-MUSCLE FIBRES*

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It has been apparent for some time that diverse forms of electrical responsiveness to directly applied stimulation are to be found in different fibres of the same muscle in crustaceans (Fatt & Katz, 1953*a*). Crustacean muscle fibres also show marked differences in passive electrical properties; these have been studied in detail and classified for a leg muscle of the crab *Carcinus maenas* by Atwood (1963). The differences are so pronounced that it is probable they are neither fortuitous nor due to damage and deterioration. Instead they indicate that differences in muscle fibre properties are utilized to perfect functional control of the muscle. Furthermore, Cohen (1963) and Dorai Raj & Cohen (1964) have been able to show that one crustacean muscle, the accessory flexor of *Cancer magister*, contains fibres with different histological appearances.

In addition, there is considerable variation in responsiveness to neural stimulation. When the single 'slow' excitor (S) and 'fast' excitor (F) axons supplying certain muscles are isolated and stimulated individually the relative magnitudes of the S post-synaptic potentials (S p.s.p.s.) and F post-synaptic potentials (F p.s.p.s.) in a given fibre may range from large F, small S through identical F and S to small F, large S; in some fibres either the F or S response may be entirely absent (Hoyle & Wiersma, 1958*a*; Atwood, 1963). These findings may reflect differences in the density of innervation of individual muscle fibres by the two excitor axons. Variations in electrical response to stimulation of the inhibitor (I) axon are also found.

Since the S, F, and I axons all innervate most of the muscle fibres, a wide range of tension control may be achieved in the individual fibre and the membrane excitation–contraction coupling relations in crustacean muscles thereby acquire special interest. Unfortunately, however, the

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complexities outlined above prevent firm conclusions being drawn from the results of traditional crustacean nerve-muscle experiments in which the tension developed is measured by attaching a transducer to the next most distal joint, so that the effort of the whole muscle is recorded. A complete picture of the functioning of a crustacean muscle can be obtained only by studying individual muscle fibres of all the various kinds found in that muscle and making an integrated appraisal of the results. To this end it is necessary to be able to record the electrical and mechanical responses of individual muscle fibres during neuronal excitation. So far no technique has been devised by which such information can be obtained.

Orkand (1962) has shown that it is possible to record the force developed in single muscle fibres of a short-length crayfish muscle, when they are stimulated by current applied through an intracellular glass-capillary micro-electrode. This method uses the individual stimulation provided by intracellular electrodes, but the tension is recorded by links to the whole muscle. Thus although the electro-mechanics of individual fibres can be studied, correlated information about their responses to nerve stimulation cannot be obtained.

The present studies were undertaken to devise a method which would permit the measurement of tension in single innervated crustacean muscle fibres in response to neuronal stimulation. This aim was accomplished, and an investigation was made of electrical and mechanical responses to indirect stimulation through the motor axons as well as responses to directly applied current pulses.

METHODS

The work reported here was done on two muscles of the walking legs of the edible Pacific crab *Cancer magister* Dana obtained from Puget Sound, Washington and Charleston, Oregon. These muscles were the 'closer' (adductor of the dactylopodite), which receives S, F, and I axons, and the 'stretcher' (reductor of the propodite) which receives a single motor axon similar in most respects to the S closer axon, and two inhibitors (cf. Wiersma, 1941).

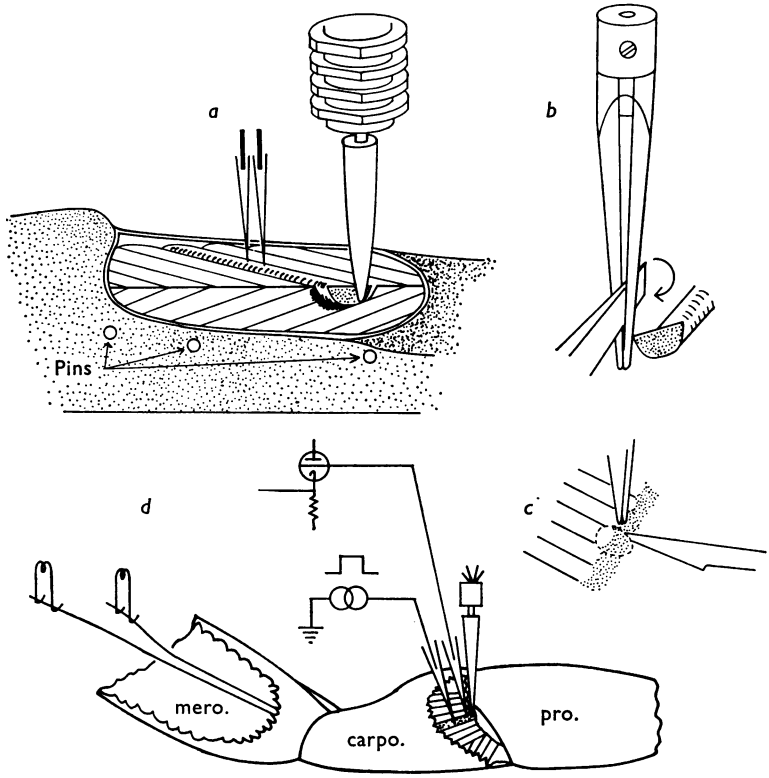
A device for recording tension from single muscle fibres was constructed by attaching the very fine tips of a pair of forceps directly to the peg of an RCA 5734 transducer tube (Text-fig. 1). The tips are cut off Dumoxel no. 5 forceps and further reduced by filing. They are caused to be pressed together at the extreme tips only, by attaching them to a small wedge at their bases. The wedge is in turn attached to a small metal cylinder drilled with a hole which fits tightly over the transducer peg, and further secured with a small transverse screw. A force of more than 30 g is required to open the forceps tips when properly made. The transducer may be housed in a metal heat sink carried on a micromanipulator. The tips of the forceps device can be opened by inserting a fine blade between them and rotating it through 90 degrees (Text-fig. 1).

In an experiment, the walking leg from an experimental animal was dissected to expose the leg nerve in the meropodite. The excitor and inhibitor axons to the desired muscle were prepared as described by Wiersma (1941). The axons were stimulated by pairs of fine platinum-wire electrodes.

The preparation was placed in a dish containing cold saline of the following composition (m-mole/l.): Na^+, 476; K^+, 8; Ca^{2+}, 20; Mg^{2+}, 12; Cl^-, 538; HCO_3^-, 10. The leg and joints were held firmly in position with the aid of pins placed through them into a sculptured wax block in the dish. The dish and its contents were maintained at 12° C by means of a Peltier cooling unit (Ferroxcube, New York) placed under the dish.

The muscle was exposed by removing overlying parts of the shell, care being taken to avoid damage to the muscle fibres. The further procedure depended on the location of the muscle fibre to be examined. The tendon of the closer runs through most of the propodite as a flattened, tongue-shaped strip with its broad sides facing the walls of the joint. At its distal end, however, it becomes flattened in the plane at right angles to the main axis before joining the dactyl. The most distal muscle fibres are attached in a band, side by side, whilst in the rest of the muscle they are attached one behind the other in herring-bone fashion.

To prepare a proximal fibre in one of the surface layers the fibre is first selected and its immediate neighbour distally cut away. This is followed by removing its partner on the



Text-fig. 1. Diagram illustrating technique of preparing single muscle fibres for individual tension recording *in situ*. Details in text. *a*. General view of an exposed closer muscle with prepared fibre (shaded) clamped by forceps tips. Pins through shell and tendon prevent movement of main part of the muscle. *b*. To show details of construction of the forceps tips and means of opening them to clamp on small piece of dissected tendon attached to single muscle fibre. *c*. Technique of undercutting tendon attachment to prepare distal fibres. Knife-blade tip follows interrupted line under fibre. *d*. Diagram of the preparation of the stretcher muscle showing a single fibre of the muscle's central region held by the transducer forceps.

opposite side of the tendon and also the partner of the fibre removed first. This procedure exposes a small piece of tendon (Text-fig. 1 *a*). The forceps-tip device described above is then manipulated into position and clamped on to the exposed piece of tendon, the tips occupying the region from which paired muscle fibres on each side of the tendon have been removed. The tendon is then cut, starting distally and working under the selected muscle fibre, until the attachment of this fibre is freed from the tendon, but remains clamped by the forceps tips. The bulk of the muscle is prevented from moving by driving fine entomological pins through the walls of the leg and across the tendon.

Selected fibres in the most distal part of the closer can be prepared without removing any of the neighbouring fibres, by cutting the cuticle underlying the attachment, thereby leaving a piece of tendon just large enough to be grasped by the forceps (Text-fig. 1 *c*). Muscle fibres lying in the body of the muscle can be reached and prepared only after dissecting away all the overlying fibres. This procedure usually damages the preparation severely and, successful results with deep-lying fibres are seldom obtained.

In the stretcher muscle, the portion of the shell overlying the most distal and lateral muscle fibres is removed together with the associated hypodermis by careful dissection (Text-fig. 1 *d*). The exposed muscle fibres have their origins on the lateral wall of the carpopodite and are inserted near the junction of the stretcher tendon with the propodite. Single-fibre preparations can be obtained as described for the most distal closer muscle fibres. Fibres prepared in this way often still retain much of their motor innervation, as judged by comparison of electrical responses to stimulation of the axon before and after the operation.

Direct stimulation of the semi-isolated fibres is carried out by passing current through a micro-electrode inserted into the fibre, as described by other workers (Watanabe, 1958; Orkand, 1962). Changes in membrane potential are monitored by means of a second micro-electrode.

Forces down to 0·005 g could be registered by the transducer device. The output voltage was linear over the force range encountered and the recording almost ideally isometric.

The *in situ* lengths of the fibres in the closer muscle ranged from 5 to 9 mm, i.e. a few times longer than the space constant (λ). These muscle fibres are consequently too long to permit quantitative studies on the relation between membrane potential and tension when depolarizing pulses are applied through a single internal micro-electrode (cf. Orkand, 1962). Nevertheless, significant differences between fibres may readily be discerned by comparing their responses to a depolarizing pulse.

The fibres in the stretcher muscle averaged about 5 mm in length and were found to have space constants of 2–3·4 mm. Thus it was possible to change the membrane potential over most of the length of some of these fibres by passing current through a micro-electrode inserted near the centre of the fibre. A better comparison of mechanical responses to direct and indirect stimulation could be made in these fibres.

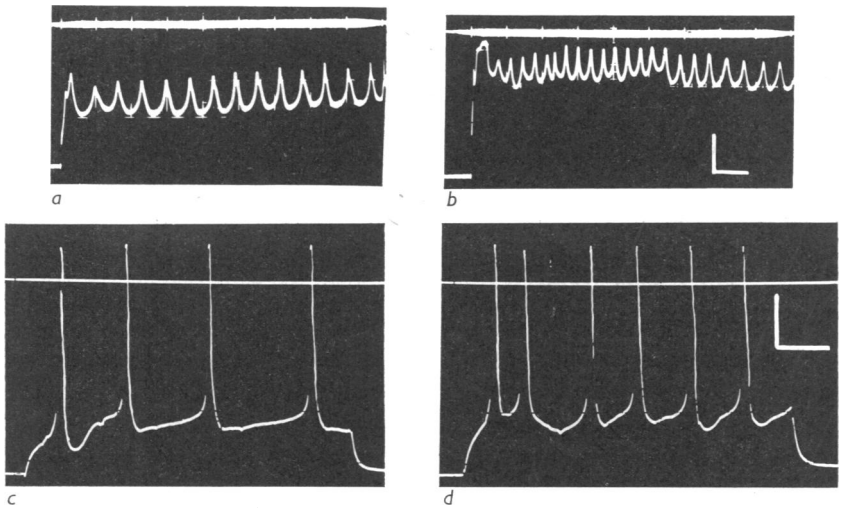
In some experiments on stretcher muscle fibres it was desired to change the ionic composition of the physiological saline. For this purpose the volume of solution surrounding a fibre was minimized (to about 0·4 ml.) by constructing a chamber of soft wax around the semi-isolated fibre and forceps tips. The test solution was flushed through the compartment from a syringe. Approximate control over the rate of change of the solution could be exercised by adjusting the rate of discharge of the syringe. It was estimated that rapid discharge effected complete change of the saline in about 5 sec.

RESULTS

Types of electrical response to depolarizing current pulses

A preliminary survey of the electrical responses to direct intracellular stimulation of fibres from different parts of the closer and stretcher muscles was undertaken to determine the kinds of fibre present under

optimal conditions, after subjecting the fibres to the least possible damage. The electrical characteristics of the muscle fibres could be studied within 5 min of severing the limb, whilst up to half an hour was required before the mechanical responses could be examined in addition, owing to the time needed for dissection and micro-manipulation.



Text-fig. 2. *a*, *b*. Large graded responses to long depolarizing pulses in a closer muscle fibre. Upper trace coincides with zero-membrane potential. *c*, *d*. Response of a closer muscle fibre giving all-or-nothing spikes to long depolarizing pulses, at lower *c* and higher *d* levels of depolarization. The upper trace corresponds to zero membrane potential. Calibrations: 20 mV; 100 msec. Spikes retouched.

The survey showed that both muscles contained many fibres which gave graded membrane responses to depolarizing current pulses. Similar fibres have been described in other crustacean muscles by Fatt & Katz (1953*a*), Fatt & Ginsborg (1958) and Werman, McCann & Grundfest (1961). Characteristically these fibres showed oscillations in the membrane potential with sufficient depolarization. With increasing depolarization the magnitude of the oscillations at first increased, but as the maintained membrane potential was made to approach zero, the oscillations became smaller (Text-fig. 2). They varied in size from 3 to 35 mV in different fibres. In some fibres the oscillations persisted with maintained depolarization, whereas in other fibres they died away rapidly. Many fibres gave a single graded response only, no matter how strong the current or how long it was passed.

These fibres are of common occurrence in crustacean muscles and will be referred to as *gradedly responding fibres*. Typically, they do not give

all-or-nothing spikes without special treatment (e.g. high external ion calcium-ion concentration or addition of barium ion; Werman *et al.* 1961).

In both closer and stretcher muscles of some crabs a characteristic kind of fibre was found which gave large, single or repetitive spike discharges in response to adequate depolarization. The spikes were all-or-nothing and frequently showed an overshoot of the zero membrane potential level (Text-fig. 2*c*, *d*). Fibres having these properties were not seen in crabs which were about to moult, or which had recently moulted, nor were they usually found in female crabs. They were always found in the more vigorous male crabs, and they formed approximately 20 % of the fibres examined in the muscles of certain of these animals.

It will be convenient to designate these fibres as *all-or-nothing responding*, in relation to their membrane activity. Usually they were distinguished by a sharp threshold for spike production, though some were seen to produce graded responses to weak stimulation, when fatigued, or during prolonged depolarization.

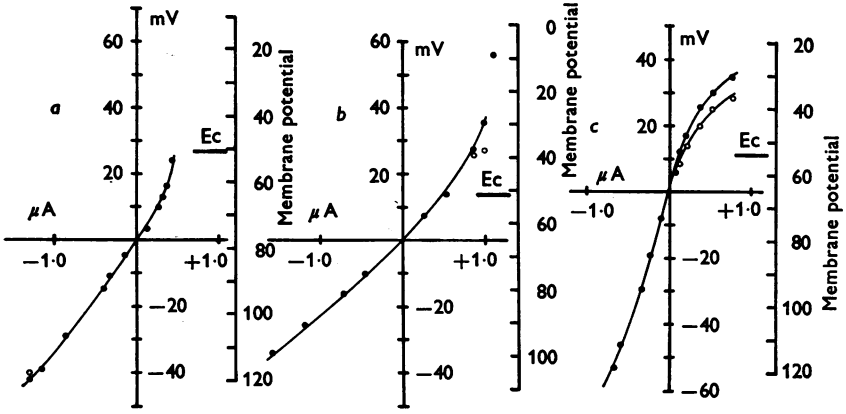
In each muscle some of the fibres examined did not give oscillations or graded electrical responses to depolarization. Some showed a rapid, brief, initial depolarization which superficially resembled a graded response. However, this 'response' always occurred at the same time, immediately following the onset of depolarization, and was attributable to marked 'delayed rectification' (cf. Hodgkin, Huxley & Katz, 1949; Burke & Ginsborg (1956). A few of the fibres which did not give graded responses showed no delayed rectification, but only simple electrotonic responses to applied depolarization.

The fibres exhibiting delayed rectification or electrotonic responses only, will be referred to as *passively responding fibres*, in regard to electrical properties.

Three principal kinds of fibre may be recognized for descriptive purposes, based upon the reactions of the membrane to depolarizing current pulses. These are: all-or-nothing spiking, gradedly responding and passively responding. The distinctions between the extremes are well marked, but in the graded category a wide range of responsiveness was encountered. No regular distribution within the joint of fibres of the various types was seen in the closer. In the stretcher it was noted that passively responding fibres occurred more commonly at the edges of the muscle. In both muscles the sampling was confined to the most readily accessible fibres; therefore a complete description of these muscles cannot be given yet.

A more precise comparison of the three main kinds of muscle fibre can be made by plotting the membrane-voltage responses against the applied depolarizing or hyperpolarizing current for each kind (Text-fig. 3). The passively responding fibres are distinguished by a fall in maximum mem-

brane voltage response with increasing outward current, indicating a predominance of rectification processes. The all-or-nothing responding fibres show a critical-spike threshold, whereas the gradedly responding fibres show variable oscillations and small spikes at depolarizations in excess of about 20 mV.



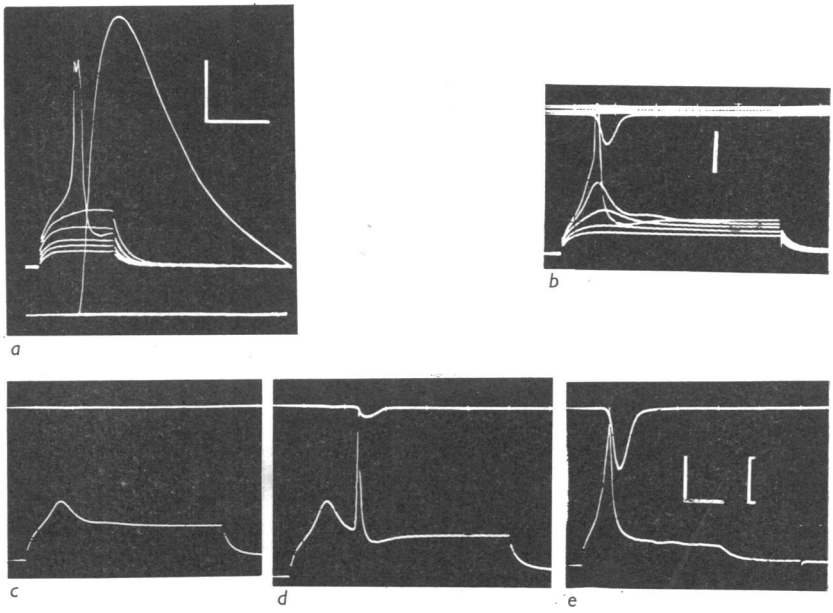
Text-fig. 3. Membrane voltage–current plots for all-or-nothing spiking fibre *a*, a gradedly responding fibre *b*, and a passive responding fibre *c*, all from the stretcher muscle. In each graph the positive abscissa represents depolarizing current, the positive ordinate represents membrane depolarization, the right-hand voltage scale indicates the absolute value of the membrane potential, and the short bar represents the depolarization level at which contraction of the fibre was seen (Ec). The filled circles represent the initial membrane response to the applied current; open circles represent the final steady level of membrane potential. The value of Ec in *a* is only approximate, as no contraction was seen at depolarizations less than those eliciting spikes.

Tension responses to depolarizing current pulses

The membrane potential at which a contraction just occurs will be referred to as the threshold for excitation of contraction and designated Ec. Resting potential will be designated Em. The value of Ec was determined by visual observation of the fibre under a binocular microscope. Two micro-electrodes, one passing current and the other recording potential were placed with their tips close together, and the impaled region observed during stepwise increases in depolarization. It was sometimes found that after a number of subthreshold current pulses had been delivered at a frequency of 1 per sec, a contraction could be detected. The extent of depolarization required to reach the threshold for contraction, Ec, could apparently be decreased by up to 2 mV by passing depolarizing current across the membrane or by damage caused by electrode penetration. Minimal tension was usually recorded by the transducer at the same level

of depolarization at which it could be observed visually, provided the depolarizing electrode was not near the proximal end of the fibre.

The relations between membrane potential and tension showed much variation. For descriptive purposes, the fibres will be divided into the three classes established above on the basis of electrical responsiveness.

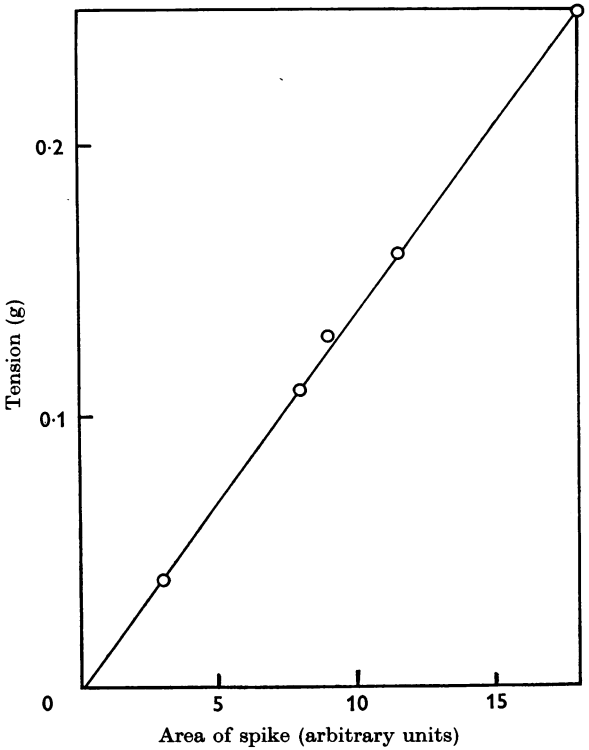


Text-fig. 4. *a*. Membrane potential (upper trace) and tension (lower trace) recorded from a stretcher fibre giving all-or-nothing spikes. No tension was recorded at depolarizations below the spike threshold. Resting potentials: 70 mV. Calibration: 20 mV; 100 msec, 0·5 g. *b–e*. Tension and depolarization responses in two closer muscle twitch fibres (*b* and *c–d*); slight deterioration associated with preparation for tension recording. Upper traces register zero membrane potential when flat, then tension. Note especially relationship of tension to duration of spike. Graded response in *d* just exceeds threshold for contraction (Ec.) Calibration: 20 mV; 100 msec, 0·5 g (spikes retouched).

All-or-nothing spiking fibres. When fibres of this type were freshly prepared no tension was developed by depolarizations smaller than those required to initiate all-or-nothing spikes (Text-figs. 3, 4). With each spike a brisk twitch occurred. In view of the appearance of tension only in response to the large spikes it is not possible to say for these fibres exactly at what membrane potential the threshold for contraction was exceeded. Spikes were initiated in different fibres at membrane potentials between 63 mV (maximum-least threshold) and 48 mV (minimum-highest threshold).

As all-or-nothing spiking fibres began to deteriorate, the responses

became less sharply defined and graded responses occurred in addition to the all-or-nothing spikes. An example of this behaviour in a closer muscle fibre is given in Text-fig. 4. At first the graded responses did not give any tension. From this we may infer that the threshold for tension development (Ec) was at a membrane potential further removed from the resting potential than that reached by the largest graded responses which just failed to evoke a spike, which was 43 mV. With further ageing of the preparation, graded responses reaching lower levels of membrane potential than this produced contractions.



Text-fig. 5. Relation between tension and area of spike above threshold for tension development in a spiking fibre of a closer muscle (same fibre as in Text-fig. 4*c–e*).

When all-or-nothing fibres in good condition were subjected to prolonged, strong depolarization and were observed visually, the frequency of the repetitively firing spikes quickly declined to zero and the twitching ceased. The fibres did not give contractural responses. The same fibres when re-tested later in the same way gave shorter trains of spikes, and a contractural response became apparent. The membrane potential at which the latter occurred was about 50 mV, appreciably closer to the resting

potential than the earlier Ec. Such fibres could not be distinguished, at this stage, from those initially giving large graded responses.

A possible interpretation of these results is that the threshold for contraction moves closer to the resting potential as the excitable properties of the membrane change from all-or-nothing spiking to gradedly responding.

In many spiking fibres it was noted that the spikes varied somewhat in duration (3–8 msec) and also, to a small extent, in height. A corresponding variation was noted in the sizes of the twitches. For spikes reaching identical levels of membrane potential those with greater duration gave rise to more tension in the same muscle fibre. In one closer muscle fibre which gave both graded responses and large spikes, the value of Ec was clearly established. When the product of time and potential above threshold of the spikes in this fibre was plotted against tension, a straight line was obtained (Text-fig. 5), indicating a simple relation. This observation corresponds with those of Orkand (1962), who noted that in single crayfish fibres a large spike sometimes gave rise to a smaller contraction than a small spike and suggested that this was due to longer duration of the smaller one.

Gradedly responding fibres. This category includes the majority of the fibres encountered in both the closer and stretcher muscles. It is a broad one, encompassing fibres which gave only small oscillatory responses even when strongly depolarized, and also fibres which gave fairly large, but variable spike-like responses.

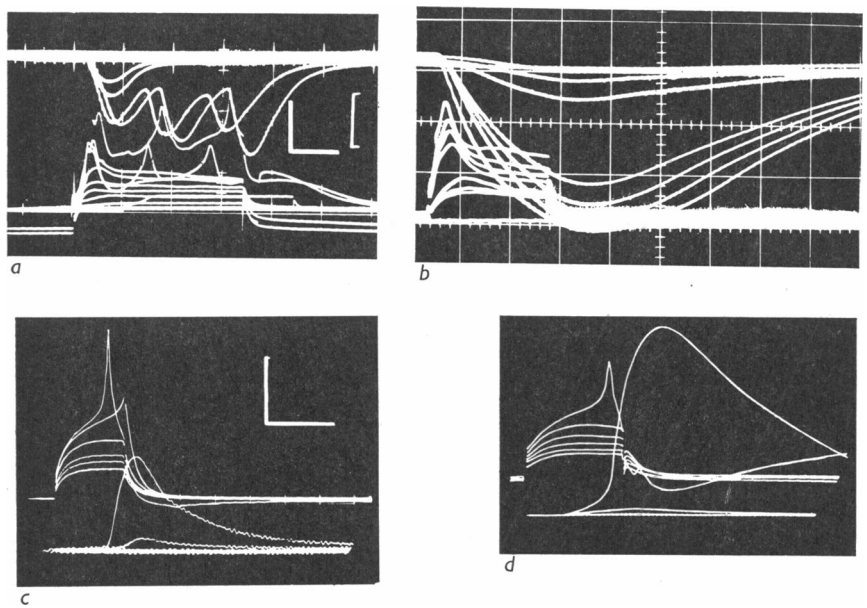
Fibres in this category generally gave two kinds of contractile response, phasic and tonic, the former associated with graded spikes and the latter with maintained depolarizations. There were, however, marked differences in the speeds of the contractions even when the electrical responses were similar. The extremes may be labelled simply *fast-follower*, if tension rise was rapid in response to quick depolarization, and *slow-follower* if the tension rise was slow. Extreme slow-followers did not give phasic contractions.

Fast-follower fibres. The latency of the mechanical response to depolarization past Ec was not more than 2 msec. In the fastest-following cells a peak in the response to the potential was followed by a peak in tension after a lag of as little as 10 msec (range, 10–60 msec). In these fibres maximum contraction and relaxation rates were high. The onset of relaxation followed the termination of the depolarizing pulse with a delay as brief as 8 msec (Text-fig. 6).

Slow-follower fibres. The maximum rate of contraction in these fibres was relatively slow, and the rate of relaxation quite slow (average 0·3 g/sec), about 60 times slower than relaxation in the fastest twitch fibres. The termination of depolarization was not immediately followed by

relaxation and tension continued to rise for 50–60 msec after termination of the pulse (Text-fig. 6).

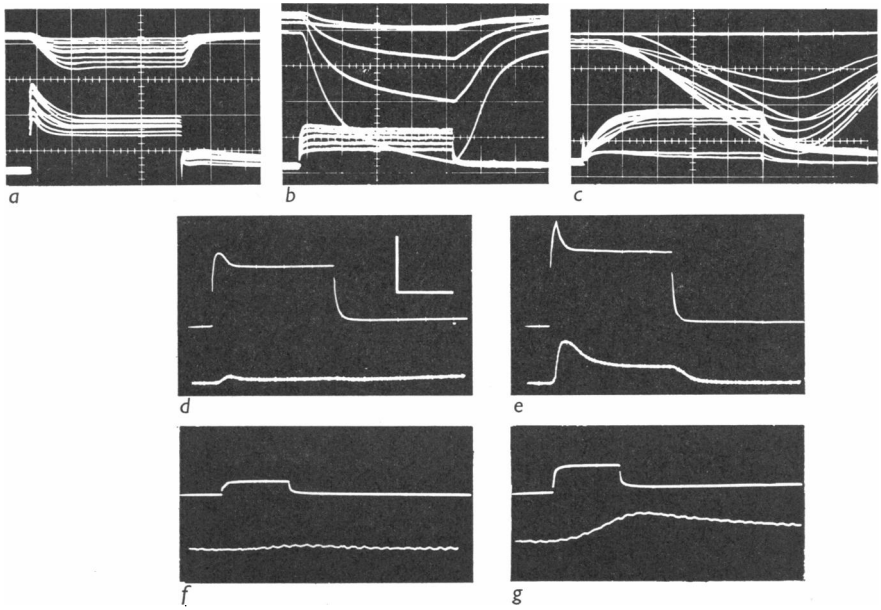
The threshold membrane potential for elicitation of contraction in gradedly responding fibres varied from 45 to 64 mV. The fibres with the larger graded responses were more often of the fast-follower type, and had lower values for Ec (i.e. higher thresholds for contraction), than many of the fibres giving small oscillations.



Text-fig. 6. *a, b.* Gradedly responding fibres in the closer muscle. Tension development in response to depolarization in fast-follower *a* and slow-follower *b* fibres. Note the rapid following of tension upon membrane-potential change in *a*. Upper traces register: first, zero membrane potential, then tension; lower traces register membrane potential. Calibration: 20 mV; 100 msec, 0·5 g. *c, d.* Membrane depolarization (upper traces) and tension (lower traces) in two gradually-responding stretcher muscle fibres. Although membrane responses were similar, the relative speeds of the tension responses suggest that *c* is a fast-follower and *d* is a slow-follower. Resting potentials: *c*, 71 mV; *d*, 68 mV. Calibration: 20 mV; 100 msec, 0·1 g.

Passively responding fibres. Fibres in this category of membrane response were also divisible into fast-followers and slow-followers with respect to their contractile behaviour. Tension responses of passively responding fibres of fast-follower type are shown in Text-fig. 1*a* (closer) and Text-fig. 7*d, e* (stretcher). In the closer fibre, tension reached steady levels about 60 msec after maintained plateaux of depolarization were established. Relaxation started within 10 msec of the termination of the

depolarizing pulses. The stretcher fibre showed marked delayed rectification, and the tension response followed the changes in membrane potential faithfully and with very little delay. A noteworthy feature in this fibre is the high threshold for tension development.



Text-fig. 7. *a–c*. Tension development (upper traces) in response to depolarization, in passively responding fibres of the closer muscle. *a*, fast-follower; *b*, intermediate; *c*, slow-follower. Calibration: vertical scale, 20 mV and 0·1 g/div. Horizontal scale, 100 msec/div. *d–g*. Passively responding fibres of the stretcher muscle, showing responses of a fast-follower (*d*, *e*) and a slow-follwer (*f*, *g*). Note the different membrane-potential thresholds for contraction. Tension, lower traces; membrane potential, upper traces; resting potentials, 63 mV (*d*) and 71 mV (*f*). Calibration: vertical, 20 mV, 0·1 g; horizontal, 100 msec (*d*, *e*) and 400 msec (*f*).

By contrast, the slow-follower cells in both closer (Text-fig. 7*c*) and stretcher (Text-fig. 7*f*, *g*) required over 500 msec to reach a plateau of tension during a steady depolarization, and usually gave increasing tension for at least 1 sec. Relaxation often required about 2 sec in the extreme slow-followers of the stretcher (Text-fig. 7*f*). Often the threshold for contraction was only 5–10 mV removed from the resting potential.

Many of the fibres encountered showed mechanical responses of kinds which were intermediate between the more pronounced fast- and slow-followers (Text-fig. 7*b*). A wide range of contraction speeds was present in fibres of both muscles.

In the stretcher muscle it was noted that the extreme slow-followers

were of comparatively small diameter (100–200 μ) and had higher membrane resistance than the rest of the fibres.

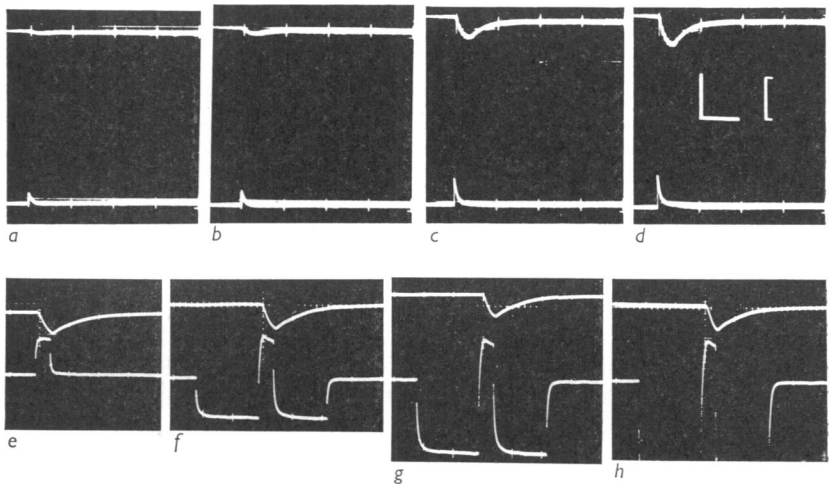
Approximate determinations of membrane resistance for eight slow-followers in stretcher muscles gave an average value of 1070 Ω cm^2. The average for eight fast-followers was 940 Ω cm^2. The range of variation was so large, and the uncertainties in determining these values so evident, that no significant difference could be established. However, membrane resistance of the extreme slow-followers was evidently higher than the average values. In other respects also, these fibres showed considerable differences from the fast-followers and less extreme slow-followers. The extreme fast-followers had comparatively low membrane resistances, but precise values for membrane-cable constants could not be obtained on account of the large diameters of most of the fibres (200–400 μ).

A few fibres found in the closer muscle were distinguished by the ability to respond to very short depolarizations at the threshold level (0·03 msec compared to about 1–3 msec for most fibres). The extent of depolarization required to produce contraction in these fibres was only a few mV; apparently the resting potential, Em, was only slightly greater than Ec, both being close to 65 mV. The time constants of these fibres were short. Their tension responses to brief depolarizations were twitch-like (Text-fig. 8) but the relaxation rates were relatively slow. The presence of a few such fibres could significantly affect the total response pattern of the muscle, and their characteristics are sufficiently distinct, to justify assigning a special category to them. It is proposed to call them *supersensitive* fibres.

The virtual coincidence of Ec and Em in these fibres brings into focus the hypothesis that the contractile response is related to the membrane potential. A test was made in which the membrane potential was altered artificially by passing a long-duration hyperpolarizing pulse and then passing a shorter depolarizing one from the altered membrane-potential level to beyond the threshold for contraction, through the same electrode. Similar contractions were obtained when the peak of the short depolarizing pulse reached the same level of membrane potential and stayed there for the same duration, whatever the level from which the pulse started (Text-fig. 8). Similar results were obtained at different locations on the same fibre. Thus the absolute level of membrane potential was the initial factor determining tension development in these fibres.

Results with passively responding fibres show a very steep relation between tension and membrane potential in the region between Ec and zero. This is partly due to a naturally steep relation between these quantities and partly to an increased length of the muscle fibre coming into contraction with increasing depolarization.

The responses of fibres sampled from a number of closer muscles are summarized in Table 1.



Text-fig. 8. *a–d.* Supersensitive fibre. Tension developed in response to brief (0·3 msec) pulses at increasing strengths. Calibration: 20 mV; 100 msec, 0·1 g. Resting potential 55 mV. *e–h.* Experiment to demonstrate that tension is a function of absolute level of membrane potential. Electrically inexcitable fibre was given long hyperpolarizing pulses of various magnitudes and depolarized briefly during the course of the hyperpolarizing pulse (registered on lower traces). Similar contractions (upper traces) were evoked when the same membrane potential was reached, irrespective of the level of initial membrane potential.

Histology

Cohen (1963) and Dorai Raj (1964) have found that in the small accessory flexor muscles (proximal and distal heads) of *Cancer magister* there are fibres having several distinctive histological appearances. The extremes are a short-sarcomere (3 μ) type with a punctate appearance in cross-section on the one hand, and a broad-sarcomere ($> 10\ \mu$) type in which the fibrils are clumped together, on the other. A variety of intermediate types was seen. These results made it seem possible that the physiological differences observed during the present work might also be correlated with histological differences.

General sections of the closer muscle revealed fibres of different types (Pl. 1), including some which were similar in appearance to those reported by Cohen (1963) and Dorai Raj (1964). Accordingly, individual fibres were tested physiologically and then prepared for histological study. Preliminary studies showed that the all-or-nothing spiking, gradedly responding fast-follower, and passively responding fast-follower fibres are all virtually identical in histological appearance in transverse section. The

TABLE 1. Summary of the results obtained in a sample of fibres in closer muscles of *C. magister*. Classification is based only on electric excitability and tension responses. Results for the stretcher muscle were similar except that no supersensitive fibres were found, and passively responding fibres were more common

Fibre type (electrical responsiveness)	Tension development i, fast-follower ii slow-follower	Approx. %	Mechanical response to prolonged depolarization	Resting potential (mV)	Time constant (msec)	Ec (mV)	Maximum* rate of force development (kg cm^{-2} sec^{-1})	Maximum* rate of relaxation (kg cm^{-2} sec^{-1})
All-or-nothing spike	i	0–20	Series of twitches only when fresh. Few twitches plus tonic contraction when deteriorating	70–90	30–40	42 (when fresh) falling to 50	40	24
	ii	None found	—	—	—	—	—	—
Graded								
Large	i	30	Few twitches plus tonic contraction which fades rapidly	65–85	30–50	56 (45–62)	16	10
	ii	None found	—	—	—	—	—	—
Small	i	15–20	Weak phasic contractions plus prolonged contraction fading slowly	65–80	30–50	56 (45–62)	8	5
	ii	15–20	Tonic contraction only, fading slowly	65–80	50	60 (56–66)	2	1
Passive								
Normal	i	10	Tonic contraction only, fading slowly	68–80	20–50	60 (54–66)	6	3
	ii	10	Tonic contraction only, maintained indefinitely	60–70	50–100	58 (54–64)	1	1
Supersensitive	i	5	Phasic contraction, plus longer contraction fading rapidly	64, 66	15–30	Close to resting potential	8	2
	ii	None found	—	—	—	—	—	—

Total % fast-follower = 70–75 %
slow-follower = 25–30 %

* These figures are approximate.

fibres are large in diameter and have evenly dispersed fibrils. There are few invasions by sarcolemmal elements. The sarcomere length is variable, but relatively short (3–6 μ).

Interspersed among the fibres with closely packed, evenly dispersed fibrils are others having varying degrees of invasion by sarcolemmal elements. One type, in which the subdivision of the fibre is particularly pronounced, may be clearly distinguished. These fibres are present in groups of four or five, though it is impossible to decide what are the true boundaries of these cells from the sections. These fibres are associated with extreme slow following of tension upon membrane-potential changes. The fibres also have very broad sarcomeres (10–14 μ), in which H-zones can easily be distinguished.

In addition to the two extreme types, various intermediates are present. These correspond to moderately slowly contracting, gradedly responding fibres and perhaps constitute the norm for crustacean muscle.

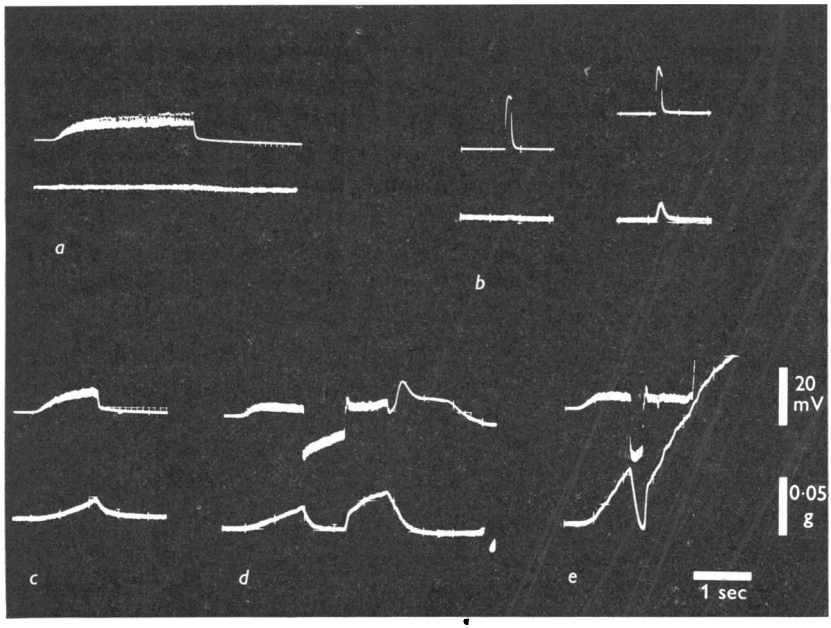
Association of membrane potential and tension in innervated fibres

Development of a suitable technique for observing the tension responses of single innervated muscle fibres during stimulation of the motor axons permitted a more precise examination of the relation between indirectly produced tension and the associated electrical activity than has been attempted in previous work on responses of whole muscles (Hoyle & Wiersma, 1958*a*, *b*). For these experiments the relatively short fibres of the stretcher muscle proved most suitable, since the membrane potential over much of the fibre could be altered by current passed through a micro-electrode.

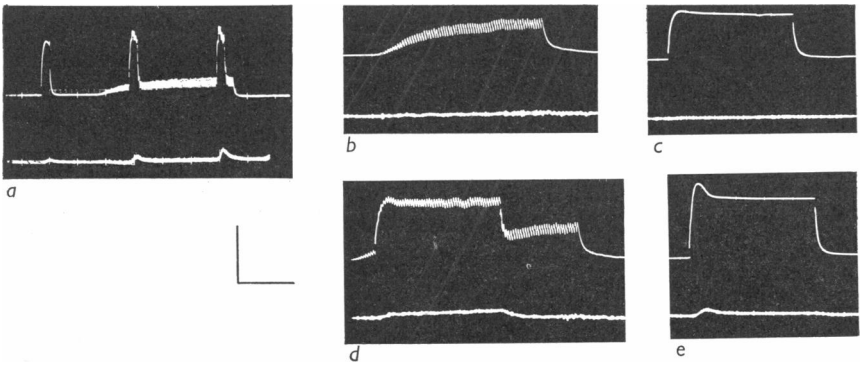
Isolation of single stretcher muscle fibres for tension recording with retention of much of the motor innervation could be accomplished fairly readily, but great care was necessary to avoid artifacts introduced by contraction of the rest of the muscle. Precautions which could be taken included fixation of the tendon by means of pins inserted through it into the surrounding shell.

In the successful preparations, stimulation of the isolated single motor axon at frequencies of 10–40 per sec did not produce contraction even though depolarizations of 10–15 mV were common. These fibres were of the passively responding, or occasionally of the gradedly responding types, and the electrical responses to nerve stimulation were in the form of facilitating and summing post-synaptic potentials (Text-figs. 9, 10).

Although the stimulation of the motor axon was ineffective in eliciting tension at frequencies below 40 per sec in most of these fibres, contractions could be obtained by first lowering slightly the membrane potential by adding saline with higher than normal content of potassium chloride



Text-fig. 9. Responses of a semi-isolated stretcher muscle fibre (passively responding slow-follower) to stimulation of the excitor axon at 50/sec. before *a* and after *c*, *d*, *e* depolarization with excess potassium. In *b* the shift of membrane potential from 70 to 56 mV by the potassium treatment is illustrated; less depolarization is required for tension production after the shift. Hyperpolarizing pulses, causing relaxation of tension, were given during nerve stimulation in *d* and *e*. Note that in *e* the resting potential is slightly lower than in *d*, and that the tension response is larger. The electrode pulled out of the fibre in *e*.



Text-fig. 10. Responses of two passively responding stretcher muscle fibres (*a*, and *b–e*) to stimulation of the motor axon at 50/sec and to passage to depolarizing pulses. In *a*, pulses given during axon stimulation produce more tension than pulses of the same magnitude given before axon stimulation. In *b*, axon stimulation fails to produce tension, but when a subthreshold depolarizing pulse *c* is given simultaneously, tension results *d*. Total depolarization in *d* exceeds the Ec level *e*. Resting potentials: (*a*) 69 mV; (*b*) 70 mV. Calibration: vertical, 20 mV and 0·05 g; horizontal, 1 sec (*a*) and 400 msec (*b–e*).

(Text-fig. 9) or by passing depolarizing current (Text-fig. 10). The motor axon was then stimulated at a frequency which was previously without effect. When the membrane potential was shifted closer to Ec by treatment with potassium chloride, contraction could be evoked by fairly low frequencies of stimulation applied to the motor axon (Text-fig. 9*b*, *c*). The tension responses so obtained could be abolished completely by hyperpolarizing the membrane with an inward current pulse during nerve stimulation (Text-fig. 9*d*, *e*).

Another experiment which was performed with these fibres involved stimulation of the axon at a frequency below that required for tension production, and simultaneous application of a depolarizing pulse of insufficient size to attain Ec. These two depolarizations summed to a level in excess of Ec and evoked contraction (Text-fig. 10). Continued stimulation of the motor axon after cessation of the depolarizing pulse did not result in a continued contraction. The combined depolarization necessary to produce tension was similar to that required when a depolarizing pulse alone was employed.

These experiments indicate that nerve stimulation evokes tension in the muscles fibres only when depolarization attains the Ec level.

Neuromuscular transmission and contraction in different muscle fibres

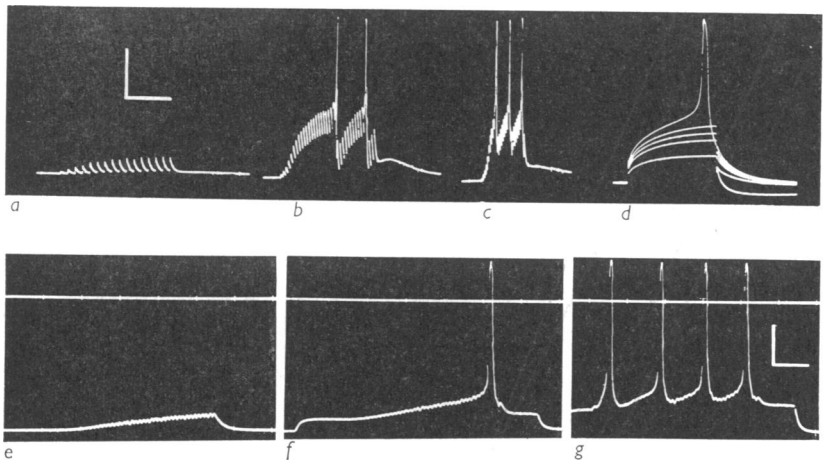
On the basis of the different types of membrane responsiveness and contractile properties described previously in this account, one would expect considerable variation in the electrical and mechanical responses to stimulation of the motor and inhibitor axons in both closer and stretcher muscles. Attempts were therefore made to sample these responses in various fibres of the two muscles. It was seldom possible to prepare for tension recording semi-isolated fibres which were still innervated by all the motor and inhibitor axons; in some cases damage may have occurred to the fine terminations of one or more axons, but in many cases there was no reason to suspect damage and it was concluded that not all axons supply endings to each individual muscle fibre. The latter situation was more evident in the closer muscle, in which it was frequently found that certain fibres did not respond to stimulation of the common inhibitor axon or to one of the two motor axons. Similar findings have been described in the closer muscles of this and other species (Hoyle & Wiersma, 1958*a*; Florey & Hoyle, 1961; Atwood, 1963).

The responses to stimulation of the axons will be classified according to the kind of electrical and mechanical responsiveness obtained from direct stimulation with depolarizing current.

All-or-nothing spiking fibres. These fibres were easily recognized because of their large spikes, but they formed a small percentage of the fibres in the

majority of the muscles examined. In all instances such fibres had deteriorated to the extent of giving some large graded responses instead of only all-or-nothing spikes by the time they had been prepared for tension recording.

Examples of the responses to motor-axon stimulation of fibres in the closer and stretcher muscles are given in Text-fig. 11. These recordings were made before preparation of the fibres for tension measurements. In the stretcher, the single excitatory axon evoked post-synaptic potentials (p.s.p.s.) of 5–10 mV when stimulated at a low frequency (below 15 per sec). At frequencies of stimulation of 20–40 per sec the facilitation and



Text-fig. 11. All-or-nothing spiking fibres of the stretcher (*a–d*) and closer (*e–g*). Stimulation of the single excitor axon to the stretcher was given at 6/sec (*a*), 19/sec (*b*), and 30/sec (*c*); the fibre was also stimulated directly (*d*). Resting potential: 68 mV. Calibration: vertical, 20 mV; horizontal, 1 sec (*a–c*) and 100 msec (*d*). In *e–g* the F closer axon was stimulated at 100 per sec for 0·4 sec. In *e* the summed, facilitated, p.s.p.s. failed to elicit spikes. A small decrease in membrane potential achieved by passing a depolarizing pulse *g* gave rise to four spikes. Upper trace registers zero membrane potential. Calibration: 20 mV; 100 msec.

summation of the p.s.p.s. during a train of impulses produced depolarizations large enough to initiate all-or-nothing spikes. A transient positive after-potential followed each spike. As the frequency of stimulation was raised the rate of spiking increased (cf. Dorai Raj, 1964). Spikes evoked by direct stimulation were of the same size and occurred at the same membrane potential level as those produced by indirect stimulation in the same fibres (Text-fig. 11).

In closer muscle fibres similar features were observed during nerve stimulation. Excitatory p.s.p.s. were small and required a good deal of facilitation before they fired a spike. Of eleven all-or-nothing spiking

fibres examined in the closer, six responded to only the fast axon, four to both axons and one to the slow axon only. As far as could be determined, these fibres were quite unresponsive to stimulation of the inhibitor axon. Even large displacements of the membrane potential from rest did not cause an appearance of inhibitory potentials in most of these fibres.

Although these fibres did not produce spikes or tension after they had been prepared for tension recording, it was observed visually that indirectly evoked spikes produced twitches in these fibres before they were isolated.

In spiking fibres of the closer muscle which responded to both F and S axons the F p.s.p.s. were larger than the S p.s.p.s. and the stimulation of the S axon was not observed to evoke any form of contraction. Thus it is possible that the S axon may not be independently functional in these fibres but may normally act in association with the F axon by providing a background depolarization preparatory to the firing of the latter. The simulataneous action of S and F axons would lead to spike production in those fibres in which none occurred during the action of the F axon alone, and to the firing of spikes sooner in those capable of spike responses to it alone.

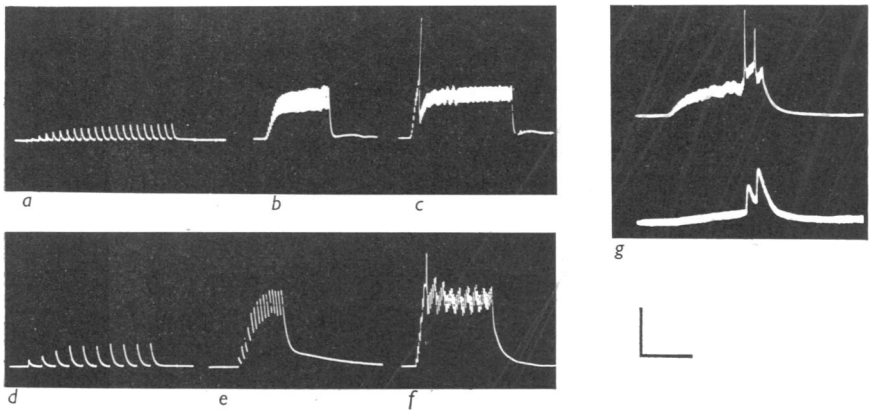
The possibility cannot be excluded that some of the spiking fibres in the closer muscle, perhaps those deeper in the muscle, were capable of responding to single F axon impulses with very large p.s.p.s. or with spikes. Two observations which raise this possibility are: (1) the occurrence of a small twitch response in the muscle as a whole when single shocks were delivered to the F axon (this was seen only in some muscles); and (2) the discovery of fibres giving large electrical responses to F axon stimulation in closer muscles of other crabs (Hoyle & Wiersma, 1958*a*; Atwood, 1963).

Gradedly responding fibres. These fibres were commonly found in both the stretcher and closer muscles. In the stretcher, the electrical responses to indirect stimulation at frequencies below 15 per sec were generally similar to those of fibres giving all-or-nothing spikes, although in a few fibres the excitatory p.s.p.s. were much larger and produced considerable depolarization even at fairly low frequencies (Text-fig. 12). At higher frequencies of stimulation (20–50 per sec) graded membrane responses and oscillations occurred. Quite often these fibres produced one or two large graded responses at the start of a train of impulses, then smaller oscillations; this behaviour is similar to that observed by Wiersma & Bobbert (1961) in muscle fibres of *Eupagurus*.

Tension records from semi-isolated, gradedly responding fibres of the stretcher showed that twitch contractions of variable magnitude were evoked by the graded membrane responses (Text-fig. 12). In some records a tonic tension response was also seen, although it was not always clear

that this tension was due solely to the semi-isolated fibre. In many cases the membrane potential at which the graded responses were evoked was close to the Ec level (cf. Text-figs. 3, 6).

In the closer muscle the gradedly-responding fibres were almost always innervated by both F and S axons. Neither axon gave large p.s.p.s. to single shocks, but during a train of stimuli facilitation occurred. The F p.s.p.s. usually grew in size at about twice the rate of the S p.s.p.s. in the same fibre, but there was otherwise no significant difference between them

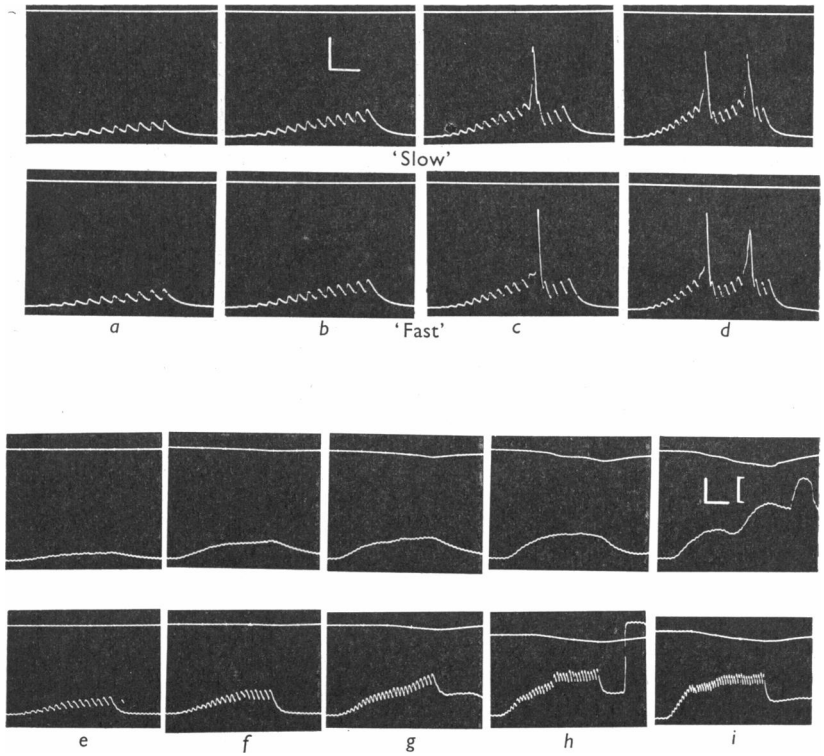


Text-fig. 12. Gradedly responding fibres of the stretcher muscle (*a–c*, *d–f* and *g*). The single excitor axon was stimulated at 6/sec (*a*), 25/sec (*b*), and 35/sec (*c*); at 3/sec (*d*), 13/sec (*e*), and 24/sec (*f*); and at 60/sec (*g*). Tension appears in the lower trace in (*g*). Resting potentials: *a*, 65 mV; *d*, 68 mV; *g*, 67 mV. Calibration: 1 sec, and 20 mV = 0·1 g.

and sometimes even this differentiating feature was lacking. In Text-fig. 13, records from a fibre showing virtually identical responses to F and S axons are presented. In this fibre both F and S axons evoked large graded responses with sufficient depolarization but in some fibres this type of response could not be produced indirectly, even though the summed plateau of depolarization passed the threshold for production of large graded responses as determined by direct electrical depolarization. This, together with the loss in ability to produce graded responses during a train of stimuli (Text-fig. 12), suggests that membrane excitability is sometimes decreased during indirect stimulation, perhaps by the parallel shunt for the normal membrane afforded by the activated synaptic regions, which may be of considerable area in crustacean muscle fibres.

In closer fibres, as in the stretcher fibres, both tonic and phasic tension responses were seen, the latter associated with the large graded responses and the former related to the plateau of depolarization caused by the summed p.s.p.s. In some of the gradedly responding fibres repetitive

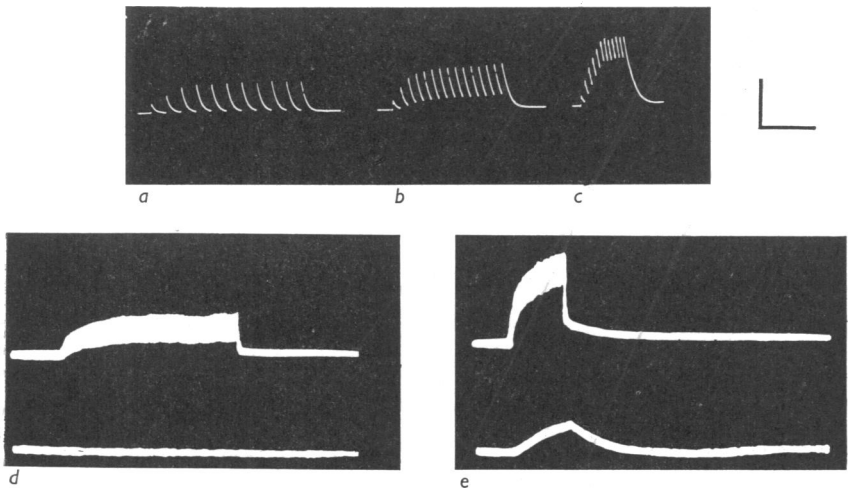
stimulation of the S axon gave rise to a smooth depolarization with little evidence of individual synaptic potentials, whereas in the same fibres the F axon gave good-sized p.s.p.s. An example of the tension responses of such a fibre is given in Text-fig. 13. Similar tension was developed by stimulating the F axon at such a frequency that the mean depolarization achieved was about the same as that associated with a given S axon response. This constitutes evidence that both S and F axons caused contraction in this fibre through membrane depolarization.



Text-fig. 13. Gradedly responding fibres of the closer muscle. *a–d.* Relative facilitation rates during F and S axon stimulation; the large e.p.s.p.s. had almost identical facilitation rates to S and F axons. Stimulation at 20, 25, 30 and 40/sec. *e–i.* Comparison of tension development in response to S and F axon stimulation in small gradedly responding fibre. The S axon evoked small p.s.p.s. with a long decay time, so that summing led to a smooth depolarization. The frequencies of stimulation of S and F were similar and progressively increased. Calibration: 20 mV; 100 msec, 0·2 g.

Passively responding fibres. A wide range in size of excitatory p.s.p.s. was encountered in these fibres in both stretcher and closer muscles. In the former muscle comparatively large p.s.p.s. were encountered in some of the small-diameter, extreme slow-follower fibres. At frequencies of

10–15 per sec total depolarizations of 30–40 mV sometimes occurred; even at about 5 per sec the electrical responses were large enough to pass the Ec level (Text-fig. 14). The individual p.s.p.s. had slow time constants of decay (70–200 msec). In other fibres, and particularly those known to be of fast-follower type, the electrical responses to indirect stimulation were similar to those seen in gradedly responding and spiking fibres at low frequencies of stimulation but even at very high frequencies of stimulation there was no evidence of even small-graded, direct, membrane responses.



Text-fig. 14. Passively responding fibres of the stretcher muscle. *a–c*, records from an extreme slow-follower during stimulation of the excitor axon at 3/sec (*a*), 6/sec (*b*), and 12/sec (*c*). *d–e*. Records from a slow-follower during stimulation of the excitor axon at 30/sec (*d*) and 60/sec (*e*). Tension, lower traces. Resting potentials; *a*, 64 mV; *d*, 65 mV. Calibration: vertical, 20 mV and 0·1 g; horizontal, 1 sec (*a–c*) and 3 sec (*d–e*).

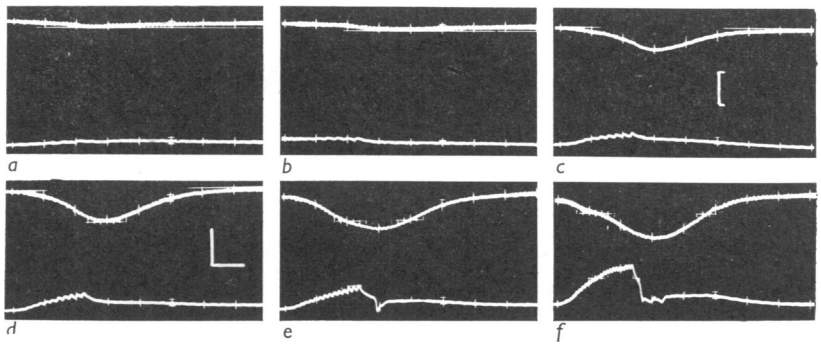
Tension responses of passively responding stretcher fibres are shown in Text-figs. 9, 10 and 14. In semi-isolated fibres it was necessary to stimulate the excitatory axon at frequencies above 40 per sec to obtain tension without chemical or direct electrical lowering of the membrane potential in addition. In order for tension to be observed, the depolarization had to exceed the directly determined Ec level. Some deterioration of the nerve terminals probably occurred during isolation, and in the normal fibres contraction might occur at somewhat lower frequencies.

The characteristic features of fast-follower (Text-fig. 10) and slow-follower (Text-figs. 9, 10, 14) tension responses were observed during both direct and indirect stimulation in the same fibres.

In the closer muscle most of the inexcitable fibres studied responded to the S axon only and gave only small p.s.p.s. The depolarizations evoked

in response to trains of excitation were frequently smooth, with little visible evidence of individual p.s.p.s. A few, however, gave large p.s.p.s. These responded only to the S axon.

Both fast- and slow-follower types of fibre gave good contractions to motor-axon stimulation. Most of the fast-followers were innervated by both axons; stimulation of each in turn resulted in similar rapidly developing contractions, and the axon producing the larger depolarization at a given frequency evoked the stronger contraction. The slow-followers examined were usually innervated only by the S axon, and had relatively large membrane time constants. Tension development was slow (Text-fig. 15) and continued after stimulations of the S axon was stopped.



Text-fig. 15. Tension development in passively-responding slow-follower fibre of the closer. Fibre responded to S axon but not to F. Stimulation rates (per sec): 25, 30, 50, 60, 75, 100. Calibration: 20 mV; 100 msec, 0·2 g. Note: tension continues to develop after cessation of p.s.p.s.; slow relaxation rate.

Only two of the supersensitive fibres in the closer muscle were obtained as semi-isolated fibres with their innervation intact. They were innervated by the S axon but probably not by the F. They gave small p.s.p.s. which facilitated little, but showed summation to quite a high level of depolarization at high frequencies of stimulation. Tension was observed when the membrane was depolarized by as little as 1 mV during stimulation of the S axon. Tension changes followed rapidly membrane-potential changes in these fibres, reaching a plateau in only 30 msec after attainment of steady depolarization. In this respect these fibres are matched only by the all-or-nothing twitch fibres. This is interesting in view of their completely unresponsive membrane, compared with the all-or-nothing spike membrane of the former.

Inhibition

Few successful preparations showing inhibition of indirectly evoked contractions were obtained. This appears partly to be due to a high sensitivity of the inhibitory nerve terminals to mechanical damage but it is

also now apparent that only a fraction of the muscle fibres receive inhibitory axon innervation.

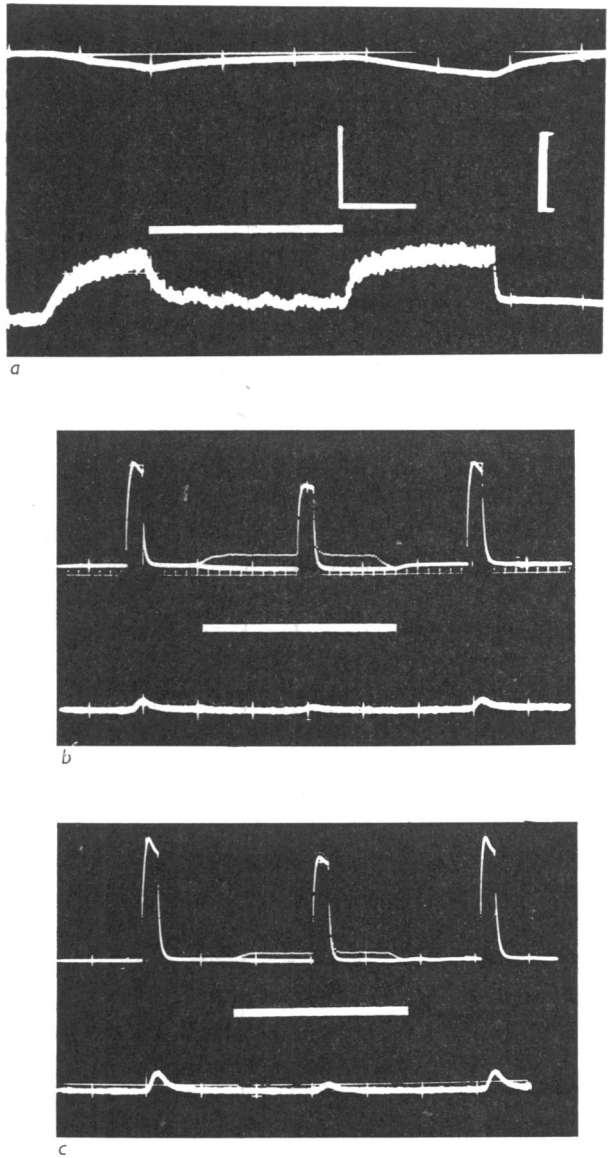
One successful preparation was obtained of a passively responding slow-follower fibre in the closer muscle which remained innervated by S and I axons (Text-fig. 16). In this fibre the effect of inhibitory stimulation was marked. The Ec value was close to the resting potential of 64 mV. An appreciable attenuation of the S p.s.p.s. and pronounced polarization (almost to the resting potential level) resulted when the I axon was stimulated during a train of S p.s.p.s. The tension was almost completely abolished during the inhibitory action.

The inhibition of tension has been attributed to a raising of the mean membrane potential level (Fatt & Katz, 1953*b*). A test of this hypothesis was made in semi-isolated stretcher muscle fibres still innervated by the specific inhibitor axon. These fibres were of the passively responding slow-follower type. In one case tension was developed in response to a depolarizing pulse of 17 mV (Text-fig. 16). When the specific inhibitor axon was stimulated during passage of outward current normally sufficient to elicit contraction, the membrane was rapidly polarized by several millivolts and a reduction in absolute size of the membrane-voltage response also occurred, indicating increased membrane conductance. The combination of these two effects reduced the depolarization to a level barely equal to Ec, and tension practically disappeared. An increase in strength of the stimulating current resulted in a larger depolarization both before and during inhibition and in a larger contraction during inhibition. In all cases the membrane depolarization and contraction returned to their original levels after release of the inhibition.

During continuous stimulation of the inhibitory axon the reduction of membrane depolarization and of tension became more pronounced with duration of the train of stimulation. It is apparent from this observation, as from previous work (Wiersma, 1961), that facilitation of inhibitor action occurs during prolonged stimulation. However, as long as the mean depolarization exceeded Ec, the contraction did not disappear completely.

Contractures of single fibres evoked by a high external potassium level could be relaxed by stimulation of the inhibitor axon. Repolarization accompanied the reduction of the maintained tension (cf. Boistel & Fatt, 1958). When the inhibition was released, depolarization and tension returned.

All the experiments on inhibition in single fibres are compatible with the view that the inhibitor action is confined to the muscle-cell membrane, and that the relative values of the membrane potentials and the Ec level determine whether or not tension is developed.



Text-fig. 16. Inhibition. *a*. Inhibition of S axon-evoked responses in passively responding slow-follower fibre in the closer. Stimulation rates: excitor, 200/sec; inhibitor, 150/sec. Tension, upper trace. *b*, *c*. Effects of stimulation of the specific inhibitory axons of the stretcher on tension production of an isolated passively responding muscle fibre (tension, lower traces). The inhibitor axon was stimulated at 30/sec during passage of depolarizing current pulses in *b* and in *c*; depolarizing current was stronger in *c*, and tension was reduced to a lesser extent by the inhibitor action. In all records the white bars between the membrane potential and tension traces indicate stimulation of the inhibitory axon. Resting potential (*b*, *c*), 67 mV. Calibration: vertical, 20mV, 0·2 g (*a*), 0·1 g (*b*, *c*); horizontal, 100 msec (*a*), 1 sec (*b*, *c*). In *a* the upper trace is centred on zero membrane potential.

Depolarization by potassium ions

By changing the potassium concentration of the solution surrounding a semi-isolated fibre, it was possible to depolarize the membrane and to observe tension responses. These experiments were performed on the relatively short stretcher-muscle fibres, which could more easily be depolarized uniformly by this method. The membrane-potential changes along the length of some of these fibres were checked by simultaneous recording with two micro-electrodes placed in different locations; the time courses and magnitudes of the membrane-potential shifts were closely similar in different places in the same fibre.

Tension occurred when the potassium-induced depolarization exceeded the values for Ec established by direct stimulation with outward current. Above the Ec level, there was a steep relation between membrane depolarization and tension, as found by Hodgkin & Horowicz (1960) in frog 'twitch' fibres. Passage of hyperpolarizing current during the contracture resulted in reduction or elimination of the tension, according to the degree of membrane polarization.

In fast-follower fibres the tension changes followed membrane-potential changes quite rapidly, as when depolarizing current was used. Much slower tension responses were seen in fibres known to be slow-followers. Since potassium depolarization was uniform, the characteristics of the tension responses must reflect a genuine contractile difference between fast- and slow-followers, and not an artificial condition such as a variable degree of spread of depolarization from a stimulated region.

Fast-following fibres were found to relax spontaneously in high potassium solutions; this process began about half a minute after application of a solution containing 45–75 m-mole/l. potassium ion. With lower potassium concentrations (25–40 m-mole/l.) tension was maintained at lower levels for a longer time (cf. Hodgkin & Horowicz, 1960). That relaxation depended on depolarization past the Ec level and not merely on the presence of excess potassium ion was shown in experiments in which the membrane was strongly hyperpolarized by a steadily maintained inward current during application of excess potassium. In these circumstances depolarization was not sufficient to exceed Ec and no tension was developed. After 3–6 min, well in excess of the time required for relaxation of steadily depolarized fast-follower fibres, the inward current was stopped. The membrane potential then fell past the contraction threshold and tension was developed. Subsequently, relaxation occurred at about the same rate as in fast-followers immersed in a high potassium solution without pre-treatment with hyperpolarizing current.

It was found that some of the slow-follower fibres, particularly certain

of the closer fibres, could maintain tension for many minutes when steadily depolarized past the Ec level. Thus phasic and tonic fibres exist in the closer and stretcher muscles, as in the accessory flexor muscle of the same species (Atwood & Dorai Raj, 1964).

The effect of treating spiking fibres with high potassium salines, even for a brief time, was to abolish the ability to give all-or-nothing spikes. After treatment such fibres resembled the passively responding fibres. Most had fast-follower characteristics, but a few were found which were similar to some of the less extreme slow-followers, in that they continued to develop tension rather slowly during a maintained depolarization of 100–500 msec and also showed slow relaxation. The value for Ec in spiking fibres which had been rendered inexcitable by potassium treatment was frequently found to be within a few millivolts of the level at which spikes were initiated before treatment. This does not necessarily mean that in the normal, spiking fibre, the value for Ec was the same as that determined after treatment with high potassium (see above).

It was noted that fast-followers which had relaxed in a solution of supranormal potassium concentration could still be made to contract by passing outward current pulses. In such fibres several millivolts of depolarization were needed to elicit tension. The value of Ec in these fibres appeared to have shifted from its original value to a new level a few millivolts removed from the membrane potential established in the high-potassium solution. Tension responses were much weaker in these fibres for a given amount of depolarization past Ec, but this effect was partly caused by a fall in membrane resistance and hence in the fibre-space constant. In both all-or-nothing spiking fibres and in passively responding fibres, the value of Ec is apparently shifted by changes which alter membrane excitability or resting potential.

Changes in external calcium ion concentration

The potassium contractures of the single muscle-fibre preparations were found to depend upon the presence of external calcium ions. After a muscle fibre had been soaked for 1–3 min in calcium-free saline, application of solutions containing high potassium concentrations and no calcium failed to evoke a contracture. If a few drops of calcium-containing saline were then added, contracture appeared. The results were similar to those which Frank (1960) obtained on frog skeletal muscle.

Similarly, treatment with saline of progressively lower calcium content reduced the tension developed in response to stimulation with outward current. Increasingly larger depolarizations were necessary to elicit tension as the calcium concentration was reduced. At the same time, many of the passively responding fibres developed the ability to produce graded mem-

brane responses. This effect is similar to that recently described in barnacle fibres by Hagiwara & Naka (1964) who found that at low external calcium concentrations the membrane potential for spike initiation was moved closer to the resting potential.

When treatment with very low calcium concentrations was continued for a sufficient length of time, all tension response disappeared and the membrane became inexcitable. Provided the fibre was not allowed to remain too long in zero calcium, the ability to produce tension was restored by addition of normal saline. The value of Ec soon became similar to the original. The graded responses which were evoked in passively responding fibres whilst bathed in low calcium salines were absent on returning to normal saline.

DISCUSSION

The findings of the present experiments are consistent with the hypothesis (Fatt & Katz, 1953*b*) that the excitor and inhibitor axons normally control tension in crustacean muscle fibres indirectly, by alternating the membrane potential. The proposal of a more direct action on excitation-contraction coupling mechanisms or on the contractile process itself, as was proposed by Hoyle & Wiersma (1958*b*) to account for the conflicting data obtained by studying tension in whole muscles but electrical activity from single fibres, was not borne out. The experiments on semi-isolated single muscle fibres are of particular significance in this regard, for in these fibres stimulation of the excitatory axon(s) could produce considerable electrical activity (evidence for plentiful release of transmitter substance) without causing contraction. Only when the depolarization exceeded Ec, the threshold membrane potential for contraction as determined by direct stimulation, did contraction occur. Similarly, when the inhibitor axon was stimulated, the contraction resulting from direct or indirect stimulation was suppressed completely only when the activating depolarization was reduced below the Ec level. Artificial inhibition of neurally induced contractions could be brought about by passing inward current across the membrane.

Another feature of crustacean muscles which was emphasized by the present work is the great variety of fibres present within a single muscle. This variety has now been found in *C. magister* in the stretcher, the closer, the accessory flexor (Atwood & Dorai Raj, 1964) and the extensor (Atwood, 1964). In all these muscles membrane responsiveness ranges from all-or-nothing spiking to passively-responding or inexcitable, and contractile responsiveness from fast-following and phasic, to slow-following and tonic. The several innervation patterns of these muscles indicate that the number and type of axons going to crab muscles does not determine whether or

not tonic and phasic fibres will be present. Indeed, many problems of the inter-relations between nerve and muscle during ontogeny remain to be deciphered. In the closer, there is a definite tendency for slow-following fibres to receive most or all of their innervation from the S axon, whereas the fast-following and spiking fibres tend to be especially innervated by the F axon. However, the stretcher receives a single excitor axon and yet contains a similar spectrum of muscle fibres so a 'trophic influence' of the different excitor axons on the muscle fibres is rendered questionable.

The pattern of differential innervation in the closer goes a long way towards explaining the features of 'fast' and 'slow' contractions recorded from the whole muscle as well as paradoxes in associating electrical activity with tension. Since a majority of slow-following fibres are activated by S axon stimulation, the over-all contraction will be slow to develop, especially at low frequencies. Since the spiking fibres and fast-followers are activated mainly by the F axon, excitation of the latter gives a rapidly rising contraction in response to quite brief bursts.

In the stretcher it seems likely that the mechanical response of the muscle at low frequencies of stimulation is attributable to contraction of a few extreme slow-follower fibres, for these proved to be the only ones found which gave electrical responses large enough to exceed Ec at these frequencies. In some of these fibres Ec was close to 60 mV, and the resting potential was about 65 mV. At frequencies of stimulation of 4–6 per sec, when contraction of the muscle as a whole could be detected, the p.s.p.s. reached 5–10 mV after facilitation. At higher frequencies the other passively responding fibres and the gradedly responding and spiking fibres would be recruited, giving more rapid tension development and greater total force. The contraction of the muscle as a whole, therefore, is composed of component contractions of widely different types at higher frequencies of stimulation of the excitor axon.

The scheme adopted for classification of the different types of muscle fibre uses membrane responsiveness as the primary feature (Table 1). This scheme is one of convenience. There is good reason to believe that membrane responsiveness is easily altered in these fibres, and that conversion from one type to another may occur. Alteration of calcium and potassium ions in the external medium had pronounced effects on membrane excitability, as did ageing or deterioration of the preparation. A striking feature was the relative proportions of the different kinds of fibres in muscles of different animals. Only highly aggressive, between-moult male crabs were found to contain relatively high proportions of spiking fibres and in female crabs none could usually be detected. This finding opens up the possibility that hormonal and other long-term changes can determine to

some extent the membrane properties of the fibres and cause a conversion from one kind to another.

The value of Ec also appeared to be influenced by ionic changes and there was some evidence that changes in membrane excitability were accompanied by changes in Ec. Certainly Ec showed great variability in these fibres. Some of the passively responding slow-followers and the supersensitive fibres of the closer had values for Ec within a few millivolts of the resting potential, whereas in spiking fibres Ec was removed from the resting potential by 20–40 mV.

A marked fundamental difference between fibres lay in the maximum speeds of contraction and manner of following membrane potential changes. There was no indication that fibres could change from one contractile form to the other, although in each case membrane responsiveness could be altered. An even more prominent, and perhaps more fundamental, feature was the gross histological appearance of the fibres. Two extreme types were found, corresponding very roughly to the Fibrillen- and Felderstruktur fibres of vertebrates (Krüger, 1949; Gray, 1958). The contraction rates were correspondingly fast and slow. The division is not complete, however, and there are many intermediates. It is possible that these correspond with fibres having intermediate speeds of contraction. The relations are often obscured by variations in membrane responsiveness.

The supersensitive fibres of the closer present a special problem. The near co-incidence of the resting potential and the threshold for contraction allies them primarily with slow-followers, but they give very quick contraction, and tension follows membrane potential quickly. They have a markedly short time constant compared with most of the other fibres. The presence in crustacean muscles of fibres with Ec very close to Em can explain the occurrence of contractions associated with minute electrical changes (Hoyle & Wiersma, 1958*a*, *b*).

It is evident that a crustacean muscle can be extremely complex in its constitution. This complexity exists at several levels—in structure (which may be related to contraction speed), in membrane properties (cf. Atwood, 1963), in the detailed pattern of innervation (particularly in muscles receiving more than one excitor axon), and possibly also in excitation–contraction coupling mechanisms. The latter could be based on anatomical difference (e.g. channels of the transverse tubular system—Ruska, Edwards & Caesar, 1958) or chemical differences.

Functionally, the diversity of structure and other properties in one muscle may be related to the need to achieve a wide range of tension control peripherally, and in turn dependent on the fact that only a few motor axons are available.

SUMMARY

1. The electrical responsiveness of single fibres of the closer and stretcher muscles of walking legs of the crab, *Cancer magister* was studied. Three main categories were recognized; all-or-nothing spiking, gradedly responding and passively responding.

2. The tension developed in response to depolarization was correlated with the electrical changes. Two principal forms of relation were seen, a *fast-following* of tension upon membrane-potential changes or a *slow-following*. Various intermediate types were also seen.

3. All-or-nothing spiking fibres gave only twitch contractions when in fresh conditions. Gradedly responding fibres gave both phasic and tonic contraction. Passively responding fibres gave mainly tonic contractions.

4. With prolonged depolarization all-or-nothing spiking fibres rather quickly ceased to contract. Passively responding fibres gave very long contractions and gradedly responding ones were intermediate.

5. A few fibres were found in which a minute depolarization led to a considerable contraction. They were termed supersensitive fibres.

6. Most of the muscle fibres have an even distribution of fibrils as seen in cross-section. A minority have a clumped appearance; the latter also show extensive deep invaginations from the sarcolemma complex. It is considered that the latter fibres are the extreme slow-followers.

7. For any individual fibre tension is related to the product of membrane potential exceeding the threshold membrane potential for contraction, Ec and time.

8. The range of values for Ec encountered was from 42 mV for an all-or-nothing fibre to 66 mV for a passively responding one.

9. The electrical and mechanical responses to nervous excitation of single muscle fibres were also studied.

10. Tension was elicited during stimulation of the motor axons only when membrane depolarization exceed Ec. The inhibitor axons abolished tension when membrane depolarization was reduced below Ec.

11. The results are consistent with the view that membrane depolarization caused by excitatory synaptic action is the initial cause of events leading to tension development.

12. In the closer muscle, slow-contracting fibres tend to be innervated by only the S axon, whereas fast-contracting muscle fibres tend to be innervated by only the F axon. Many fibres do not respond to the inhibitor axon.

13. The functioning of the whole muscle is a complex result of the summing of widely different neuromuscular events in fibres having a variety of properties.

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EXPLANATION OF PLATE

Pl. 1. Histology of fibres of the closer muscle of walking leg of *Cancer magister*. Material fixed in Bouin sectioned at 7·5 μ and stained with Masson trichrome. Calibrations = 100.

a. Portion of transverse section showing 'Felderstruktur-type' fibre at upper right (between arrows) with extensive sarcolemmal invasion and clumping of fibrils. Other fibres have more evenly dispersed fibrils.

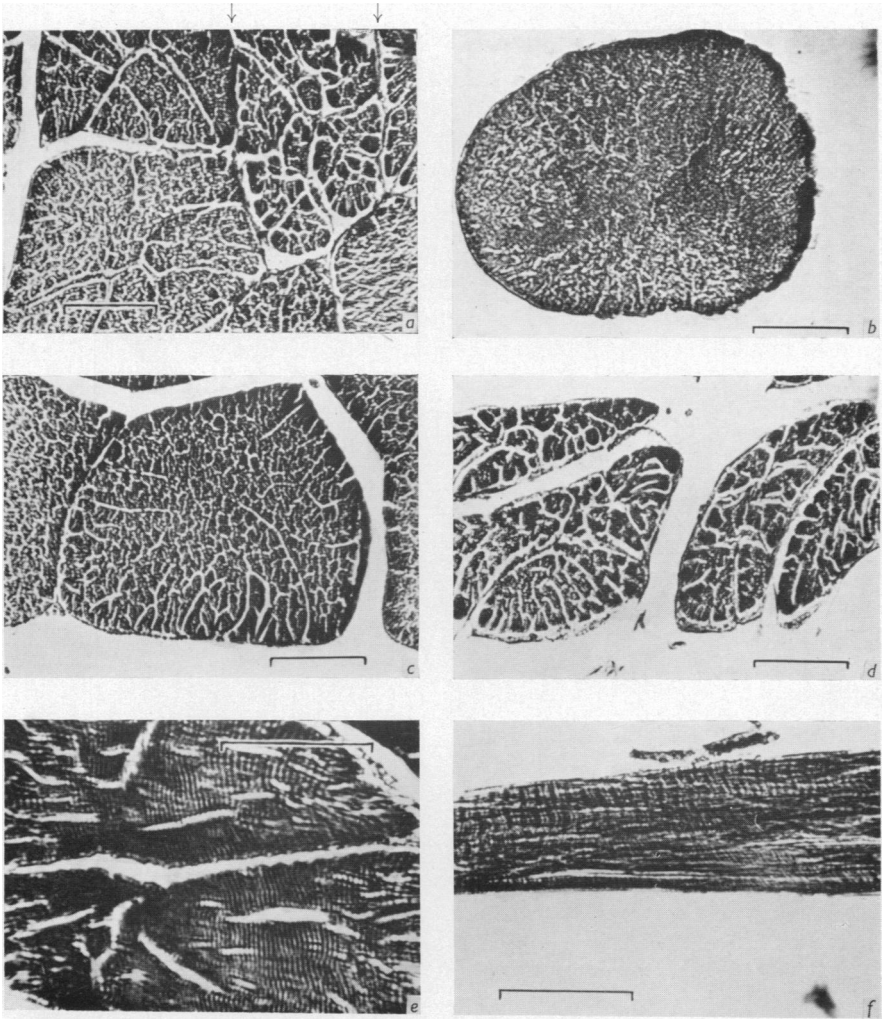
b. Transverse section through large, isolated 'Fibrillenstruktur-type' muscle fibre crammed with fibrils. Such fibres commonly give quick contractions.

c. Transverse section through intermediate-type fibre showing moderate invasion by sarcolemmal elements.

d. Transverse section through extreme 'Felderstruktur-type' muscle fibre with extensive sarcolemmal invasion. Such fibres give slow contractions.

e. Longitudinal section through average fibre (comparable to majority of fibres in *a* (upper left). Note short sarcomere lengths (3 μ).

f. Longitudinal section through fibre of 'Felderstruktur-type' (*d*). Note broad A-bands and sarcomere length (10–12 μ).



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(*Facing p.* 482)