THE NATURE OF "SLOW" AND "FAST" CONTRACTIONS IN THE SKELETAL MUSCLES OF INSECTS

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C O N T E N T S

APPENDIX

Becht, G., Hoyle, G. and Usherwood, P.N.R.
"Neuromuscular transmission in the coxal muscles of the cockroach."
J. Ins. Physiol. 4, 191-201.

Hill, R.B. and Usherwood, P.N.R.
"The action of 5-hydroxytryptamine and related compounds on neuromuscular transmission in the locust Schistocerca gregaria."
J. Physiol. 157, 393-401.

Usherwood, P.N.R.
"Spontaneous miniature potentials from insect muscle fibres."
Nature 191, 814-815.

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GENERAL INTRODUCTION

Interest in the neuromuscular physiology of insects dates back to the late nineteenth century. Rollet (1884) published an account of the mechanical responses of the skeletal muscles of Hydrophilus and Dytiscus in which he observed that the duration of the twitch response of the muscles of Hydrophilus was greater, by over a factor of three, than the twitch duration of the muscles of Dytiscus. Conclusions drawn from earlier experiments on vertebrate preparations greatly influenced him in the appraisal of his results. He suggested that the rapid contracting muscles of Dytiscus might be compared with vertebrate white muscle; the more slowly contracting muscles of Hydrophilus with vertebrate red muscle.

Although the gross properties of insect muscle preparations are essentially similar to those of vertebrate muscle preparations, it is perhaps unfortunate that early workers treated their insect preparations as if they were similar to the frog gastrocnemius muscle, since it makes the task of interpreting their results in the light of present day knowledge more difficult. Hoyle (1957) suggested that we might interpret their results on the assumption that they had stimulated only the "fast" nerve fibres of their preparations. However, this suggestion

could be very misleading if the preparations were innervated by "slow" as well as by "fast" motor axons.

Application of a single supramaximal shock to the innervating motor nerve of an insect muscle evokes a simple mechanical response from the muscle described as a twitch contraction. Between the application of the stimulus and the beginning of visible contraction there is a latent period of variable duration. The twitch consists of a contraction phase followed immediately by a relaxation phase. In the early experiments on insect muscle the contractions were recorded kymographically by causing the muscle to raise a lever which wrote on a moving drum. With this recording technique the precise form of the twitch must have been influenced by the mechanical properties of the lever.

Marchal (1910) found that the larval muscles of Bombyx mori gave twitch responses which were almost fifty times as long in total duration as the twitch responses of the muscles of the imago. Once again comparisons were made with vertebrate preparations. Marchal suggested that the slow contracting larval muscles were similar to vertebrate smooth muscle.

The first account of tonic contractions in insect muscle was published by von Buddenbrock (1920) who claimed to have

demonstrated the existence of a special tonus mechanism in Dixippus muscle able to maintain a contraction with a lower rate of energy utilization than that required to initiate it. The rates of oxygen uptake with the animal in two different cataleptic states, (1) lying on its back with legs folded up against its body and (2) standing with legs extended, agreed to within 1% and he concluded that muscles active in the standing position must be "tonus" muscles. Pringle (1939) suggested that this conclusion might well be at fault, since with the type of antagonistic musculature found in the insect leg any position from complete flexion to complete extension may be obtained with the muscles in a considerable state of tonic contraction.

It was not until Rijlant (1932) recorded two electrical responses pertaining to active contraction and tonus respectively in the leg muscles of the insects Dytiscus and Hydrophilus that a relationship between the electrical and mechanical responses of insect muscle was first suggested. In these muscles reflexly-evoked action potentials about 250 micro-volts in height were associated with vigorous mechanical activity. These potentials occurred in short high-frequency bursts and showed little signs of facilitation. During normal postural activity smaller potentials were recorded. These were about 100 micro-volts in magnitude and occurred

rhythmically at a frequency of 2-40/sec. Rijlant assumed that the large and small potentials originated from two types of muscle fibre.

Over ten years elapsed before any further progress in the study of insect neuromuscular physiology was made. Then in the early nineteen thirties a large body of data was published: Heidermanns (1931) on the wing muscles of Aeschna; Solf (1931) on the leg muscles of Decticus and Gryllotalpa; Kraemer (1932a and b) on the leg muscles of Decticus and Lucanus; Cremer (1934) on the wing muscles of Aeschna. In all these investigations the recording techniques employed cast considerable doubt on the validity of the results obtained. In many cases little or no anatomical data on the preparation ~~was~~ were available to the experimenter and few quantitative results of value were obtained. Perhaps some of the more interesting results were obtained by Heidermanns (1931). He found that stimuli of subthreshold intensity applied to the surface of some of the thoracic muscles of Aeschna at first failed to elicit any noticeable mechanical response. Later, however, under continued stimulation, a contraction appeared which increased in magnitude with each succeeding stimulus. It is possible that Heidermanns was, on this occasion, stimulating a "slow" motor axon.

Several workers have commented on the relationship between the intensity of stimulation and the amplitude of the mechanical response elicited. Heidermanns (1931) found a direct proportionality for the wing muscles of Aeschna. Solf (1931), however, concluded that in the normal unfatigued extensor tibiae muscle of Decticus the excitation thresholds for all the muscle fibres are similar and that the mechanical response of the muscle as a whole is all-or-none. Kraemer (1932b) found four distinct steps in the mechanical response of the extensor trochanteris of Dytiscus with increasing intensity of stimulation.

The possibility of double innervation of insect muscle was first suggested by Friedrich (1933). At stimulation intensities below threshold he claimed to have demonstrated peripheral inhibition in the leg muscles of Dixippus either as a slight relaxation of the resting muscle or as an increased rate of relaxation following active contraction. Although considerable doubt has been cast both on the validity of his results (Becht, 1959) and on the validity of his interpretations (Pringle, 1939), he was nevertheless the first person to attempt stimulation of insect muscle through the innervating motor nerve.

The first major advance in elucidating the mechanisms

involved in neuromuscular transmission in insects was made by Pringle (1939) when he demonstrated a double motor innervation for the extensor tibiae muscle of the meta-thoracic leg of Periplaneta americana. The recording techniques employed throughout his experiments were far superior to those employed by earlier workers. External electrodes were placed on the surface of the extensor muscle to record the electrical responses of the muscle to reflex stimulation and to indirect electrical stimulation. Small (100μV) potentials, occurring as a more or less regular series of spikes at a frequency of about 30/sec, were recorded to reflex stimulation of the muscle. Against this background of small spikes Pringle observed short bursts of larger spikes. The larger spikes were about 2 mV in amplitude, and occurred at a frequency of about 75 c/sec. The smaller spikes were associated with tonic contractions of the muscle whereas the larger spikes were accompanied by brief tetanic contractions.

Pringle was able to obtain both tetanic and tonic responses of the cockroach muscle through stimulation of the innervating motor nerve. His method of reproducing the tonic response, to the exclusion of the tetanic response, by allowing the motor nerve to partially dry out has been criticized by Becht (1959). Nevertheless the mechanical

response obtained using this rather drastic method was similar in every respect to the tonic contraction recorded previously from the intact preparation following reflex stimulation.

Pringle assumed that the two types of electrical responses recorded from the extensor muscle occurred in the same muscle fibres and that these fibres were capable of both tonic and tetanic contractions. This assumption has been criticized by Becht (1959) on the grounds that the muscle might be a compound structure, the several parts of which could yield different mechanical and electrical responses. As an alternative explanation Becht suggested that the two responses could be located in two different sets of muscle fibres.

Further attempts to correlate the electrical and mechanical events of nerve-muscle activity in insects were made by Roeder and Weiant (1950). They used the tergal remotor muscle of the cockroach to record simultaneously the isometric tension developed and the electrical response evoked following indirect stimulation through the innervating motor nerve. Unfortunately in the use of the piezo-electric technique they were handicapped to some extent and were unable to determine the linearity and frequency

characteristic of the mechanical response. Their investigations were mainly confined to the electrical events of neuromuscular activity, although they did demonstrate that the remotor muscle acted as a single motor unit with a mechanical response not unlike that performed by the extensor tibiae muscle of the cockroach to "fast" axon stimulation (Pringle, 1939).

Hoyle and a number of co-workers using the intracellular micro-electrode technique on insect nerve-muscle preparations obtained an excellent account of the events leading up to and including contraction of the femoral muscles of the locust Locusta migratoria migratorioides. Hoyle (1955b and c) clearly demonstrated that the different types of mechanical response obtained from the extensor tibiae muscle of the locust leg are correlated with the innervation of the muscle. "Slow" or tonic contractions are evoked by stimulation of a "slow" motor axon whereas "fast" or twitch-tetanic contractions result from stimulation of a "fast" motor axon. Furthermore, many of the fibres of the extensor tibiae muscle of the locust respond to both "fast" and "slow" axon stimulation. It was concluded that a single muscle fibre may be innervated by more than one motor axon.

The intracellular technique was used also by Wilson

(1954) to record the electrical responses in the flexor tibiae muscle fibres of the cockroach following motor nerve stimulation. To maximal, indirect, electrical stimulation non-facilitating "fast" electrical responses varying in magnitude from 40-80 mV were recorded from many of the fibres of the flexor muscle. "Slow" responses consisting of small facilitating potentials, varying in magnitude from 8-20 mV, were recorded from other fibres. Wilson was unable to obtain both types of response from the same muscle fibre and concluded therefore that the two types of activity represent the responses of two different types of fibres. An analogy was drawn with the "slow" and "fast" muscle fibres of certain amphibian muscles (Kuffler and Williams, 1953). Hoyle (1957) has severely criticized Wilson's results on the basis that no attempt was made to relate the innervation of the muscle to the electrical recordings obtained and suggested that Wilson's observations were possibly from muscle fibres with either high or low resting potentials; the so-called "slow" responses were therefore possibly reduced "fast" responses obtained from muscle fibres with low resting potentials.

Hoyle suggested that if Wilson had raised the potassium level whilst recording from fibres with large resting potentials he would have probably converted the "fast" responses

of these fibres into "slow" responses. In other words, all Wilson's observations were possibly responses to "fast" axon stimulation from both high and low resting potential muscle fibres. Although Wilson's observations could have resulted possibly from poor technique, the possibility that the control of muscle contraction, in some insect muscles at least, is myogenic and not neurogenic has been suggested by a number of other insect physiologists.

Ewer (1957) found that certain prothoracic muscles of Acathacris, when directly stimulated by low-frequency shocks, did not respond with twitch contractions. He had previously reported a similar condition in the nymphal musculature of this insect (1954). The results of further experiments on the thoracic muscles of acridids, this time on the muscles of L. migratoria, led him to the conclusion that there are functional differences between the various thoracic muscles. Muscles which gave a large twitch response to a single direct stimulus were termed "twitch" muscles, whereas muscles which gave an appreciable contraction to high-frequency stimulation only were termed "tonic" muscles. Some of the muscles responded with either a twitch or a tonic contraction according to the intensity of stimulation. Ewer called these "twitch-tonic" muscles.

The validity of these conclusions is open to doubt for

Fig. 21. *Some characteristics of muscle types.*

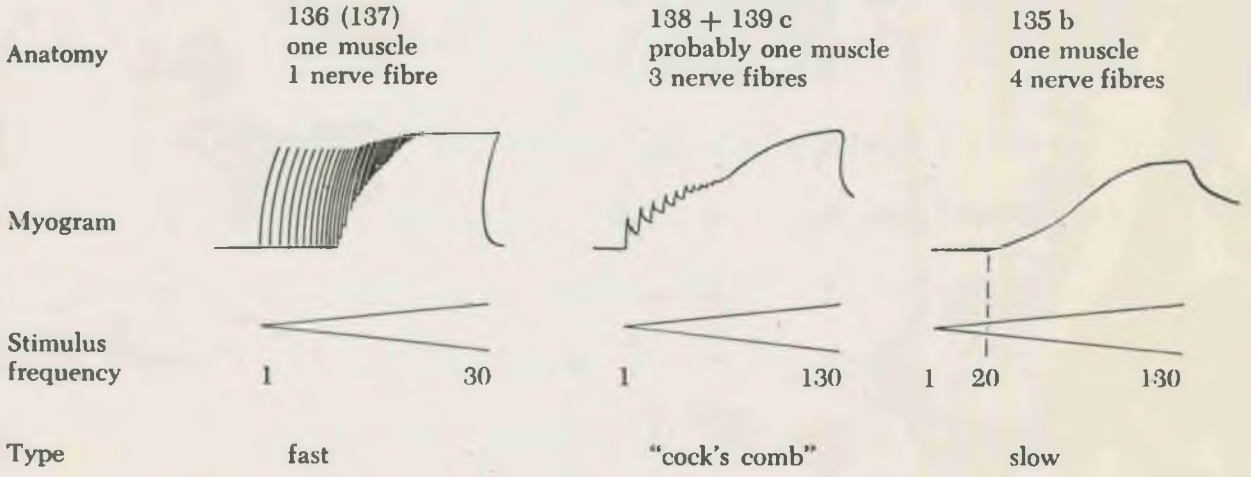


Fig. 1. (After Becht, 1959).

Ewer made no attempt to stimulate the muscles indirectly through the innervating motor nerves. It is possible that his recordings were the result of differences in motor nerve innervation to the various thoracic muscles; the muscles which gave tonic responses are possibly innervated by motor axons of the "slow" type.

Hoyle (1955b and c) has already demonstrated that in other skeletal muscles of the locust the type of mechanical response performed is governed by the type of motor nerve innervation to the muscle. Muscles innervated by "fast" motor axons are capable of performing "fast" mechanical responses whilst muscles innervated by both "fast" and "slow" motor axons are capable of performing both "fast" and "slow" mechanical responses. On the basis of Ewer's conclusions the mechanical response of the thoracic muscle of the locust is governed by some other factor possibly inherent in the muscle itself, i.e. control of contraction is myogenic and not neurogenic.

Further evidence for myogenic as opposed to neurogenic control of contraction in insect muscle was forwarded by Dresden (1956), Becht and Dresden (1956) and Becht (1959). These authors studied the mechanical responses of the meso-thoracic coxal muscles of the cockroach P. americana using an isotonic recording system.

They concluded from their results that the coxal muscles are divisible into three classes on the basis of differences in the mechanical responses of the different muscles to motor nerve stimulation (Fig. 1). Some of the muscles, e.g. 136 and 137 (Carbonell, 1947), gave a large twitch response to a single maximal shock of the innervating motor nerve, showed high twitch/tetanus ratios for isotonic excursion and fatigued very quickly under sustained tetanic stimulation. These muscles were designated "fast". On the other hand, muscle 135b gave only a minute response to a single maximal stimulus, had a low or very low twitch/tetanus ratio for isotonic excursion and fatigued only slightly under tetanus. Because the rate of shortening of muscle 135b was much slower than the rate of shortening of the rest of the coxal muscles, Becht and Dresden termed it a "slow" muscle. The isotonic myograms obtained by Becht and Dresden from the third class of muscles were, in many respects, similar to those obtained from the "slow" muscle 135b but at the same time characteristics of the myograms obtained from their "fast" muscles were present. They suggested that the "fast" and "slow" functions are localized in different muscle fibres and that these muscles should be termed "mixed". Furthermore, according to Becht and Dresden the alkaloid ryanodine inhibits the mechanical response of the "fast"

muscles or muscle fibres before it affects the mechanical response of the "slow" muscles or muscle fibres.

If the results and conclusions of Becht and Dresden are accepted, therefore, three types of muscle are present in the coxa of the cockroach, "fast", "mixed" and "slow". However, Becht and Dresden could not rule out the possibility that the different mechanical responses of the various muscles were explicable in terms of differences in their physiology of synaptic transmission between nerve and muscle. This possibility was examined by Becht, Hoyle and Usherwood (1960). These authors made intracellular recordings of the electrical events in the fibres of the different coxal muscles during graded electrical stimulation of the innervating motor nerves. The results obtained from this investigation showed clearly that if any differences in the mechanical properties of the different coxal muscles do exist, then they are probably not associated with any differences in the electrical properties of the muscles or in synaptic transmission and may therefore reside in the contractile processes of the muscle fibres themselves or in the mechanism coupling excitation to contraction.

Smit (1958) has shown that Becht and Dresden's "slow" and "mixed" cockroach muscles contain more lipids and

possess a stronger reducing capacity for methylene blue than the "fast" muscles. Recently George and Bhakthan (1961) have shown that there is more lipase activity in the "slow" and "mixed" muscles than in the "fast" muscles.

Small histological differences between the different muscles of the cockroach coxal segment have been described by Smit (1958). He has also measured the sarcomere lengths of the different coxal muscles and has found that the sarcomere lengths of the "fast" muscles are shorter than those of the "slow" muscles (personal communication).

It is perhaps not surprising that of the other arthropod groups the most extensively studied have been the Crustacea. The large size and the plentiful supply of the decapod crustaceans make them excellent preparations for neuromuscular-physiological investigations. Lucas (1917) was the first to postulate two types of contraction mechanisms for a single arthropod muscle. His work on the crayfish Astacus led him to the conclusion that two independent systems were located in the same muscle. From histological and anatomical studies it soon became clear that many decapod muscles are innervated by more than one nerve fibre, and it is certain that the same muscle fibres can react to impulses of different motor axons. Furthermore, it has been established recently that many of the flexor muscle

fibres of the carpopodite in the legs of Panulirus interruptus are able to respond to all four motor axons that innervate this muscle (Furshpan, 1955). As yet, however, the condition in certain insect muscles where only a fraction of the muscle fibres are innervated by the "slow" axon (Hoyle, 1955c) has not been found in crustacean muscles. In all instances where the crustacean muscle is dually innervated all the muscle fibres apparently receive branches from both motor axons.

Perhaps the main distinctive feature of the decapod crustacean neuromuscular system is the presence of peripheral inhibitory axons. If these axons are stimulated simultaneously with an excitatory axon, the mechanical response which would result if the latter alone were stimulated is either partially or completely inhibited.

Peripheral inhibition in the insects has been reported on a number of occasions. Friedrich (1933), working with Dixippus morosus, claimed to have demonstrated peripheral inhibition in the tibial muscle of the metathoracic leg. He found that after severance of the ganglion, low intensity stimulation of the motor nerve to the metathoracic leg relaxed the tibial muscles beyond their normal resting length.

Ripley and Ewer (1951) found that stimulation of the

levator tarsus muscle of the metathoracic leg of L. migratoria at an intensity about three times above threshold caused a marked reduction in the mechanical response. Hoyle (1955c) repeated their experiments using more refined techniques and was able to demonstrate that the effect is not true inhibition but an artifact resulting from failure to excite the nerve. He suggested that electrode polarisation resulting from the high voltage employed might be responsible for this failure. Finally, Becht and Dresden (1956) and Becht (1959) noted that an increase in the intensity of stimulation induced a rather marked decline of tension in some but not all of the coxal muscles of the mesothoracic leg of P. americana.

The experiments described in this thesis were made in an attempt to resolve some of the conflicting opinions of the control of contraction in the skeletal muscles of insects. Are the contractions of the coxal and femoral muscles of the cockroach and the thoracic muscles of the locust controlled myogenically as suggested by Becht and Dresden, Ewer and Wilson, and if so then how are the differences in contraction properties between the various muscles correlated with their histological properties? Furthermore, if these muscles are controlled myogenically then how do their histological and neuromuscular

transmission properties differ from those of the femoral muscles of the locust which are thought to have a neurogenic control mechanism?

The electrical and mechanical responses of the coxal and femoral muscles of the cockroach, the femoral muscles of the locust and some of the thoracic muscles of the locust have been re-examined with modified stimulating and recording techniques. Many of the results of these experiments which are described in Section II have been accepted for publication by the Journal of Insect Physiology.

An attempt has been made to correlate directly the mechanical and electrical events of neuromuscular transmissio in the different muscles of the coxa and femur of the cockroach by recording the mechanical and electrical responses of the muscles simultaneously, following graded-electrical stimulation of the innervating motor nerves.

The action of ryanodine on the "fast" and "slow" electrical and mechanical responses of the extensor tibiae preparation of Schistocerca has been studied in detail. Some of the results, i.e. those which are relevant to the main theme of the thesis, are included in Section II. The main results of this investigation have been submitted as a separate paper for publication in the Journal of Physiology.

Experiments were performed on the muscles of four members of the Orthoptera; Periplaneta americana, Blaberus giganteus, Schistocerca gregaria and Locusta migratoria. P. americana, the American cockroach, has already received considerable attention from anatomists and physiologists alike and a large amount of useful data ~~is~~ are available on the structure and function of the locomotory muscles. Furthermore, it is easily bred in the laboratory and requires little attention once it has become established. One main limiting factor is its small size. For comparative purposes an attempt was made to find an alternative and larger species from the same insect group. During a short visit to this country Dr G. Becht of Nijmegen, Holland introduced the author to some specimens of the giant cockroach B. giganteus. This insect is one of the largest members of the cockroach family, some specimens measuring as much as 55 mm in overall length. It is an indigenous species of warmer climates and is found in Central and South America (Rehn and Herbard, 1927). Apart from a short study on its life history (Piquett and Fales, 1953) studies on the anatomy and physiology and behaviour of B. giganteus have not been made previously.

At that time it seemed an exciting possibility as an experimental medium. Further specimens were imported from

the United States and bred with some considerable success in the laboratory. Dr C.C. Hassett of the United States Chemical Center at Maryland was responsible for kindly supplying the insects on which the culture was founded.

The first experiments on Blaberus met with little success and although many of the main difficulties have been overcome in the course of the investigations, there are still some left outstanding. For this reason only a few of the more satisfactory results from the Blaberus experiments will be described.

The anatomy and histology of the coxal muscles of Periplaneta and Blaberus, the femoral muscles of Schistocerca and some of the thoracic muscles of Schistocerca and Locusta have been re-examined in the light of earlier work in this field. The results of these studies, in which particular emphasis was laid on the innervation properties of the different muscles, are described in Section I of this thesis.

An Appendix to the thesis includes reprints of some of the papers already published by the author. Results described in the first paper were obtained when the author was working in conjunction with Dr G. Hoyle and Dr G. Becht. At that time the author was still learning the micro-electrode

technique and played only a minor part in the investigation. However, the author has since repeated and extended all of the experiments described in that paper. The results for the second paper were obtained when the author was working in conjunction with Dr R.B. Hill, a visiting Post-Doctoral Fellow from the United States. The majority of the experiments described in this paper were performed by the author, who has since extended the investigations.

SECTION I. ANATOMY AND HISTOLOGY

Hoyle (1957) laid down certain requirements which he considers should be fulfilled if the results of experiments on insect nerve-muscle apparatus are to be of comparative value. Among these is the requirement that the arrangement and innervation of the muscle fibres under examination must be known. In this Section an attempt is made to meet this requirement. Although some of the data described ~~is~~ are derived from the results of previous investigators in this field, a large proportion ~~is~~ are derived from original observations made by the author.

METHODS

Complete serial sections of the metathoracic femurs of adult specimens of Periplaneta, Blaberus and Schistocerca were made. The topography of the muscles was reconstructed from the slides and checked by dissection under the binocular microscope. The technique for preparation of the slides was as follows.

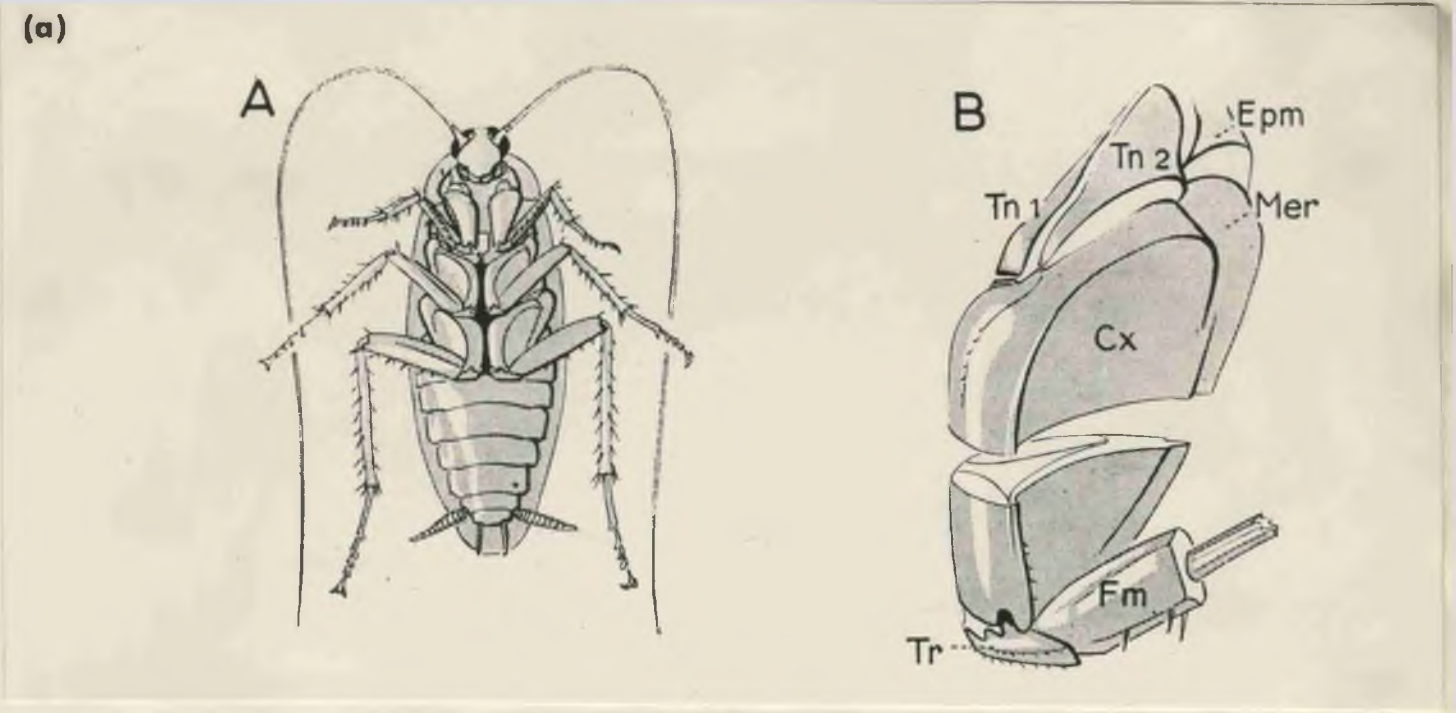
Newly-moulted adult specimens of these insects were anaesthetized with carbon dioxide and decapitated. The metathoracic leg of either side was removed from the animal and the femur separated from the rest of the leg at the

coxo-trochanteral and femoro-tibial junctions. The separated femoral segments were fixed in alcoholic Bouin for 24 hours, dehydrated through the alcohols and finally embedded in ester wax (Steedman, 1947). Serial sections of the femur were cut at 10μ on a Cambridge microtome and stained either with Ehrlich's haematoxylin and eosin or by Wigglesworth's iron haematoxylin method (1953).

Most dissections were performed on anaesthetized preparations under saline, although a few dissections were performed on preparations fixed in 70% alcohol.

The structure and arrangement of the coxal muscles of Periplaneta and Blaberus and the thoracic muscles of Schistocerca were examined in less detail. However, the methods employed were essentially similar to those described above.

Sarcomere "rest" lengths have been measured in the muscle fibres of the coxal muscles of Periplaneta and Blaberus. The muscles of the mesothoracic coxal segment of anaesthetized adult male or female specimens were fixed in situ, in either 70% alcohol for 12 hours or 4% neutral formalin for 24 hours. The apodeme attachments to the trochanter were severed before fixation to ensure that all the muscles were fixed at a constant reference length, the "equilibrium length".



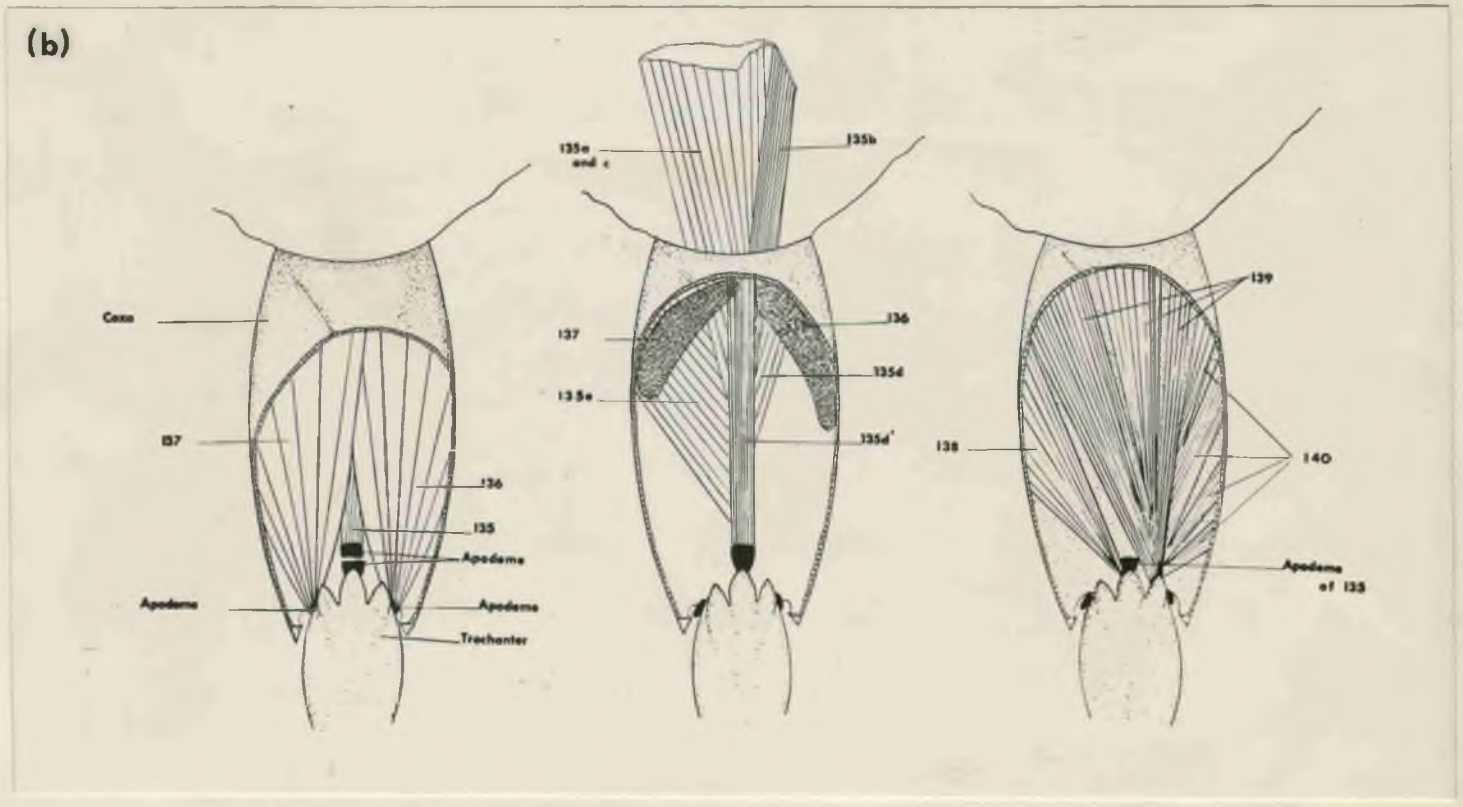


Fig. 2a. A. The cockroach, Periplaneta americana (L) seen from the ventral side.
B. The left mesothoracic leg seen ventrally.
Epm = Epimeron
Mer = Meron
Tn1, Tn2 = first and second trochanter
Cx = coxa
Tr = trochanter
Fm = femur. (After Becht, 1959)

Fig. 2b. Muscles of the coxa of the mesothoracic leg of Periplaneta, shown diagrammatically in dorsal view. The flexor muscles are not illustrated in the two left-hand drawings. In the right-hand drawing the extensor muscles are not shown.

After fixation the muscles were extracted from the coxa. Fragments were isolated and examined in water, under polarised light. The sarcomere lengths, i.e. the distance between adjacent Z-lines, were measured with a calibrated eyepiece. About ten readings were taken from each muscle preparation and the results from a number of preparations of each muscle were collated (Tables 1, 2 and 3).

RESULTS

(1) The mesothoracic coxal muscles of the cockroach

The arrangement and innervation of the coxal muscles of Periplaneta have been studied by a number of investigators. Pringle (1939) examined the muscles in the metathoracic leg and gave an account of their innervation. Carbonell (1947) gave an extensive description of the musculature of all thoracic coxal segments but did not describe the innervation of the muscles. A much fuller account was published in a series of papers by Nijenhuis and Dresden. In 1953 they described the mechanism of motion of the second thoracic leg and some supplementary anatomical data relating to this segment. In 1955 they extended their investigations to the course of the motor nerves in the coxal segment. Finally, in 1958 they completed their observations with an account of the number of motor axons innervating each mesothoracic coxal muscle.

In the present investigations the description, definition and numbering of the muscles have been derived from the studies of Carbonell (1947), Pringle (1939) and Dresden and Nijenhuis (1953, 1958). The muscles studied were the extensors 136, 137, 135a and c, 135b and 135d and e, and the flexors 138, 139c, 139a and b, and 140. The motor nerve innervation to these muscles has been re-examined by the author and the results obtained are in full agreement with those published earlier by Dresden and Nijenhuis (1958).

Extensor Muscles

Muscle 136. A broad muscle which originates on the posterior dorsal wall of the coxa, near the rim. Its fibres converge to an apodeme which is attached to a small dentiform apophysis on the medio-proximal edge of the trochanter.

Muscle 137. The anterior coxal extensor of the trochanter. This muscle arises from the meso-ventral part and the meso-ventral angle of the coxa. It is separated from muscle 136 by the muscle group 135 and like muscle 136 it is attached to the trochanter on a small dentiform apophysis at the medio-proximal edge of the trochanter.

Muscles 136 and 137, which receive a common nerve

supply of one large axon from nerve 5 ramus 1, are white in colour due to the absence of pigmentation.

Muscle 135. The main extensor of the trochanter, muscle 135, is the most powerful muscle of the mesothorax and leg. It originates on several parts of the mesothorax and coxa; its branches converge to a broad spoon-shaped apodeme inserted on the median dentiform apophysis of the trochanter.

1. 135a. The tergal branch of muscle 135 originates on the antero-lateral part of the tergum and its fibres converge to the apodeme about midway down the coxa. Muscle 135a is supplied by three motor axons from nerve 4 ramus 3.

2. 135c. The basalar muscle of the forewing is attached anteriorly to the apodeme which arises from the anterior edge of the basalar plate. It inserts posteriorly on the broadest portion of the trochanteral apodeme. Muscle 135c is innervated by one motor axon contained in nerve 4 ramus 3.

3. 135b. The sternal branch of muscle 135 originates on the downward-bent flange of the sternal arm and inserts on the trochanteral apodeme at the level of insertion of 135a. Muscle 135b is a small band-shaped muscle innervated by nerve 4 ramus 2b which supplies it with at least four axons.

4. 135d. The coxal branch of muscle 135 originates on the coxal wall between muscles 136 and 137. Part of 135d is band-shaped and is attached posteriorly to the trochanteral apodeme close to the trochanteral apophysis, i.e. the muscle extends throughout the length of the coxa. The other part of 135d consists of a small group of fibres which insert at an angle on the proximal posterior face of the apodeme. Muscle 135 is innervated by nerve 5 ramus 1b which supplies it with one large and three small axons.

5. 135e. The second coxal branch of muscle 135 originates in the anterior part of the coxal rim, near the coxo-trochantinal articulation. The fibres of 135e are attached at an angle to the anterior border of the trochanter apodeme. Innervation is through nerve 5 ramus 1a which supplies 135e with one large and three smaller axons.

Flexor muscles

Muscle 138. The anterior coxal flexor of the trochanter muscle 138 is a slender, weak muscle which originates on the anterior wall of the coxa. The fibres converge to a slender apodeme which is attached to the lateral edge of the trochanter. Muscle 138 is innervated by seven motor axons from nerve 3b.

Muscle 139. The main coxal flexor of the trochanter,

muscle 139, has several origins on the coxal wall. Its fibres converge to an apodeme attached to the lateral wall of the trochanter. This muscle has been divided into three units.

1. 139a. Origin on the posterior wall of the coxa, toward the meral angle. It is innervated by seven nerve fibres contained in nerve 6b.

2. 139b. Origin on the anterior part of the coxal rim and on the ridge which limits the meron. Muscle 139b is innervated by nerve 6b.

3. 139c. Origin on the anterior wall of the coxa and coxal rim. This muscle is innervated by nerve 3b.

Muscle 140. The posterior coxal flexor of the trochanter is a rather small muscle, with four branches (a, b, c, and d), which originates on the posterior wall of the coxa. All the branches converge to an apodeme which is attached to the trochanter close to the point of attachment of muscle 139.

The structure and innervation of the coxal muscles of Blaberus are essentially similar to those of the coxal muscles of Periplaneta.

Table 1. Average sarcomere length (μ) + S.e. of mean for muscle fibres of the coxal muscles of P. americana fixed in 70% alcohol at equilibrium length.

Muscle	At equilibrium length	At minimum body length	At minimum body length (after Smit)
135a and c	4.3 ± 0.08 (68)	4.9 ± 0.25 (19)	(1.6 ± 0.5) *
137	4.2 ± 0.05 (138)	4.5 ± 0.05 (50)	(1.9 ± 0.13) *
136	3.8 ± 0.32 (20)	4.5 ± 0.04 (100)	(1.9 ± 0.11) *
135d'	3.7 ± 0.05 (135)	4.9 ± 0.07 (43)	(1.7 ± 0.46) *
135d	3.9 ± 0.04 (123)	5.4 ± 0.11 (56)	(1.9 ± 0.27) *
135b	3.5 ± 0.07 (49)	5.5 ± 0.08 (193)	(2.8 ± 0.55) *
135e	3.7 ± 0.04 (171)	5.1 ± 0.12 (75)	(1.9 ± 0.14) *
135e'	3.5 ± 0.1 (45)	-	(-)
138	4.0 ± 0.13 (32)	5.9 ± 0.8 (35)	(-)
139c	3.9 ± 0.03 (35)	4.4 ± 0.05 (56)	(-)
139a and b	4.2 ± 0.02 (10)	4.8 ± 0.12 (100)	(-)
140	4.3 ± 0.10 (40)	6.4 ± 0.12 (54)	(-)

Figures in brackets indicate number of readings taken. Measurements in asterisked brackets are after Smit (personal communication).

Table 2.

Average sarcomere rest length (μ) + S.e. of mean for muscle fibres of the coxal muscles of P. americana fixed in 4% formalin at equilibrium length.

Muscle	Sarcomere rest length
137	3.1 ± 0.2 (14)
136	3.1 ± 0.05 (31)
135 a and c	3.4 ± 0.13 (20)
135b	3.1 ± 0.07 (54)
135d	3.1 ± 0.05 (31)
135d'	3.0 ± 0.05 (33)
135e	2.9 ± 0.08 (21)
135e'	3.5 ± 0.1 (40)
138	-
139c	3.5 ± 0.12 (21)
140	3.4 ± 0.09 (40)
139 a and b	3.5 ± 0.07 (65)

Figures in brackets indicate number of readings taken.

Table 3.

Average sarcomere rest length (μ) + S.e. of mean for muscle fibres of the coxal muscles of B. giganteus fixed 24 hours in 4% formalin at equilibrium length.

Muscle	Sarcomere rest length
137	3.5 ± 0.02 (40)
136	3.3 ± 0.03 (30)
135a and c	4.2 ± 0.17 (12)
135b	3.3 ± 0.1 (14)
135d	3.3 ± 0.05 (30)
135d'	3.3 ± 0.07 (30)
135e and e'	3.9 ± 0.05 (40)
138	3.1 ± 0.06 (17)
139c	3.1 ± 0.05 (17)
140	3.1 ± 0.04 (20)
139a and b	-

Figures in brackets indicate number of readings taken.

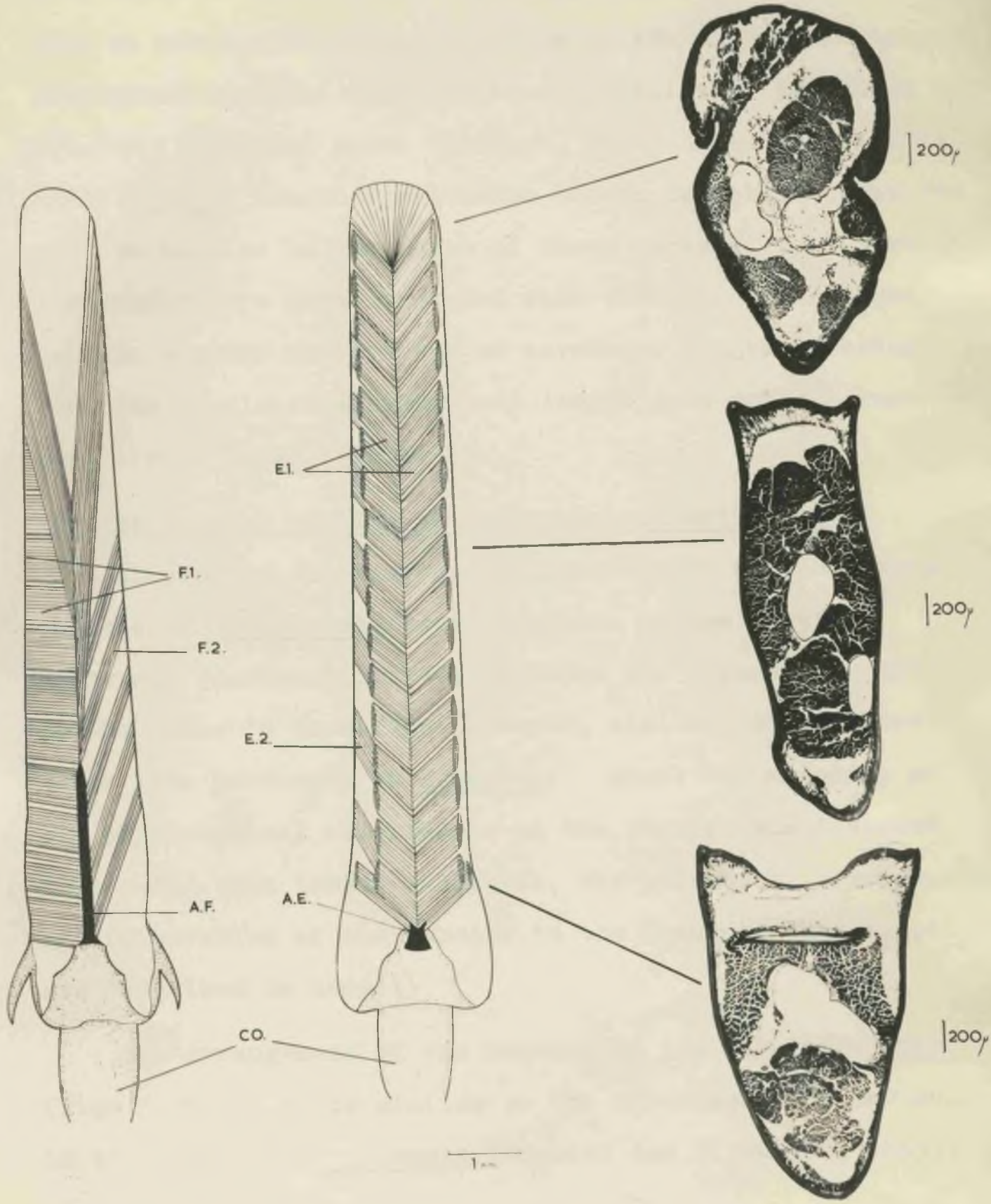
A general picture of the musculature in the mesothoracic coxal segment of Periplaneta is illustrated in Fig. 2b.

Striation. Slight variations in the sarcomere lengths of the different coxal muscles were recorded. The values for sarcomere rest length, measured at equilibrium length, ranged between 3.5-4.3μ after alcohol fixation (Table 1) and 2.9-3.5μ after formalin fixation (Tables 2 and 3). In other Orthoptera sarcomere rest lengths ranging between 2.7-4.8μ have been recorded from the skeletal muscles (Tiegs, 1955). Weis-Fogh (1956) gave a value of 3.6μ for the sarcomere rest length of the locust flight muscle fixed at equilibrium length in formalin. He showed also that changes in sarcomere rest length occur in this muscle during stretch. Edwards, Santos, Santos and Sawaya (1954) gave values of 4.19μ and 4.33μ respectively for the sarcomere rest lengths of the flight muscle and femoral muscles of P. americana, and Kawaguti and Nakamura (1960) gave a value of about 3μ for the sarcomere rest length of the extensor and flexor tibiae muscles of the house-cricket (Gryllus mitratus) at equilibrium length.

It is concluded from these results that the sarcomere rest lengths of the various coxal muscles of both Periplaneta and Blaberus do not differ significantly from each other or

Fig. 3. Arrangement of the muscle units in the metathoracic femur of the cockroach Blaberus giganteus with photographs of transverse sections through the regions indicated. Sections stained in Ehrlich's haematoxylin and eosin and cut at 10μ.

A.E.	-	Apodeme of extensor tibiae.
A.F.	-	Apodeme of flexor tibiae.
CO.	-	Tibia.
E.1.	-	Anterior units of the extensor tibiae.
E.2.	-	Posterior units of the extensor tibiae.
F.1.	-	Anterior units of the flexor tibiae.
F.2.	-	Posterior units of the flexor tibiae.


E.1.
F.1.
F.2.
E.2.
A.F.
A.E.
CO.
200µ
200µ
200µ
1 mm

from those of other insect skeletal muscles. Any differences that do exist bear little relation to the different contraction rates recorded from these same muscles by Becht and Dresden (1956) and Becht (1959). It is possible that the ratio *in situ* length/equilibrium length is not the same for all the muscles in the coxae of these insects, i.e. some of the muscles are more stretched than others. This might explain why the differences of sarcomere length recorded from the musclesat minimum body length were not apparent at equilibrium length (Table 1).

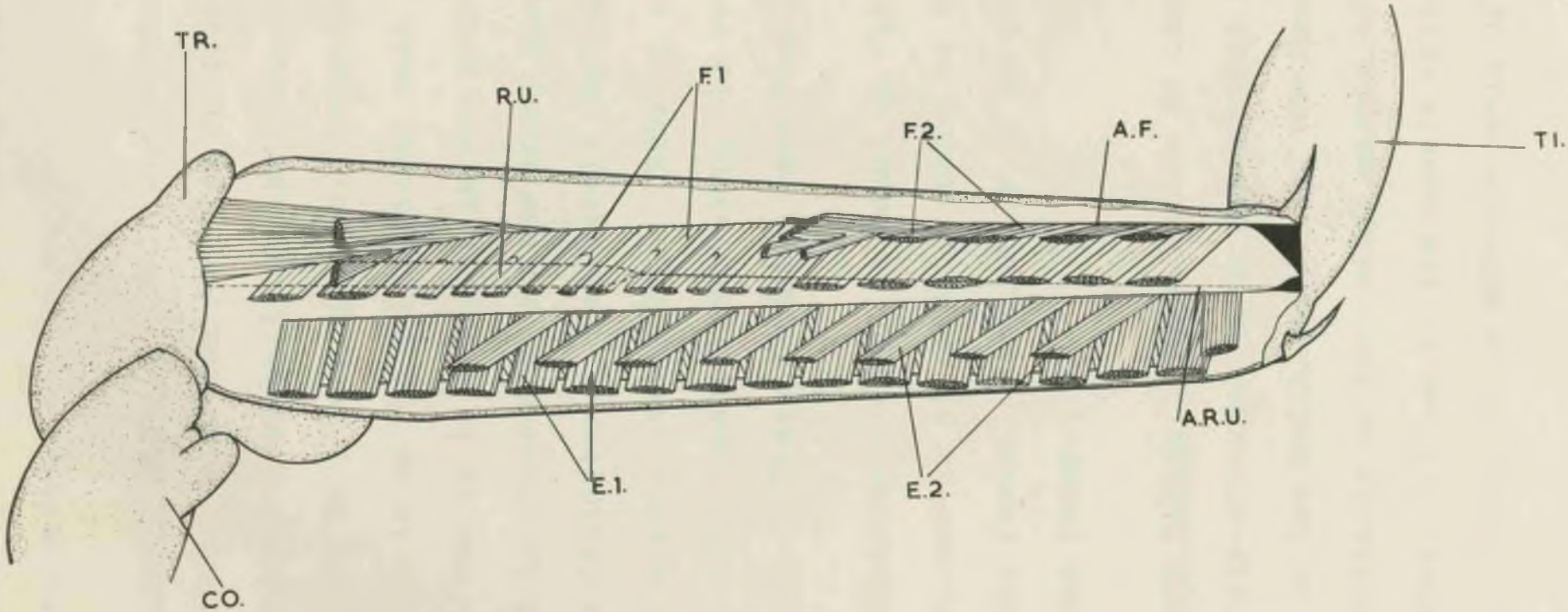
(2) The femoral muscles of the cockroach and locust

Studies of the anatomy and innervation of the femoral muscles of *Periplaneta* have been made on two previous occasions (Carbonell, 1947; Dresden and Nijenhuis, 1953). To the author's knowledge, however, similar studies have never been performed on *Blaberus*. Since the majority of the physiological experiments on the femoral muscles were made using this larger cockroach, the structure, arrangement and innervation of the muscles in the femur of this insect are described in detail.

The arrangement of the muscles in the femur of *Blaberus* (Figs 3, 4 and 5) is similar to the arrangement of the muscles in the femur of *Periplaneta* (Dresden and Nijenhuis, 1953).

Fig. 4. Arrangement of the femoral muscles in the metathoracic leg of *Blaberus giganteus*, viewed posteriorly.

A.F. - Apodeme of flexor tibiae.
A.R.U. - Apodeme of retractor unguis.
CO. - Coxa.
E.1. - Anterior units of extensor tibiae.
E.2. - Posterior units of extensor tibiae.
F.1. - Anterior units of flexor tibiae.
F.2. - Posterior units of flexor tibiae.
R.U. - Retractor unguis.
TI. - Tibia.
TR. - Trochanter.


TR.
R.U.
F.1
F.2.
A.F.
T1.
A.R.U.
E.1.
E.2.
CO.

Three muscles, a flexor, an extensor and a retractor unguis are always present.

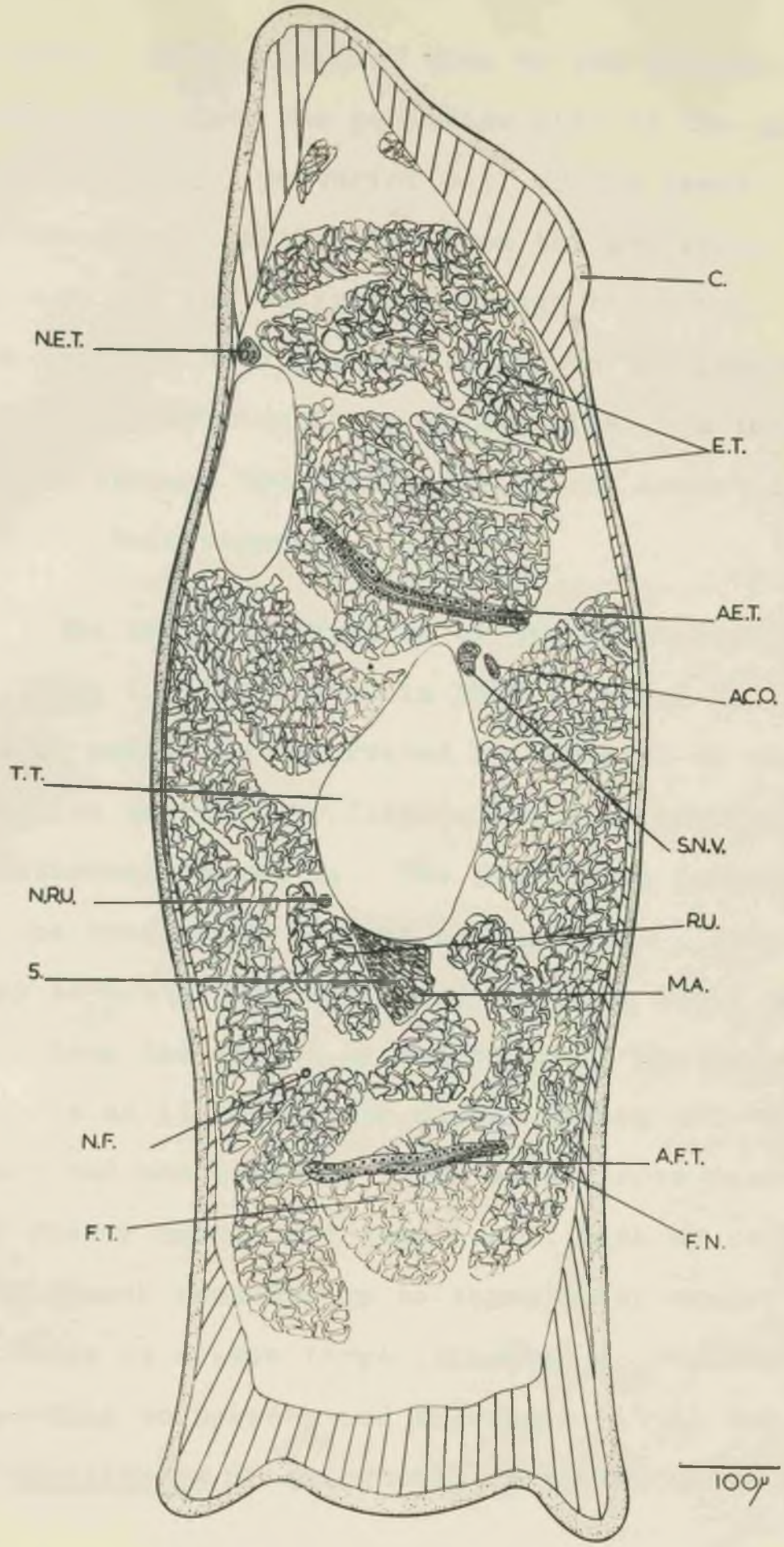
Retractor unguis. This muscle consists of a small number of fibres which run parallel to the walls of the femur. It is attached proximally to the posterior wall of the femur and distally to a long apodeme. The distal attachment of the apodeme is on the medio-distal wall of the tibia. The retractor muscle is situated in the posterior region of the femur and is innervated by two motor axons from nerve 5, one large (diameter approximately 20μ) and one medium (diameter approximately 7μ). Dresden and Nijenhuis (1958) found two motor axons innervating the retractor unguis of Periplaneta. These findings have been checked and confirmed.

A chordotonal organ is found on the opposite side of the femur, and is attached distally to the postero-lateral wall of the femur. Proximally it is attached to the anterior wall of the femur.

Flexor tibiae. This muscle is composed of approximately 26 pinnately-arranged units which arise from a common apodeme situated in the medial region of the femur. The apodeme inserts distally on the medial margin of the tibia. Approximately fifteen of the muscle units are distributed along the length of the anterior wall of the femur and are

Fig. 5. Diagrammatic transverse section of the metathoracic femur of B. giganteus, cut about half way along the femur to show position of nerves and muscles.

A.C.O.	-	Apodeme of the chordotonal organ.
A.E.T.	-	Apodeme of extensor tibiae.
A.F.T.	-	Apodeme of flexor tibiae.
C.	-	Cuticle.
E.T.	-	Extensor tibiae.
F.N.	-	Motor branch innervating flexor tibiae.
F.T.	-	Flexor tibiae.
M.A.	-	Motor axons of the "motor" branch of nerve 5.
N.E.T.	-	Motor branch innervating extensor tibiae.
N.F.	-	Motor branch innervating flexor tibiae.
N.R.U.	-	Motor branch innervating retractor unguis.
R.U.	-	Retractor unguis.
S.N.V.	-	Sensory branch of nerve 5.
T.T.	-	Tracheolar trunk.
5.	-	Nerve 5.


C.
N.E.T.
E.T.
A.E.T.
A.C.O.
T.T.
S.N.V.
N.R.U.
R.U.
S.
M.A.
N.F.
A.F.T.
F.T.
F.N.
100μ

inserted on the anterior side of the apodeme. Four or five units arise from the posterior side of the apodeme and are inserted on the posterior wall of the femur. The remainder of the flexor units arise from the posterior face of the apodeme and run obliquely through the femur. Six of them are attached at different levels to the femoro-tibial junction, three units on either side. A further unit passes through the trochanter to its insertion on the medial wall of this segment.

The motor innervation of the metathoracic leg of Blaberus is illustrated in Figs 5, 6 and 7. The extensor tibiae muscle is innervated by nerve 3b of the metathoracic ganglion whereas the flexor muscle is innervated by the metathoracic nerve 5. The two nerves join to form a plexus in the trochanter segment of the limb. From the plexus they separate once again and continue their course uninterrupted, down the length of the femur. The crural nerve 5 divides as it leaves the plexus giving off two branches, one motor and one sensory. The motor nerve passes close to the flexor muscle and supplies it with up to fifteen branches Each branch contains up to three motor axons; at least one of these is always large (diameter approximately 20μ). According to Dresden and Nijenhuis (1958) the flexor muscle of Periplaneta is innervated by eleven nerve axons altogether

Fig. 6. Diagram showing distribution of motor nerves to the metathoracic leg of B. giganteus.

C.O. - Coxa.

F.E. - Femur.

M.G. - Metathoracic ganglion.

M.N.5. - Motor branch of nerve 5.

N.E.T. - Motor nerve innervating extensor tibiae.

N.3a. - Metathoracic nerve branch 3a.

N.3b. - Metathoracic nerve branch 3b.

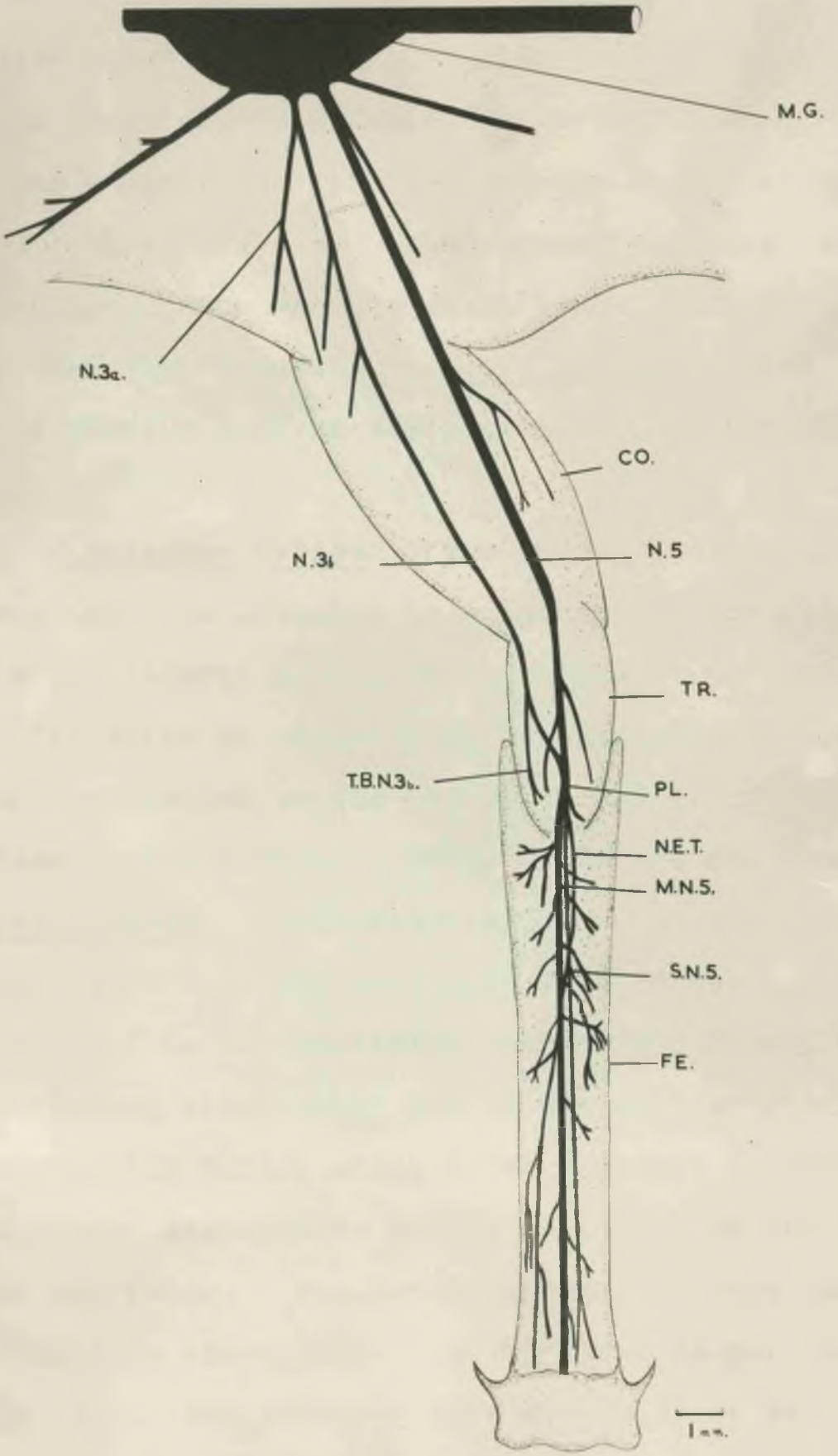
N.5. - Metathoracic nerve branch 5.

PL. - Plexus in trochanter of nerve 5 and nerve 3b.

S.N.V. - Sensory branch of nerve 5.

T.B.N.3b.- Trochanteral branch of nerve 3b.

TR. - Trochanter.


M.G.
N.3a.
CO.
N.3b
N.5
TR.
T.B.N.3b.
PL.
N.E.T.
M.N.5.
S.N.5.
FE.
1 cm.

five large (diameter 10-20μ) and six medium (diameter 5-10μ). The axons are distributed in nerve branches, which arise from a number of points along the length of the femoral portion of nerve 5. Each branch contains two motor axons, one large axon and one small axon. The innervation of the flexor tibiae muscle of Periplaneta has been re-investigated. The results confirm the conclusions of Dresden and Nijenhuis (1958)

Extensor tibiae. The 26 units of which this muscle is composed are attached to a common apodeme which is inserted on the lateral margin of the tibia. The two distal bundles, which arise on either side of the apodeme, are short, thick units attached to the posterior and lateral walls of the femur respectively. These units are not found in Periplaneta. Approximately eight muscle bundles arise at intervals from the posterior face of the apodeme and are inserted on the posterior wall of the femur, the insertions extending along about 70% of the wall. The remainder of the muscle units, which arise somewhat laterally from the apodeme, dichotomise and are inserted on the lateral wall of the femur. The total number of fibres in the extensor muscle is about 3000. Motor nerve 3b passes down the femur to supply the extensor muscle with up to seven motor axons of varying diameters (Fig. 7a and b). According to Dresden

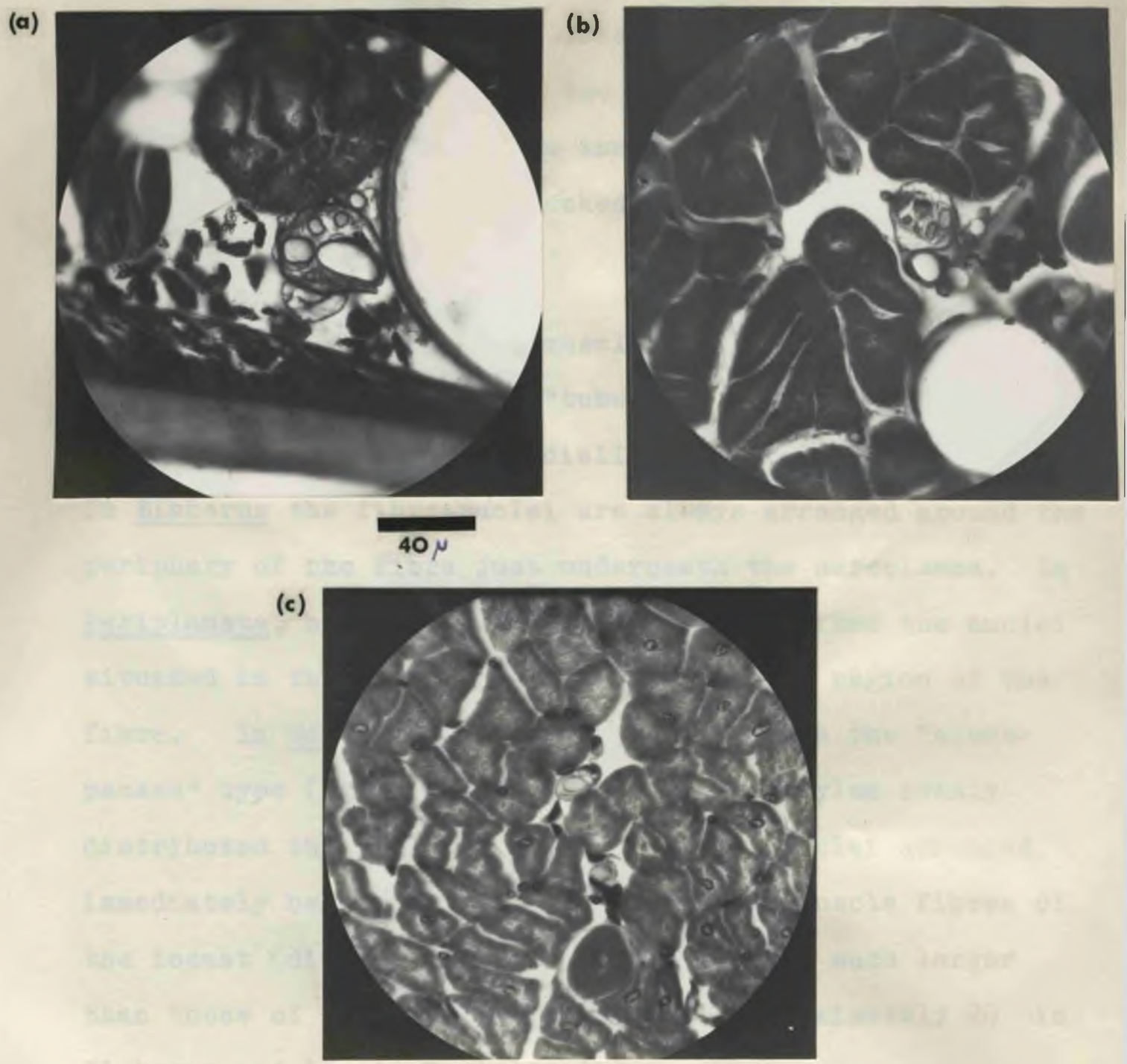


Fig. 7. Motor nerve innervation and histological structure of the femoral muscles of B. giganteus and P. americana. (a-b) Transverse sections of the motor nerve innervating the metathoracic extensor tibiae muscle of B. giganteus. About seven motor axons of varying diameters are seen. (c) Motor innervation to the flexor tibiae muscle of P. americana. This section is through one of the many branches of nerve 5 which supply this muscle. Note in both muscles the "tubular" muscle fibres.

and Nijenhuis the extensor tibiae muscle of Periplaneta is innervated from nerve 3b by two very large axons (diameter + 20μ) and approximately two small axons (diameter 2-5μ). These findings have been checked and confirmed in the present investigation.

The fibres of the leg muscle of both Blaberus and Periplaneta are all of the "tubular" type (Tiegs, 1955) with the sarcostyles arranged radially around a central lumen. In Blaberus the fibre-nuclei are always arranged around the periphery of the fibre just underneath the sarcolemma. In Periplaneta, however, it is not unusual to find the nuclei situated in the central, clear sarcoplasmic region of the fibre. In Schistocerca the fibres resemble the "close-packed" type (Tiegs, 1953) with the sarcostyles evenly distributed throughout the fibre and the nuclei arranged immediately beneath the sarcolemma. The muscle fibres of the locust (diameter approximately 50μ) are much larger than those of the cockroach (diameter approximately 20 in Blaberus and 15μ in Periplaneta).

The structure and innervation of the muscles found in the metathoracic femoral segment of the locust have been described in detail by Hoyle (1955b) and Campbell (1961). The dorsally-placed extensor muscle is about 1.6 mm long and occupies the greater part of the femur.

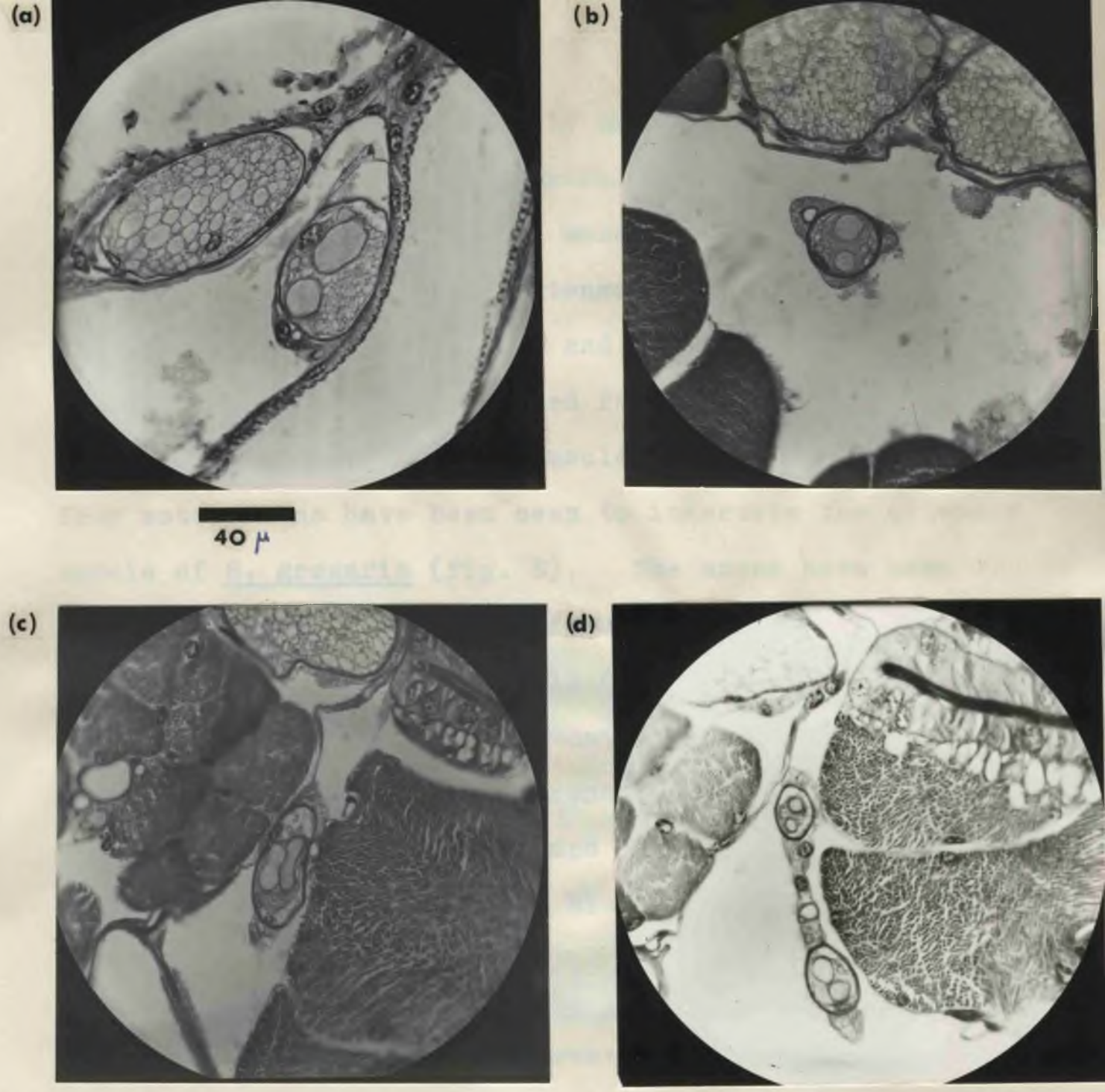


Fig. 8. Transverse sections of motor nerve innervating the extensor tibiae muscle of the metathoracic leg of Schistocerca gregaria. (a) At a point just beyond fusion of nerves 3b and 5. The motor branch with the four motor axons is shown in the right hand side. (b) At the trochantero-femoral junction. (c) Point of division of nerve into two branches, about one third the way along the femur. (d) After division of motor nerve into two branches. In (a) note the four motor axons accompanied by a large number of small, presumably sensory axons.

The muscle fibres are pinnately arranged and are attached at an angle of 30-40° to a centrally placed apodeme. The total number of fibres in the muscle is about 3500. Hoyle (1955b) suggested that the extensor muscle is supplied with two motor axons from nerve 3b and one from nerve 5. However, Hoyle (1955c) obtained four types of electrical responses from the extensor muscle. In the present study four motor axons have been seen to innervate the extensor muscle of S. gregaria (Fig. 8). The axons have been traced throughout the length of the femur. One axon is large, one is medium and two are small (Fig. 8). About halfway down the femur nerve 3b dichotomises and at this point it is possible that one of the branches contains only three motor axons (Fig. 8d). Perhaps it was from this region of the femur that Hoyle obtained his histological data.

(3) The thoracic muscles of the locust

The arrangement and innervation of the thoracic muscles of the locust have been described in detail by a number of insect anatomists. Among these, Albrecht (1953) published a detailed account of the structure and arrangement of the skeletal muscles of Locusta but his description of the nervous innervation to these muscles has proved inadequate. Campbell (1961) has repeated and extended Albrecht's investigations to give an excellent account of the

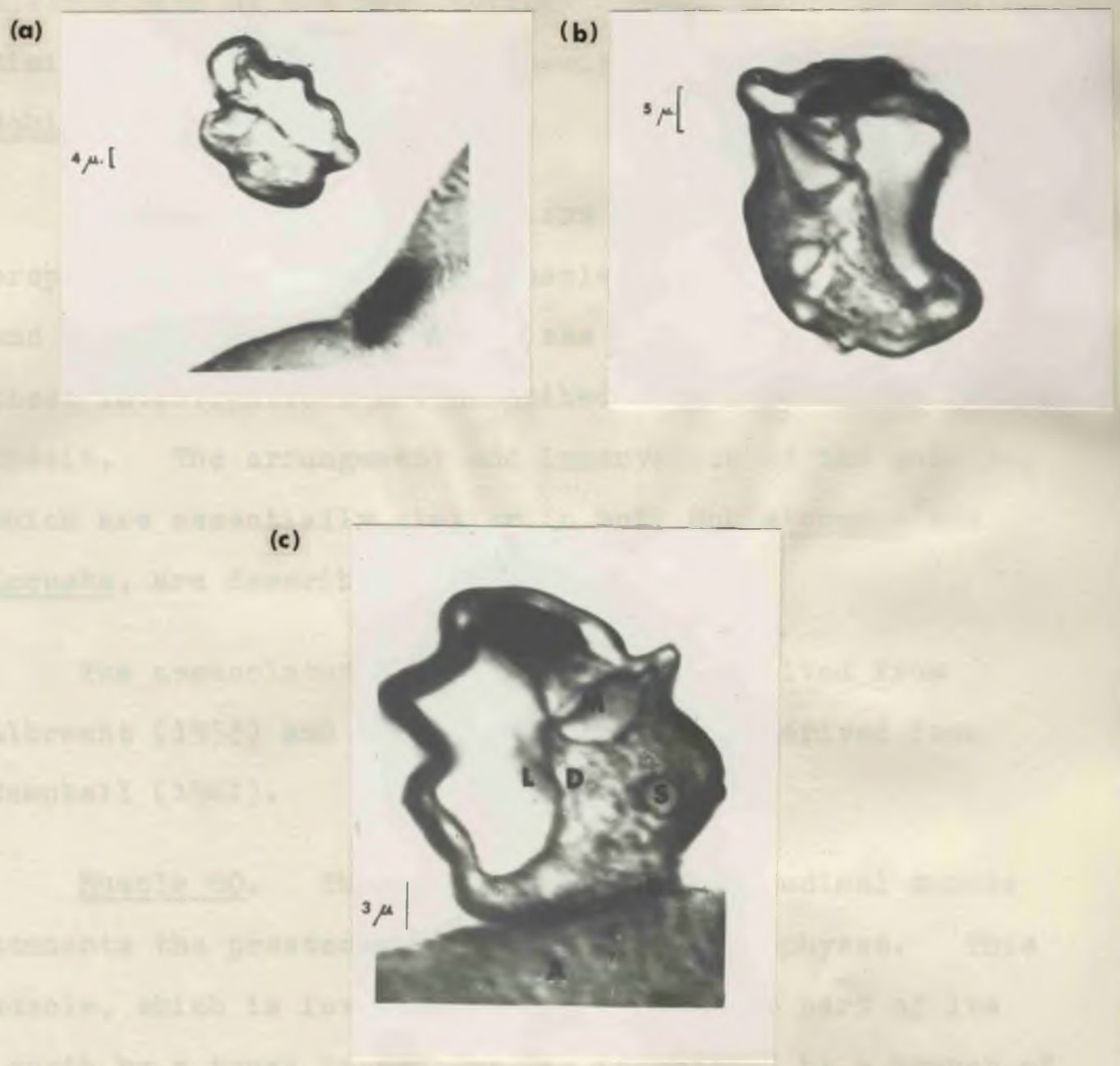


Fig. 9. Transverse sections of the motor nerve innervating muscle 92. Sections stained in Ehrich's haematoxylin and eosin and obtained at different points along the nerve. (a) Near the mesothoracic ganglion; (b) about half-way between ganglion and muscle 92; (c) near muscle 92.

A - muscle fibre.
B - groove in surface of muscle fibre to accommodate motor nerve.
D - tunicated sheath around motor axons.
L - large motor nerve fibre (probably "fast").
M - medium motor nerve fibre (probably "slow").
S - small motor nerve fibre (probably "slow").

distribution of the mesothoracic nerves of this insect. Similar information on the closely related locust Schistocerca is not available.

Physiological investigations of the neuromuscular properties of some thoracic muscles of both Schistocerca and Locusta have been made by the author. The results of these investigations are described in Section II of this thesis. The arrangement and innervation of the muscles, which are essentially similar in both Schistocerca and Locusta, are described below.

The nomenclature of the muscles is derived from Albrecht (1953) and that of the nerves is derived from Campbell (1961).

Muscle 60. The second ventral longitudinal muscle connects the prosternal and mesosternal apophyses. This muscle, which is invested along the greater part of its length by a tough integument, is innervated by a branch of the prothoracic recurrent nerve.

Muscle 81. The longitudinal dorsal muscle is a powerful muscle extending between the first and second phragmata. It is innervated by a branch of the 1st mesothoracic nerve.

Epm epimeron
Eps episternum
PIR pleural ridge
SA sternal apophysis
Spn spina
v suture dividing episternum

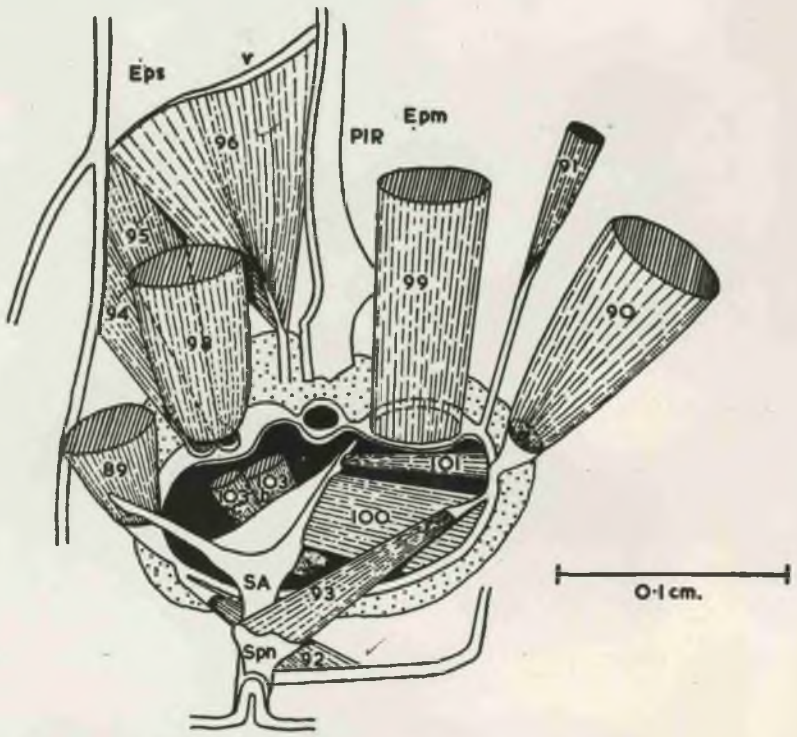


FIG. 71. Musculature of mid coxa

Fig. 10. (After Albrecht, 1953).

Muscle 87. The third ventral longitudinal muscle connects the first spina to the anterior edge of the mesosternal apophysis. In Locusta this muscle is innervated by the mesothoracic recurrent nerve but in Schistocerca it appears to be innervated by the 3rd mesothoracic nerve which supplies it with three motor axons, one large, one medium and one small.

Muscle 92. The anterior rotator of the coxa is a fan-shaped muscle arising from the sternellar ridge and inserted on the anterior angle of the mesothoracic coxa. This muscle is innervated by nerve $3C_2$ which supplies it with three motor axons, one large, one medium and one small (Fig. 9).

Muscle 96. The third coxal abductor muscle is fan-shaped and arises from a ridge of episternum to insert on the coxal membrane anterior to the pleural articulation. It is innervated by a branch of the 3rd mesothoracic nerve.

Muscle 99. The depressor-extensor muscle of the forewing lies against the wall of epimeron 2 and inserts dorsally on the sub-alar plate of the wing base. Ventrally the muscle is inserted on the wide basicostal plate of the meral region. This muscle is innervated by a branch of mesothoracic nerve 4.

FIG. 68. Musculature of pterothorax, inner view of left side

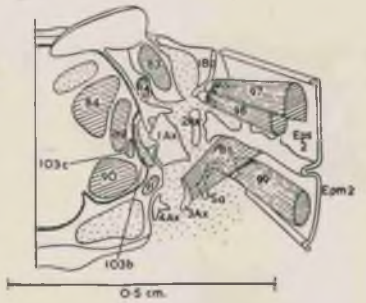
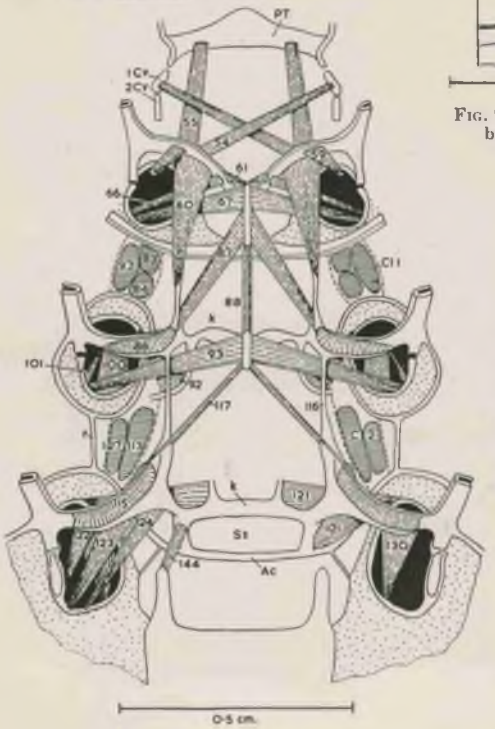


FIG. 70. Inner view of mesothoracic wing-base and adjacent regions showing muscle origins and insertions



Ac	antecosta
1Ax–4Ax	first to fourth axillary sclerites
1Ba	first basalare
C11, C12	callosities of prothoracic sterа
1Cv, 2Cv	first and second cervical sclerites
Epm 2	epimeron
Eps 2	episternum
k	furcal suture
PT	posterior arm of tentorium
r	pleurosternal suture
Sa	subalare
Ss	spinasternite

FIG. 69. Endoskeleton of thoracic sterna, showing muscle attachments

Fig. 11. (After Albrecht, 1953).

The arrangement of these muscles in the thorax of Locusta is illustrated in Figs 10 and 11.

SECTION II. PHYSIOLOGY

GENERAL METHODS

Stimulation of the preparations was by rectangular pulses controlled as to strength, delay and duration. The pulses were generated by a double-channel, rectangular-pulse stimulator (Parker, unpublished). The output of the stimulator was isolated from earth by means of a radio-frequency coupling unit (Schmidt and Dubbert, 1949). Paired silver-silver chloride stimulating electrodes were prepared from 40 gm silver wire tapered electrolytically (Roeder, 1940) and insulated to the tips with shellac varnish. The electrodes were mounted on Palmer rackwork.

Intracellular electrical recordings were made with the aid of 3M KCl-filled glass capillary micro-electrodes (Ling and Gerard, 1949; Nastuk and Hodgkin, 1950) of from 10-20M Ω resistance. The micro-electrodes were used in conjunction with conventional display circuitry consisting of a balanced-cathode follower (Bishop, 1949), a DC pre-amplifier (Copeland, 1952) and an oscilloscope (Cossor model 1049).

Extracellular nerve and muscle potentials were recorded with the aid of paired silver-silver chloride electrodes connected to an AC pre-amplifier.

Mechanical recordings were made with an RCA 5734

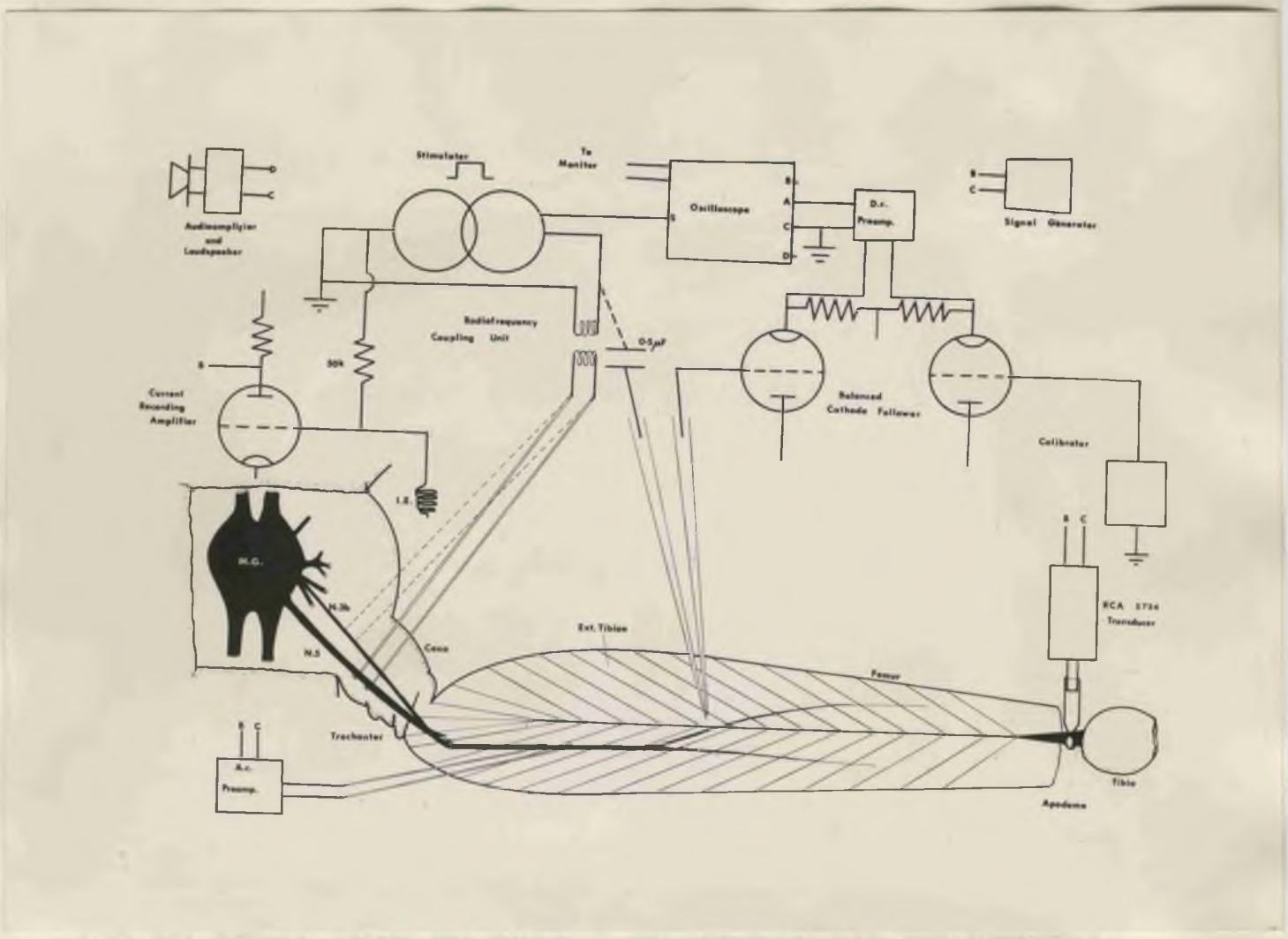


Fig. 12. Simplified plan of circuits used in the experiments described in this thesis. In some experiments a further recording circuit with its own balanced cathode follower input stage was used in conjunction with the circuits shown above to record the electrical activity of a single cell intracellularly at two different points along the length of the cell.

mechano-electronic transducer. The plate shaft of the transducer was connected by a short glass hook to the apodeme of the muscle under examination. Mechanical activity of the larger muscle preparations was also recorded kymographically with the aid of isotonic and auxotonic lever systems.

The stimulator, R.F. coupling unit, DC pre-amplifier, AC pre-amplifier, cathode follower, an audio-amplifier, and the associated power supplies were built by the author.

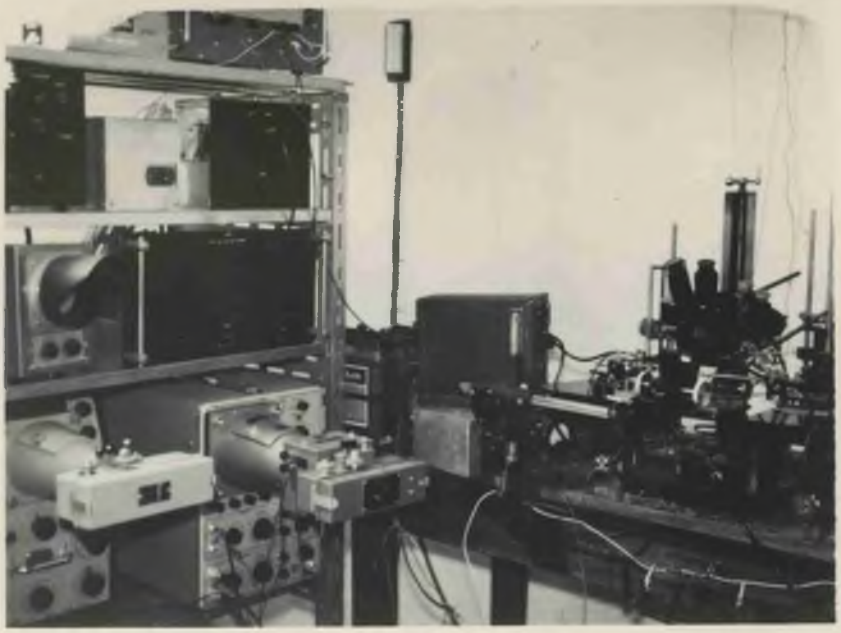
A simplified plan of the stimulating and recording circuitry is illustrated in Fig. 12. Photographs of the apparatus are shown in Fig. 13a and b.

Experimental salines

Periplaneta americana. The saline used for all experiments on Periplaneta had the following composition:- 158 mM NaCl, 10.8 mM KCl, 9 mM $CaCl_2$, 0.2 mM $NaHCO_3$, 0.08 mM NaH_2PO_4 made up to one litre with distilled water (Becht, 1959). The bicarbonate-phosphate buffer maintained the saline at about pH 7.2. Similar values for pH have been recorded from the haemolymph of Periplaneta.

Schistocerca gregaria. For all experiments on Schistocerca, saline of the following composition was used:- 140 mM NaCl, 10 mM KCl, 2 mM $MgCl_2$, 6 mM NaH_2PO_4, 4 mM $NaHCO_3$

(a)



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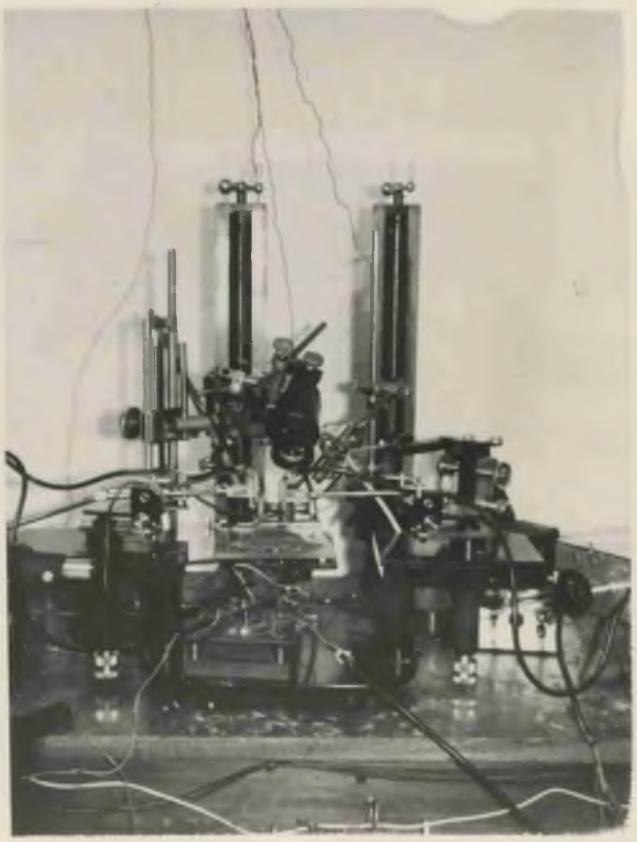


Fig. 13(a) General view of apparatus. On the right are the micromanipulator, electrode assemblies, transducer, lamp and binocular microscope.

(b) General view of micromanipulator and electrode assemblies.

2 mM $CaCl_2$ to one litre with distilled water (Hoyle, 1953c). The pH of the saline (about 6.9) closely approximates that of the haemolymph.

Blaberus giganteus. Attempts to obtain satisfactory recordings from the femoral muscles of Blaberus using the cockroach saline designed by Becht (1959) were not successful In this saline the condition of the preparation remained satisfactory for only about 30 minutes. It was necessary therefore to develop a workable saline which would maintain the preparation in good condition throughout the duration of the experiments. On the broad assumption that the haemolymph bathing the body tissues of the insect provides an optimum external ionic environment for the normal functioning of the nerve-muscle apparatus, an analysis was made of the ion content of the haemolymph of Blaberus and from the results of this analysis it has been possible to design a saline in which the normal functions of the excitable tissues are maintained for considerable periods.

The sodium and potassium concentrations of the haemolymph of animals of either sex were determined photometrically using an Eel flame photometer. Considerable difficult was experienced in obtaining samples large enough for the assays because the haemolymph quickly coagulated in the air. This difficulty was avoided to some extent if the animal had

Table 4.

Concentration of sodium and potassium ions in the haemolymph of adult Blaberus of either sex. (Results in mg. eq/L ± standard error mean.)

Animal	Sodium	Potassium
1	235.0 ± 2.8	33.8 ± 1.5
2	233.0 ± 1	34.0 ± 1
3	133.0 ± 1	10.7 ± 0.1
4	151.0 ± 3.7	10.6 ± 0.9
5	112.0 ± 9.6	9.2 ± 1
6	183.0 ± 3	5.7 ± 2.5
7	153.0 ± 3	32.0 ± 1
8	150.0 ± 3	30.0 ± 1
9	134.9 ± 1.5	10.0 ± 1
10	128.6 ± 2	9.1 ± 1
Mean ± S.e.	161.4 ± 8.2	18.5 ± 3.8

Table 5.

The melting point of haemolymph from adult Blaberus.

Sample	Sex	Melting point (°C)	Conc. NaCl (mM)
1a	Male	-0.65	181.9
1b		-0.66	180.8
2	Male	-0.625	175.2
3	Female	-0.73	199.3
Mean		-0.66	184.3

previously been exposed for some time (about one hour) to temperatures below 0°C. On recovery to a temperature of about 10°C, an incision was made in the cuticle separating the coxal segment from the thorax. Any haemolymph which accumulated at this point was extracted, measured in a micro-pipette, diluted with a known volume of distilled water and placed in the photometer. The sodium and potassium ion concentration of each sample was checked against control solutions containing known quantities of these ions. The results given in Table 4 are characterized by a rather high degree of individual variation despite the similarity in diet of the individuals used.

Values for the concentration of sodium in the haemolymph of Blaberus were similar to the values of 164.2 mM/l and 157.4 ± 28 mM/l obtained by Asperen and Esch (1956) and Treherne (1961) respectively for the haemolymph of Periplaneta. Treherne (1961) obtained the value of 12.3 ± 2.0 mM/l and Asperen and Esch (1956) a value of 7.4 mM/l for the potassium concentration in the haemolymph of Periplaneta.

Blaberus is usually an herbivorous insect but if food is scarce it may become carnivorous. The animals used in the present experiments were fed on a diet of apples, dog biscuits and water. Tobias (1948) found that the sodium

and potassium concentrations in haemolymph obtained from Periplaneta specimens fed on a similar diet to this were 169 ± 46 mM/l and 27.1 ± 5 mM/l respectively.

The total ionic concentration of Blaberus haemolymph was determined by the melting point technique (Jones, 1941). The melting points of frozen haemolymph samples were compared with those of known NaCl standards. The haemolymph was removed from the abdominal region of the animal in a fine capillary tube. The tube was sealed with paraffin and the haemolymph frozen over carbon dioxide snow. Haemolymph samples from three cockroaches were tested. The results are given in Table 5.

An average value of -0.66°C for the melting point of Blaberus haemolymph calculated from the above experiments compares with the value of -0.897°C obtained by Treherne (1961) for the melting point of the haemolymph of Periplaneta.

Attempts to obtain values for the ionic concentration of magnesium, calcium, phosphate and bicarbonate in the haemolymph were unsatisfactory. Instead experiments were made to determine the optimum concentrations for these ions in the saline by varying the ions individually whilst maintaining the total concentration and the pH constant. By noting the effect of these ionic variations on the electrical

responses of the muscles it was possible to arrive at an approximate optimum saline. It must be remembered, however, that marked variations in the ionic composition of insect haemolymph occur normally (Tobias, 1948; Hoyle, 1953c; Treherne, 1961) and that any saline chosen is, within limits, arbitrary. Measurements of the pH of Blaberus haemolymph were obtained with a Pye portable pH meter. An average value of 6.9 was recorded. The final composition of the saline used for all experiments on Blaberus was 161.4 mM NaCl, 18.5 mM KCl, 9 mM $CaCl_2$, 6 mM NaH_2PO_4, 4 mM $NaHCO_3$ to one litre with distilled water.

EXPERIMENTAL TECHNIQUES AND RESULTS

(1) The mesothoracic coxal muscles of the cockroach

Experiments were made on the mesothoracic coxal muscles of the cockroach P. americana. A few recordings taken from the mesothoracic coxal muscles of B. giganteus were essentially similar to those taken from the coxal muscles of P. americana. Adult specimens of either sex were employed for the experiments. Following the numbering of Carbonell (1947) the muscles examined were 136, 137, 135a and c, 135b, 135d and e (extensors); 138, 139a, b, and c, and 140 (flexors).

The cockroach was lightly anaesthetized with carbon

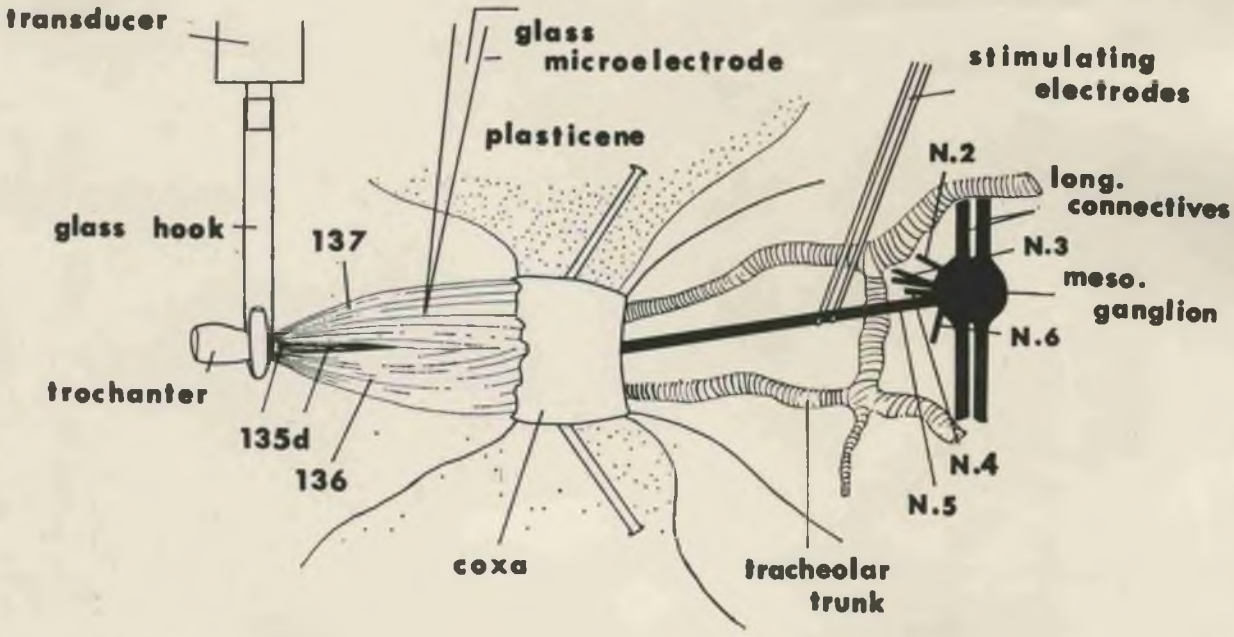


Fig. 14. Arrangement for recording the isometric mechanical and the intracellular electrical responses of a typical preparation of the coxal muscle group 136 and 137.

dioxide and partially embedded on its dorsal surface with plasticine. The mesothoracic coxa on one side was gently pulled out at right angles to the thorax and fixed down securely in plasticine, with its ventral surface uppermost, leaving the trochanter free to move. The mesothoracic ganglion was exposed and all motor and sensory nerves to it were severed.

The cut motor nerve innervating the coxal muscle under examination was placed over a pair of silver-silver chloride stimulating electrodes. Sometimes experiments were performed with the relevant motor nerve still attached to the mesothoracic ganglion, thus enabling studies on reflex stimulation to be made (Fig. 14). The distal part of the cuticle on the ventral surface of the coxal segment was carefully removed together with the underlying hypodermis to expose the coxal muscles and their distal attachments to the trochanter. The coxal muscles not under examination were carefully removed from the coxa to eliminate any errors in recording that they might introduce. All parts of the limb distal to the trochanter were removed and the hinged joints connecting the coxa to the trochanter were severed. After the coxa had been cut back a little, a glass hook, attached to the plate shaft of an RCA 5734 mechano-electronic transducer, was placed under the apodeme of the muscle to

be examined and the muscle was pulled out to the maximum length it normally occupied in the coxa. A longer glass hook was used for the smaller coxal muscles. The myograms obtained from the coxal muscles using this recording technique were very nearly isometric, in contrast to the isotonic myograms obtained by Becht and Dresden (1956) and Becht (1959).

Stimulation was by rectangular pulses of variable magnitude, frequency, duration and delay. Recordings of electrical events from the coxal muscles were obtained using glass intracellular micro-electrodes filled with 3M KCl.

Isolated preparations

A number of experiments were also performed on isolated muscle preparations. The required muscle, with its attachment to the trochanter still intact, was dissected together with its innervating motor nerve out of the coxa and firmly fixed down on to a cork plate by a pair of small pins passing through its proximal cuticular attachment. It was then connected to the transducer with a glass hook and pulled out to the length it normally occupied in the coxa.

All experiments were performed in a room thermostatically controlled at 19°C.

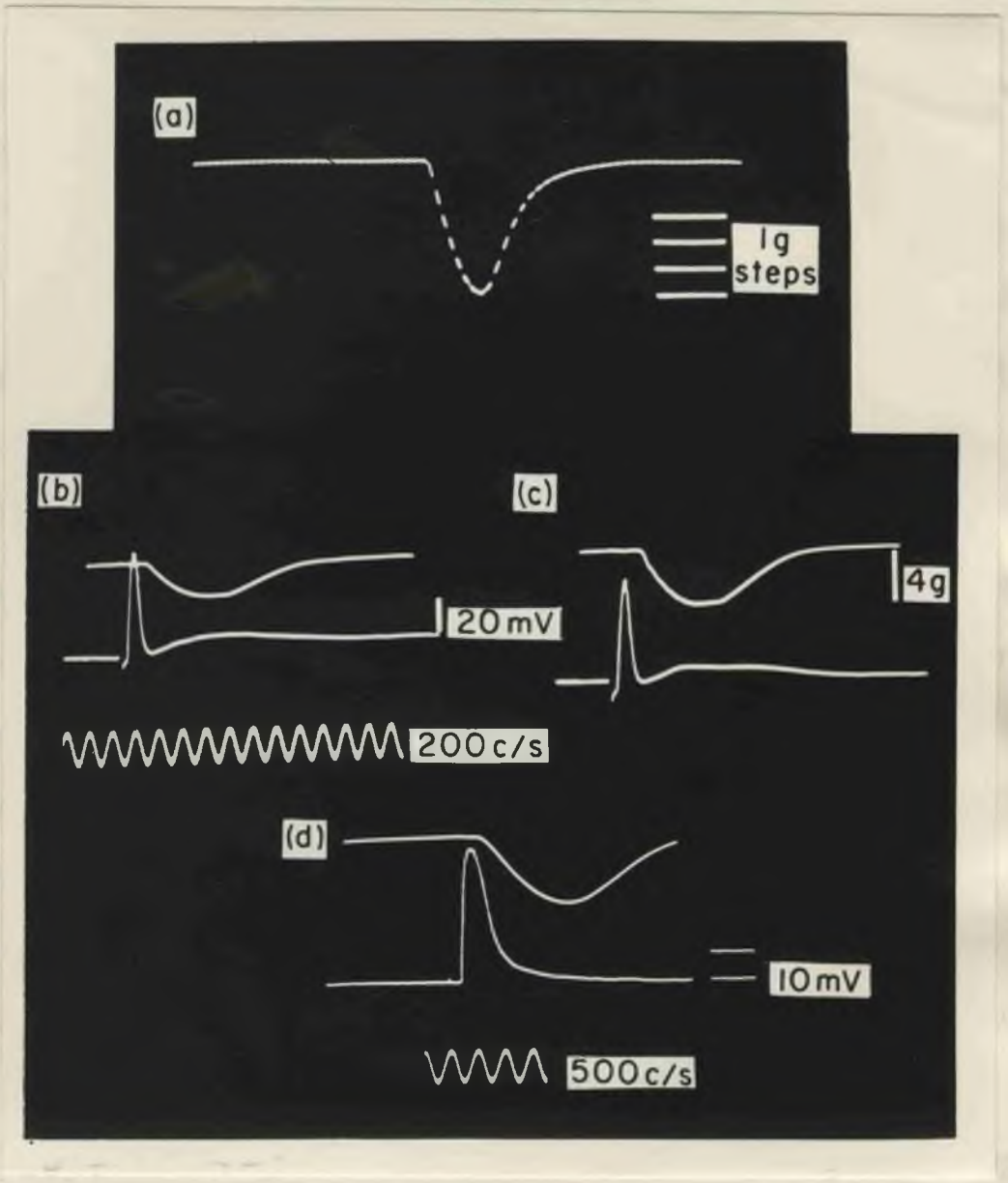


Fig. 15. Mechanical and electrical responses of muscle group 136 and 137. (a) Isometric twitch response to maximal indirect stimulation. Record modulated at 500 c/sec. (b-d) Simultaneous recordings of the electrical response (lower trace) and the mechanical response of three different preparations, to single supramaximal stimuli applied to the innervating motor nerve. The electrical responses, recorded intracellularly from single muscle fibres, are typical of responses to "fast" axon stimulation. Tension calibration same for (b-c). Time calibration same for (b-c) at 200 c/sec. Small deflections on relaxation phase of mechanical response in (b) and (c) are artifacts introduced by the recording system.

Extensor muscles

Muscles 136 and 137. Because of the common nerve supply to these muscles they were treated as a single motor unit in most experiments. The normal mechanical response of this muscle group to a single indirect supramaximal stimulus consists of a twitch contraction lasting about 45 msec and developing a peak tension of up to 7.5 gm (Fig. 15). Becht and Dresden obtained an isotonic twitch duration of about 160 msec for this muscle group. At frequencies of stimulation above about 20 c/sec the individual responses begin to fuse until at a frequency of about 50 c/sec a smooth tetanic contraction is obtained. The peak tetanic tension for a preparation in good condition is only slightly in excess of that of the peak twitch tension. The twitch/tetanus force ratio of the preparation under these conditions is slightly less than unity at about 1/1.2-1/1.5. The peak twitch tension for a poor preparation is much less than normal and under these conditions a twitch/tetanus force ratio as low as 1/6 has been recorded for 136 and 137.

Under sustained tetanic stimulation the mechanical response of this muscle group fatigues quickly and falls to zero. In a number of otherwise quite normal preparations of this muscle group, fatigue of the mechanical response

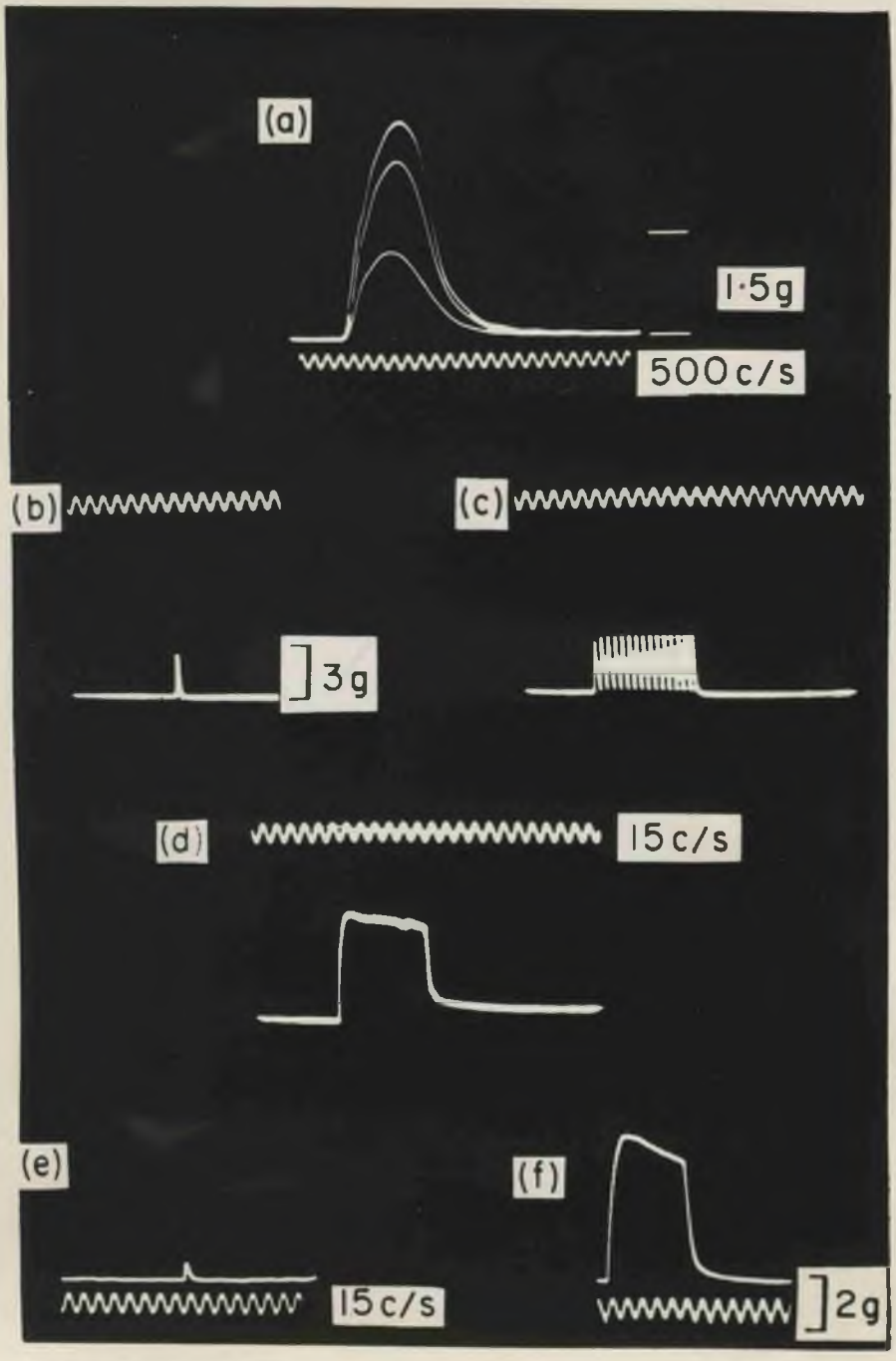


Fig. 16. Isometric myograms of muscle group 135a and c. (a) Typical response of muscle 135a to graded stimulation of nerve 4. Note that durations of the three twitch responses are similar. Oscillations on traces are possibly artifacts introduced by the recording system. (b-d) Effect of stimulation frequency on the maximal response of 135a and c; (b) 1 c/sec; (c) 30 c/sec; (d) 50 c/sec. (e-f) Isometric responses of a different preparation, which was possibly in poor condition, to a stimulation frequency of (e) 1 c/sec and (f) 50 c/sec. Note lower peak twitch tension and lower twitch/tetanus force ratio for this preparation.

has been recorded at frequencies of stimulation as low as 7 c/sec and this fatigue is sometimes associated with a decline in the magnitude of the electrical response.

Becht (1959) reported that this preparation sometimes gave a contractural response following a tetanic contraction. This phenomenon has not been observed in the present experiments.

Simultaneous recordings of mechanical and electrical events have been made in a number of preparations of 136 and 137 (Fig. 15). The mechanical responses have always coincided with the appearance of electrical events of the "fast" type, i.e. large post-synaptic junctional potentials evoking varying degrees of electrical response from the muscle fibre membrane (Hoyle, 1955a and b).

Muscles 135a and c. These two large extensor muscles were also treated as a single motor unit in the majority of the experiments. On the basis of the different motor innervations of the two muscles they behave differently to graded electrical stimulation. Muscle 135c responds at all intensities of stimulation above threshold with a single contraction height. On the other hand, graded stimulation of muscle 135a results in the appearance of three discrete contraction heights (Fig. 16a).

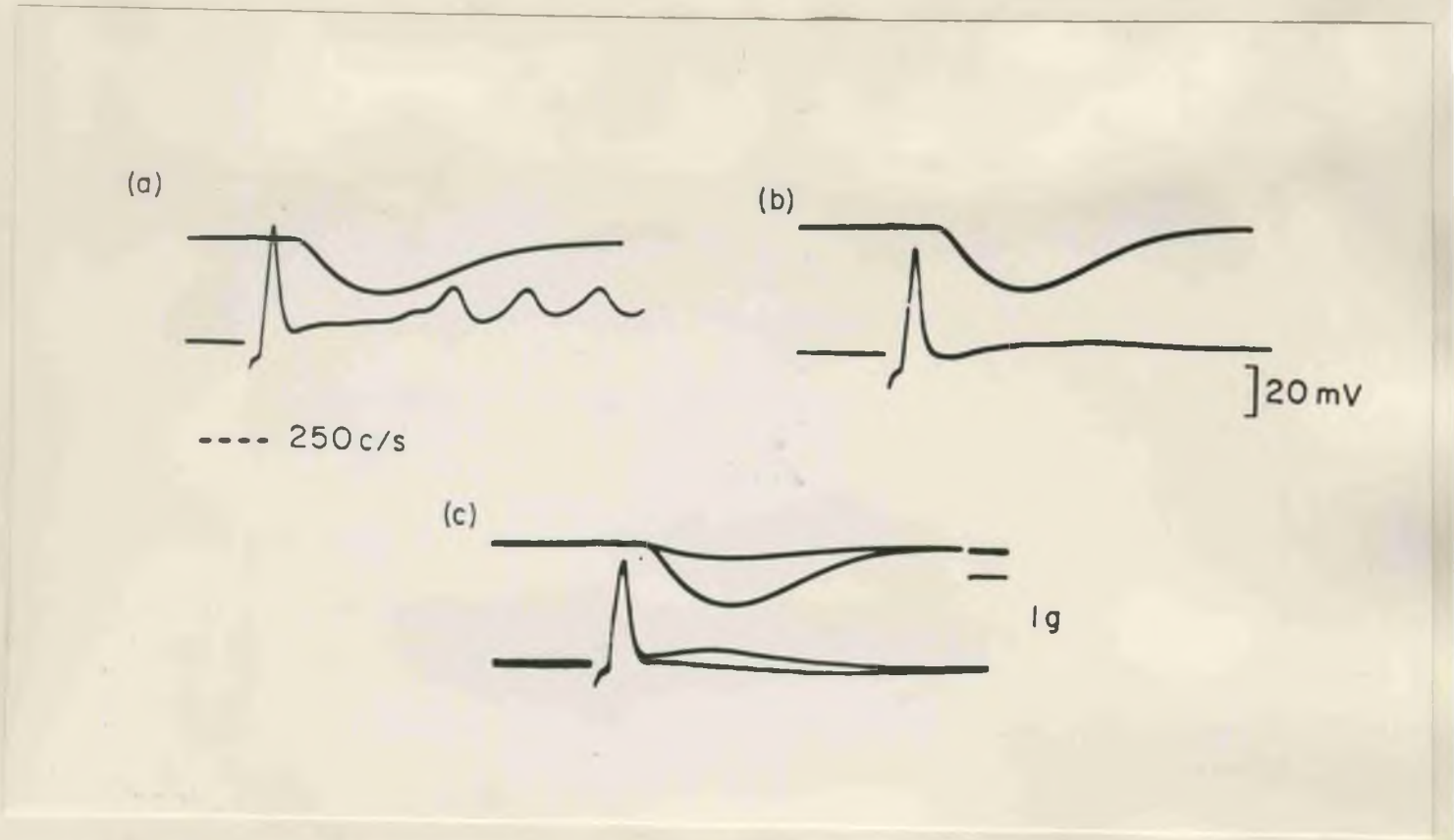


Fig. 17. Simultaneous recordings of electrical and mechanical responses from a preparation of muscle group 135a and c to low frequency motor nerve stimulation. The electrical changes are typical of responses to "fast" axon stimulation and were recorded from three different muscle fibres of the preparation. In (a) the electrical response of the fibre is followed by three injury potentials resulting from damage of the fibre membrane by the micro-electrode during the twitch contraction. In (c) the electrical response of a single fibre to graded stimulation showing that, at least in this case, the increment in tension on increasing the stimulus strength is not accompanied by an increase in the electrical activity of the muscle fibre from which the electrical recording is obtained.

The maximal response of muscle group 135a and c to a single shock consists of a large twitch response which lasts for about 45 msec and produces a peak tension of from 2-5 gm. The twitch/tetanus force ratio of about 1/2-1/3 for this muscle group is on the average slightly less than that of 136 and 137 since at relatively high frequencies of stimulation a small amount of mechanical summation occurs (Fig. 16). The mechanical response fatigues quickly under sustained tetanic stimulation and sometimes even at much lower frequencies of stimulation. However, the rate of fatigue of the mechanical response of 135a and c is never as marked as that of 136 and 137.

Intracellular recordings of electrical events from single muscle fibres of 135a and c have shown clearly that the mechanical responses of this muscle group are associated exclusively with electrical events of the "fast" type in all the muscle fibres (Fig. 17). Furthermore, the four contraction heights obtained from this preparation by graded stimulation are due to the bringing into play of four distinct groups of muscle fibres, each group innervated by a separate motor axon from nerve 4. The maximal response of 135a and c consists of the summed responses of the four motor units. Of some importance is the possibility that variation of the number of motor units active at any moment

not only achieves a certain degree of control over the tension developed but partially offsets the adverse effects of fatigue.

Muscles 135d and e. This muscle group, classified as "mixed" by Becht and Dresden (1956) proved to be especially interesting. Becht and Dresden obtained a fairly large isotonic twitch response from this preparation, following a single indirect maximal stimulus, but unlike the twitch response of their "fast" muscle, which had a relatively short duration, the response of 135d and e often lasted for well over one second. This prolongation of the twitch response was found to be due mainly to a large increase in the duration of the relaxation phase. At relatively low frequencies of stimulation, considerable mechanical summation occurred to give a characteristic "cock's-comb" myogram. Becht and Dresden also found that the twitch/tetanus ratio for isotonic excursion of 135d and e was considerably lower than that of their "fast" muscle and that the mechanical response fatigued only partially under sustained tetanic stimulation.

In the present set of experiments the isometric recordings obtained from this preparation were completely different from those obtained isotonically by Becht and Dresden. By applying carefully graded electrical stimulation

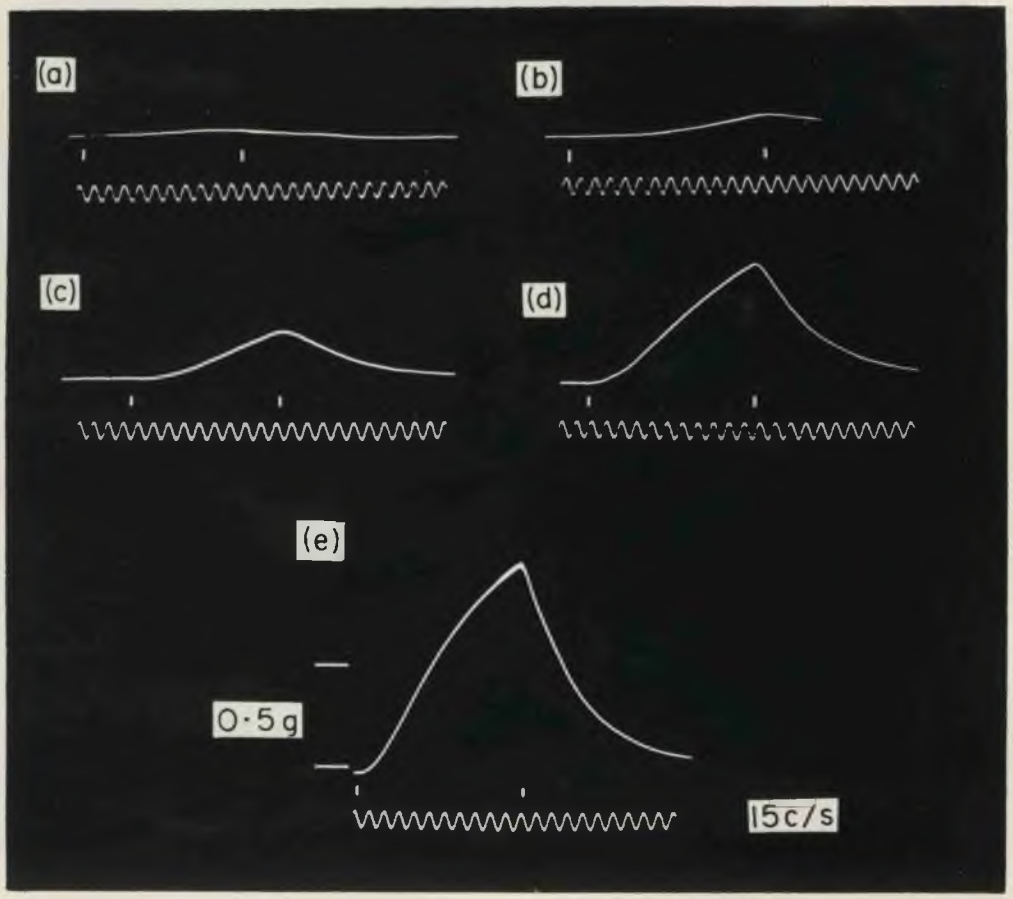


Fig. 18. Mechanical response of muscle group 135d and e to stimulation of the "slow" innervating axon at a variety of frequencies. (a) 10 c/sec; (b) 25 c/sec; (c) 35 c/sec; (d) 80 c/sec; (e) 125 c/sec.

to the innervating motor nerve, four distinct contractions have been obtained with increasing intensities of stimulation. The first contraction to appear was appreciable in most experiments only at high frequencies of stimulation. If the preparation was in good condition the response at this stimulation intensity to a single shock consisted of a minute contraction, but in the majority of preparations little mechanical activity was recorded below a stimulation frequency of 15 c/sec. At higher frequencies of stimulation a smooth tonic contraction was obtained, with the rate of rise of tension and the total tension developed related to the frequency of stimulation. With a stimulation frequency of about 150 c/sec a peak tension of up to 1 gm has been obtained at this intensity (Fig. 18). The response, which showed little signs of fatigue under sustained stimulation at 150 c/sec, was characterized further by its slow rate of relaxation following cessation of stimulation. A slightly higher stimulation intensity usually brought into play a second "slow" type of mechanical response which summated with the lower threshold response. However, the excitation thresholds for these two contractions were often so close together that it was impossible to decide whether they were really two. These "slow" responses were very similar to those obtained by Pringle (1939) from the extensor

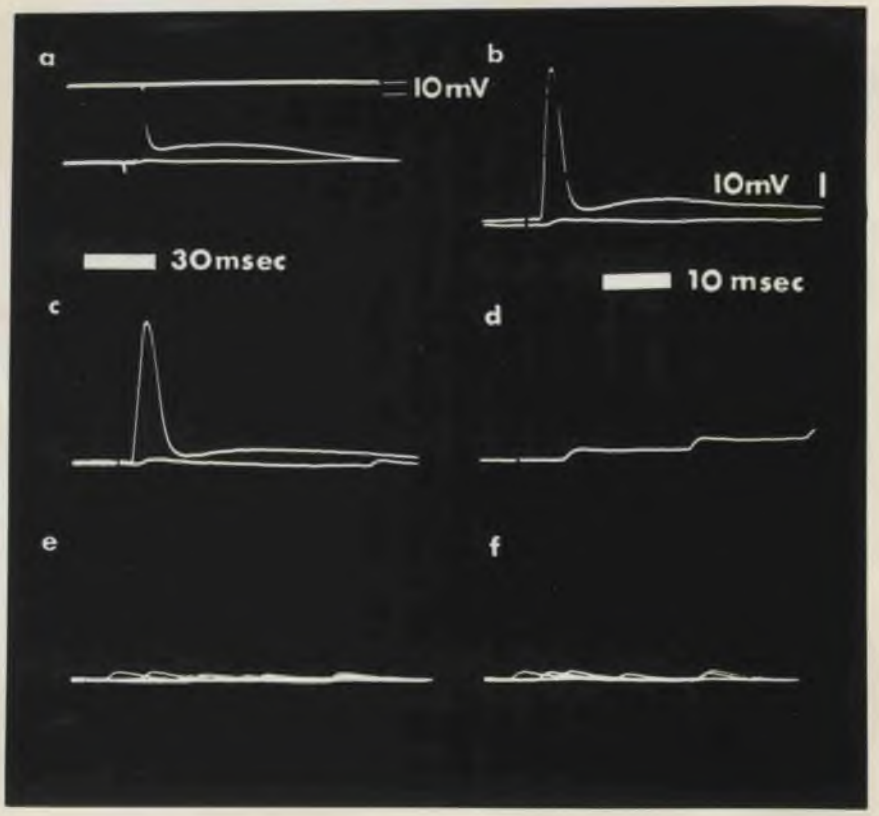


Fig. 19. Electrical responses of muscle fibres of muscle 135d and e. (a-c) Responses to separate stimulation of the "slow" axon and the "fast" axon. Critical width of square pulse was used to separate the more slowly-conducted impulse of the "slow" axon from that of the "fast". (d-f) Reflexly-evoked "slow" axon responses. Time calibration at 300 c/s. Voltage calibration same for (b-f).

tibiae muscle of the cockroach and by Hoyle (1953, 1955c) from the extensor tibiae muscle of the locust.

The electrical activity of the muscle fibres of 135d and e during stimulation at these lower intensities consists of small junctional potentials (Fig. 19) which show considerable facilitation with repetitive activation. The parallel between the electrical and mechanical responses of muscles 135d and e at these stimulation intensities and the "slow" electrical and mechanical responses of the locust extensor tibiae muscle (Hoyle, 1953, 1955c) is very close.

At higher intensities of stimulation the mechanical and electrical responses of 135d and e are markedly different from those recorded at the lower intensities. Large twitch contractions are obtained to low frequency stimulation (Fig. 20). If the condition of the preparation is good, they have a duration of about 50 msec and produce a peak tension of 0.5-2 gm. At high frequencies of stimulation only slight mechanical summation occurs, with the result that the tetanic response obtained at a stimulation frequency of about 50 c/sec produces a peak tension which is only about double that of the twitch. Associated with the mechanical response exhibited by 135d and e at these higher stimulation intensities, electrical events of the "fast" type appear in a large number of the muscle fibres.

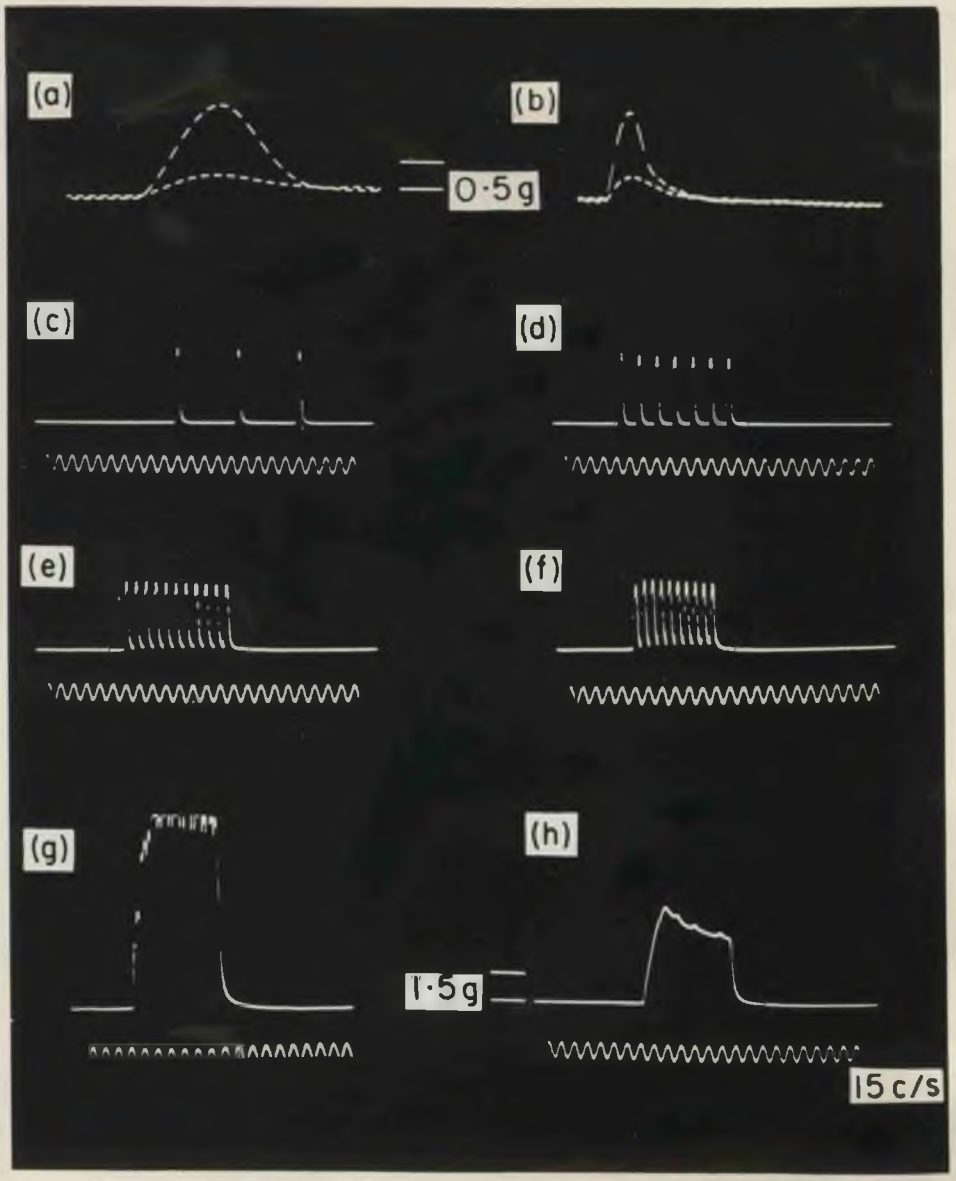


Fig. 20. Mechanical response of muscle group 135d and e to electrical stimulation of the two "fast" motor axons which innervate it. (a-b) Isometric twitch response to low frequency stimulation; (a) modulated at 500 c/sec; (b) modulated at 250 c/sec. (c-h) Effect of stimulation frequency on the maximal mechanical response of 135d and e; (c) 8 c/sec; (d) 14 c/sec; (e) 20 c/sec; (f) 25 c/sec; (g) 35 c/sec. All at the same sweep speed. The gain was reduced before taking record (h). Tension calibration for (a) and (b) also applies to (c-g) and is 0.5 gm. Tension calibration for (h) is 1.5 gm. Time calibration for (c-h) is 15 c/sec.

Furthermore, the appearance of each of the two twitch contraction heights obtained from this preparation by graded stimulation coincides with the successive appearance of "fast" electrical responses in two different sets of muscle fibres.

The fact that the excitation thresholds of the two "fast" axons are usually higher than those of the two "slow" axons means that it is impossible to obtain records of the effects of "fast" axon stimulation to the exclusion of the "slow". However, this difficulty is partially offset, for any one frequency of stimulation, by the fact that the tensions developed by "fast" axon stimulation are far greater than those developed by "slow" axon stimulation, with the result that interference by the "slow" response is usually not too marked. It is fairly safe to assume that the myograms obtained from good preparations by stimulation at the threshold for the "fast" axon or axons will be similar essentially to myograms obtained through stimulation of the "fast" axon or axons alone. At a stimulation frequency of 50 c/sec the total peak isometric tension developed by a typical preparation of muscle group 135d and e following stimulation at the maximal "fast" axon threshold was about 4.5 gm whereas the peak tension developed by stimulation at the "slow" axon threshold was only 0.2 gm.

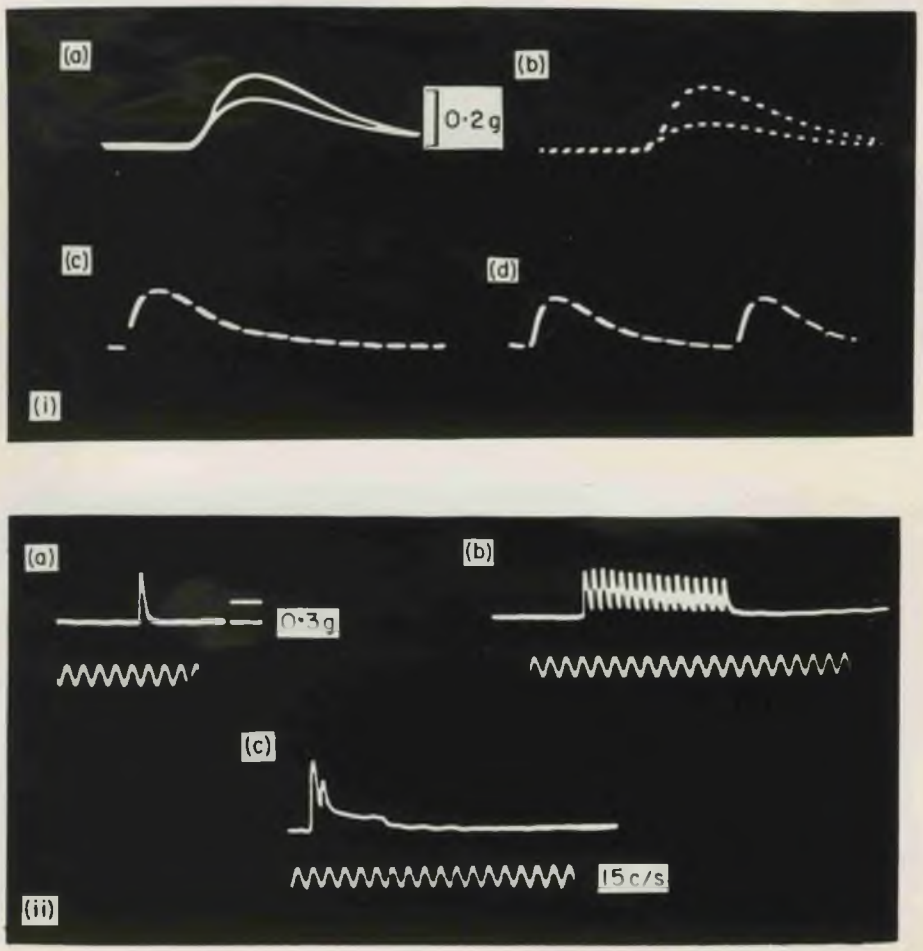


Fig. 21. (i) Mechanical response of muscle 135b to stimulation of the "fast" motor axons which innervate it. (a) and (b) illustrate the responses of two 135b preparations to stimulation of, first one, and then both, innervating "fast" axons with single shocks; (b) is modulated at 500 c/sec. (c) and (d) illustrate reflexly-evoked mechanical responses to "fast" axon stimulation and are modulated at 250 c/sec. Tension calibration same for all records.

(ii) Mechanical response of 135b to maximal stimulation of the motor nerve at a variety of frequencies:- (a) 1 c/sec; (b) 10 c/sec; (c) 50 c/sec. Preparation with a high twitch/tetanus force ratio. Note in (c) intermittent phases of recovery during fatigue of the tetanic response.

If the preparation is initially in a poor condition the myograms obtained from this muscle group are entirely different. The total tension developed through maximal stimulation of the innervating motor nerve is only slightly greater than the tension obtained by stimulation at the "slow" axon threshold. Myograms obtained from such a preparation are not unlike the "cock's-comb" myograms recorded by Becht and Dresden for this muscle and consist of the diminished "fast" response superimposed upon an apparently normal "slow" response.

It is concluded from these results that the muscle group 135d and e is innervated by at least four motor axons. Two of these axons are of the "slow" type and two are of the "fast" type. The maximal response of this preparation consists of the summated responses to stimulation of the four motor axons and apart from differences in motor nerve innervation there is no evidence to support the suggestion that this muscle group differs in any way from muscle groups 136 and 137 and 135a and c.

Muscle 135b. Muscle 135b was the slowest muscle encountered by Becht and Dresden (1956) and they were unable to record from it kymographically any response to low frequency stimulation. It is significant, however, that

Becht and Dresden could see small twitch contractions under the microscope on these occasions. At high frequencies of stimulation they recorded a slow shortening of muscle 135b, with an equally slow return of the muscle to its resting length following cessation of stimulation. Intracellular micro-electrode recordings of electrical events in the fibres of muscle 135b during graded electrical stimulation of nerve 4 have demonstrated the occurrence of two types of electrical response, "fast" and "slow", respectively (Becht et al., 1960). About 40% of the fibres are dually innervated, receiving branches from both "slow" and "fast" motor axons. On the basis of the mechanical recordings obtained by Becht and Dresden from muscle 135b the problem posed by the dual innervation established for this muscle by Becht et al. (1960) is extremely interesting. It would require that the type of mechanical response obtained from this muscle was the same following "fast" axon and "slow" axon stimulation. Since it has previously been shown that muscle group 135d and e, which also receives an innervation from "fast" and "slow" motor axons, gives different isometric mechanical responses to "fast" axon stimulation and to "slow" axon stimulation, attempts were made to resolve the problem by recording simultaneously the electrical and mechanical events of neuromuscular transmission of muscle 135b.

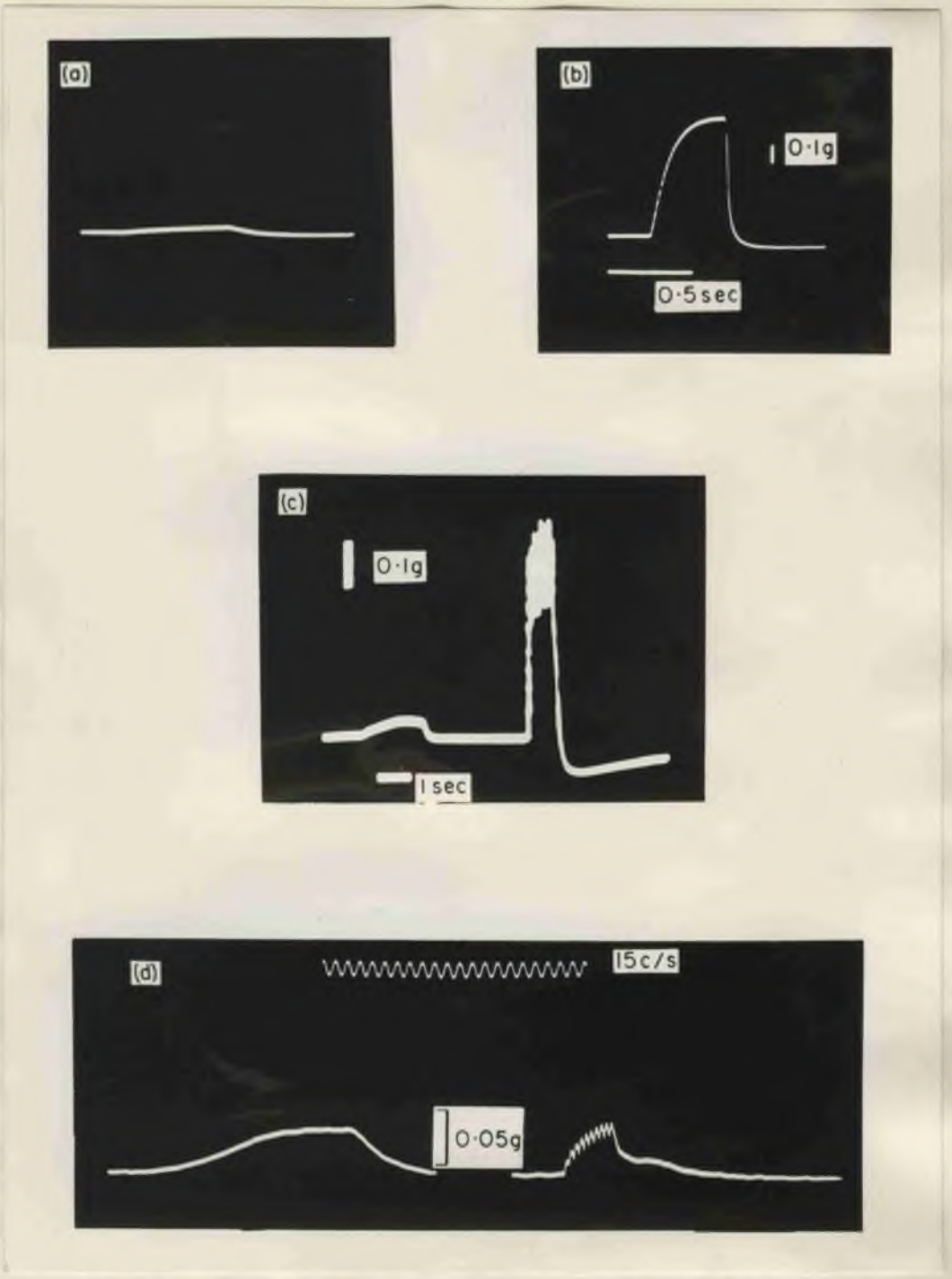


Fig. 22. Comparisons of the mechanical responses of muscle 135b to "fast" and "slow" axon stimulation. (a) Response to "slow" axon at a stimulation frequency of 50 c/sec. (b) Response of the same preparation to maximal stimulation at 50 c/sec. (c) Response of another preparation to stimulation of the "slow" axon (on the left) and the "fast" axon, at a frequency of 30 c/sec. (d) Response of a preparation, possibly in poor condition, to "slow" axon stimulation (on the left) and to "fast" and "slow" axon stimulation at a frequency of 30 c/sec. Note in the right hand record the diminished "fast" response superimposed upon the relatively large "slow" response. The small hump on the relaxation phase of this record is possibly an artifact introduced by the recording system.

At low frequencies of stimulation two isometric twitch heights were recorded from muscle 135b by graded stimulation of the innervating motor nerve (Fig. 21). If the nerve is stimulated at frequencies above about 20 c/sec a third response appears at slightly lower intensities of stimulation than those required to evoke the two twitch or "fast" responses (Fig. 22). This lowest threshold response was similar in many respects to the "slow" responses of muscle group 135d and e. It was characterized by a slow rate of tension rise and a slow rate of relaxation on cessation of stimulation. At stimulation frequencies of about 150 c/sec the response still showed little signs of fatigue, and was capable of developing a peak tension of up to 0.1 gm.

The normal response of 135b to a single maximal stimulus consists of a twitch contraction of duration no greater than 70 msec, which develops a peak tension of 0.1-0.75 gm (Fig. 21). The duration of the twitch response is longer than that obtained for the coxal muscles discussed earlier. However, preparation of muscle 135b is extremely difficult and it is possible that most of the preparations were not in perfect condition. One or two preparations did give twitch contractions with durations at least no greater than those of, for example, muscle group 135a and c (Fig. 21).

Once again, as was seen for muscle group 135d and e, the

excitation thresholds for the "fast" axon innervating muscle 135b are usually higher than the threshold for the "slow" axon, and it is necessary to make the same approximations as for 135d and e in order to obtain data on the response of 135b to "fast" axon stimulation. Bearing this in mind the twitch/tetanus force ratio for a good preparation has been estimated at about 1/4. In some preparations the ratio was as low as 1/10 but on one occasion a very high ratio of only 1/2 was obtained (Fig. 21). This suggests that the normal twitch/tetanus force ratio for muscle 135b is similar to that of muscle group 136 and 137. The fact that most preparations of muscle 135b had lower twitch/tetanus force ratios than this was due possibly to a slight deterioration in their condition rather than to any real differences in their mechanical properties. Under sustained tetanic stimulation the mechanical response of 135b falls very quickly to a low level, presumably to the level of tension developed by "slow" axon stimulation.

The main difficulties involved in obtaining reliable mechanical recordings from these coxal muscles, apart from those associated with their small size and relative inacessibility, arise from the spontaneous movements of the thorax during breathing which affect the myograms. It is desirable to secure firmly the proximal attachments of the

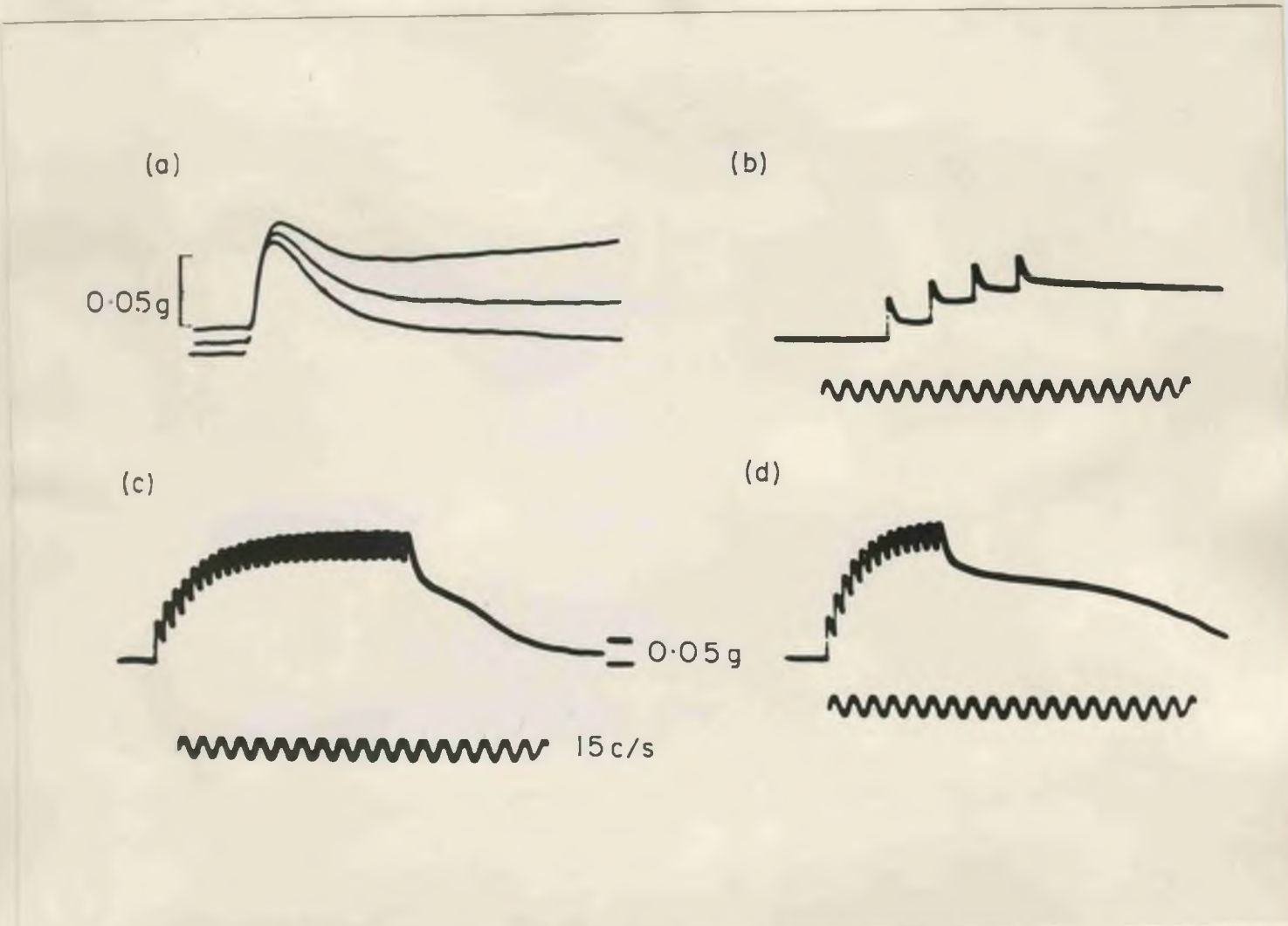


Fig. 23. Mechanical responses of muscle 135b to maximal motor nerve stimulation with the proximal attachment of the muscle only loosely fixed. (a) Three superimposed twitch contractions recorded successively to illustrate the marked variation of twitch durations under these conditions. (b-d) Effect of stimulation frequency on the maximal response of this preparation; (b) 6 c/sec; (c) 30 c/sec; (d) 40 c/sec. Tension calibration same for records (b), (c) and (d). Note mechanical summation even at relatively low frequencies of stimulation and variation in relaxation rate. Tension calibration same for (b-d).

muscle or muscle group in order to prevent these parts from moving during the contraction of the muscle. 135b was especially difficult in this respect. Its proximal attachment is a downwardly-bent flange of the sternal arm (Dresden and Nijenhuis, 1953). A number of other mesothoracic muscles also attach themselves to this portion of the endoskeleton, and since it is a relatively non-rigid structure the position which it occupies in the thorax is partly dependent upon the interplay of the muscles attached to it.

During this set of experiments the position of the flange and hence the resting length of muscle 135b were maintained approximately constant by fixing the flange into position with a pair of small pins. If this precaution is not taken, recordings from 135b are changed beyond recognition. The effect of movements of the flange on the myograms obtained from 135b is made even more apparent by dissecting away some of the thoracic muscle attached to the flange. Instead of obtaining a twitch response of duration about 60 msec, responses of up to and over 1 sec in duration are recorded. The apparent increased duration of the twitch under these conditions results mainly from an increase in the relaxation time, which leads to considerable summation of the mechanical responses at even low frequencies of stimulation (Fig. 23). It is important to note that not

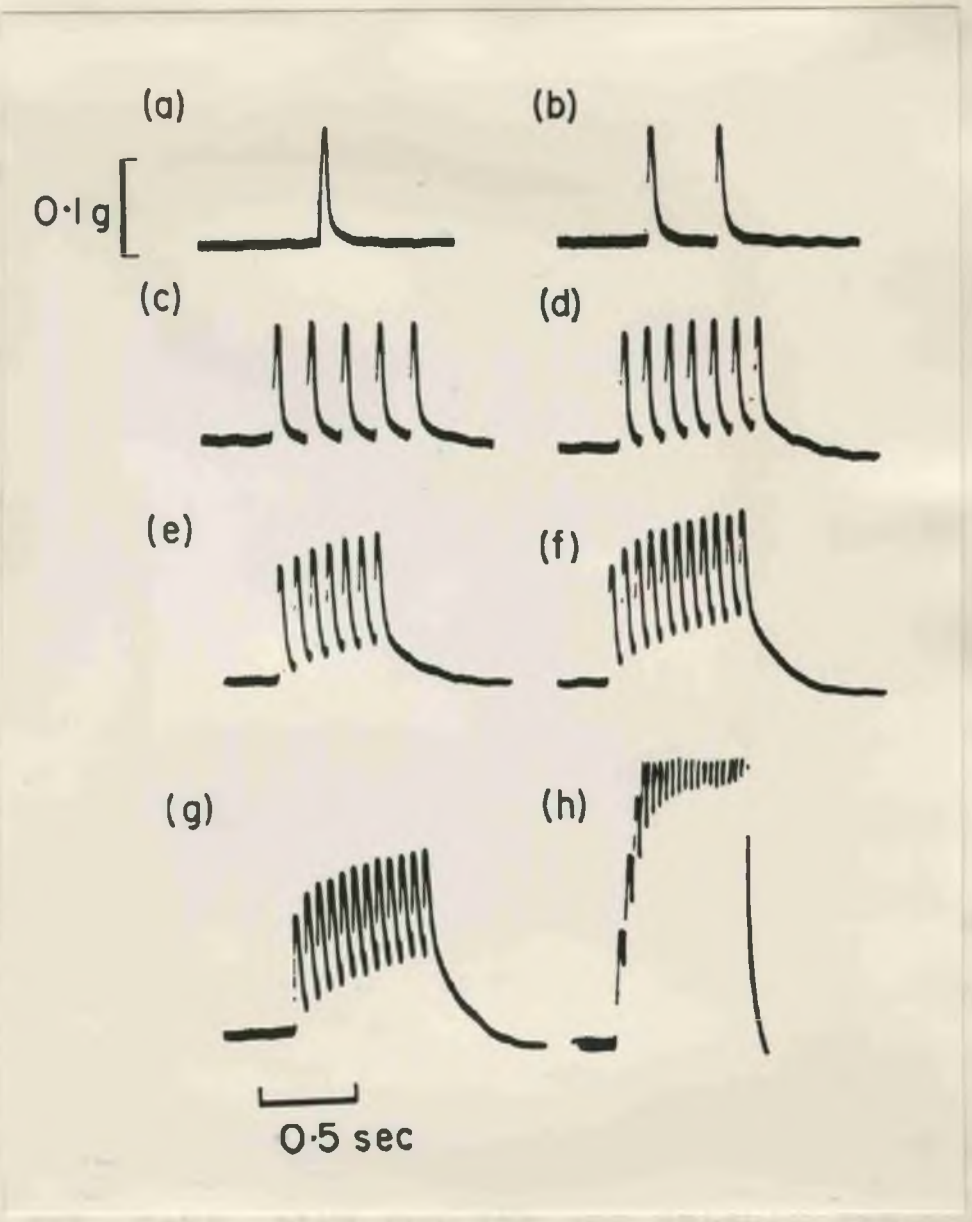


Fig. 24. Response of an isolated preparation of muscle 135b in good condition following maximal stimulation of the innervating motor nerve. The frequencies of stimulation were (a) 1 c/sec; (b) 5 c/sec; (c) 8 c/sec; (d) 10 c/sec; (e) 12 c/sec; (f) 14 c/sec; (g) 16 c/sec; (h) 30 c/sec. Note the increasing contribution of the "slow" contraction to the total tension developed by the muscle with increasing frequency of stimulation. In (h) the crests of the record are masked off.

only is the mechanical response prolonged under these conditions but it is also markedly reduced in magnitude, as would be expected if the muscle is allowed to shorten at the expense of tension development.

Isolated preparations of muscle 135b were made only with considerable difficulty and even then the results obtained were never as reliable as those obtained from the intact preparations. However, the myograms recorded from the better preparations were essentially similar to those recorded from the intact preparations apart from a slight reduction in the magnitude of the peak twitch tension of the "fast" response (Fig. 24).

Simultaneous recordings of electrical and mechanical events of muscle 135b to motor nerve stimulation have been obtained from both isolated and intact preparations. The tonic response described is associated always with electrical events of the "slow" type whereas the tetanic response coincides with the appearance of electrical events of the "fast" type. Since a large number of the muscle fibres are innervated by both "fast" and "slow" axons it is not unreasonable to assume that these fibres are capable of performing both types of contraction.

It is concluded from these results that it is possible

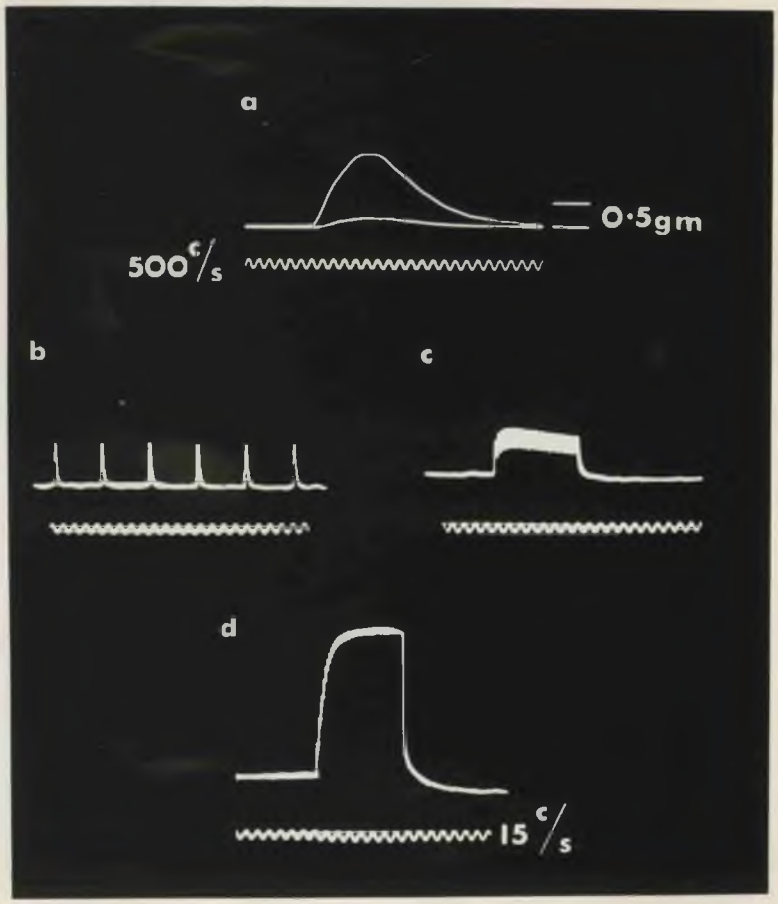


Fig. 25. Mechanical responses of muscle 139c. (a) Isometric twitch responses obtained by graded electrical stimulation of the innervating motor nerve. (b-d) Effect of stimulation frequency on the normal response muscle 139c. (b) 4 c/sec; (c) 20 c/sec; (d) 40 c/sec. Time calibration 15 c/sec. Tension calibration same for all records.

to interpret the different mechanical responses obtained from muscle 135b on the basis of its innervation by two distinct types of motor axon of the kind called "fast" and "slow". Two axons are of the "fast" kind and each one innervates a separate bundle of muscle fibres. Stimulation of either "fast" axon results in the appearance of electrical events of the "fast" type in the innervated muscle fibres, followed by what may be termed a "fast" contraction of these fibres. Muscle 135b is also innervated by a "slow" axon with a lower excitation threshold than that of either "fast" axon which, when stimulated, leads to a "slow" contraction of the muscle fibres innervated, and the appearance of electrical events of the "slow" type in these fibres. The mechanical response of 135b to maximal indirect stimulation consists of the ~~summated contractions~~ summation of two "fast" responses and one "slow" response.

Flexor muscles

Muscles 138 and 139c. This muscle group was placed in the "mixed" muscle class by Becht and Dresden (1956), together with muscle group 135d and e.

By very careful graded electrical stimulation of the innervating motor nerve three distinct twitch contraction heights have been recorded isometrically from this

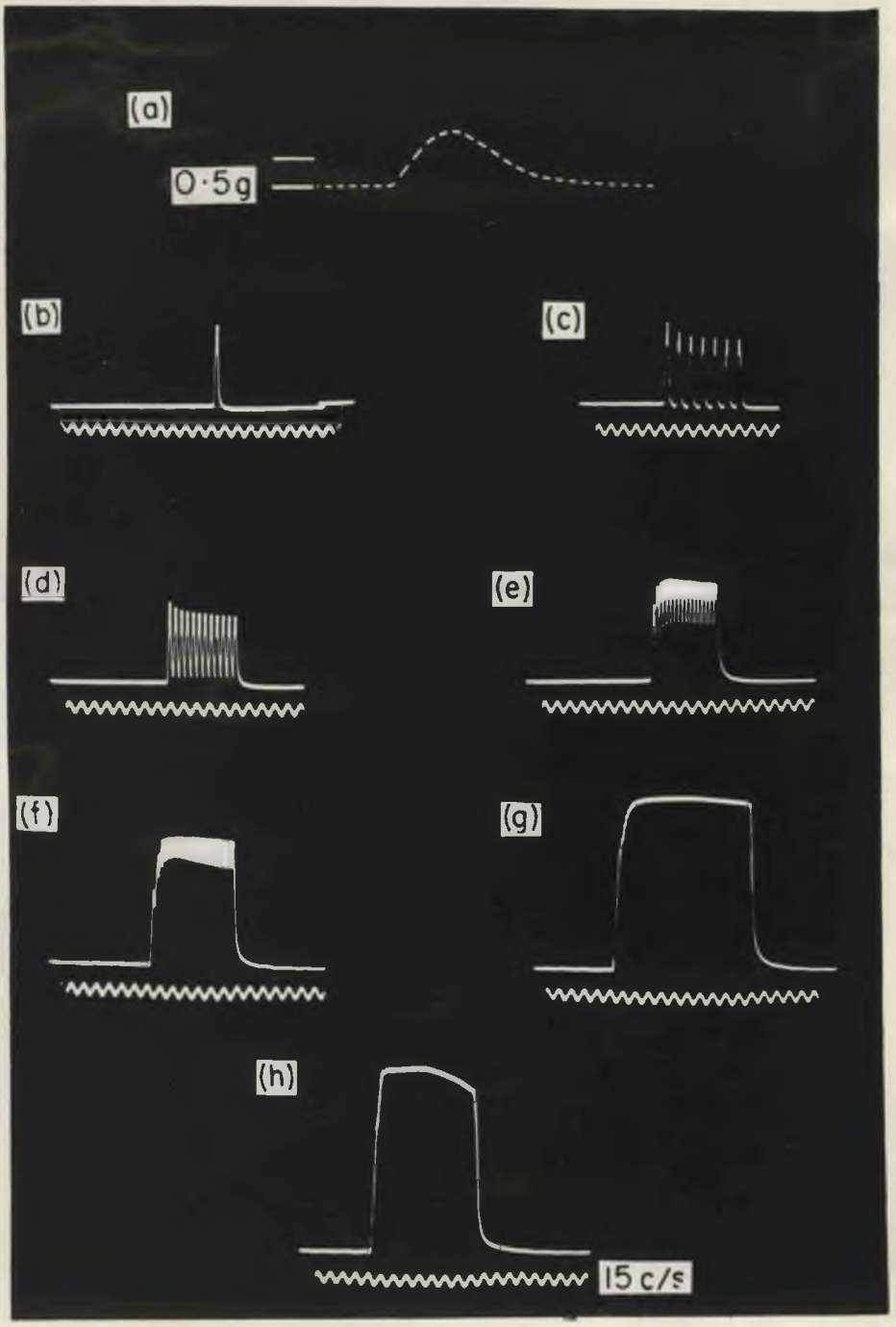


Fig. 26. Mechanical responses of muscle group 138 and 139c. (a) Isometric twitch response to a single indirect maximal stimulus. Trace modulated at 500 c/sec. (b-f) Effect of stimulation frequency on the maximal response of 138 and 139c; (b) 1 c/sec; (c) 15 c/sec; (d) 25 c/sec; (e) 35 c/sec; (f) 45 c/sec; (g) 55 c/sec; (h) 65 c/sec. Time calibration is 15 c/s.

preparation. Experiments on the individual muscles of this group have shown that muscle 139c responds to graded stimulation with two discrete contraction heights (Fig. 25). The maximal isometric response of 138 and 139c to a single stimulus lasts for no longer than 50 msec and may give rise to peak tensions of up to 2 gm. It is perhaps significant that twitch responses of duration less than 40 msec have been recorded from this preparation. These are briefer than the isometric twitch responses of Becht and Dresden's "fast" muscles. Only a small amount of mechanical summation occurs at high frequencies of stimulation, with the result that the force of the tetanic response of 138 and 139c, which appears at about 50 c/sec, is not much greater than the force of the twitch response. If the condition of the preparation is good, then twitch/tetanus force ratios as low as 1/2-1/3 are obtained (Fig. 26).

Once again the figures quoted are only approximate, since the maximal response of muscles 138 and 139c consists of the summated contractions resulting from "fast" and "slow" axon stimulation. The "slow" axon innervating this muscle group, by virtue of its lower excitation threshold, is stimulated at lower intensities than the "fast". Once again stimulation of the "slow" axon produces a tonic response of the innervated muscle fibres, which coincides with the appearance of

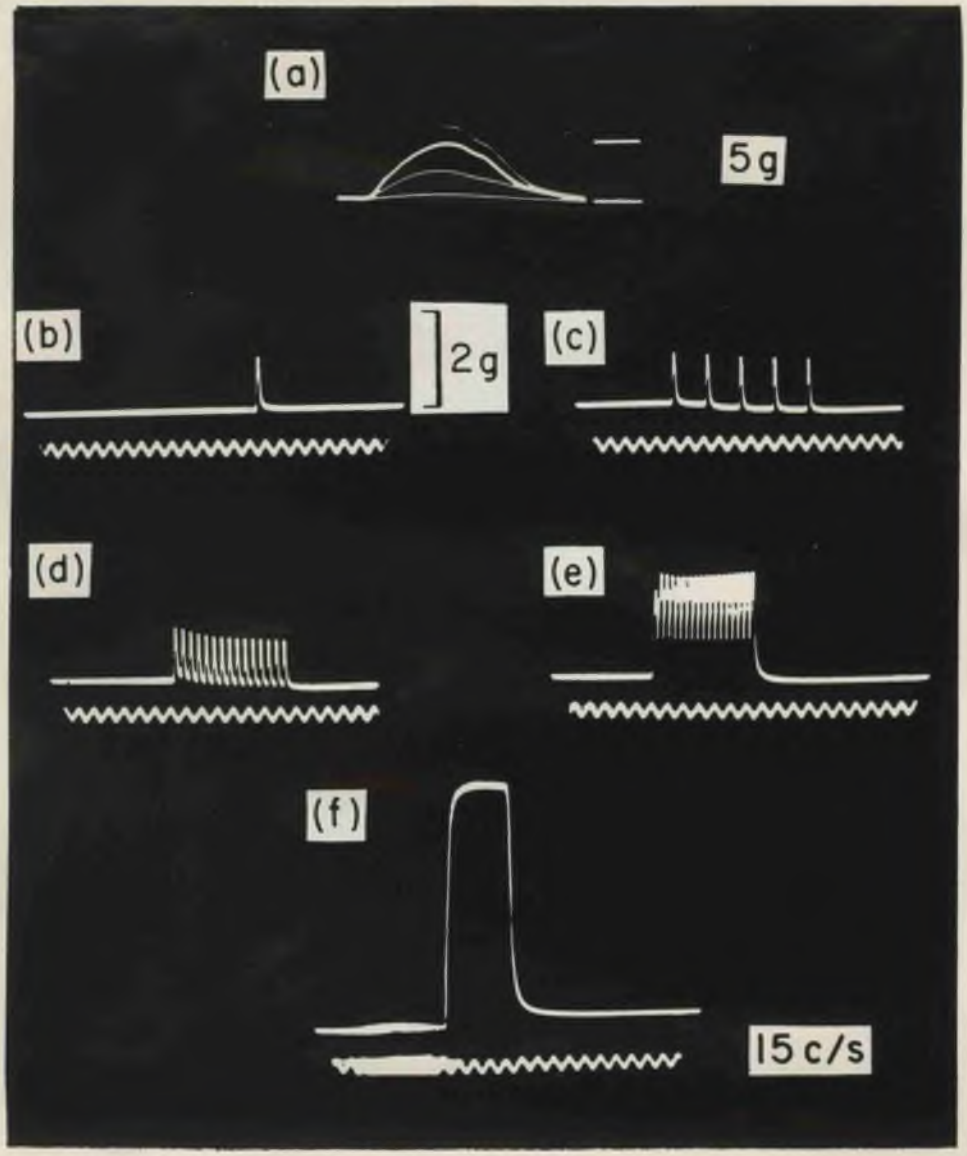


Fig. 27. Mechanical responses of muscle group 139a and b and 140 to motor nerve stimulation. (a) Isometric twitch responses obtained by graded electrical stimulation of the innervating motor nerve. The peaks on the relaxation phases of the top two traces are artifacts introduced by the recording system. (b-f) Effect of stimulation frequency on the maximal response of this muscle group; (b) 1 c/sec; (c) 7 c/sec; (d) 25 c/sec; (e) 35 c/sec; (f) 50 c/sec. Time calibration is 15 c/s. Tension calibration same for records (b-f).

electrical events of the "slow" type in those fibres. On the other hand, the twitch contractions of muscle group 138 and 139c are associated with electrical events of the "fast" type.

Considerable difficulty was experienced in separating the different electrical and mechanical responses of 138 and 139c by graded stimulation, and it is quite possible that apart from the three "fast" axons and one "slow" axon found, other "fast" and "slow" axons innervate this muscle group.

If the preparation is in poor condition, muscles 138 and 139c give isometric myograms of a "cock's-comb" character. The diminished "fast" response is superimposed upon the summated "slow" response.

Muscles 139a and b and 140. By graded electrical stimulation of the innervating motor nerve and optimum separation of the stimulating electrodes, four or five contraction heights have been recorded from this preparation. A tension of about 5 gm is developed to a single maximal shock applied to the innervating motor nerve and the total duration of the twitch response is always less than 50 msec (Fig. 27). Only slight mechanical summation occurs at higher stimulation frequencies and hence the twitch/tetanus force ratio is rather high, at about 1/1.5-1/3. The twitch and tetanic

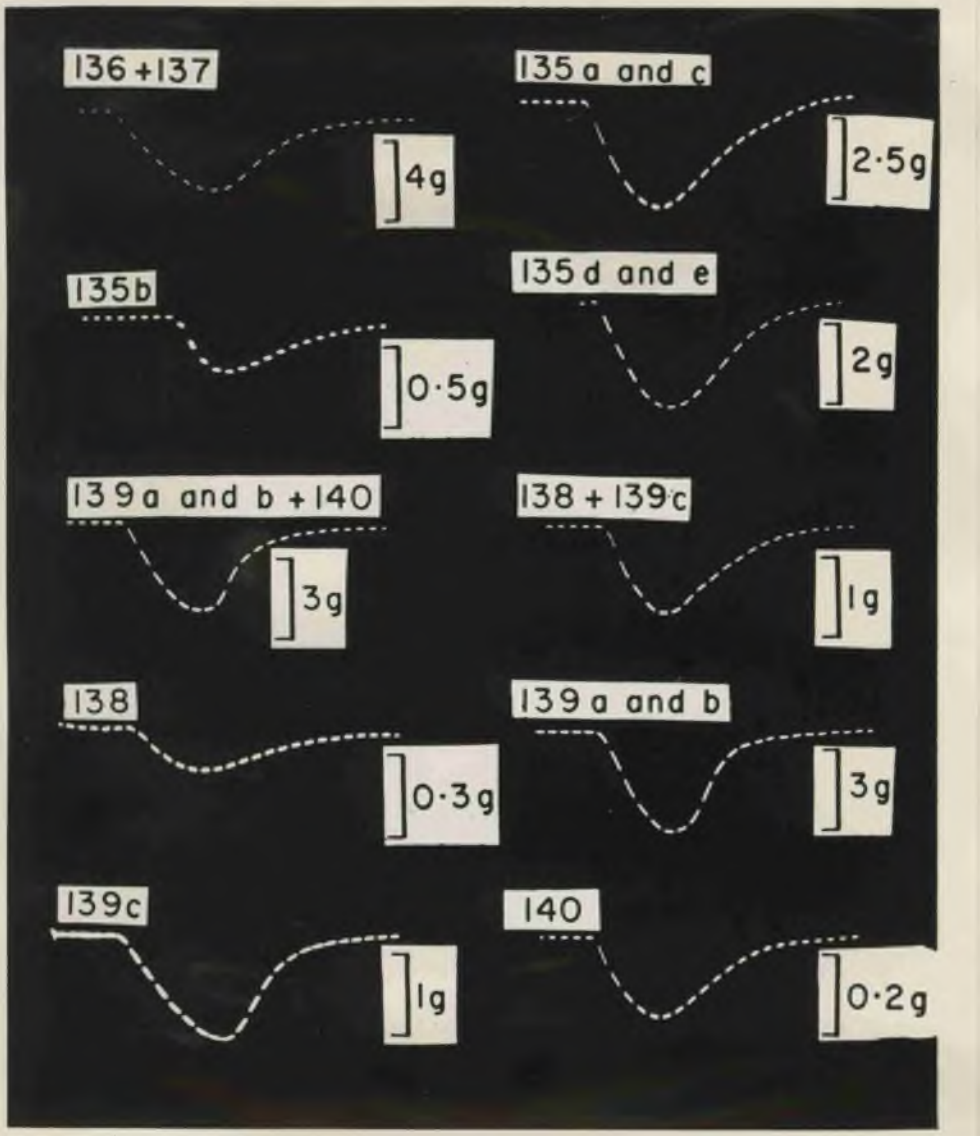


Fig. 28. Records showing total durations of isometric twitch responses recorded from the different muscles and muscle groups of the mesothoracic coxa of Periplaneta americana following maximal electrical stimulation of their innervating motor nerves. All the responses are modulated at 500 c/sec.

Muscle	Innervation (established by electrical recordings)	Mechanical responses to graded stimulation	Peak tension (gm) developed following a single maximal stimulus	Rise time of twitch (msec) ± S.e. of mean	Decay time of twitch (msec) ± S.e. of mean	Half decay time of twitch (msec) ± S.e. of mean	Estimated cross sectional area of muscle (sq. cm.)	Approximate isometric twitch tension ($gm.cm^{-2}$) at max. body length	Becht and Dresden's classification
136 + 137	1 "fast" axon (1)	1 "fast" contraction	4 - 7.5	13.5 ± 1.0 (23)	30.2 ± 2.5 (14)	10.3 ± 1.2 (14)	8.2×10^{-3}	850	"fast" muscle
135a and c	4 "fast" axons (4)	4 "fast" contractions	2 - 5	10.6 ± 0.1 (7)	33.7 ± 1.9 (6)	10.0 ± 1.6 (6)	7.2×10^{-3}	700	"fast" muscle
135d and c	2 "fast" axons (4) 2 "slow" axons	2 "fast" contractions 2 "slow" contractions	0.5 - 2	12.1 ± 1.4 (19)	41.9 ± 3.2 (11)	12.9 ± 0.5 (10)	-	-	"mixed" muscle
135b	2 "fast" axons (4) 1 "slow" axon	2 "fast" contractions 1 "slow" contraction	0.1 - 0.75	13.3 ± 0.6 (24)	71 ± 4.7 (11)	15.1 ± 1.1 (10)	8.4×10^{-4}	850	"slow" muscle
138 + 139c	3 "fast" axons (7) 1 "slow" axon	3 "fast" contractions 1 "slow" contraction	1 - 2	11.4 ± 0.7 (8)	29.5 ± 0.9 (8)	9.2 ± 1.0 (11)	3.6×10^{-3}	550	"mixed" muscle
139a and b + 140	5 "fast" axons (7)	5 "fast" contractions	2 - 5	11.7 ± 0.6 (13)	30.1 ± 0.5 (10)	9.7 ± 1.0 (7)	6.6×10^{-3}	800	"mixed" muscle

Table 6. Summary of electrical and mechanical recordings obtained from the mesothoracic coxal muscles of Periplaneta americana. In column 2 figures in brackets represent the number of motor axons innervating the muscles (histological data from Dresden and Nijenhuis (1958)). In columns 5 and 6 figures in brackets represent number of measurements made.

responses of 139a and b, and 140 are associated always with electrical events of the "fast" type in all the muscle fibres. The muscle group does not appear to be innervated by a "slow" axon.

The results obtained from the different coxal muscles of Periplaneta are summarized in Table 5 and Fig. 28.

(2) The femoral muscles of the cockroach

The electrical and mechanical responses of the femoral muscles of the cockroaches, B. giganteus and P. americana, have been examined and re-examined respectively in the light of the controversy that exists over the interpretation of the control of muscular activity in these muscles (Pringle, 1939; Wilson, 1954; Becht, 1959). Recordings were taken from the extensor and flexor tibiae muscles of the pro-, meso- and metathoracic legs of these insects. The metathoracic muscles were studied in detail. The results are compared with those obtained from the extensor tibiae muscle of the metathoracic segment of S. gregaria.

The animal under examination was anaesthetized with carbon dioxide and partially embedded on its back with plasticine in a petri dish. The leg under examination was pulled out at an angle to the body and fixed down with wax. The wax, prepared by Dr H.F. Steedman, consisted of 90%

flexo wax - a hydrocarbon with a high melting point (55-58°C) and adhesiveness but with suppleness and rigidity; and 10% M.S2 resin - a cyclic ketone synthetic resin. The femur was fixed down in the petri dish with ventral and dorsal supports. With this rigid arrangement it could be safely assumed that the major part of the force developed during isometric activity of the femoral muscles was recorded at the distal end of the apodeme.

The hinged joints connecting femur to tibia were severed and the distal portion of the femoral cuticle removed to expose the apodemes of the femoral muscles at their points of attachment to the tibia. The cuticle, together with the underlying hypodermis on the ventral part of the femur, was carefully removed. The apodeme of the muscle under examination was slipped over a glass hook attached to the plate shaft of a transducer and the muscle was held at the length at which it just failed to show any resting tension. In these muscles this length corresponds to the minimum body length (m.b.l.). The motor nerve supply to the leg was exposed in the thorax and paired stimulating electrodes were placed under the appropriate nerve bundle. For reflex stimulation of the muscle the nerve supply was left in contact with the ganglion; for electrical stimulation the motor nerve was severed from the ganglion. To reduce unwanted

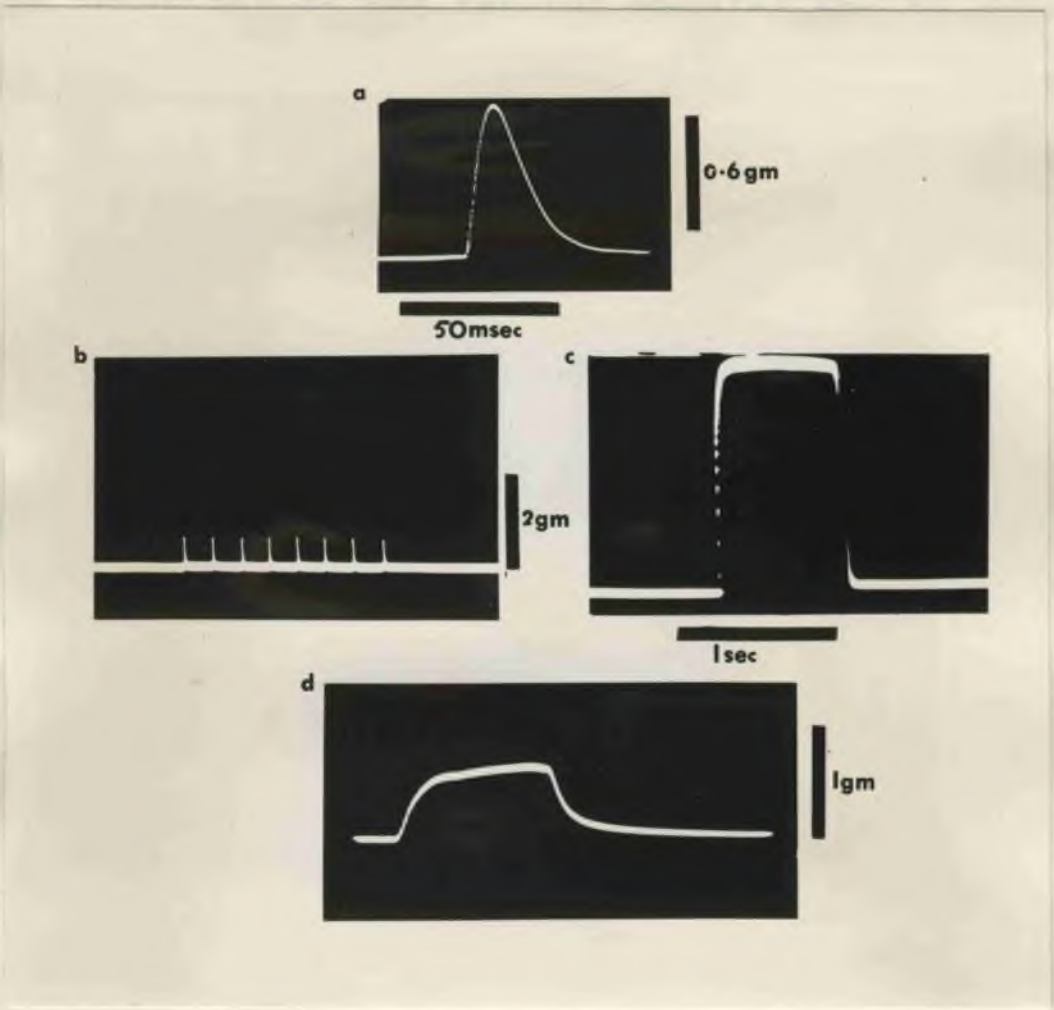


Fig. 29. Isometric mechanical responses of the metathoracic flexor tibiae muscle of Blaberus recorded at m.b.l. (a) Response to a single maximal stimulus. (b) Maximal response to stimulation at 5 c/s. (c) Maximal response to stimulation at 100 c/s. (d) Response to stimulation of "slow" axon at 100 c/s. Time calibration same for (b-d). Tension calibration same for (b-c).

spontaneous movements of the preparation to a minimum, all other nerves to the ganglion were severed.

The functions of the femoral muscles are to extend and flex the tibial segment, i.e. the flexor and extensor muscles are antagonistic. A change in the position of the tibia from complete extension to complete flexion involves changes in the lengths of the flexor and extensor muscles. With the tibia fully extended the extensor muscle is at the minimum length that it can occupy in the body; with the tibia fully flexed the extensor muscle is at the maximum length it can occupy in the body. The changes of length in the flexor muscle are the reverse of those described for the extensor. To examine the effect, if any, that these changes of resting length have on the tension that the muscle is able to develop during activity, the resting and active tensions of the extensor tibiae muscles of B. giganteus and S. gregaria were recorded for a variety of lengths between and including the maximum and minimum body lengths.

The resting and isometric active tensions were recorded with an RCA 5734 mechano-electronic transducer. The transducer was firmly attached to the moveable arm of a Prior micro-manipulator; the plate shaft of the transducer was attached directly to the muscle apodeme as described above. Fine movements of the micro-manipulator arm, and therefore

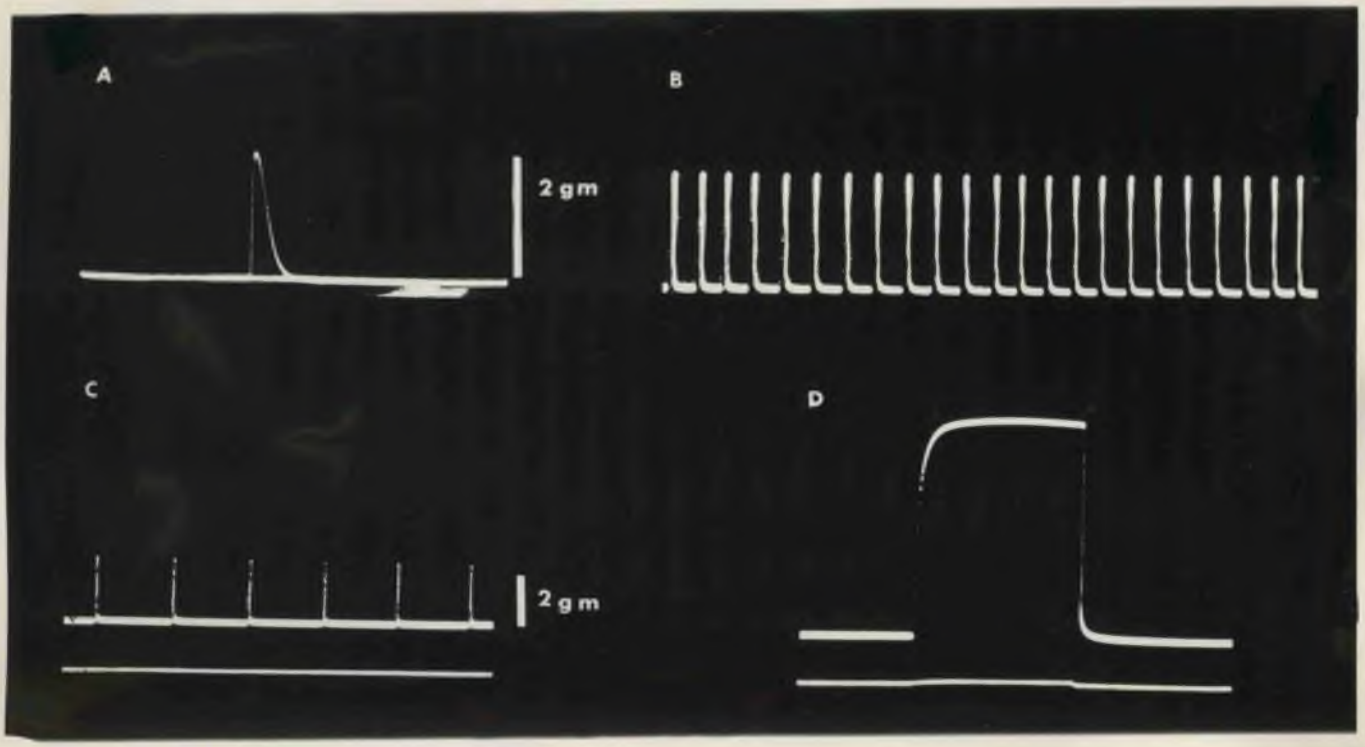


Fig. 30. Mechanical responses of the metathoracic flexor tibiae preparation of B. giganteus to maximal electrical stimulation of the innervating motor nerve. (A) Response to a single shock. (B) Response, to a stimulation frequency of 1 c/sec, with little variation in the height of the twitches. (C-D) Response of a different preparation to maximal stimulation at (C) 0.4 c/sec and (D) 100 c/sec to show difference in twitch and tetanic responses. All recordings made with muscle held at minimum body length.

of the transducer, along the long axis of the muscle could be calibrated accurately to 0.1 mm using the built-in vernier scale on the micro-manipulator.

The length of the muscle was altered in 0.1 mm steps and three recordings of the passive and active tensions developed by the muscle at each length were made. After each length-change the muscle was allowed one minute in which to equilibrate before any recordings were made.

Flexor tibiae. Graded electrical stimulation of the motor nerve has demonstrated that in Blaberus and Periplaneta the flexor tibiae muscle is divisible into five "fast" motor units. Each motor unit is innervated by a separate "fast" motor axon, each axon with its own discrete threshold of stimulation. Microscopical examination of the muscles during stimulation has shown that the bundle of muscle fibres in the posterior ventral part of the femur are innervated by the motor axon with the lowest stimulation threshold, whereas the anterior bundles situated ventrally in the femur are innervated by the motor axon with the highest stimulation threshold.

A single maximal indirect electrical stimulus evoked a brisk twitch contraction with a rise time of about 14 msec and a half decay time of about 21 msec (Fig. 29). At m.b.l. the peak twitch tension of the metathoracic flexor muscle of

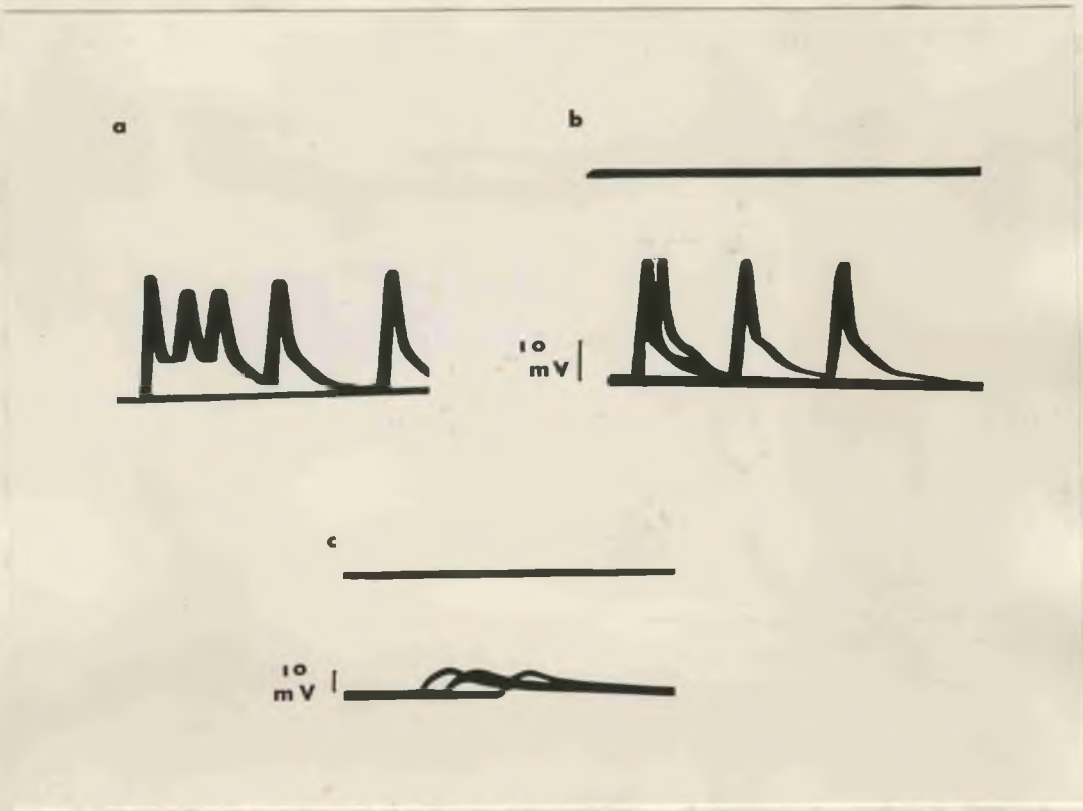


Fig. 31. Intracellularly-recorded responses to reflex stimulation of the metathoracic flexor tibiae preparation of B. giganteus. Records (a) and (b) from two different fibres showing responses to "fast" axon stimulation only. Note refractoriness of the responses and the presence of mechanical artifacts on the phase of repolarisation of the action potentials. (c) Fibre responding to "slow" axon stimulation only. Upper traces of records (b) and (c) indicate zero membrane potential. Voltage calibration same for (a) and (b). All traces lasted for 50 msec.

Blaberus averaged about 2 gm. Lower values for peak twitch tension were recorded from the metathoracic flexor muscle of Periplaneta at m.b.l. although the temporal characteristics of the twitch were similar. At frequencies of stimulation below 5 c/sec the mechanical responsiveness of the muscle remained relatively constant (Fig. 30) but at stimulation frequencies above 5 c/sec it showed signs of fatigue with a gradual decline in the magnitude of the twitch responses. Fusion of the mechanical responses occurred about 20 c/sec and was complete at about 100 c/sec to give a smooth tetanic response which fatigued quickly under sustained stimulation. Twitch/tetanus force ratios ranging between 1/3-1/10 have been recorded from these preparations (Figs 29 and 30).

Intracellular recordings from single muscle fibres during "fast" mechanical activity demonstrated that electrical responses of the "fast" type always accompanied the twitch and tetanic responses described above, i.e. "fast" mechanical responses of the muscle were always associated with "fast" electrical responses in both nerve and muscle.

The "fast" electrical responses, which ranged between 50-90 mV in magnitude, consisted of large post-synaptic responses with spikes which often overshot the zero membrane potential (Figs 31 and 32). They were recorded from a majority of the flexor muscle fibres and lasted for 3-7 msec. Resting

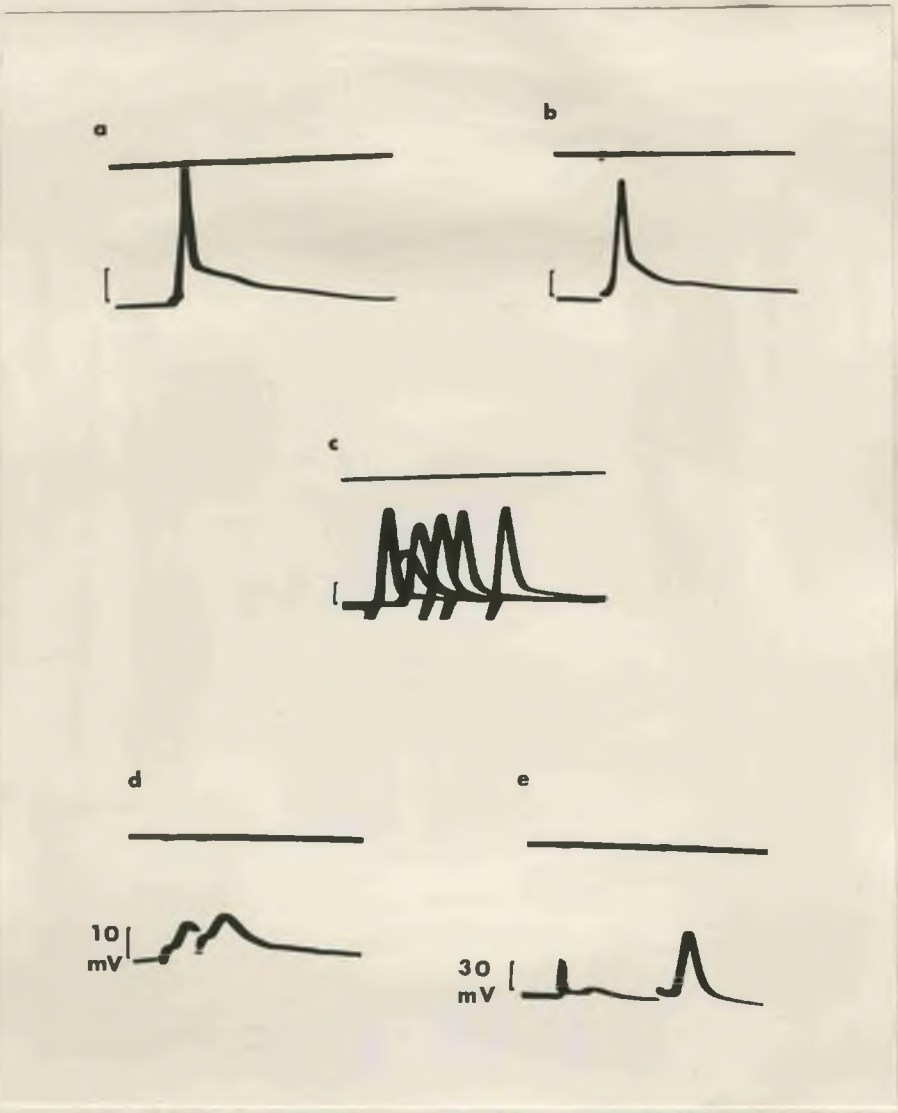


Fig. 32. Intracellularly-recorded electrical responses to indirect electrical stimulation from the extensor and flexor tibiae muscle fibres of the metathoracic leg of B. giganteus. (a) "Fast" response from a fibre of the flexor muscle to maximal stimulation of metathoracic nerve 5. (b) "Fast" response from an extensor muscle fibre to maximal stimulation of nerve 3. (c) Superimposed responses of a single fibre of the extensor muscle to stimulation of nerve 3 with two maximal shocks. The distance between the two shocks was gradually decreased. Note marked refractoriness of the "fast" response. (d) "Slow" muscle potentials evoked from a fibre of the flexor muscle following stimulation of nerve 5 with a pair of shocks. Note summation of the post-synaptic responses. (e) Responses of a single fibre of the flexor muscle to stimulation of nerve 5 with a pair of shocks of different intensities. The first stimulus evoked a "slow" response whilst the second, larger, stimulus evoked a "fast" response. Vertical calibrations in (a-d) all equal to 10 mV. Duration of all traces was 50 msec. Upper trace of records (a-d) represent zero membrane potential.

potentials ranging between 50-70 mV were recorded from these fibres.

At lower stimulation frequencies than those required to elicit the "fast" responses the flexor muscle performed a different type of mechanical response (Fig. 29) which was not unlike the "slow" mechanical response recorded from the locust extensor tibiae preparation following stimulation of its "slow" axon (Hoyle, 1955c). The "slow" contraction of the cockroach flexor muscle was apparent at stimulation frequencies above 20 c/sec although in a few preparations minute contractions of the muscle were recorded to single shocks. The magnitude and rate-of-rise of the "slow" response increased with increasing frequency of stimulation. A peak tension of 1 gm was recorded from the Blaberus preparation at 150 c/sec. Under prolonged high-frequency stimulation at the "slow" axon threshold the muscle showed little signs of fatigue. It is possible that the flexor tibiae of Blaberus is innervated by more than one "slow" motor axon, for two "slow" mechanical responses were recorded from some preparations of this muscle.

Electrical responses recorded from the muscle fibres during "slow" mechanicalactivity were post-synaptic potentials ranging in magnitude from 5-30 mV and in duration from 30-40 msec (Figs 31 and 32). A few of the larger responses

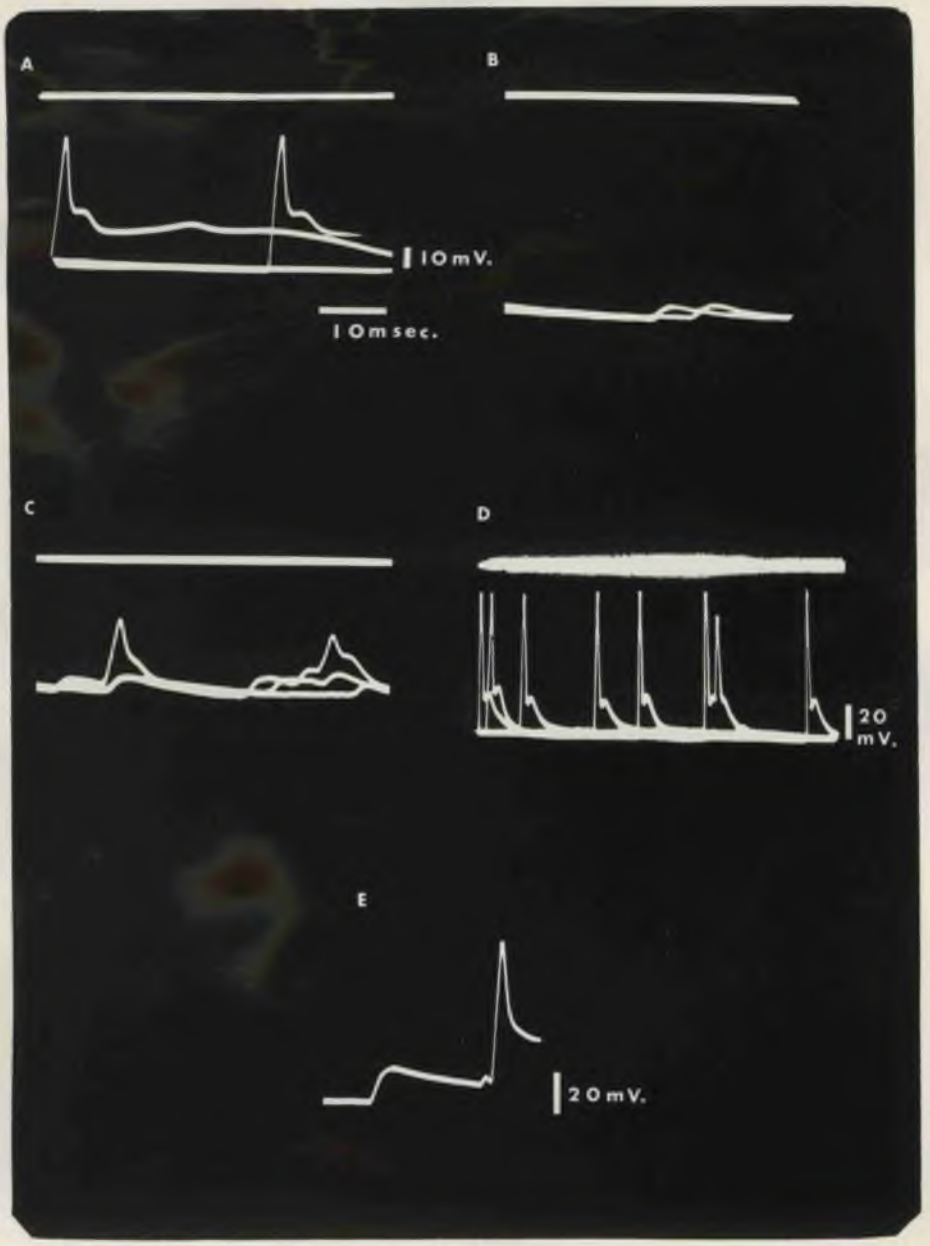


Fig. 33. Intracellularly-recorded potentials from the extensor and flexor tibiae muscles of the metathoracic femur of Periplaneta, following reflex stimulation, with the motor nerves in contact with the metathoracic ganglion. (A-B) Two fibres from the flexor muscle; (A) responded only to "fast" axon stimulation; (B) responded only to "slow" axon stimulation. Note differences in resting potential and compare with records a-f of fig. 34; also Wilson (1954). In (A) the "fast" responses are accompanied during repolarisation by mechanical artifacts. (C) "Fast" and "slow" potentials recorded from a fibre of the extensor muscle. The "slow" post-synaptic potentials summated to give a local, small spike response. (D) Extensor muscle fibre showing responses only to "fast" axon stimulation. Note refractoriness of these responses. (E) "Fast" and "slow" responses recorded from a fibre of the flexor muscle. Voltage calibration same for (A-D). Time calibration same for all traces except for record (D) which was recorded on a slower time base. Upper trace of each record represents zero membrane potential.

evoked small local active responses from the membrane. The resting potentials of fibres showing "slow" electrical and presumably mechanical activity ranged between 40-70 mV.

About 30% of the flexor muscle fibres showed both "fast" and "slow" electrical activity and presumably were capable of performing both "fast" and "slow" mechanical activity. Only about 5% of the muscle fibres examined failed to respond to stimulation of the "fast" axons innervating the flexor muscle.

In general the resting potentials of these fibres were lower than those of fibres showing only "fast" activity. However, exceptions to this were found, e.g. one fibre showing only "slow" activity had a resting potential of 90 mV (Fig. 33).

"Fast" electrical responses have never been recorded intracellularly from muscle fibres during "slow" mechanical activity. The "slow" mechanical responses were always accompanied by electrical activity of the "slow" type.

During reflex activity of the flexor muscle of the cockroach, the dually-innervated fibres respond continually to the spontaneous discharge of the "slow" axon. The frequency of discharge of the "slow" axon may be increased by gently stroking the anal cerci; the "slow" potentials then

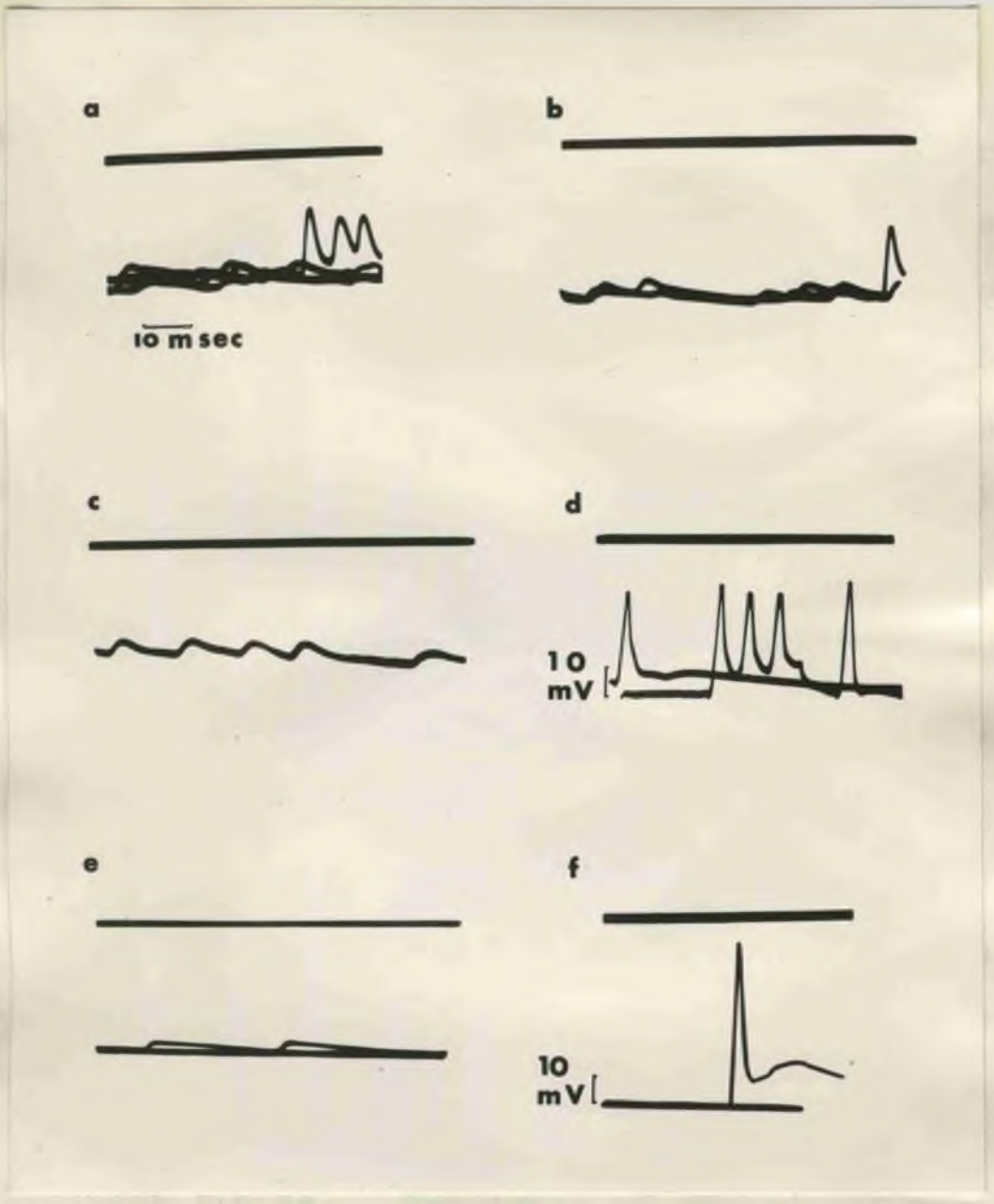


Fig. 34. "Fast" and "slow" electrical responses recorded from the metathoracic tibial-extensor muscle of P. americana following reflex stimulation through the motor nerve. Records (a) and (b) obtained from fibres responding to both "slow" and "fast" axon stimulation. Note summation of the "slow" responses and refractoriness of the "fast". Records (c) and (e) show fibres responding to "slow" axon stimulation only; records (d) and (f) show fibres responding to "fast" axon stimulation only. Note lower resting potential in records (c) and (e). Upper trace of each record represents zero membrane potential. Time calibration same for all records. Voltage calibration same for (a-e).

facilitate and summate and small local spike responses appear on the summed post-synaptic responses. If the anal cerci are stimulated vigorously "fast" electrical responses appear in the same muscle fibres in short high-frequency bursts superimposed on the background response to the "slow" axon. Records obtained from fibres during reflex stimulation of the "fast" axon illustrated clearly the refractory properties of the "fast" response (Fig. 31).

It is concluded from these results that at least a small part of the cockroach flexor tibiae muscle is dually innervated, i.e. it receives branches from a "slow" as well as from a "fast" motor axon. Furthermore, it seems possible that the dually innervated fibres are able to perform both "slow" and "fast" contractions.

Extensor tibiae. Two types of mechanical response have been recorded from the extensor tibiae muscles of the pro-, meso- and metathoracic legs of Periplaneta and Blaberus. Stimulation of metathoracic nerve 3b with a single maximal shock resulted in a brisk twitch contraction of the extensor muscle with a rise time to peak tension of about 15 msec and a half-decay time of about 15 msec (Fig. 35). The peak tension developed at m.b.l. following a single maximal stimulus ranged between 0.5-1.5 gm.

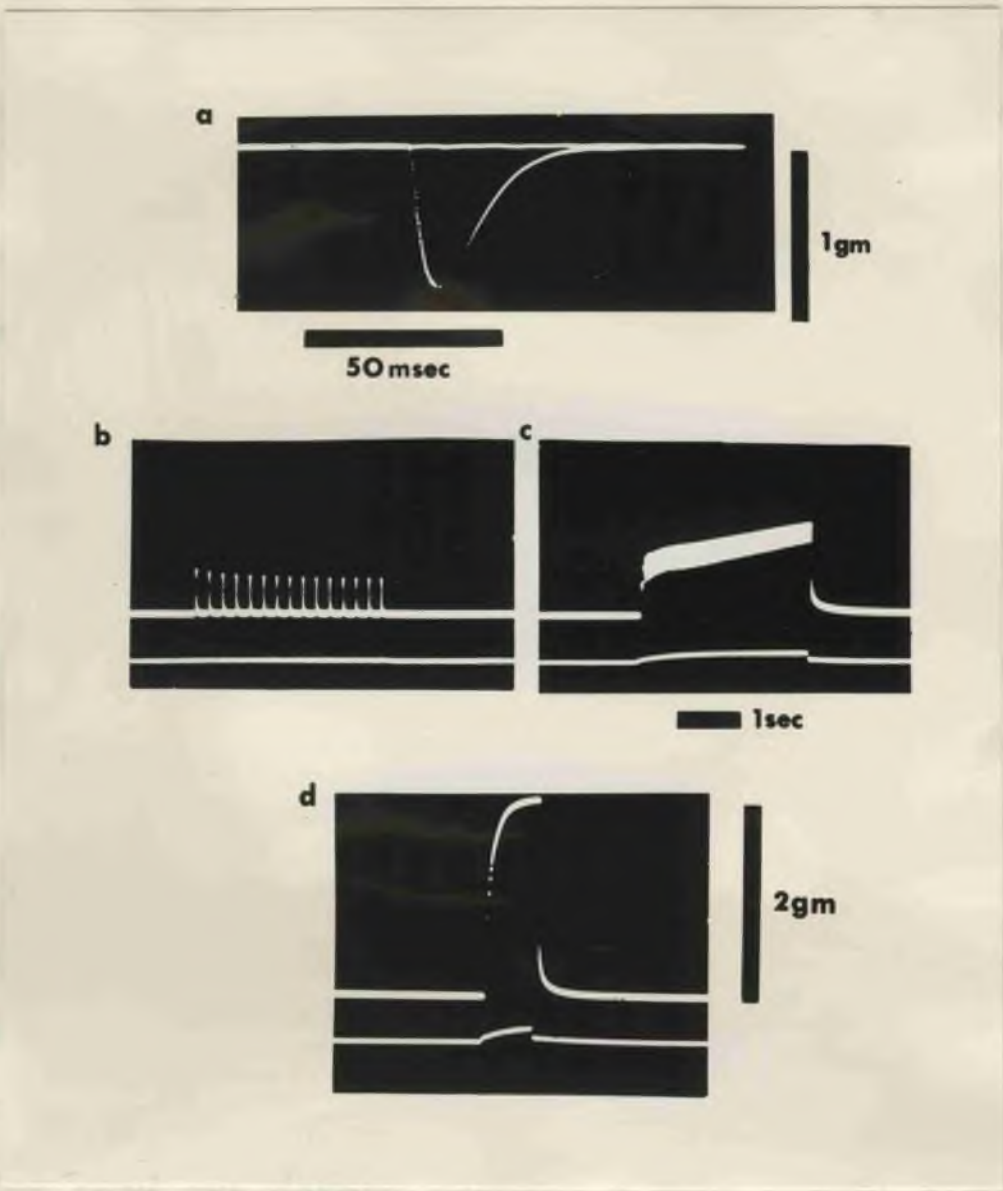


Fig. 35. Mechanical responses of the metathoracic extensor tibiae preparation of Blaberus recorded isometrically at m.b.l. (a) Isometric twitch contraction to a single maximal stimulus. (b-d) Effect of stimulation frequency on the maximal response; (b) 5 c/s; (c) 50 c/s; (d) 100 c/s. Time and tension calibrations same for records (b-d). Note in (b) fatique of twitch response with repeated stimulation. Note in (c) the fused twitches superimposed upon a "slow" response.

This "fast" response was always all-or-none in character, the muscle acting as a single "fast" motor unit, and was always accompanied by an electrical response of the "fast" type in most of the muscle fibres examined (Figs 32 and 34). Fusion of the twitch responses occurred at stimulation frequencies above 20 c/sec and at about 100 c/sec a smooth tetanic response was obtained (Fig. 35). Twitch/tetanus force ratios ranging between 1/5-1/10 were usual for preparations of these muscles.

With stimulation frequencies exceeding 20 c/sec and at a stimulation intensity lower than that required to elicit the "fast" response a typical "slow" mechanical response, which developed a higher peak tension than the "slow" response of the flexor muscle, was recorded from the extensor muscle (Fig. 36). The larger "slow" response recorded from the extensor muscle may be correlated with the fact that, compared with the flexor muscle, a much higher proportion (about 50%) of the extensor muscle fibres are dually innervated.

The electrical responses of the motor nerve innervating the extensor tibiae preparation of Blaberus to reflex and electrical stimulation have been studied with the aid of extracellular electrodes. Three types of potential, classified according to size, were recorded from nerve 3b.

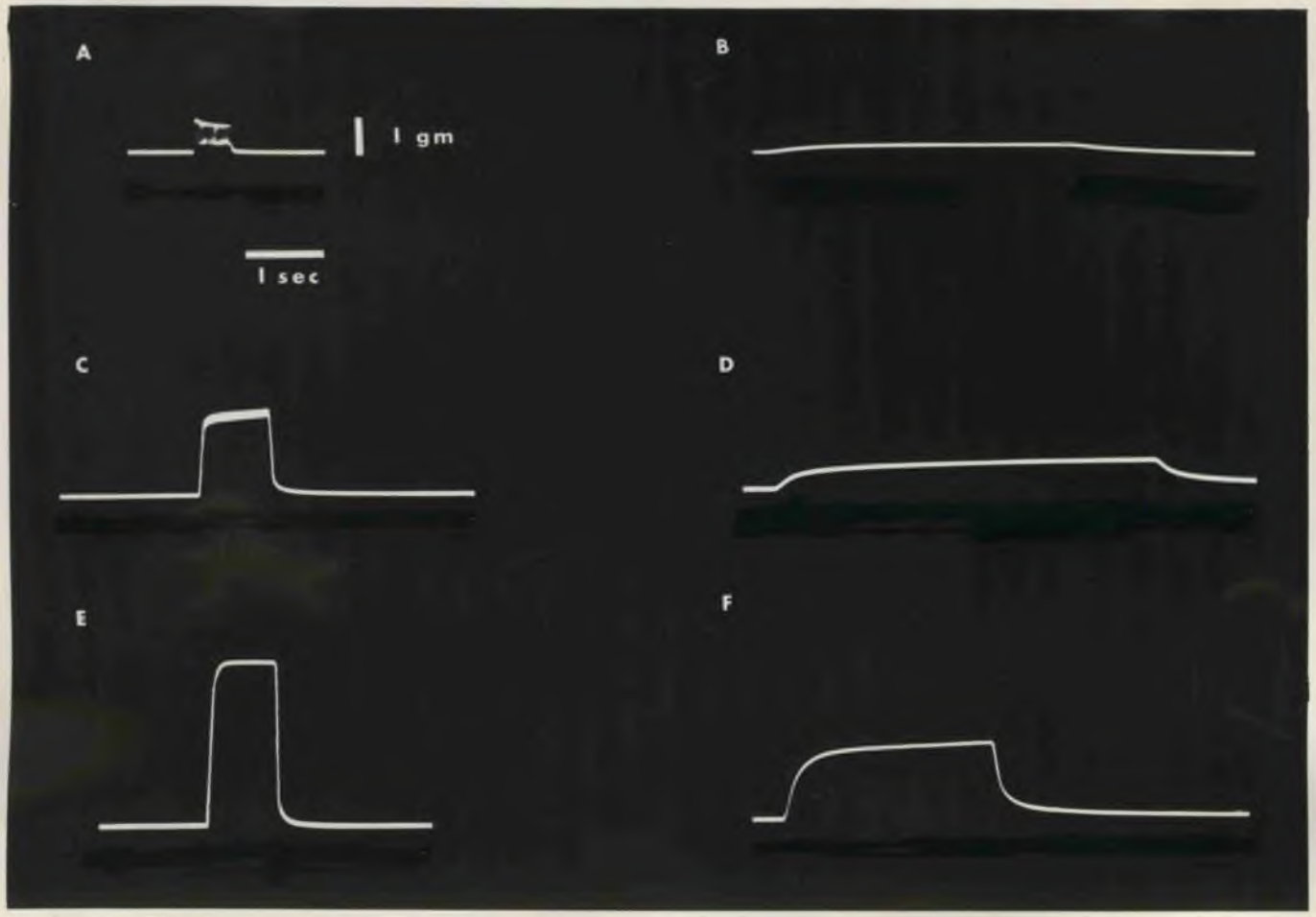


Fig. 36. Effect of stimulation frequency on the mechanical responses of the metathoracic extensor tibiae muscle of B. giganteus to "fast" and "slow" axon stimulation. "Fast" responses illustrated in records (A), (C) and (E). The preparation was stimulated at 30 c/sec; (C-D) 50 c/sec; (E-F) 100 c/sec. Time and tension calibrations same for all traces. All recordings made at minimum body length.

Simultaneous recordings of the electrical events of nerve and muscle have shown that the largest nerve potentials are associated with large electrical responses, i.e. typical "fast" responses, in most of the muscle fibres. Smaller electrical responses consisting of pure post-synaptic potentials are associated with the smaller nerve potentials.

Comparisons with the locust. The electrical and mechanical responses of both flexor and extensor tibiae muscles of the metathoracic segment of the locust have been described (Hoyle, 1955c). The flexor tibiae muscle of Schistocerca is divisible into three or four motor units of the "fast" type. Each unit is innervated by a separate "fast" motor axon. This means that three or four degrees of twitch or tetanic tension may be obtained from this muscle by bringing the units into play, one after the other. In this respect this muscle of the locust is similar to the homologous muscles in Blaberus and Periplaneta. However, unlike the homologous muscles in the cockroach, the extensor tibiae muscle of the locust is not innervated by a "slow" motor axon.

In many insect preparations it is possible to correlate the number of innervating motor axons with the number of electrical and mechanical responses recorded from the muscle

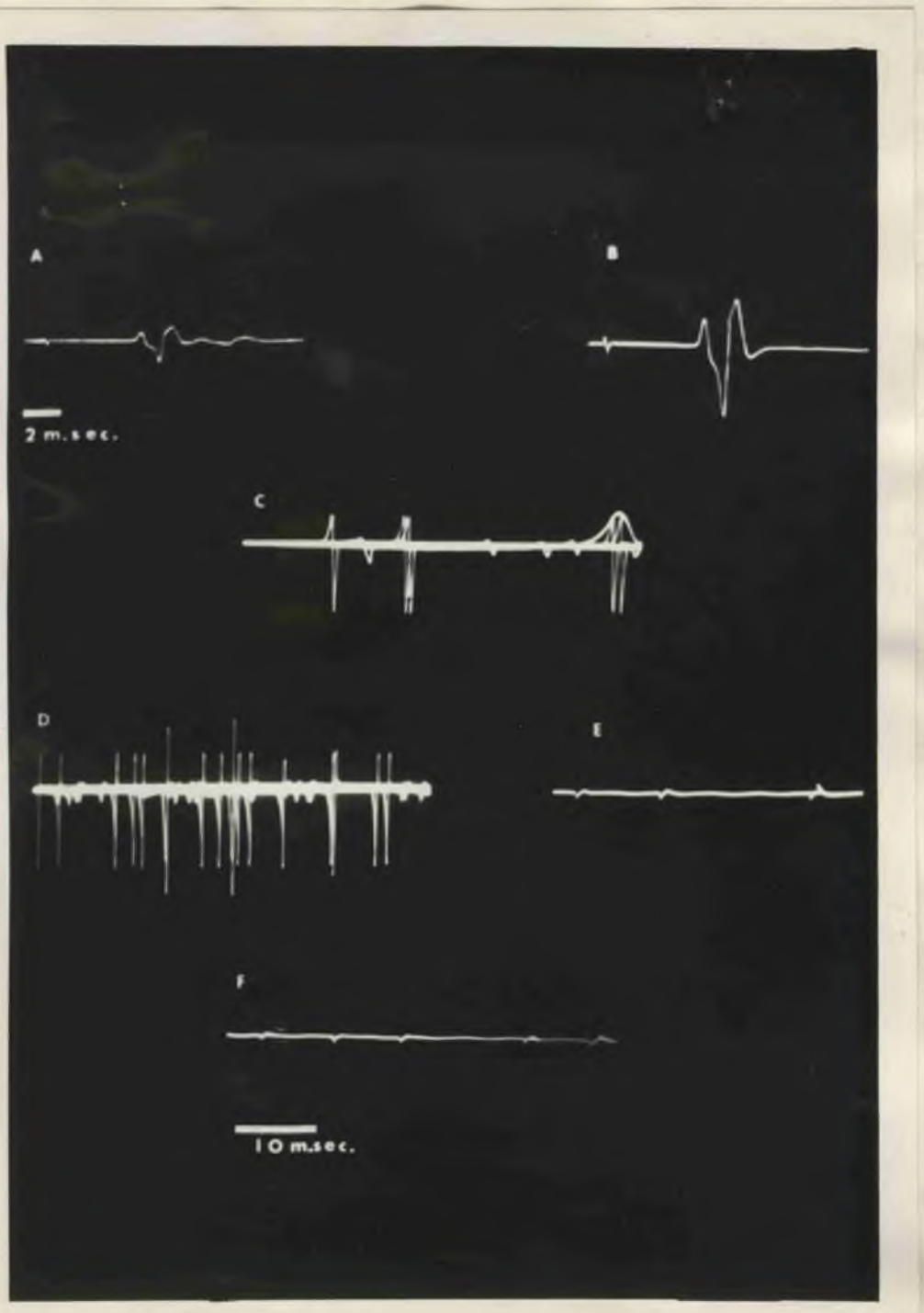


Fig. 37. (A) and (B) Responses recorded from the femoral part of the extensor tibiae nerve of Schistocerca to stimulation of nerve 3b and 5 respectively. (C-F) Successive records obtained extracellularly from the motor nerve innervating the extensor tibiae muscles of the metathoracic leg of Schistocerca. Discharge of the motor axons was evoked by coating the metathoracic ganglion with nicotine. Time calibration for (B) as for (A). Time calibration same for (C-F).

to indirect-electrical and reflex stimulation. A notable exception to this rule was demonstrated by Hoyle (1955c). He recorded four types of electrical response from the supposedly triply-innervated extensor tibiae muscle of the locust. He suggested that one of the motor axons supplied some of the motor fibres with two types of end-plate. In the present experiments histological examination of the motor nerve innervating the extensor muscle of Schistocerca has shown that the greater part of the muscle is innervated by four and not three motor axons. It should be possible therefore to record four different electrical responses from the motor nerve. To test this the metathoracic ganglion was painted with a dilute solution of nicotine for excitation, and impulses travelling down the motor branch at nerve 3b + 5 were recorded in the femur. Four different potentials were recorded using this technique (Fig. 37).

The "fast" electrical responses of both the locust and cockroach muscle consist of large depolarisations which often overshoot the zero potential base line. Larger action potentials were recorded from the muscle fibres of Periplaneta than from the fibres of Blaberus and Schistocerca. It was not possible to determine the duration of these "fast" responses with any exactness because of the vigorous contractions which accompanied the electrical responses. These

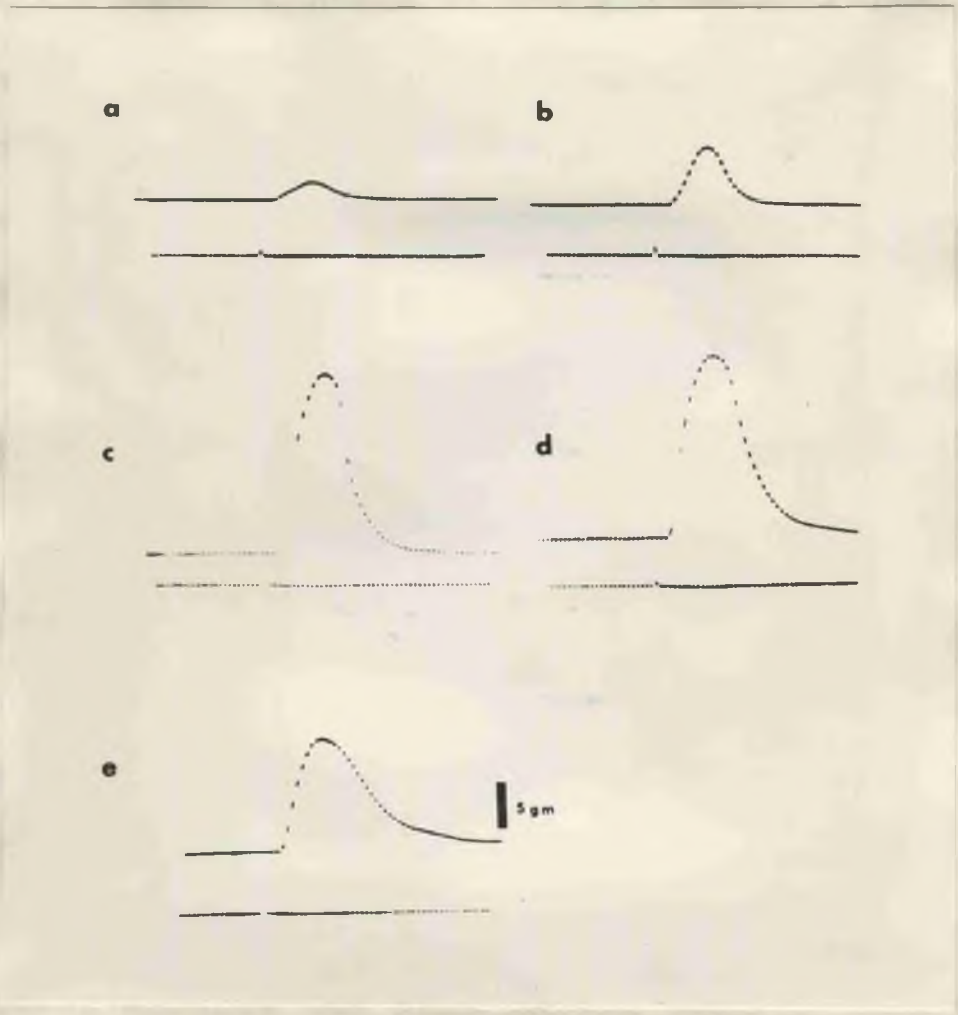


Fig. 38. Effect of muscle length on the magnitude and form of the isometric "fast" twitch response of the metathoracic extensor tibiae muscle of B. giganteus. (a) Muscle at minimum body length (m.b.l.) i.e. approximately 11 mm. (a) m.b.l.; (b) m.b.l. + 0.5 mm; (c) m.b.l. + 1.0 mm; (d) m.b.l. + 1.5 mm; (e) m.b.l. + 2.0 mm. Maximum body length was about 12.0 mm. Note increased peak twitch tension at muscle lengths greater than m.b.l. and up to about maximum body length. Above maximum body length a lower peak tension was recorded. (e) Note also increased duration of twitch response at muscle lengths greater than maximum body length. Tension calibration same for all records. All records modulated at 500 c/sec.

contractions led to the appearance of large mechanical artifacts on the falling phase of the action potentials. It appears, however, that the duration of the "fast" electrical response of the cockroach is about half that of the "fast" electrical response of the locust, i.e. approximately 5 msec compared with approximately 10 msec. These differences had been reported previously by Hoyle (1957) and it is possible that they are causally related to the differences in duration between the "fast" mechanical responses of the extensor tibiae muscles of these animals.

The values for peak twitch tension per unit cross-section of muscle for the femoral muscles of the locust and the cockroach (Table 7) are much lower than those recorded for the coxal muscles of the cockroach (Table 6). However, in many of the coxal muscles the peak twitch tension does not differ greatly from the peak tetanic tension, whereas in the femoral muscles not inconsiderable differences between twitch and tetanic peak tensions were recorded. It is important to note, however, that recordings from the coxal muscles were made with the muscles at their maximum body lengths. It has also been shown that the peak twitch tension developed by the femoral muscles is considerably increased at lengths greater than equilibrium or minimum body length (Fig. 38). Fig. 39 illustrates the effect of stretch on the peak isometric twitch tension of the metathoracic extensor tibiae preparation of

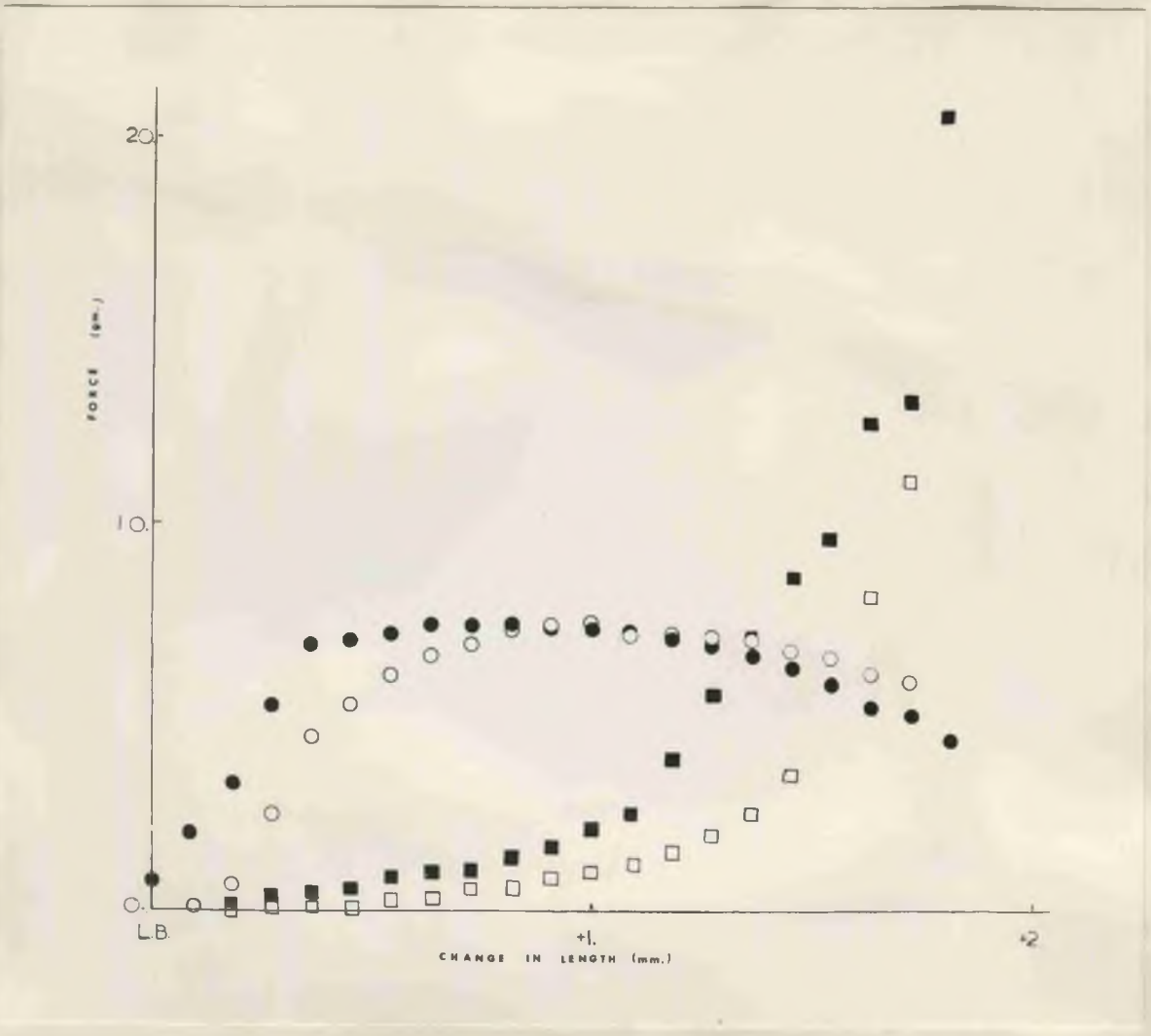


Fig. 39. Tension-length curve for the extensor tibiae muscle of the metathoracic leg of B. giganteus muscle at rest and during activity.

● Total tension, less resting tension, of muscle during twitch activity, reading m.b.l. - m.b.l. + 1.8 mm.

○ Total tension less resting tension of muscle during twitch activity reading m.b.l. + 1.8 mm - m.b.l.

■ Total tension of muscle at rest reading m.b.l. - m.b.l. + 1.7 mm.

□ Total tension of muscle at rest reading m.b.l. + 1.7 mm - m.b.l.

L.B. = m.b.l. (minimum body length.) Note different tension-length curves for decreasing and increasing muscle lengths. The force plotted represents tension recorded at the transducer. To obtain the real force developed by the muscle all values must be multiplied by the cosecant of the angle of insertion (about 35°) of the muscle fibres. See also, fig. 40.

Blaberus. The relation between force and passive stretch (squares) has a steep slope which is nearly constant at lengths above 110% body length. In one preparation (Fig. 39) the passive tension was about 0.75 gm at about 110% of the minimum body length and the active tension was about 7.5 gm. These values are to be compared with 0 gm for the passive tension and about 1 gm for the active tension of the muscle at minimum body length. A small increase in length resulted in a large increase in active tension, i.e. the peak twitch tension. Further increases in length did not lead to any further increases in active tension. These results are significant for an increase of muscle length of about 10% led to an increase of peak twitch tension from about 150 gm cm^{-2} to about 760 gm cm^{-2}. Furthermore, the length of the extensor muscle at 110% m.b.l. approximates closely to the maximum length that it is able to attain in the femur. Unfortunately it has not been possible to record accurately the change of peak, tetanic tension with changes of muscle length, partly because the muscle fatigued very quickly even at relatively low frequencies of stimulation and partly because the stimulation threshold of the "fast" axon was usually higher than the stimulation threshold of the "slow" axon, i.e. it was not possible to stimulate the "fast" axon without stimulating the "slow" axon as well. This means that the peak tension of about 1630 gm cm^{-2}

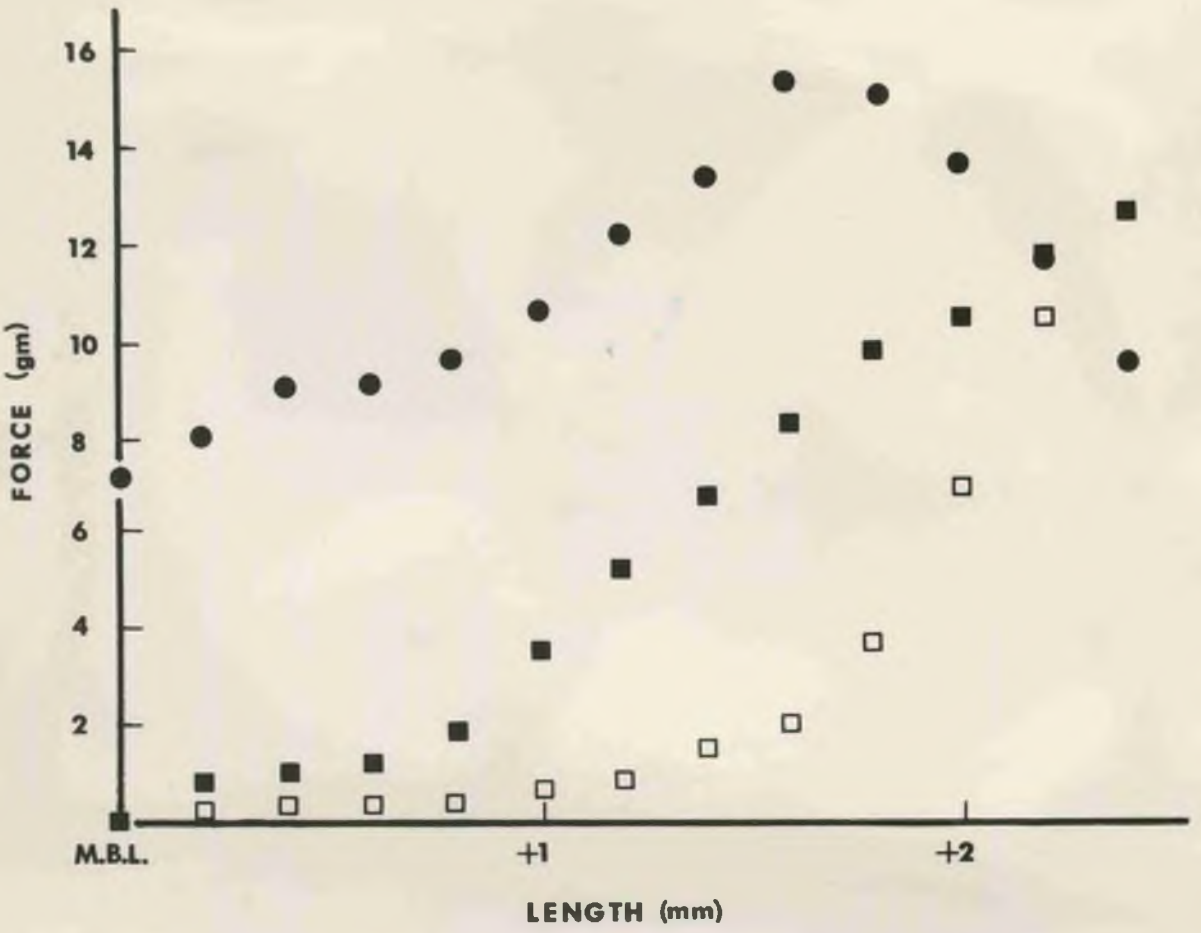


Fig. 40. Tension-length diagram for the extensor tibiae preparation of the metathoracic leg of B. giganteus.

● Active force to a stimulation frequency of 100 c/sec.

■ Passive force reading m.b.l. - m.b.l. + 2.4 mm.

□ Passive force reading m.b.l. + 2.4 mm - m.b.l.

M.B.L. = minimum body length (about 11 mm). Maximum body length was about 12 mm.

recorded from the extensor tibiae muscle of Blaberus to maximal stimulation at 100 c/sec (Fig. 40) was developed by the combined activity of the "fast" and "slow" systems. At low frequencies of stimulation the magnitude of the "slow" component was negligible and it could be safely assumed that the peak tension recorded at these frequencies approximated closely to the peak tension that would have appeared if the "fast" axon had been stimulated alone.

With the extensor tibiae preparation of the locust it is possible to record the effect of high-frequency "fast" axon stimulation to the complete exclusion of interference from the "slow" response because the "fast" and the "slow" axons innervating this muscle are located in different nerve trunks. Fig. 41 illustrates the average peak twitch tension and passive tension of eight different preparations of this muscle. At minimum body length the peak twitch tension averaged 54 gm cm^{-2}. With a twitch/tetanus force ratio of 1/10 (Hoyle, 1957) the estimated peak tetanic tension is about 520 gm cm^{-2}. At 107% m.b.l. the peak twitch tension recorded averaged 213 gm cm^{-2}, whereas at 110% m.b.l. an average value of 326 gm cm^{-2} was recorded for the peak twitch tension. In Fig. 42 the average peak tensions, for eight different muscles, developed at a stimulation frequency of 20 c/sec are plotted against muscle length. At 113% body

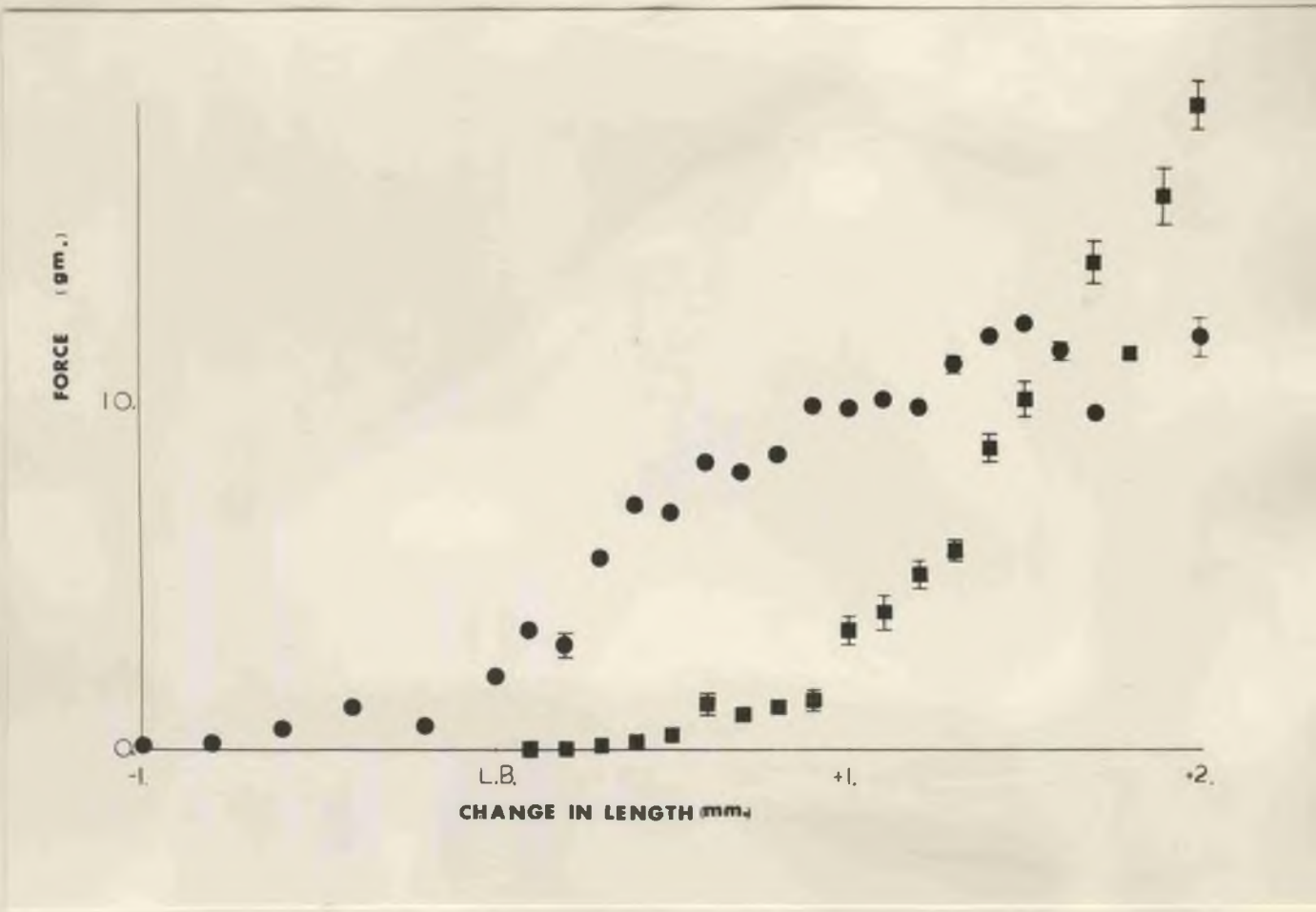


Fig. 41. Average tension-length curves from the extensor tibiae muscle of Schistocerca, at rest and during "fast" twitch activity. Graphs show the averaged results from eight different muscle preparations. L.B. = minimum body length (about 16 mm). Maximum body length is about m.b.l. + 1.5 mm.

● Peak twitch tension ± S.e. of mean x 0.2 for muscle lengths m.b.l. - 1 mm to m.b.l. + 2 mm.

■ Resting tension ± S.e. of mean x 0.2 for muscle lengths m.b.l. - 1 mm to m.b.l. + 2 mm.

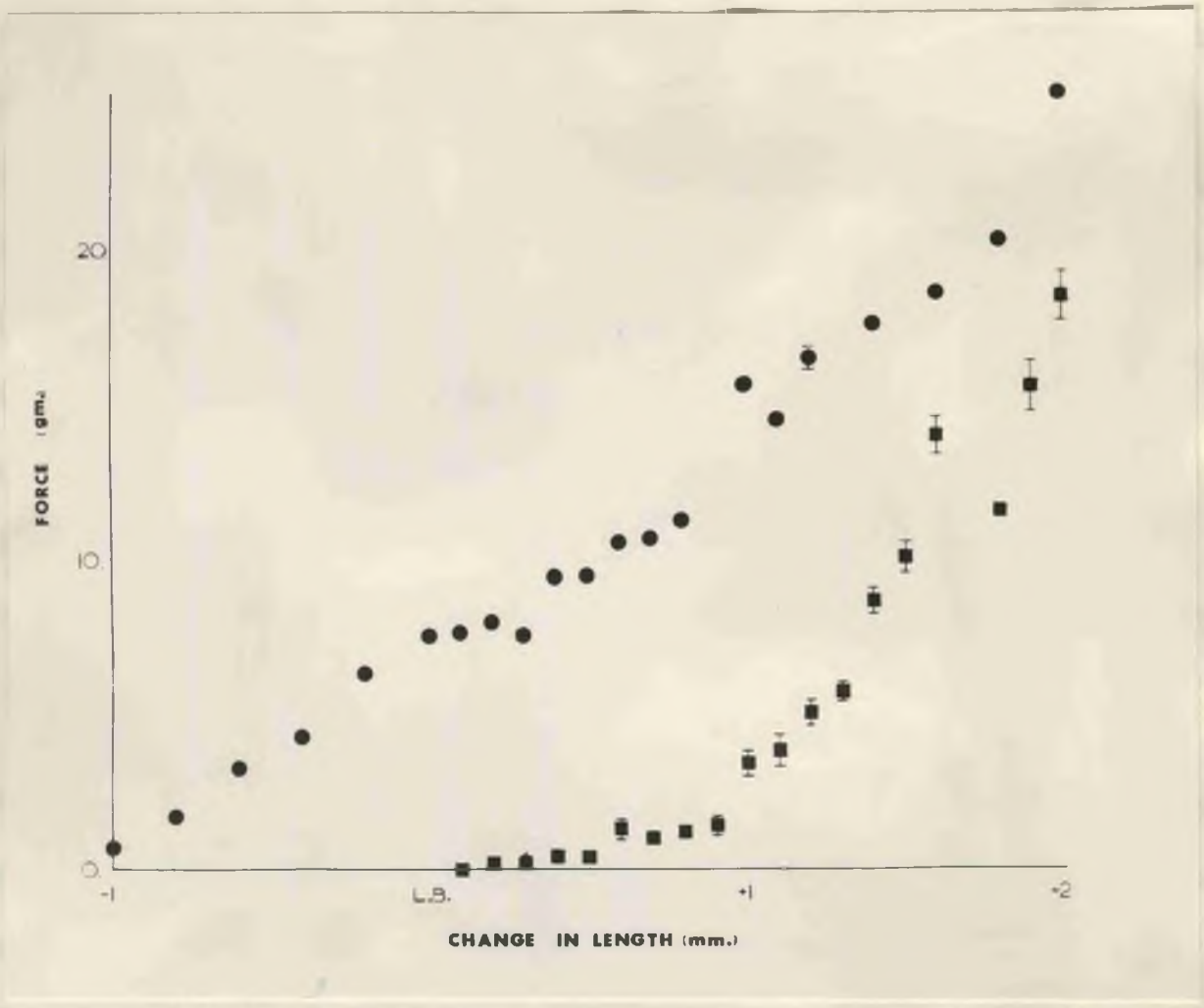


Fig. 42. Average tension-length curves for eight different extensor tibiae preparations of S. gregaria. ■ Tension ± S.e. of mean x 0.2 of muscle at rest. ● Tension ± S.e. of mean x 0.2 of active muscle following stimulation of the "fast" motor axon at a frequency of 20 c/s. L.B. = minimum body length (about 16 mm).

length the total peak tension recorded was well over 20 gm. This compared with a peak tension of under 10 gm recorded at minimum body length. From these results it may be assumed that the total maximum force that the muscle can develop to "fast" axon stimulation is dependent on the length of the muscle at the time of recording. At lengths slightly greater than minimum body length, and which roughly correspond with the maximum body length, the extensor tibiae muscle of the locust gives an active tension per unit muscle cross-section which does not differ considerably from that developed by other insect muscles studied (Weis-Fogh, 1956; Buchthal, Weis-Fogh and Rosenfalck, 1957).

Hoyle (1955c) reported that the rate of relaxation of the locust extensor tibiae muscle was the same following both "fast" and "slow" mechanical activity. In the present experiments on the cockroach it has been found that the rate of relaxation of the femoral muscles following a "fast" mechanical response is usually faster than the rate of relaxation following a "slow" response. A re-examination of the relaxation rates of the "slow" and "fast" responses of the locust extensor tibiae muscle gave conflicting results. In some preparations the total duration of the relaxation phase of the "fast" response was less than that of the "slow". In other preparations, however, the rates of relaxation were almost the same.

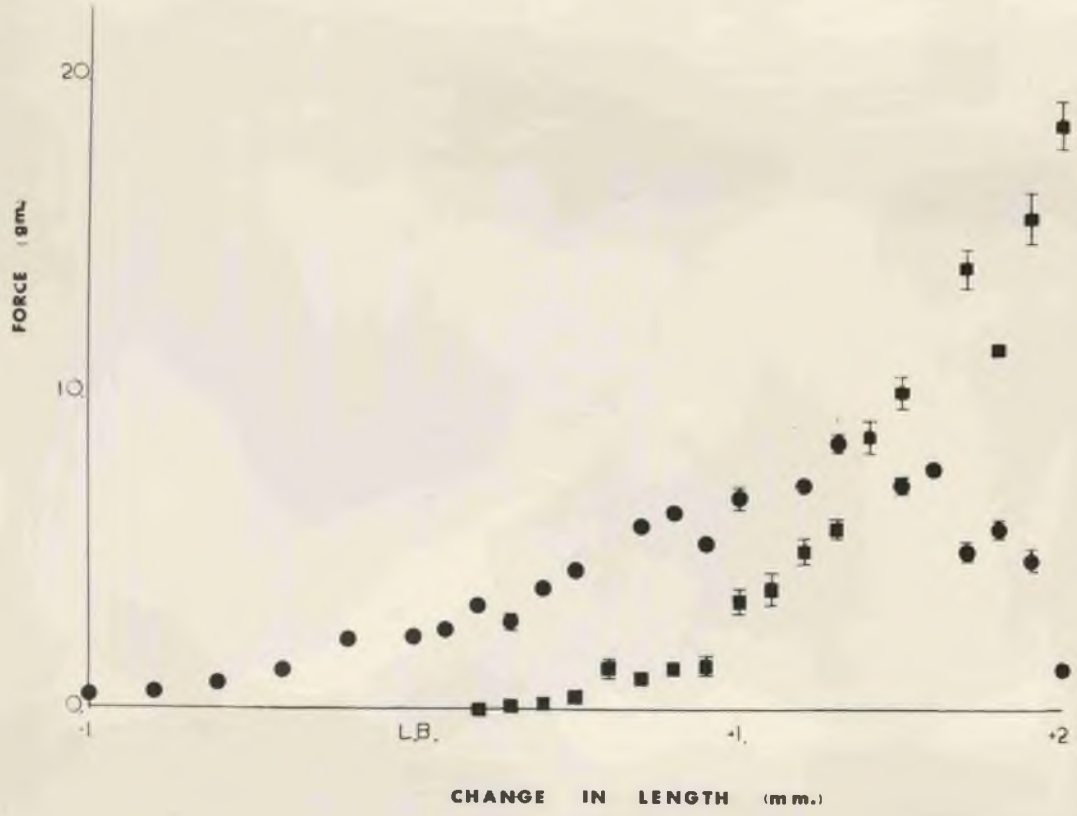


Fig. 43. Average tension-length curves for eight different extensor tibiae muscles of the metathoracic leg of S. gregaria during activity following stimulation of the "slow" axon at a frequency of about 150 c/sec, and at rest. L.B. = minimum body length (about 16 mm). ● Total active tension ± S.e. of mean x 0.2 during "slow" axon stimulation at muscle lengths m.b.l. - 1 mm to m.b.l. + 2 mm. ■ Total rest-tension ± S.e. of mean x 0.2 for muscle lengths m.b.l. - 1 mm to m.b.l. + 2 mm. Maximum body length of muscle was about 17.5 mm.

Values shown for tension were those recorded directly at the transducer. To obtain true tension developed by muscle all values must be multiplied by the cosecant of the angle of insertion (about 35°). See also figs 41-42.

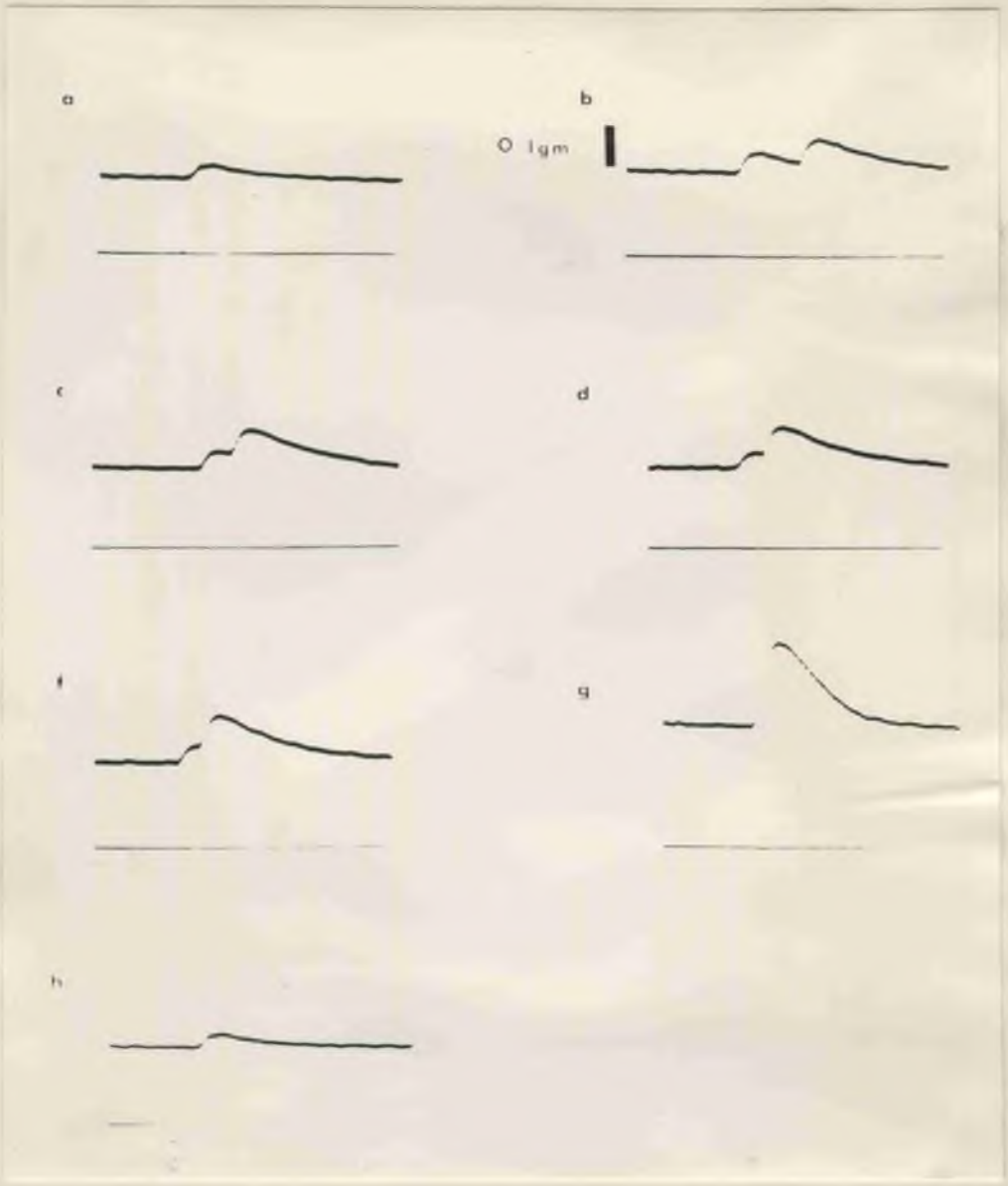


Fig. 44. "Slow" twitch contractions recorded from the extensor tibiae preparation of the locust with the muscle stretched 0.5 mm beyond its minimum body length. The "slow" axon innervating this muscle was stimulated with a pair of shocks whilst gradually decreasing the ~~distance~~ interval between the shocks. The "slow" twitches first summated and then with the shocks very close together the muscle became refractory to the second response. Duration of each trace was 1.5 sec. Tension calibration same for each record.

Preparation	Muscle	Innervation (histological data)	Innervation (Electrical data)	Mechanical responses to graded stimulation	Peak tension (gm) developed following a single maximal stimulus (recorded at transducer)	Rise time of twitch (msec) ± S.e. of mean	Decay time of twitch (msec) ± S.e. of mean	Half decay time of twitch (msec) ± S.e. of mean	Estimated cross sectional area of muscle (sq. cm.)	Approximate isometric twitch tension ($gm.cm.^{-2}$) at minimum body length	Approximate isometric twitch tension ($gm.cm.^{-2}$) at maximum body length
Schistocerca	Metathoracic extensor tibiae	1 large axon 1 medium axon 2 small axons	1 "fast" 3 "slow"	1 "fast" 2 "slow"	1 - 2	56 ± 1.52(9)	119 ± 4.2 (9)	61 ± 2.4 (9)	4.5×10^{-2}	54	330
Schistocerca	Metathoracic flexor tibiae	-	4 "fast"	4 "fast"	-	-	-	-	-	-	-
Blaberus	Metathoracic extensor tibiae	1 large axon 1 medium axon 2 small axons 2 + very small axons	1 "fast" 1 + "slow"	1 "fast" 1 + "slow"	0.5 - 1.5	15.3 + 0.3(9)	33 + 7.2(9)	15.1 ± 1.3(9)	1.2×10^{-2}	152	760
Blaberus	Metathoracic flexor tibiae	-	5 "fast" 1 + "slow"	5 "fast" 1 + "slow"	0.5 - 3 gm	13.8 + 1.9(5)	51.7 ± 17.6(5)	21.3 ± 1.8(5)	-	-	-
Periplaneta	Metathoracic extensor tibiae	2 large axons 2 small axons	1 "fast" 2 "slow"	1 "fast" 1 "slow"	-	-	-	-	-	-	-
Periplaneta	Metathoracic flexor tibiae	-	5 "fast" 1 "slow"	5 "fast" 1 "slow"	-	-	-	-	-	-	-

Table 7. Summary of electrical and mechanical recordings obtained from the metathoracic femoral muscles of P. americana, B. giganteus and S. gregaria. In columns 7, 8 and 9 figures in brackets represent number of measurements made.

At muscle lengths greater than the minimum body length the "slow" mechanical response of the locust extensor muscle is increased (Fig. 43) and the response to a single shock is recordable as a minute twitch contraction. These "slow" twitches summate at higher frequencies of stimulation to give a maintained tonic response (Fig. 44). Small twitch contractions to "slow" axon stimulation have been seen also in the femoral muscles of Blaberus and Periplaneta.

(3) The thoracic muscles of the locust

Intracellular recordings were made from the muscle fibres of muscles 60, 81, 87, 88, 92, 96 and 99 of the locusts S. gregaria and L. migratoria during electrical and reflex stimulation. The mechanical responses of these muscles to graded electrical stimulation of the motor nerves and to reflex stimulation have been recorded visually with the aid of a binocular microscope.

Preparation of the majority of the thoracic muscles of the locust is extremely difficult especially if it is required to stimulate the muscle indirectly through the innervating motor nerve. For this reason only a few of the thoracic muscles were examined during the course of this investigation. Nevertheless the muscles that were examined included a good selection of Ewer's (1957) twitch, "tonic" and "mixed" types.

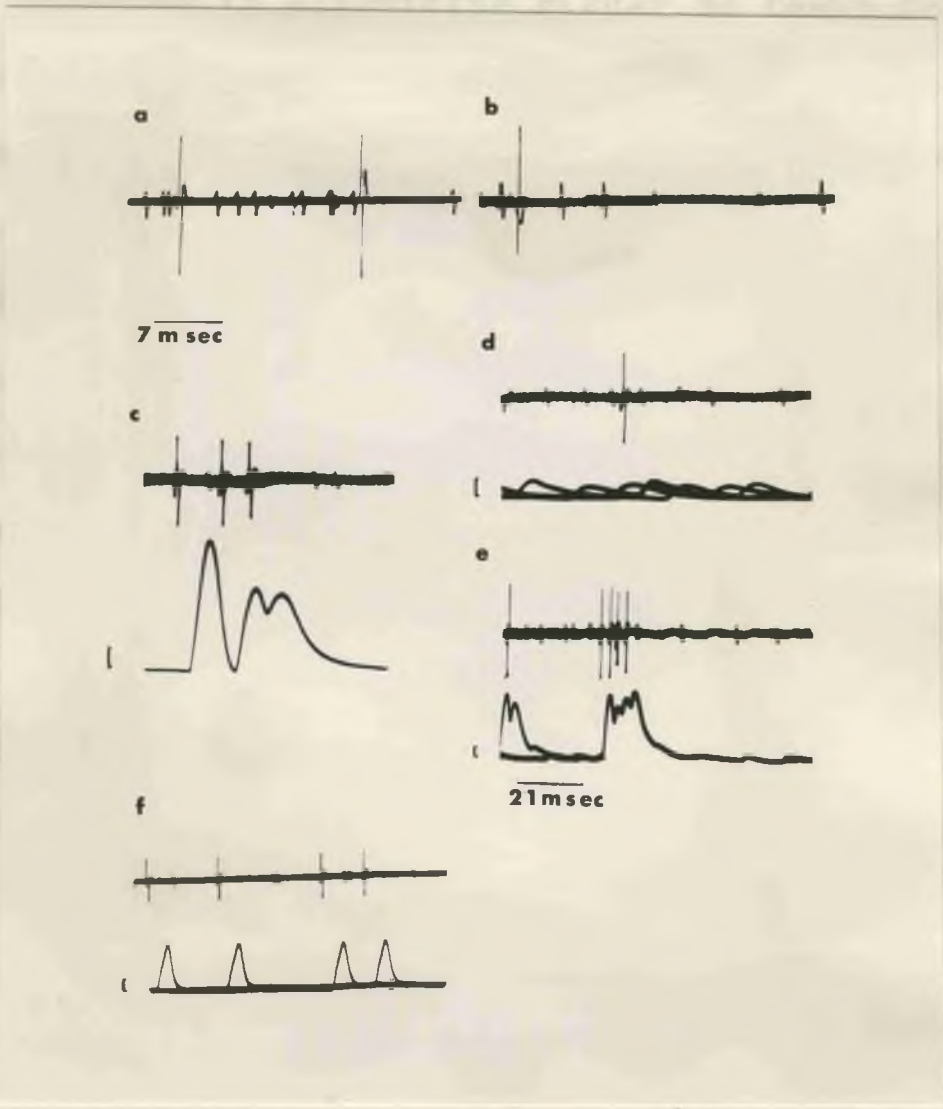


Fig. 45. Electrical responses to reflex stimulation of the nerve-muscle preparation 92. (a-b) Extracellular potentials recorded from the motor nerve $3c_2$. (c-d) Simultaneous records of extracellular nerve potentials and the intracellular muscle potentials. Record (c) shows a fibre which responded to "fast" axon stimulation only. Fibre in (d) responded to "slow" axon stimulation only. The fibre in (e) responded to discharge of both "slow" and "fast" motor axons. Time calibration same for (a-d). Vertical calibrations equal to 10 mV. Upper trace of records (c-e) represent zero membrane potential.

The animals were anaesthetized with carbon dioxide and partially embedded in plasticine either on their dorsal or ventral surfaces or on their side, according to the muscle under examination. The legs were firmly fixed down with plasticine but the abdomen was left freely moveable to facilitate respiration. The muscle under examination was exposed by dissecting away the overlying cuticle. In many cases it was necessary also to dissect away many of the adjacent muscles. The exposed muscle was surrounded by a wall of plasticine and immersed in locust saline (Hoyle, 1953c). Wherever possible the motor nerve innervating the preparation was exposed and under it was placed either a pair of stimulating electrodes or a pair of recording electrodes. For many recordings the nerve was left in contact with the thoracic ganglion in order to obtain responses to reflex stimulation.

Three types of electrical response have been recorded intracellularly from the muscle fibres of the thoracic muscles of the locust. Every muscle and nearly every muscle fibre gave responses to maximal electrical stimulation which were typical of responses evoked in other muscles (e.g. the extensor and flexor tibiae muscles of the locust) by "fast" axon stimulation. These responses, which were similar apart from small variations no matter from which muscle or

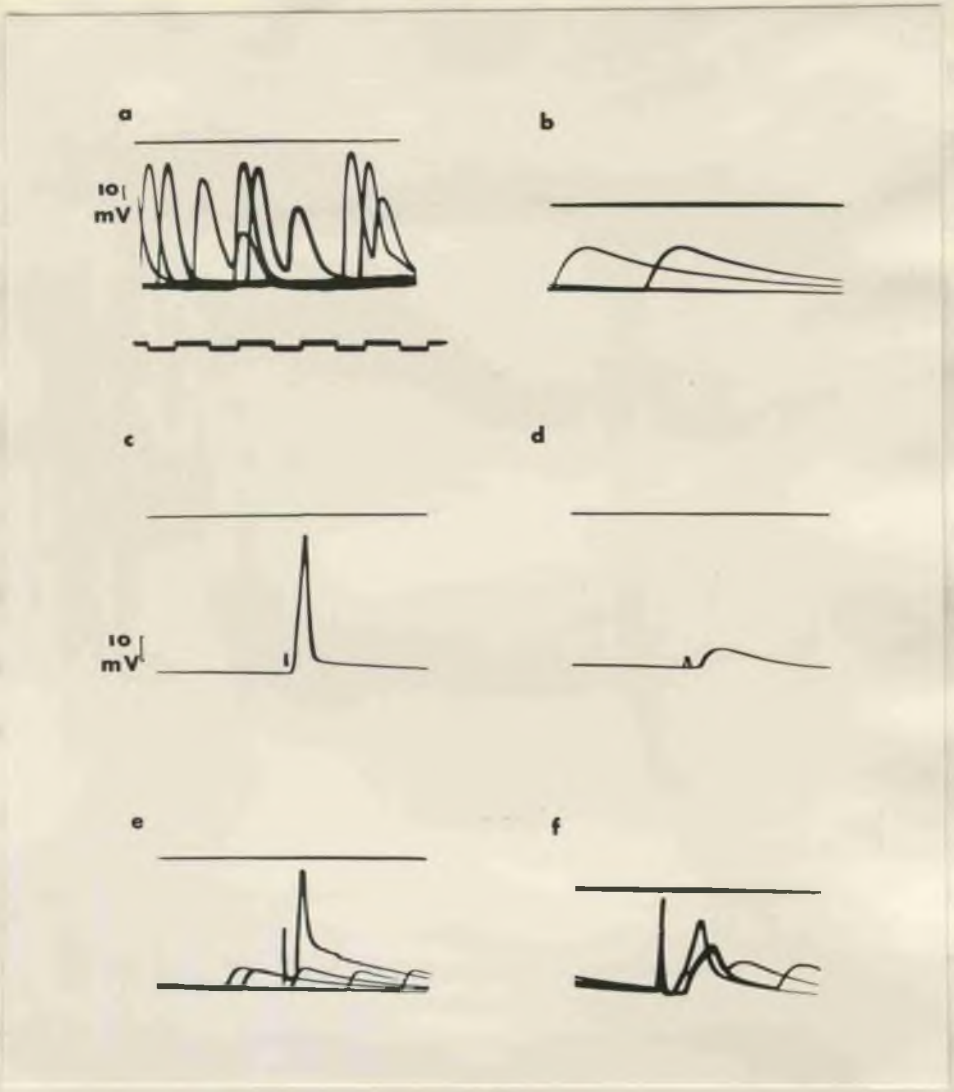


Fig. 46. Intracellularly-recorded electrical responses of muscle 92. (a) Responses to "fast" axon stimulation evoked reflexly by touching the anal cerci. (b) Responses of a surface fibre to reflex stimulation. This fibre did not respond to "fast" axon stimulation. (c-d) Responses of two different muscle fibres to indirect maximal electrical stimulation. The fibre in (c) gave a "fast" response. The fibre in (d) was apparently not innervated by the "fast" axon and gave only the "slow" response. (e) Fibre innervated by both "fast" and "slow" motor axons. (f) "Fast" and "slow" electrical responses from a single fibre of muscle 92 evoked by gradually increasing the intensity of stimulation of the motor nerve. The maximal response consisted of both "fast" and "slow" potentials. At lower stimulation intensities only the "slow" axon was stimulated. In this record the "slow" post-synaptic potential, evoked by electrical stimulation, summated with a "slow" response, evoked reflexly, to give a small, local spike response. The time calibration of 22 msec was the same for every record. Voltage calibration same for (b-f).

part of a muscle fibre they were recorded, were orthodox insect action potentials of the "fast" kind (Figs 45 and 49) consisting of large post-synaptic potentials and active membrane responses which sometimes overshot the zero membrane potential (Hoyle, 1955c). The action potentials ranged in magnitude between 45 and 75 mV. Whenever the responses were closely placed, e.g. during reflex stimulation, refractoriness appeared and the second and subsequent responses were rather small (e.g. Fig. 46a). During reflex activity the "fast" responses occurred in short high-frequency bursts and were always associated with vigorous mechanical responses of the muscles. High-frequency electrical stimulation of the "fast" axon evoked a quickly fatiguing "fast" tetanic response. There was no indication that any of the thoracic muscles examined were innervated by more than one "fast" axon.

In addition to the "fast" axon some muscles receive one or more "slow" axons which, when stimulated, evoke typical "slow" electrical responses in the muscle fibres they innervate. Two types of "slow" electrical responses, not unlike the S_{1a} and S_{1b} potentials recorded from the locust extensor tibiae preparation (Hoyle, 1955c), have been recorded from some of the thoracic muscles, i.e. muscles 92, 87, 96 and 95. In some of the muscles, e.g. muscle 92,

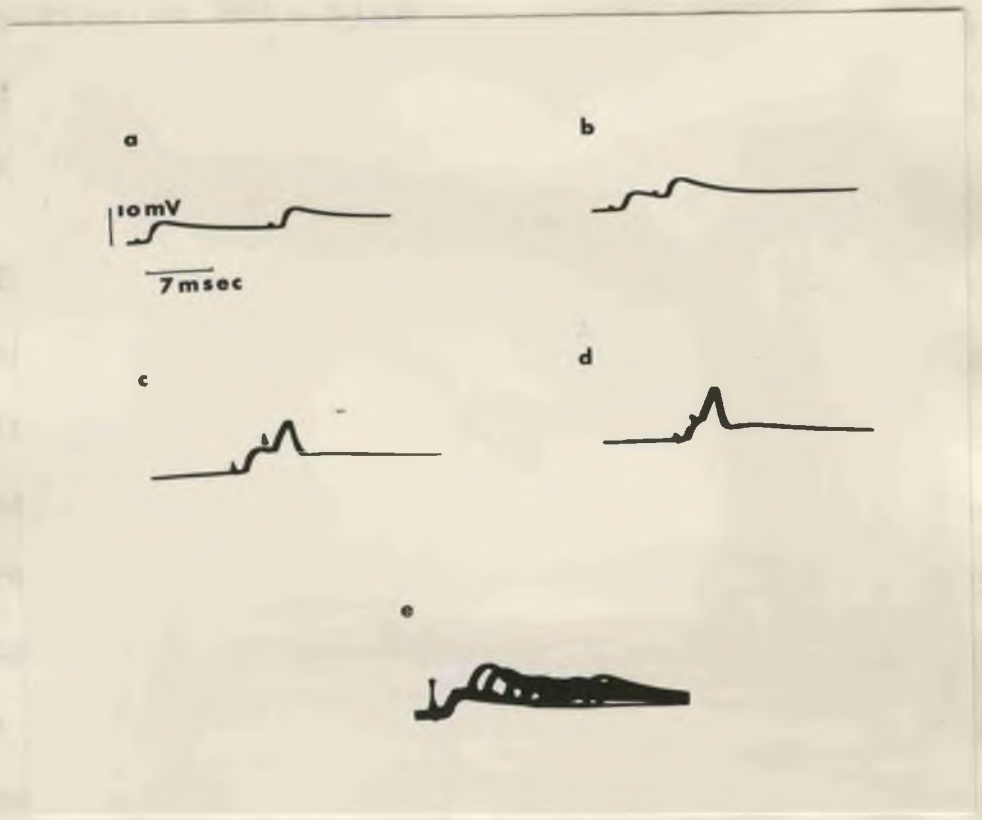


Fig. 47. (a-d) Effect of indirect electrical stimulation with a pair of shocks through the "slow" axon, whilst gradually decreasing the interval between the shocks, upon the intracellularly-recorded electrical responses of a fibre of muscle 92. (e) As for (a-d) but records superimposed on the same frame. Note summation of the "slow" post-synaptic responses to give a small spike response (d).

only a minor proportion (about 30%) of the fibres showed any signs of "slow" activity and only a fraction of the fibres showed both types of "slow" responses. In muscle 87, however, all the fibres were apparently innervated by two "slow" axons as well as a single "fast" axon. Triple innervation of this kind has not been recorded before in insect skeletal muscles although it is quite common in crustacean skeletal muscles (Furshpan, 1955).

The S_{1b}-type potentials were large post-synaptic responses, ranging between 5-35 mV in magnitude and 15-30 msec in duration, sometimes with a small secondary spike response. The magnitude of the secondary response fluctuated from response to response due possibly to variations in the magnitude of the post-synaptic potential. Stimulation of the "slow" axon evoking the S_{1b} potentials with a pair of shocks whilst gradually decreasing the interval between the shocks resulted in summation of the muscle potentials, if they were initially small (Fig. 47).

The smaller potentials also showed facilitation with repetitive stimulation. The larger responses were refractory.

Mechanical responses associated with the S_{1b}-type potentials were less vigorous than those associated with the "fast" electrical responses. Low-frequency stimulation of

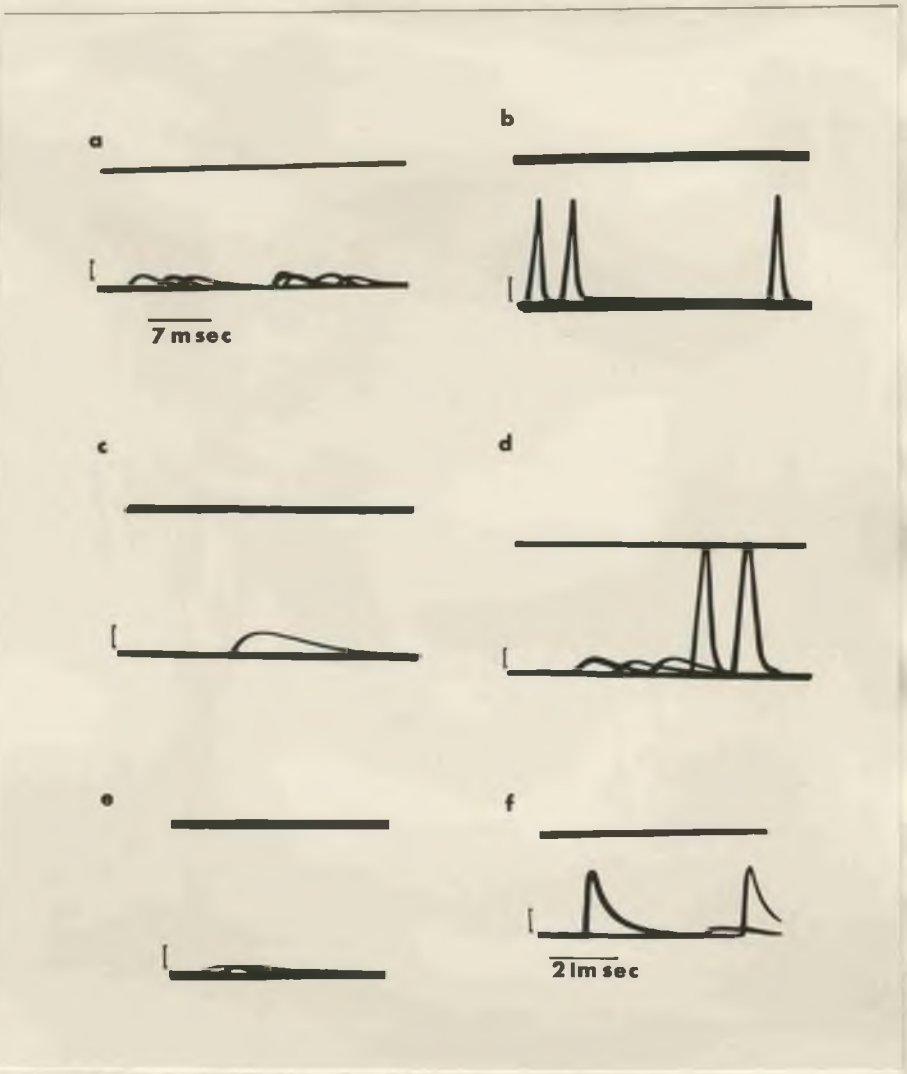


Fig. 48. Intracellular potentials recorded from fibres of some thoracic muscles of Schistocerca following reflex stimulation. (a) Large "slow" post-synaptic potentials from a fibre of muscle 96. (b) "Fast" electrical response from a fibre of muscle 95. (c) A typical large "slow" post-synaptic potential from a fibre of muscle 94. (d) A dually-innervated fibre of muscle 88 showing both "fast" and "slow" potentials. (e) "Slow" post-synaptic responses from a fibre, of muscle 96, known to be capable of "fast" electrical activity. (f) A triply-innervated fibre of muscle 95 which responded to stimulation by two different "slow" motor axons and which also responded to "fast" axon stimulation. (Fast response not shown in this record.) The time base speed in (b-e) was the same as for (a). Vertical calibrations equivalent to 10 mV. Upper trace of each record represents zero membrane potential.

the "slow" axon often led to minute twitch contractions of the innervated muscles. Usually, however, little mechanical activity was recorded below a stimulation frequency of 10 c/sec. High-frequency stimulation led to a smooth tonic contraction which fatigued only slightly under sustained stimulation.

The mechanical response associated with the smaller "slow" electrical response remains obscure. It has not been possible to separate the S_{1a} and S_{1b} responses although there is good evidence to suppose that they are evoked by stimulation of two different motor axons. For example, muscle 92 is innervated by three motor axons (Fig. 9) and both nerve and muscle give three types of electrical response to reflex stimulation (Fig. 45) and electrical stimulation. Hoyle (1955c) suggested that the S_{1a} and S_{1b} responses of the locust extensor tibiae muscle result from stimulation of a single motor axon and that this axon supplies the muscle with two types of motor ending. However, evidence has been forwarded in this thesis to show that the extensor muscle of the locust is innervated by four and not three motor axons. It appears therefore that here, as in the thoracic muscles, the S_{1a} and S_{1b} responses result from the independent activity of two different motor axons.

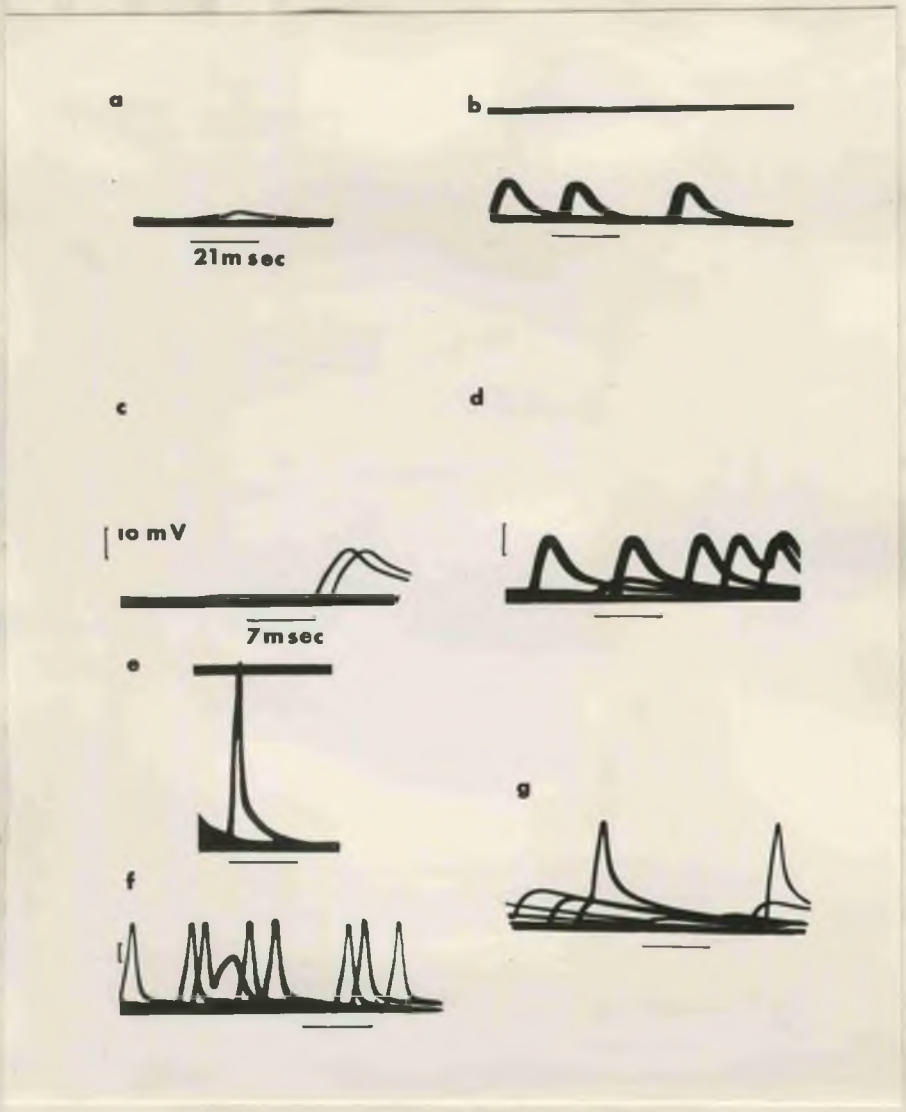


Fig. 49. Electrical responses of muscle fibres of muscle 88. (a) Responses showing summation to discharge of a "slow" axon to reflex stimulation. (b) Large post-synaptic responses probably to reflex stimulation of the larger "slow" axon. Traces (a-b) obtained from the same muscle fibre. (c-d) Large and small "slow" post-synaptic responses obtained from a single muscle fibre following reflex stimulation through the motor nerve. Responses in (d) recorded on a slower time base. (e-f) Examples of two fibres responding to "fast" axon stimulation only. Note in (g) fibre showing three types of response.

Time calibration 21 msec for (a-d) and (e-f). Time calibration of 7 msec for (c) and (g). Vertical calibrations equal to 10 mV are the same for (a-g).

Table 8.

Muscle No.	Electrical responses			Mechanical responses to graded stimulation	Ewer's results for *Locusta*	Innervation (electrical data)	Innervation (histological data)
	"Fast"	Large p.s.p.	Small p.s.p.				
92	p	p	p	Twitch Tonus	Twitch	1 "fast" 2 "slow"	1 large axon 1 medium axon 1 small axon
87	p	p	p	Twitch Tonus	Tonus	1 "fast" 1 "slow"	1 large axon 1 medium axon 1 small axon
96	p	p	p	Twitch Tonus	Twitch	1 "fast" 2 "slow"	-
95	p	p	p	Twitch Tonus	Twitch	1 "fast" 2 "slow"	-
94	p	p	p	Twitch Tonus	Tonus	1 "fast" 1 "slow"	-
88	p	p	p	Twitch Tonus	Tonus	1 "fast" 1 "slow"	-
81	p	-	-	Twitch	Twitch	1 "fast"	-
60	p	p	-	Twitch	Tonus	1 "fast" 1 "slow"	-
99	p	-	-	Twitch	Twitch	1 "fast"	-

Summary of electrical recordings made from the thoracic muscles of the locusts *Locusta migratoria* and *Schistocerca gregaria*. (p, denotes response was recorded.)

In the thoracic muscle the time course of the S_{1a}-type potentials (20-50 msec) which are small facilitating post-synaptic responses never exceeding 4 mV in magnitude was slightly longer than the time course of the S_{1b}-type responses.

A summary of the main results of these investigations is given in Table 8.

(4) The action of ryanodine on the "slow" and "fast" responses of locust and cockroach skeletal muscle

The alkaloid ryanodine, which appears to be responsible for the insecticidal action of certain dusts obtained from the tropical plant, Ryania speciosa (Rogers, Konivszy, Shavel and Folkers, 1948), is said to be an ester of alpha pyrrole carboxylic acid and ryanodole, an alcohol with an unknown terpenoid structure (Kelly, Wickingham and Wiesner, 1951). The empirical formula of the drug is given as $C_{25}H_{35}O_9N$ (Kelly et al., 1951).

According to Edwards, Weiant, Slocombe and Roeder (1948) ryanodine causes a reversible flaccid paralysis of the muscle of the cockroach, Periplaneta americana, but has little if any effect on excitation and conduction in nerves, ganglia and muscle. They suggested that ryanodine affects the energy-phosphate system of insect striated muscle causing a loss of contractile ability.

Some data have been obtained on ryanodine poisoning of vertebrate skeletal muscle. Pick and Tullius (1951) found that the drug caused a gradually increasing contracture, followed by an irreversible rigor, in the isolated abdominal muscle of the frog. They postulated that depolymerisation of the muscle proteins had taken place. Ryanodine causes an irreversible rigor of mammalian skeletal muscle also (Procita, 1954, 1956) and produces contractile failure of cardiac muscle (Procita, Shideman and Rathburn, 1952; Hillyard and Procita, 1956; Furchgott and de Gubareff, 1956). Blum, Creese, Jenden and Scholes (1957) observed that the contracture of the frog sartorius muscle, resulting from ryanodine poisoning, occurred without the accompaniment of membrane depolarisation and was preceded by partial inhibition of the twitch relaxation mechanism.

Roeder, Edwards, Weiant, Slocombe and Tabellario (1948) reported granulation and internal disintegration of the ryanodinised muscle fibres of the frog, but were unable to demonstrate similar structural changes in the ryanodinised flexor tibiae muscles of the cockroach. A further account of morphological lesions following ryanodine poisoning was given by Bodenstein (1950); in high concentrations the drug caused necrosis and complete degeneration of the muscle tissue of amphibian embryos.

A significant discovery in relation to the differences between "slow" and "fast" activity in insect skeletal muscle was made by Becht (1959) who found that the "fast" mechanical response of the cockroach coxal muscles was inhibited by the alkaloid before inhibition of the "slow" response was complete. However, Becht only recorded the mechanical responses of the coxal muscles to maximal stimulation and it is therefore possible that in his "mixed" muscles the mechanical responses to "fast" axon stimulation during the later stages of poisoning were masked by the responses to the "slow" axon. Furthermore, Becht has stated that some of his "fast" muscles gave mechanical responses to maximal stimulation even after considerable periods in the drug and that these responses were not unlike those exhibited normally by his "slow" muscles. It is possible that during the later stages of poisoning the mechanical response to "fast" axon stimulation is similar to the mechanical response to "slow" axon stimulation. The possibility that the different effects of ryanodine on the "fast" and "slow" mechanical responses of the cockroach muscle are due to different effects of the drug on the "slow" and "fast" electrical responses was not examined by Becht.

The fact that the motor nerves innervating the coxal muscles are very short in length and that the "fast" and

"slow" motor axons innervating the "mixed" muscles are contained in the same bundle unfortunately precluded any examination of the action of ryanodine on these muscles that would have been of value, for it is impossible under these conditions to separate the "fast" and "slow" systems. A few experiments were made on the coxal muscles of P. americana that are known to be innervated by one motor axon only. The results of these experiments did not of course provide a clear-cut answer to the mode of action of ryanodine on the "fast" and "slow" responses of the coxal muscles but they did show that the mechanical response of Becht's "fast" muscles 136 and 137, after treatment with ryanodine, is not unlike the mechanical response normally performed by his "slow" muscle.

The difficulties experienced in separating the "fast" and "slow" responses of cockroach muscle do not apply to the locust extensor tibiae preparation. This muscle proved excellent from the point of view of examining drug action on the "fast" and "slow" electrical and mechanical responses since the motor axons through which these responses may be elicited are situated in different nerve trunks and may be stimulated separately (Hoyle, 1955b). The electrical and mechanical responses of this preparation for a variety of drug concentrations have been examined, first separately

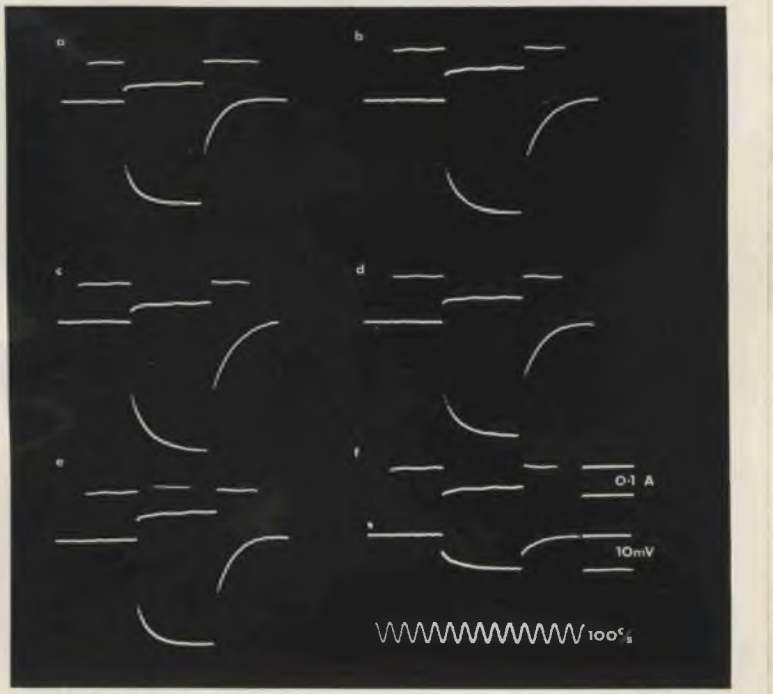


Fig. 50. Electrotonic potentials (b–f, lower traces) to hyperpolarising pulses (monitored as upper traces) after (b) 10 min; (c) 20 min; (d) 25 min; (e) 30 min; (f) 50 min in 10^{-3} M ryanodine. The response in normal saline is illustrated in record (a). Time calibration was the same for all records.

Table 9.

Action of ryanodine on the effective resting membrane resistance

Fibre	Resting resistance x 10^5(ohms) in normal saline	Resting resistance x 10^5(ohms) in 10^{-3}M ryanodine	
		Max. reading	Final reading
1	3.58	4.0 (4)	1.50 (83)
2	4.40	4.95 (4)	2.20 (48)
3	3.85	6.60 (2)	4.95 (10)
4	7.40	8.80 (2)	3.85 (24)
5	4.95	8.52 (42)	6.60 (77)
6	4.00	6.00 (20)	4.40 (120)
7	1.90	2.65 (5)	1.90 (10)
8	1.75	4.00 (81)	1.36 (120)
9	1.75	3.00 (9)	1.00 (23)
10	5.95	8.52 (42)	4.95 (110)
11	3.0	3.75 (30)	1.48 (84)
12	1.88	2.88 (10)	0.88 (23)

Bracketed numbers in column 3 indicate times (in mins.) at which the resistance of the fibres had reached a maximum. Bracketed numbers in column 4 indicate times (in mins.) when recording was stopped.

and then simultaneously, to determine the different effects of the drug on the "slow" and "fast" systems.

Pulses from a square-wave stimulator were delivered to the appropriate nerve, which was severed from the thoracic ganglion, through silver-silver chloride electrodes placed under the nerve in the thoracic region near to the metathoracic ganglion. Paired silver-silver chloride electrodes were used also to record nerve action potentials. In most experiments the frequency of stimulation was 0.1 c/sec. Muscle potentials were recorded with intracellular electrodes of 10-20mΩ resistance filled with 3M-KCl. The double micro-electrode technique (Fatt and Katz, 1951) was employed for direct stimulation of the excitable membrane of single muscle fibres. The voltage drop across a 100KΩ series resistance was used to monitor the intracellularly-applied stimulating currents. In some experiments three micro-electrodes were used, one for stimulation, the other two for recording. In these experiments the current was monitored on one oscilloscope whilst the potentials were recorded on the two beams of another oscilloscope. With 10-20MΩ micro-electrodes the time constant of the recording circuits never exceeded 100 μ sec.

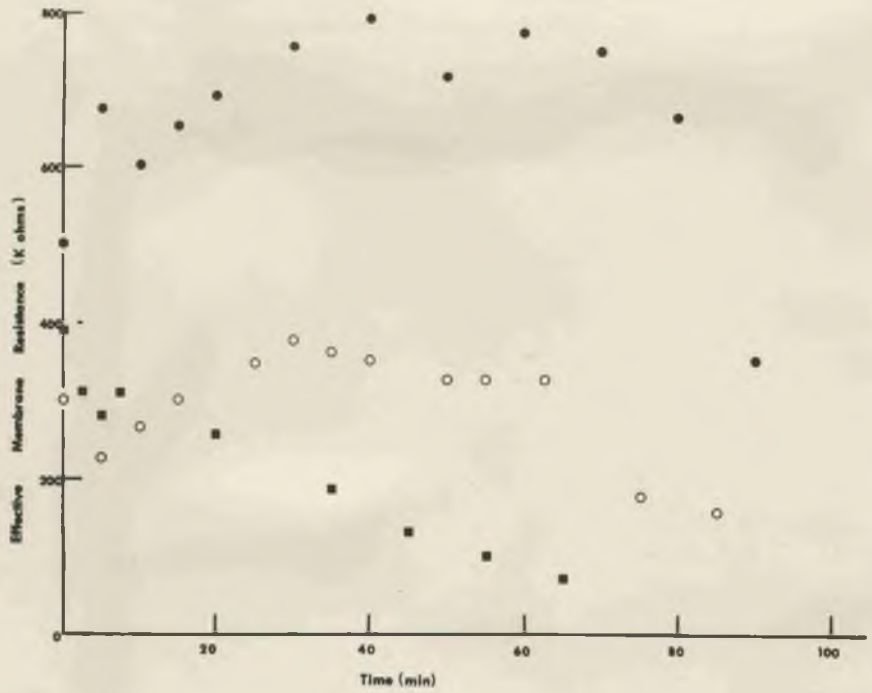
Tension development of the extensor muscle was recorded

isometrically using an RCA 5734 mechano-electronic transducer; the plate shaft of the transducer was attached directly to the distal part of the extensor muscle apodeme. Further studies of the effects of ryanodine on the mechanical responses of the locust muscle were made kymographically with isotonic and auxotonic lever systems.

Solutions of the drug were made up by dissolving the alkaloid in locust saline (Hoyle, 1953). Solutions designed to test the effect of magnesium and calcium concentrations on the progress of ryanodine poisoning were made up by substituting these ions for sodium in the saline. The experimental solutions were applied to the preparation by injection, using a fine needle. Drug concentrations ranging from 10^{-3}M to 10^{-9}M were tested and found to be effective.

Results

Resistance changes. The effective resistance between the inside and outside of the muscle fibre membrane may be determined by measuring the magnitude of the electrotonic potentials set up by either weak cathodal or anodal current pulses, provided that pulse application and current recording are carried out close together in the fibre concerned (Fatt and Katz, 1951). With the recording and stimulating



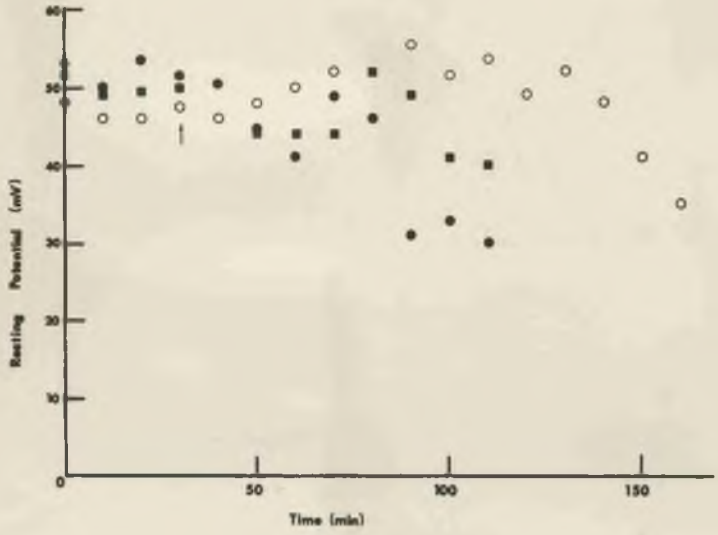


Fig. 51. (a) Changes in the effective resting membrane resistance $1/2\sqrt{(r_m r_i)}$ of three fibres following application of 10^{-3}M ryanodine. Note the relatively high resting resistance of these fibres in normal saline. All-or-none responses were recorded from all three fibres during the initial stages of poisoning.

(b) Effect of 10^{-4}M ryanodine on the resting potential of three fibres of the locust extensor tibiae preparation. The drug was added after previously soaking the fibres for 30 minutes in normal saline. All three graphs have an S-shaped curve with an initial phase of decreased resting potential followed by a phase of resting potential increase. The final phase was accompanied by a further decrease in the resting potential of the three fibres.

r_m = transverse resistance times unit length.

r_i = internal longitudinal resistance per unit length.

electrodes close together the relation between the steady potential V recorded at one electrode and the current I passing through the other electrode is expressed by $V = {}^{I}/_{2}\sqrt{(r_m r_i)}$ where $1/2\sqrt{(r_m r_i)}$ is the effective resistance between the inside and the outside of the fibre.

Brief anodal pulses were used for recording the changes of effective resting membrane resistance following application of ryanodine. Two glass micro-electrodes were inserted close together into the same muscle fibre. Anodal current pulses of about 150 msec duration were applied through one electrode and the potentials developed across the muscle fibre membrane were recorded with the other electrode. The effective resistance of the muscle fibre was determined before, and at regular intervals after treatment with ryanodine.

The effective resting membrane resistance varied from fibre to fibre. Values as low as $8.0 \times 10^{4}\Omega$ and as high as $1.2 \times 10^{6}\Omega$ have been recorded from different fibres. Treatment of the locust muscle fibre with ryanodine resulted initially in an increased resting resistance which was sometimes over twice normal (Table 9). During the later stages of poisoning a slow fall in resistance, to a value well below normal, was recorded (Fig. 50). Not every fibre examined showed an increased resistance initially

following treatment with the drug. A few fibres, usually those which had high membrane resistances in normal saline, showed a slow decline in resting resistance throughout the period of ryanodine treatment (Fig. 51). The initial stage of resistance increase was absent in these fibres.

Effect of ryanodine on length constant. Cerf, Grundfest, Hoyle and McCann (1959) and Werman, McCann and Grundfest (1961) obtained values ranging between 1.6 mm and 2.0 mm for the length constant of normal Romalea microptera muscle fibres. In Schistocerca a much wider range of values for this constant has been recorded due possibly to the equally wide range of values for resting membrane resistance normally found in the muscle fibres of this animal. An average value of 2.8 mm (1.0-3.6) has been calculated from a selection of 30 muscle fibres of a single muscle preparation. However, by selecting from a large number of preparations those fibres with resting resistances exceeding $5.0 \times 10^5 \Omega$, length constants between 3.0 mm and 11.0 mm have been obtained. The length constant of these fibres decreased in ryanodine-saline although a slight increase in resting resistance took place. In the rest of the fibres examined, a slight increase in the length constant resulted initially from ryanodine treatment. In all the fibres examined the

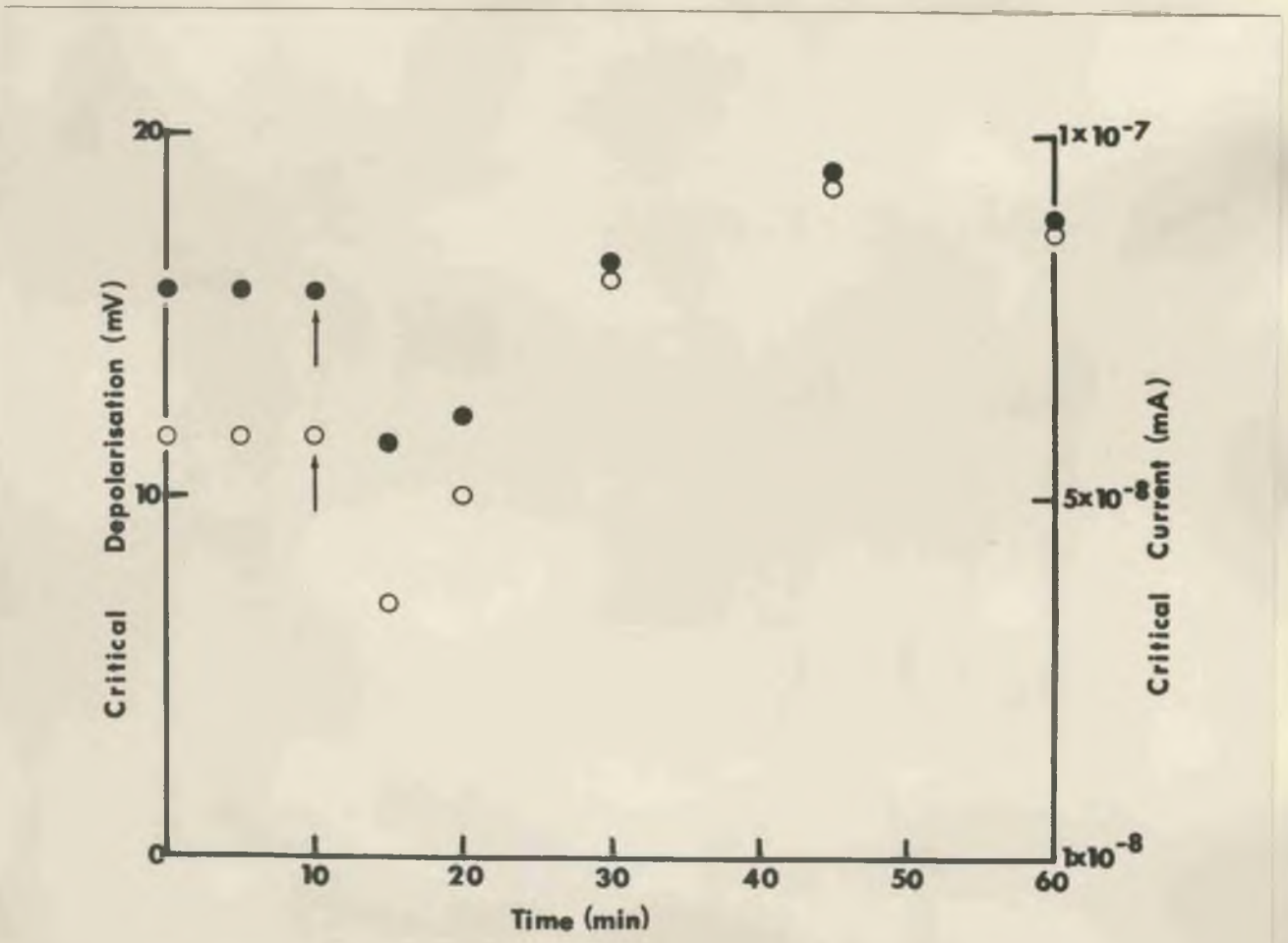


Fig. 52. Changes of critical current (hollow circles) and critical depolarisation thresholds (solid circles) in 10^{-4}M ryanodine. The alkaloid was added after the fibre had first been soaked for 10 min in normal saline.

later stages of ryanodine poisoning were accompanied by a slow decrease in the value of the length constant usually to about 1.0 mm.

The timing of the changes of resting resistance and length constant in ryanodine varied from fibre to fibre. Because of the closely-packed nature of the fibres of the extensor muscle in Schistocerca it is possible that the fibres were not all affected simultaneously by the drug.

Changes of critical depolarisation and threshold current. The changes in effective resting membrane resistance that occurred during the preliminary stages of ryanodine poisoning were accompanied by changes in the critical depolarisation and critical current thresholds. Fig. 52 illustrates these changes for two fibres of the extensor muscle. The critical depolarisation threshold, normally about 10-20 mV, decreased at first to as low as 5 mV in some fibres. In a few fibres this low threshold remained for only a few minutes before it increased to well above normal. Usually, however, the depolarisation threshold remained below normal for about 20 minutes. In a few experiments there was some indication that the later stages of poisoning were accompanied by a return of the critical depolarisation threshold to normal. Changes of the critical current threshold closely paralleled those of the

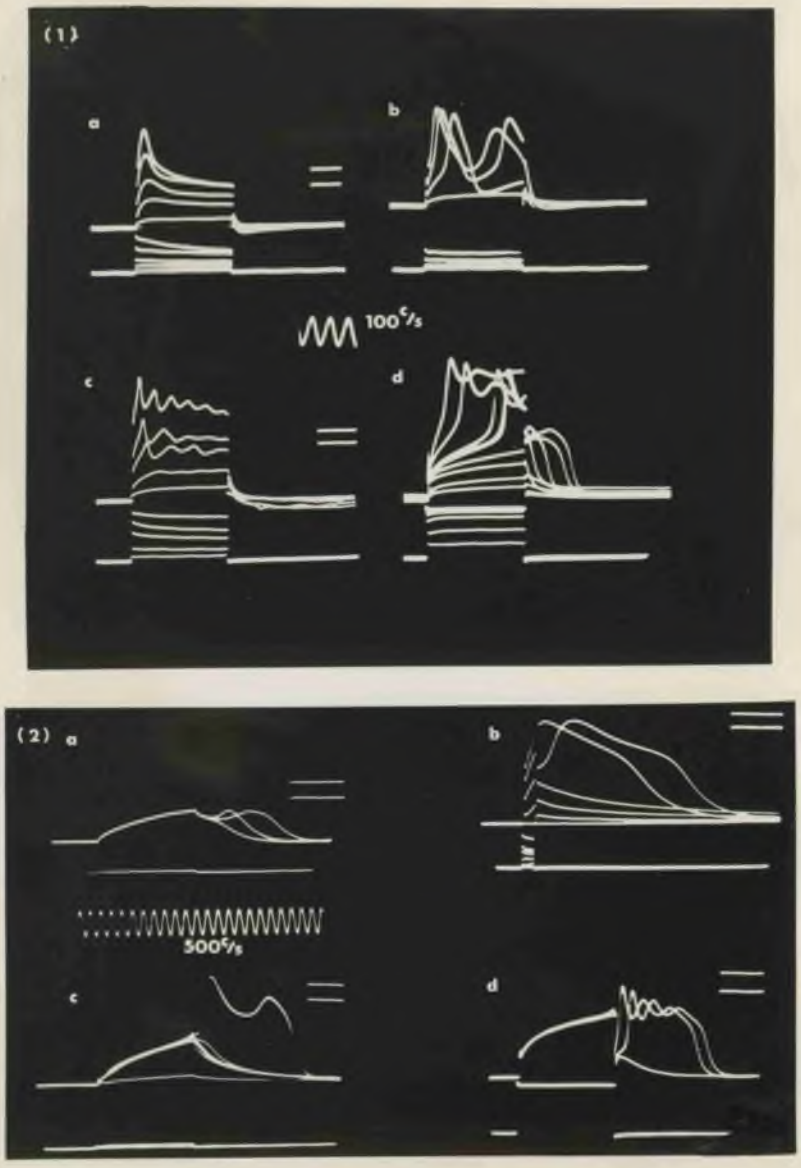


Fig. 53. (i) Active membrane responses (upper traces) to depolarising current pulses (lower traces). (a) and (c) in normal saline; note the graded spike responses. (b) and (d) in 10^{-3} M ryanodine; note the large spike responses with increased durations and in (d) the appearance of 'local' break responses at the critical depolarisation threshold.

(ii) Electrical responses of four different muscle fibres (upper traces) in 10^{-3} M ryanodine to different intensities of depolarising current (lower traces). Note persistance of potential after break of current.

depolarisation threshold. In normal saline the current threshold ranged between 0.03μA-0.1μA. During the early stages of ryanodine treatment, values as low as 0.015μA have been recorded.

Spike amplitude changes. The responses of the locust muscle fibre, in normal saline, to inward and outward current pulses have been described (del Castillo et al., 1953; Cerf et al., 1959; Hill and Usherwood, 1961). Cathodal current pulses depolarise the muscle fibre membrane with an approximately linear relationship between voltage and current only up to a depolarisation level of about 12 mV. Above this level a departure from the linear relationship is observed, with the appearance of oscillatory potential changes. These potentials are usually graded events which increase in amplitude, up to a maximum of about 35 mV, with increasing depolarisation of the muscle fibre membrane.

Hagiwara and Watanabe (1954) have demonstrated that locust muscle fibres with high resting resistances give small all-or-none responses when stimulated with supra-threshold cathodal current pulses. In the present set of experiments all-or-none responsiveness has been demonstrated in many fibres. These fibres, which were characterized further by high resting resistances and long length constants,

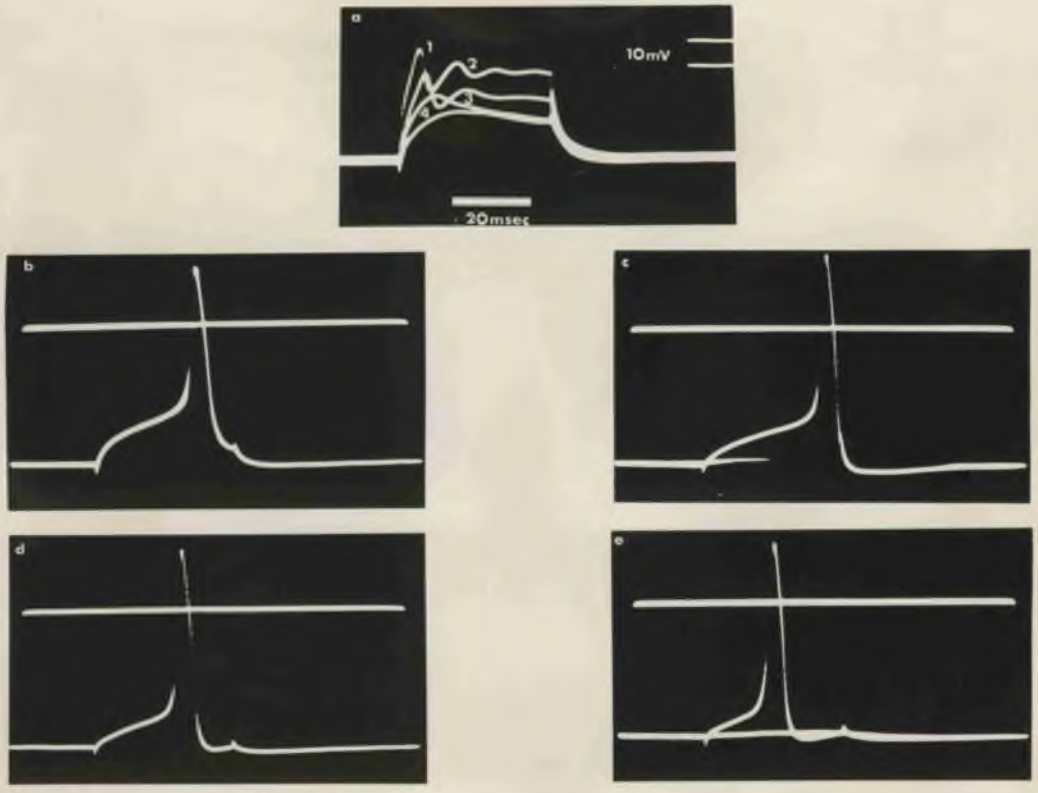


Fig. 54. Enhancement of electrically-excitable responses by ryanodine. (a) Responses of a single locust muscle fibre in normal saline to constant cathodal current pulse stimulation recorded at distances of (1) 0.1 mm; (2) 0.7 mm; (3) 1.0 mm; (4) 2.0 mm from the stimulating electrode. (b-d) Responses obtained from the same fibre after 6 minutes immersion in 10^{-5} M ryanodine - saline. Recordings made at (b) 0.8 mm; (c) 1.7 mm; (d) 2.3 mm; (e) 3.0 mm from the stimulating electrode. The upper trace of each record represents zero membrane potential. It is possible that the spike recorded in (e) was initiated near the recording electrode. In records (c) and (d) the spikes must have been initiated some distance away from the recording electrodes and may have been propagated back to the stimulated region. The membrane was less excitable at the near electrode (a), possibly due to damage.

were found to be more widely distributed in locust muscle than was indicated by Hagiwara and Watanabe (1954) but they quickly lose their all-or-none responsiveness during repeated intracellular stimulation. This change in responsiveness is associated with a decreased membrane resistance and a reduced length constant. If recordings are made from the extensor muscle without first removing the tracheolar trunk, which covers its ventral surface, a large proportion of the fibres show what appears to be all-or-none responsiveness, at least during the initial stages of recording, in contrast to the graded type of response normally associated with insect muscle fibres. The all-or-none spikes recorded in the present experiments never exceeded 30 mV in magnitude. Persistent cathodal break responses, which were small graded local responses, were recorded from these fibres by breaking the current at the critical level of depolarisation. These potentials were long, due to the long time constant (30-90 msec) of the membrane.

So far it has not been possible to demonstrate whether the all-or-none potentials are propagated without decrement, partly because of the short length of the fibres and partly because the spikes were always associated with vigorous contractions of the muscle fibre. These contractions usually resulted in damage of at least one of the three

intracellular micro-electrodes involved in stimulation and recording.

Werman et al. (1961) have suggested that the electrogenic processes of electrically excitable membranes that produce graded responses and those that produce all-or-none spikes are basically similar. It seems possible, therefore, if this concept is valid, that according to the conditions of the fibre membrane prevailing at any instant a single fibre may show either graded or all-or-none responsiveness. It is significant that Katz (1948) found some of the fibres of frog muscle, where the typical response is all-or-none, showed graded responsiveness. Furthermore, alterations in the properties of the excitable membrane of insect muscle result in the conversion of the graded electrically-excited-responsiveness of the fibres to all-or-none responsiveness. These alterations have been achieved by treatment of the muscle with tetraethylammonium ions and choline ions (Hoyle, 1962) and by substitution of the alkali earth ions for Na^+ in the saline (Werman et al., 1961). In addition to these agents it has been found that ryanodine is equally effective in this respect.

Addition of ryanodine in concentrations above 10^{-7}M led to marked changes in the responsiveness of most of the muscle fibres examined (Figs 53 and 54). Conversion

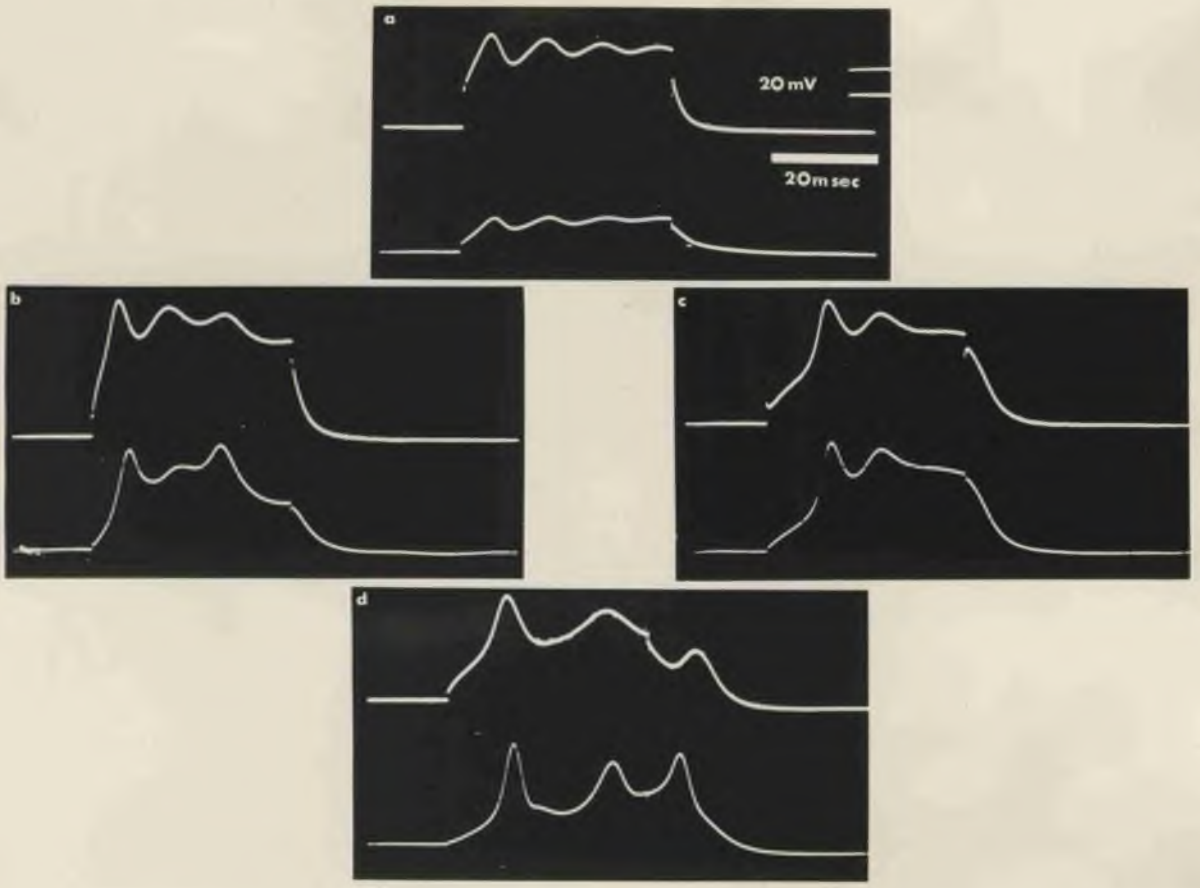


Fig. 55. Effect of ryanodine on the responsiveness of a locust muscle fibre. Responses to cathodal current pulse stimulation (a) in normal saline; (b–d) in 10^{-3} M ryanodine - saline. Stimulating and recording electrodes were 50 μ apart (top traces) and 1 mm apart (bottom traces). Recordings were made (b) 1 min; (c) 2 min; (d) 4 min after application of the drug. Note variations in latency and duration of the spikes at the different recording loci in ryanodine.

of the graded electrically-excitable response to an all-or-none response took place soon after application of the drug, when spikes with amplitudes often exceeding 80 mV were frequently recorded. When a prolonged current pulse was used for stimulation repetitive spikes were obtained. If the current was broken at the critical level of depolarisation, the membrane potential sometimes remained at the critical level for a considerable time after the break (Fig. 53). These prolonged break responses were especially evident at low stimulation frequencies and fluctuated from moment to moment.

Fluctuations in the characteristics of the membrane. Werman et al. (1961) found that substitution of Ba^{++} ions for Na^{+} ions in the saline resulted in the conversion of the normally graded electrically-excited-responsiveness of Romalea muscle fibres to all-or-none responsiveness but that there were marked irregularities in the propagation of the action potentials. During repetitive activity reversals of order of precedence were recorded at different loci on the membrane of the same fibre. Furthermore, changes in latency occurred, reflecting variations in the refractoriness and critical firing level of the membrane. These variations were distributed both spatially along the membrane and at different times at given loci in the

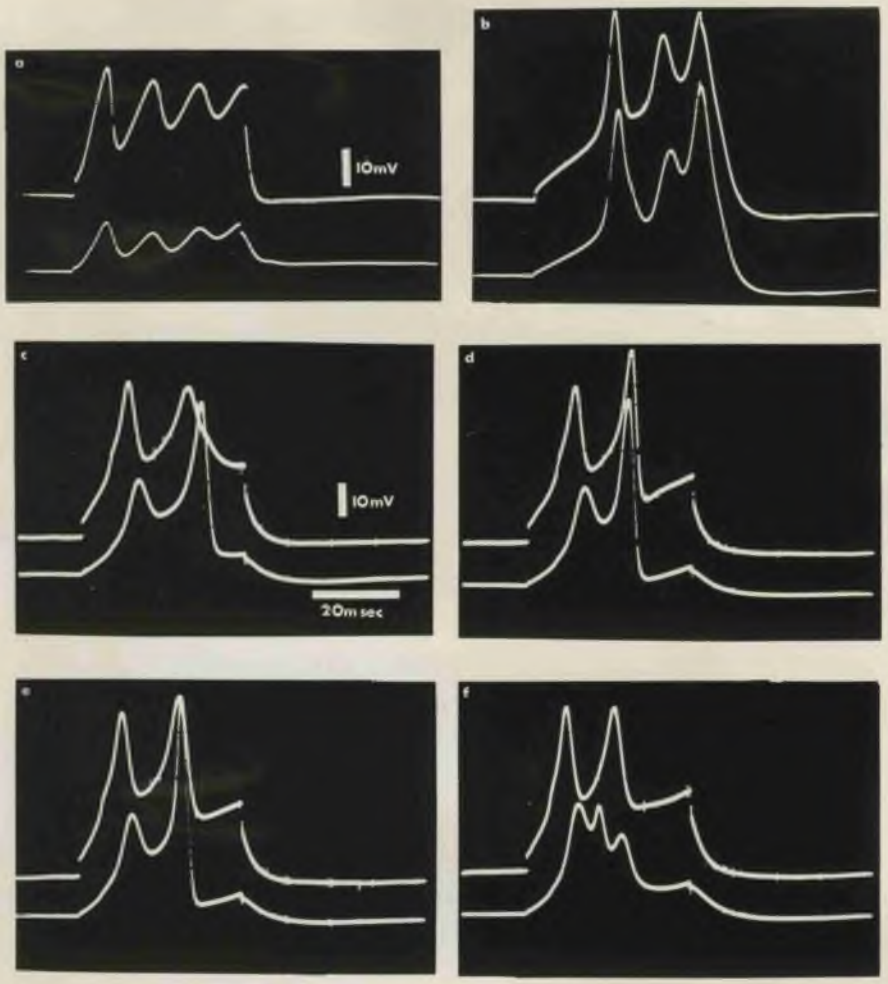


Fig. 56. (a-b) Changes in propagation of muscle spike responses in ryanodine. The responses produced by brief depolarising current pulses were recorded at two different loci, one near to and one far from the intracellular stimulating electrode. (a) Responses in normal saline; recording electrodes separated by 1 mm. (b) Responses after 10 min in 10^{-3} M ryanodine - saline; recording electrodes separated by 1.3 mm. With the higher resistance state of the membrane the spike responses in ryanodine are much less attenuated.

(c-f) Non-uniformities in the response of a locust muscle fibre in 10^{-3} M ryanodine. Note temporal and spatial differences in latency and duration of spikes recorded at the two loci. Electrode separation was 1.7 mm. Recordings were made after (c) 2 min; (d) 4 min; (c) 6 min; (f) 8 min in the drug.

Voltage calibration same for (a-b) and (c-f). Time calibration same for all records.

membrane. Werman et al. (1961) suggested that these deviations from the normal condition of decrementless propagation indicated spatial non-uniformities in the Ba^{++} treated membrane of Romalea.

Similar irregularities, perhaps not quite so marked as those obtained from the Ba^{++} membrane of Romalea, have been recorded from the muscle fibre of Schistocerca during the preliminary stages of ryanodine poisoning. In many fibres incomplete decrementless propagation of the all-or-none response induced by ryanodine was indicated following tests with three micro-electrodes, one for intracellular stimulation and two for recording (Fig. 54). One of the recording electrodes was inserted into the fibre close to the stimulating electrode; the other recording electrode was sited in the same fibre at some distance from the stimulating electrode. Irregularities were especially evident during repetitive activity (Figs 55 and 56). The state of the membrane was not only different at different points along its length but also varied from moment to moment.

It seems possible, that in certain fibres at least, ryanodine application results in the appearance of all-or-none responsiveness only in limited areas or patches on the membrane.

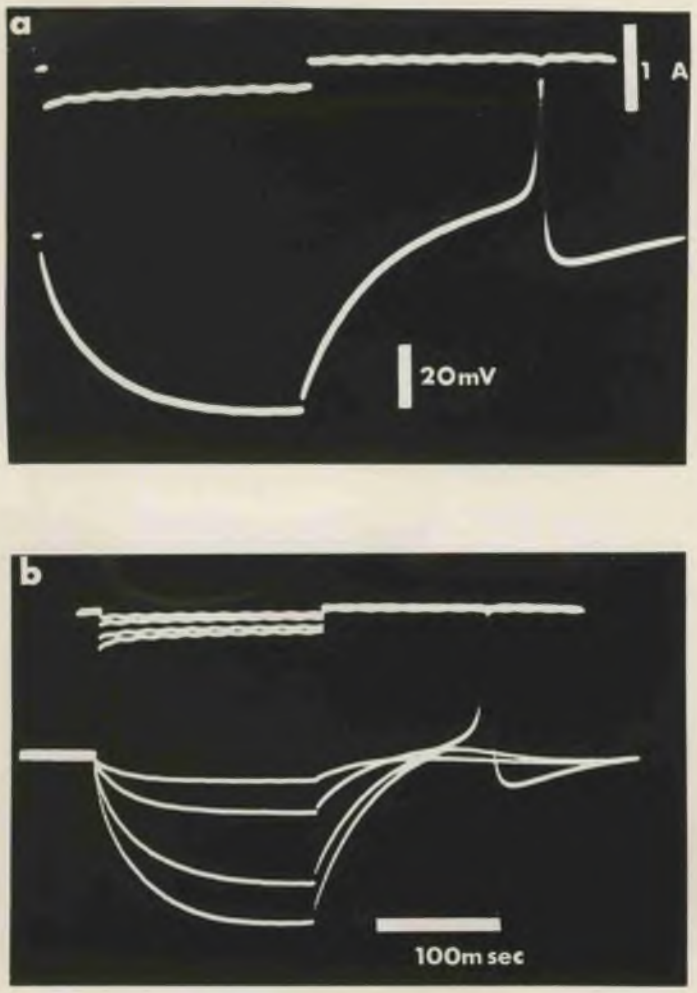


Fig. 57. Spike responses elicited from ryanodinised muscle fibres following break of anodal current (upper traces) applied to the point of recording. Note the large undershoot following the spike.

Not every fibre examined gave all-or-none spike responses to supramaximal cathodal pulse stimulation following ryanodine application. Some fibres gave responses which, although clearly augmented in character, did not differ markedly from the graded spike responses obtained from fibres in normal saline. In these fibres, which had relatively low resting membrane resistances of about 100-150 KΩ , the property of decremental propagation was maintained.

Anodal break responses. Anode break excitations were elicited from some ryanodine-treated fibres during the early stages of poisoning with drug concentrations greater than 10^{-5}M. Fig. 57 (a) illustrates the response of a ryanodinised fibre to strong anodal pulse stimulation. In (b) the response of a ryanodinised fibre to successive current pulses is shown, each pulse of slightly greater intensity than the one before. One of the pulses fails to fire an all-or-none potential and only a small graded response is elicited.

Effect of ryanodine on resting potential. A slight decrease in the magnitude of the resting potential of the locust muscle fibre was recorded initially following application of drug concentrations exceeding 10^{-5}M (Fig. 51). This decrease was followed usually by a period

of recovery. It has not been possible to determine with any accuracy the effect on the resting potential of lower drug concentrations than 10^{-5}M because low concentrations take a very long time to have any effect and also because the changes are then very small. With all the drug concentrations tested, however, the later stages of poisoning were accompanied by a progressive decline in the resting potential to as low as 30 mV in some fibres. During this period the fibres became much more opaque in appearance.

In a few of the fibres examined, treatment with 10^{-4}-10^{-3}M ryanodine resulted initially in a hyperpolarisation of the membrane of up to 10 mV.

Electrical responses to indirect stimulation

"Fast" axon

Resting potentials ranging between 50-70 mV are usually recorded from fibres of the locust extensor tibiae preparation. Responses to electrical stimulation of the "fast" axon consist of muscle action potentials about 70 mV in magnitude and 10 msec in duration.

Treatment with ryanodine results in a number of changes of the "fast" electrical response of the locust

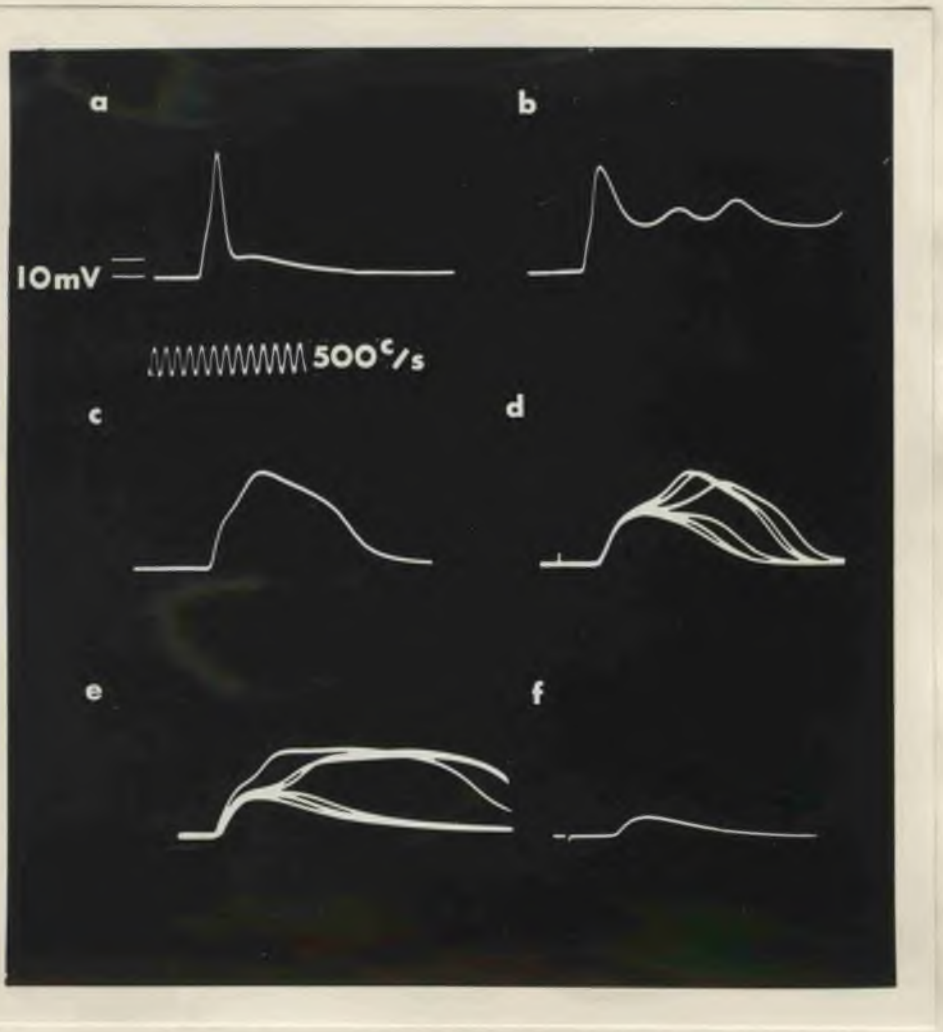


Fig. 58. Progressive effects of ryanodine on the electrical response to "fast" axon stimulation. (a) In normal saline. (b) 2 min; (c) 35 min; (d) 45 min; (e) 65 min; (f) 100 min after injection of 10^{-3} M ryanodine. (d) and (e) are recordings of a number of successive responses superimposed on a single frame to illustrate variation in the electrical response at this stage of poisoning. Stimulation at a frequency of 0.5 c/s.

muscle fibre (Fig. 58). With drug concentrations greater than 10^{-5}M the sequence of changes was as follows:- stage (1), increase in the magnitude of the action potential; stage (2), prolongation of the action potential; stage (3), formation of a pronounced plateau of repolarisation with repetitive spike formation; stage (4), depression of the action potential with loss of repetitive activity; stage (5), loss of the spike component and a slow decline of the post-synaptic potential to zero.

Stage (1). Following treatment with drug concentrations greater than 10^{-5}M an increase in the magnitude of the electrical response of the muscle fibre to "fast" axon stimulation was recorded.

Stage (2). Within a few minutes after treatment of the muscle fibre with ryanodine the rate of repolarisation of the action potential appeared markedly slower than normal with the formation of a pronounced plateau. The magnitude and duration of the plateau fluctuated considerably from one response to the next. With ryanodine concentrations below 10^{-7}M the time course of the electrical response did not at any time differ markedly from normal. With higher concentrations of the drug the prolongation of the action potential was much more marked but did not appear to be directly dependent upon the concentration of the drug employed.

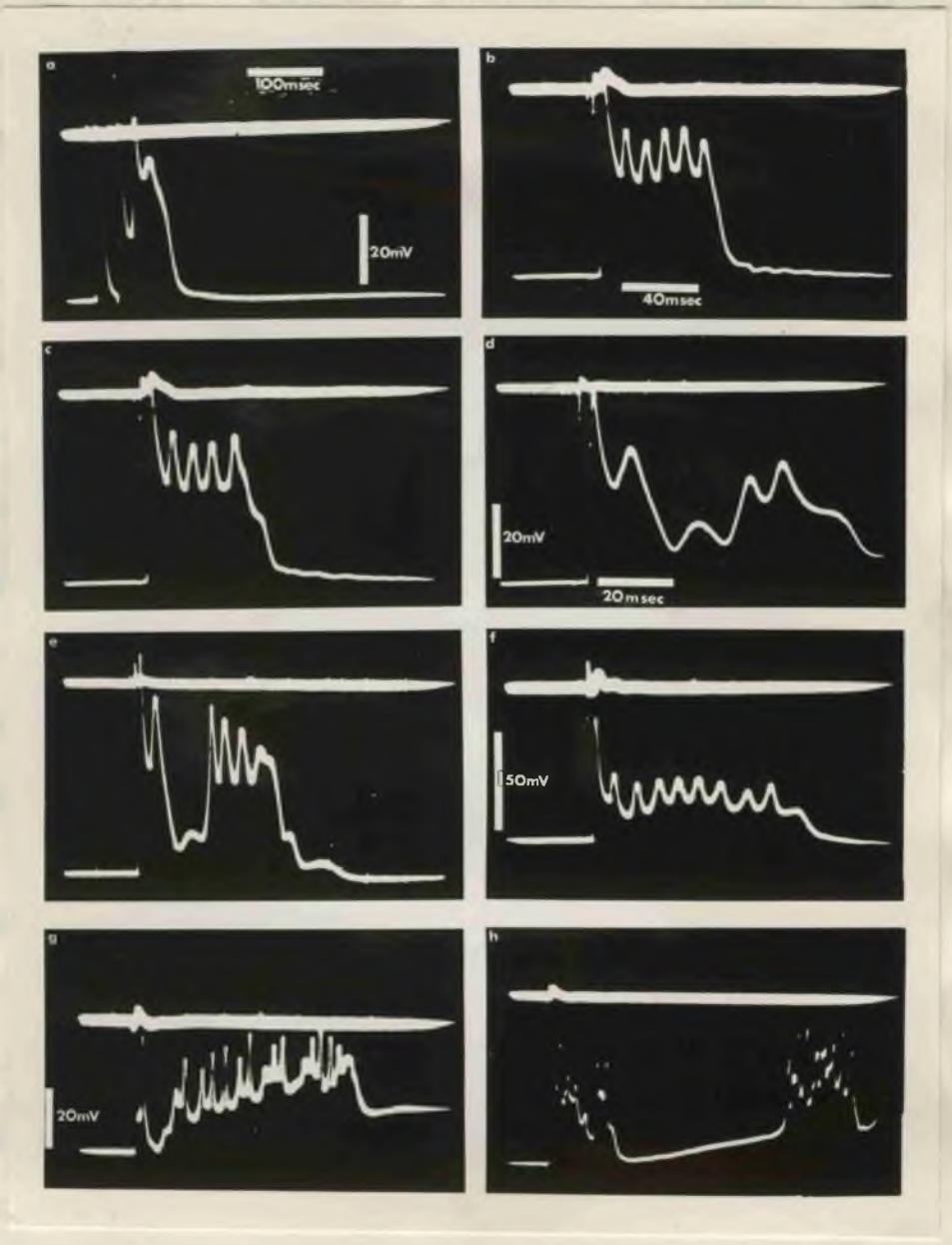


Fig. 59. Repetitive firing in ryanodine. In (a) firing is both neurogenic and myogenic in origin. In (b-h) firing is myogenic in origin only. The upper traces of each record represent zero membrane potential and register the potentials of the "fast" motor axon following stimulation of the crural nerve. In a number of the records the external electrodes used to record the nerve potentials have also partially picked up the muscle potentials.

Stage (3). Further increase in the repolarisation plateau took place and repetitive spike responses appeared. At first only a single spike followed the initial response but as the magnitude and duration of the plateau increased, an increasing number of repetitive spikes appeared. Up to 50 spike responses following a single stimulus have been recorded under these conditions (Fig. 59), with a total duration for the electrical response of well over 1 sec. The number of repetitive spike responses obtained with successive stimuli fluctuated considerably and was reduced by increasing the frequency of stimulation, possibly through fatigue of the response mechanism.

Stage (4). Later stages of poisoning were characterized by a fall in both magnitude and duration of the action potential. The associated decline of the plateau led to a reduction in the degree of spike repetition. Eventually all traces of repetition were abolished leaving a rather diminished but still slightly prolonged electrical response. As poisoning progressed the magnitude of the electrical response continued to fall, the spike component arising later and later from the post-synaptic potential (Fig. 59).

Stage (5). With the final loss of the spike component a small post-synaptic response remained. During

the following 2-3 hours the electrical response of the muscle disappeared completely but even at this late stage of poisoning the resting potential of the muscle fibres examined was still about 35-40 mV.

The action of excess magnesium. The electrical response of the locust extensor tibiae preparation to indirect stimulation through nerve 5 is rapidly depressed in saline containing raised magnesium concentrations; in 10 mM magnesium the action potential is reduced to a pure post-synaptic potential of about 10 mV (Hoyle, 1955c). In the present experiments it was desirable to study the action of ryanodine on the "fast" post-synaptic potential alone, independent of its effect on responsiveness. This was achieved by soaking the muscle for about 30 minutes in saline containing 10 mM Mg^{++} substituted for Na^{+}. When the "fast" electrical response of the muscle fibre to indirect stimulation was reduced to a post-synaptic potential of about 10 mV, ryanodine saline was perfused around it and the effects of the alkaloid on the electrical response recorded.

The action of the drug was not the same in every fibre examined. In some fibres the "fast" post-synaptic potential was augmented in magnitude and duration after only a few minutes. Soon all-or-none spike responses

appeared, with some delay, on the augmented response. In a few fibres spikes of over 80 mV were recorded.

Many of the fibres showed repetitive activity at first but as poisoning progressed, the repetitive spikes were lost and the height of the action potential gradually fell. In some fibres giving spike responses the all-or-none character of the spike was retained throughout the duration of the experiment. This was clearly demonstrated during the later stages of poisoning. As the magnitude and duration of the post-synaptic component declined the spike component suddenly disappeared presumably when the declining post-synaptic potential failed to reach the critical level of depolarisation for spike initiation. In other fibres giving initially all-or-none responses the spikes became graded in character during the later stages of poisoning. During these experiments a check on the responsiveness of the fibres was made by intracellular stimulation with cathodal current pulses.

In other fibres the effect of ryanodine on the "fast" electrical response of the magnesium-treated muscle fibre resulted in the reappearance of a graded spike response, with only slight delay. The reappearance of the spike was preceded by an increase in the magnitude of the post-synaptic response. In yet a third group of preparatio

the effect of the drug was small; the post-synaptic response was slightly larger but no electrogenic response was elicited. However, in a few of these preparations minute oscillations occurred during the prolonged recovery phase of the electrical response. It is possible that these oscillations represented either potentials picked up from the surrounding muscle fibres or they may have been very small graded responses.

The action of excess calcium. The "fast" action potential of the locust muscle fibre is augmented by the addition of excess calcium to the saline due mainly to an increase in the magnitude of the post-synaptic component (Hoyle, 1955a).

The preparation was treated with 10^{-3} M ryanodine in normal saline and left until the "fast" electrical response fell to about 10 mV owing to the action of the drug. The ryanodine-saline was then replaced by saline containing 10 mM calcium in addition. Almost immediately an increase in the magnitude of the post-synaptic potential was recorded and the spike component reappeared. After about 10 minutes the magnitude of the spike was almost the same as normal. However, this recovery of the previously-depressed electrical response lasted for only a short time after which the magnitude of the action potential fell again.

"Slow" axon

The electrical response of the extensor tibiae muscle fibre to indirect stimulation through nerve 3b consists of a post-synaptic potential varying in magnitude from less than 1 mV to up to 30 mV. Local spike responses arise from the larger potentials. The smaller potentials, which show facilitation with repetitive excitation, may have a total excursion of almost 1 minute; the larger potentials which show no facilitation with repetition have a total excursion of 15-20 msec (Hoyle, 1955b).

The action of ryanodine on the "slow" electrical response of many of the muscle fibres examined was divisible into four stages. Stage (1), augmentation and prolongation of the post-synaptic potential; stage (2), appearance of large, sometimes all-or-none, spike responses; stage (3), repetitive spike formation; stage (4), loss of repetitive activity and depression of the spike response.

Stage (1). The "slow" post-synaptic potential increased in magnitude and in duration during the first few minutes after treatment with 10^{-3} M ryanodine (Fig. 6D). In a number of preparations the height of the post-synaptic potential was more than doubled and the duration of the electrical response often reached 150 msec. The increased duration of the potential was due more to a reduction in

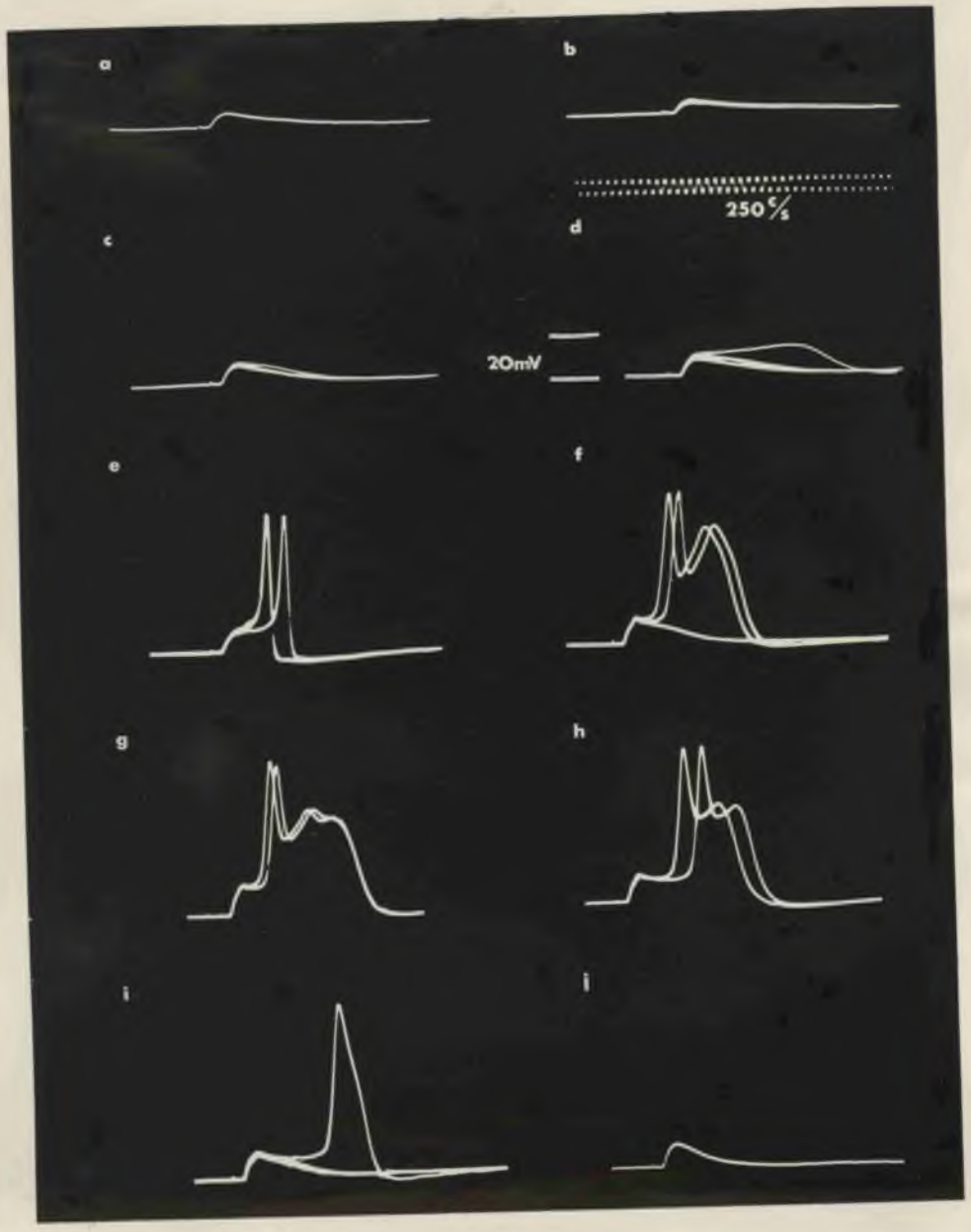


Fig. 60. Effect of ryanodine on electrical responses to "slow" axon stimulation.

(a) In normal saline. (b) 2 min; (c) 4 min; (d) 6 min; (e) 10 min; (f) 12 min; (g) 15 min; (h) 20 min; (i) 25 min; (j) 30 min after injection of 10^{-3}M ryanodine into the femur. Records (b-i) were obtained by superimposing a number of successive responses. Preparation stimulated at a frequency of 0.1 c/s. Note in (e) undershoot following the spike.

the rate of decay rather than to any change in the rise time of the response.

Stage (2). The augmentation of the post-synaptic potential was soon complicated by the appearance, in many of the fibres examined, of large secondary responses. In a few fibres small graded responses were the only type recorded. Not every post-synaptic potential was successful in eliciting an electrically-excitable response (Fig. 60). It is assumed that the reason for this was the frequent failure of the post-synaptic potential, which fluctuated in magnitude and duration from one response to the next, to attain the depolarisation threshold for secondary response initiation. The fluctuation in the magnitude and duration of the post-synaptic potential was associated with variation in the delay of the spike component. At first the rate of repolarisation of the response was relatively fast and undershoots of the resting potential of up to 10 mV followed the spike. However, as poisoning progressed, a pronounced negative plateau appeared.

The magnitudes and durations of the post-synaptic potentials did not fluctuate so much during the later stages of poisoning and almost every stimulus was successful in eliciting a spike response.

Stage (3). Further increase in the duration of the repolarisation plateau following the spike response was usually accompanied by repetitive activity. Repetitive activity of this type is usually associated with an increased membrane excitability (Werman et al., 1961). Even at relatively low frequencies of stimulation (0.2 c/sec) the magnitude and duration of the plateau varied considerably from one response to another and at higher frequencies the plateau almost disappeared. As the form of the plateau varied, so also the number of repetitive spike responses varied from one stimulus to another.

Stage (4). Later stages of poisoning were characterized by a decreased membrane excitability with a loss of repetitive activity and a decline in the magnitude and duration of the plateau. At the same time the magnitude of the electrical response fell gradually, with the spike component arising later and later from the post-synaptic potential. In some fibres loss of the spike component was a gradual process. The secondary response, if initially all-or-none in character, was first reduced and probably graded in height. In other fibres showing all-or-none spike formation loss of the spike component occurred abruptly, when the magnitude of the post-synaptic

potential fell below the depolarisation threshold for elicitation of the secondary response. In all the fibres examined the spike component eventually disappeared, leaving a rather diminished post-synaptic potential (Fig. 6D). During the final stages of poisoning the magnitude of the post-synaptic potential fell also and about 3 hours after application of the drug it was impossible to elicit an electrical response from the fibre.

In a few preparations treatment of the muscle fibres with ryanodine did not lead to the appearance of large spike responses following the "slow" post-synaptic response. An augmentation in the magnitude and duration of the post-synaptic potential of these fibres was recorded soon after treatment but it was apparently insufficient to boost the membrane potential to the level of polarisation at which secondary responses appear. It was possible, however, that these fibres differed in some way from those which showed spike activity following ryanodine poisoning. To test this possibility nerve 3b was stimulated with a pair of shocks whilst gradually decreasing the interval between them. At first two distinct "slow" electrical responses were recorded, one following each stimulus, but as the stimuli were brought closer together the responses summated. This summation often resulted in the appearance

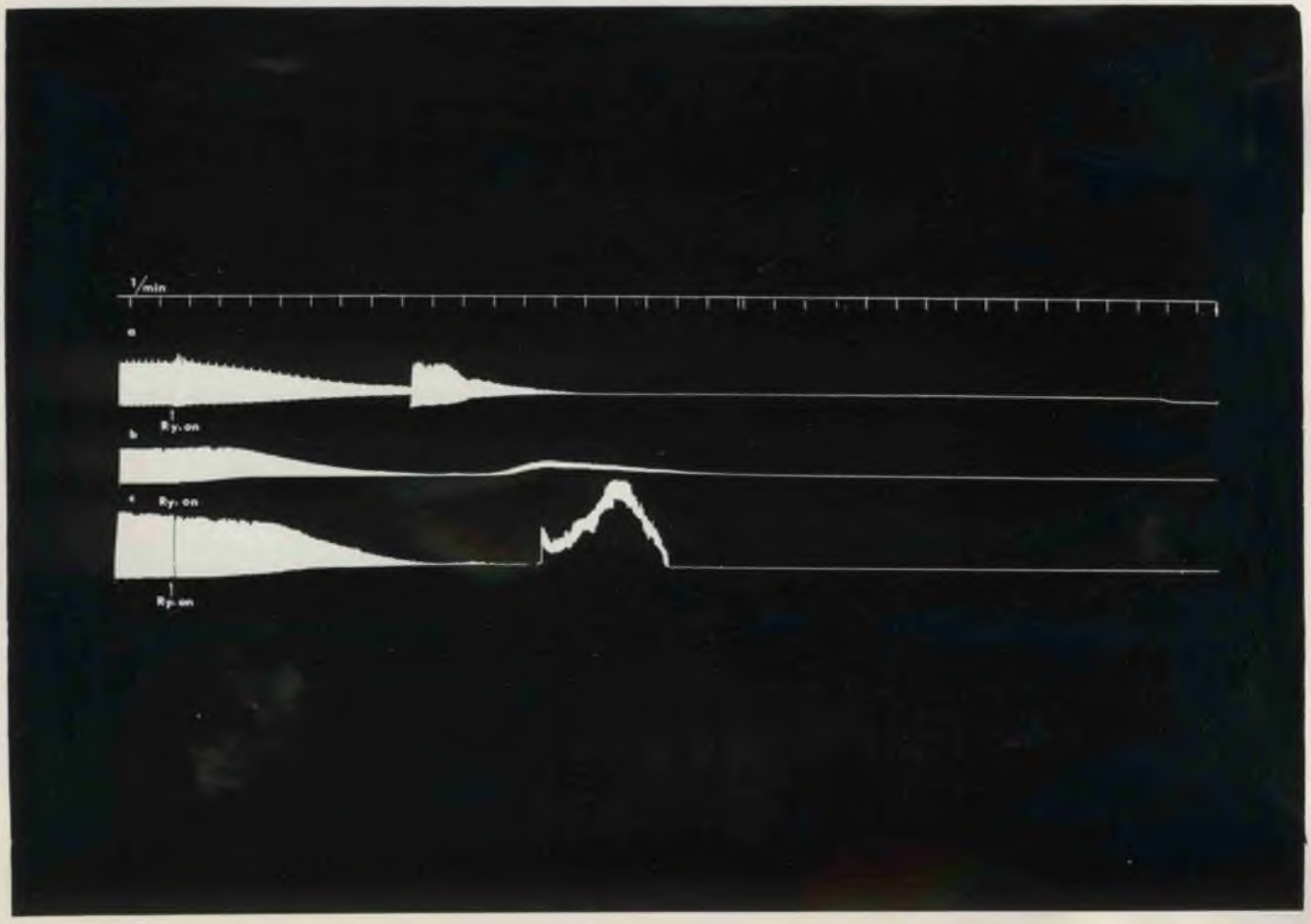


Fig. 61. Effect of 10^{-3}M ryanodine on the mechanical response of three different extensor tibiae muscle preparations. Stimulation, at a frequency of 1 c/s, was through the "fast" motor axon. Recordings were made using an auxotonic lever.

of secondary responses in the majority of the fibres which had previously failed to give them and in most fibres the secondary responses were all-or-none in character. It is suggested that the failure of some fibres to give secondary responses in ryanodine following stimulation of the "slow" axon results from the failure of the potentiated post-synaptic potential to reach the threshold depolarisation for spike initiation.

Mechanical responses to indirect stimulation

"Fast" axon

Recordings have been obtained for the extensor tibiae preparation of the locust during "fast" axon stimulation using an auxotonic lever and a kymograph (Fig. 61). The effect of ryanodine on the "fast" mechanical response appeared to be markedly dependent on the concentration of the drug and the frequency of stimulation. Treatment of the preparations with drug concentrations as high as 10^{-3}M led initially to an increased twitch height; the preparation being stimulated at frequencies less than 0.1 c/sec. This effect was recorded first by Hoyle (personal communication). Continued poisoning of the preparation resulted in a gradual decline of the twitch response and after about 3 hours the mechanical response appeared to have disappeared completely. It was not

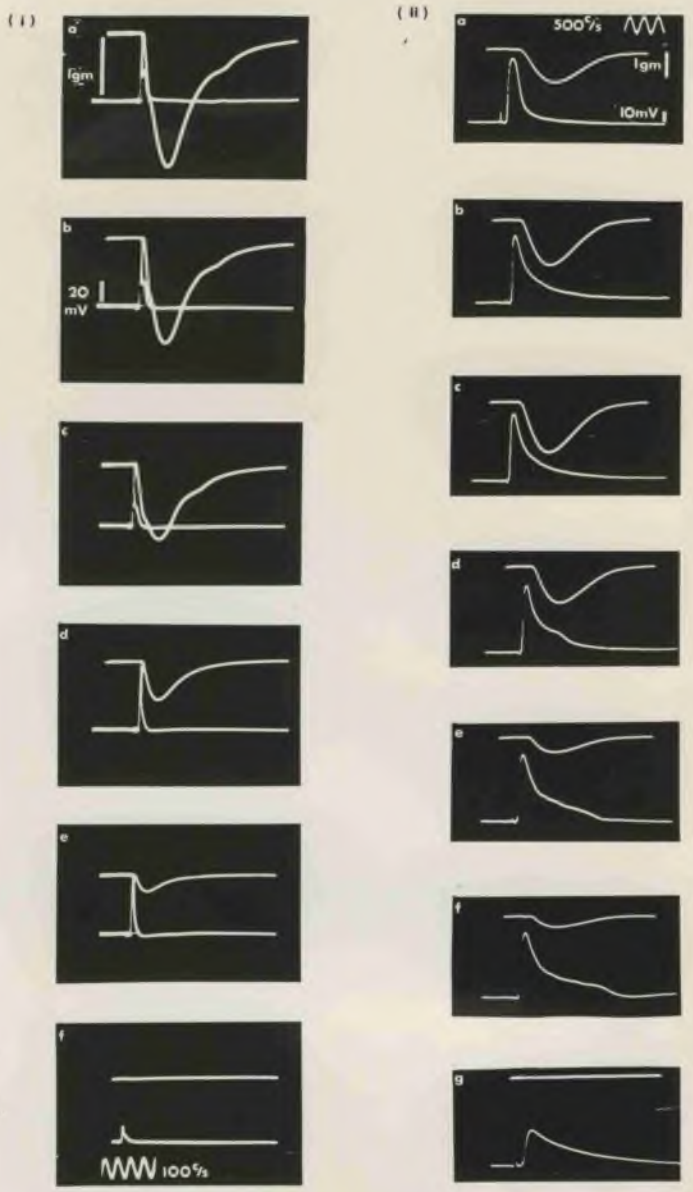


Fig. 62. The effects of ryanodine on the mechanical responses of locust and cockroach muscles.

(i) Isometric "fast" mechanical responses (upper traces) and electrical responses (lower traces) recorded from the locust extensor tibiae muscle preparation before and at intervals after treatment with 10^{-3}M ryanodine; (a) responses in normal slaine; (b) 7 min; (c) 27 min; (d) 51 min; (e) 85 min; (f) 100 min after treatment with the drug. Stimulation frequency was 1 c/s.

(ii) Isometric "fast" mechanical responses (upper traces) and electrical responses (lower traces) recorded from coxal muscle 137 of the cockroach before, and at intervals after, treatment with 10^{-3}M ryanodine; (a) responses in normal saline; (b) 3 min; (c) 6 min; (d) 15 min; (e) 18 min; (f) 21 min; (g) 40 min after treatment with the drug. Stimulation at a frequency of 0.1 c/s. Note initial potentiation of the mechanical and electrical response.

possible by means of studies on the twitch response to determine the exact time at which the mechanical response disappeared, for even when the twitch response was vanishingly small the preparation gave an appreciable contraction to stimulation at a high frequency.

During the later stages of poisoning the diminished twitch contractions were prolonged by a reduction in the rate of relaxation. This prolongation of the twitch resulted in the summation of successive responses even at relatively low frequencies of stimulation.

At frequencies of stimulation of about 1 c/sec a depression of the mechanical response was recorded initially following application of 10^{-3}M ryanodine. However, after about 10 minutes the response had increased in magnitude and in a few preparations repetitive activity occurred. During these early stages of poisoning a contractural response appeared. In many preparations the maximum contracture was recorded during the period of increased twitch height. A contractural response was not recorded with stimulation frequencies lower than 0.5 c/sec. It is suggested that the contractural response resulted from a reduction in the rate of relaxation of the muscle. Prolongation of the twitch would result in summation of successive contractions even at relatively low frequencies

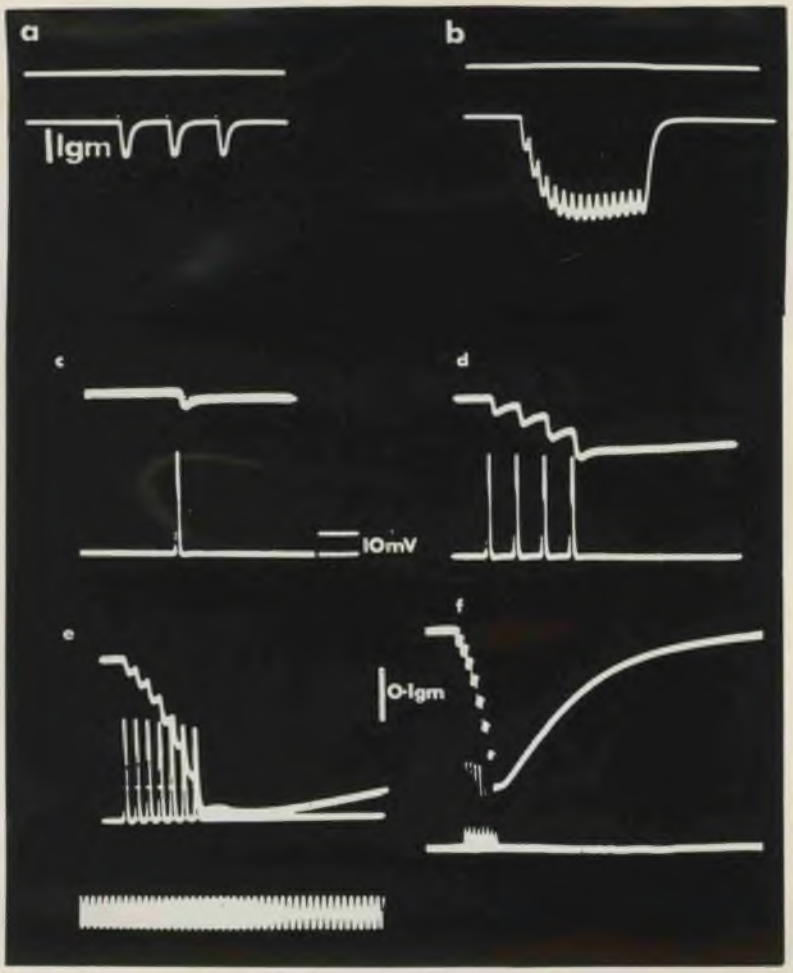


Fig. 63. Effect of ryanodine on the mechanical response of the locust metathoracic extensor tibiae muscle to "fast" axon stimulation. (a) Responses in normal saline to stimulation at 10 c/s. (b) Responses in normal saline to stimulation at 30 c/s. (c-f) Responses in 10^{-5}M ryanodine to a variety of stimulation frequencies. Note decreased rate of relaxation of the mechanical response after treatment of the muscle with ryanodine. Tension calibration same for (a-b) and for (c-f). Time calibration same for (a-e) at 50 c/s. Record (f) was obtained with a slower time base.

of stimulation. The contracture disappeared almost immediately stimulation was discontinued. It also disappeared during the later stages of poisoning as the twitch response became progressively smaller.

Experiments were also made using lower concentrations than those mentioned above. Treatment with 10^{-7}M ryanodine resulted in a slow depression of the twitch response without any phase of twitch increase.

The effects of ryanodine on the isometric mechanical response to "fast" axon stimulation were studied using a mechano-electronic transducer. Experiments were performed on the coxal muscles of the cockroach, Periplaneta americana, as well as on the extensor tibiae preparation of the locust, Schistocerca gregaria (Fig. 62). The peak twitch tension of the locust preparation fell to less than one quarter of its normal value following treatment with 10^{-9}M ryanodine, before any changes in the electrical properties of the muscle fibres were apparent. With higher drug concentrations the decline of twitch tension was much more rapid. In one experiment addition of 10^{-3}M ryanodine resulted in a decline of the peak twitch tension from more than 2 gm to less than 0.1 gm in just under a minute. In all the above experiments the frequency of stimulation was about 1 c/sec. At lower

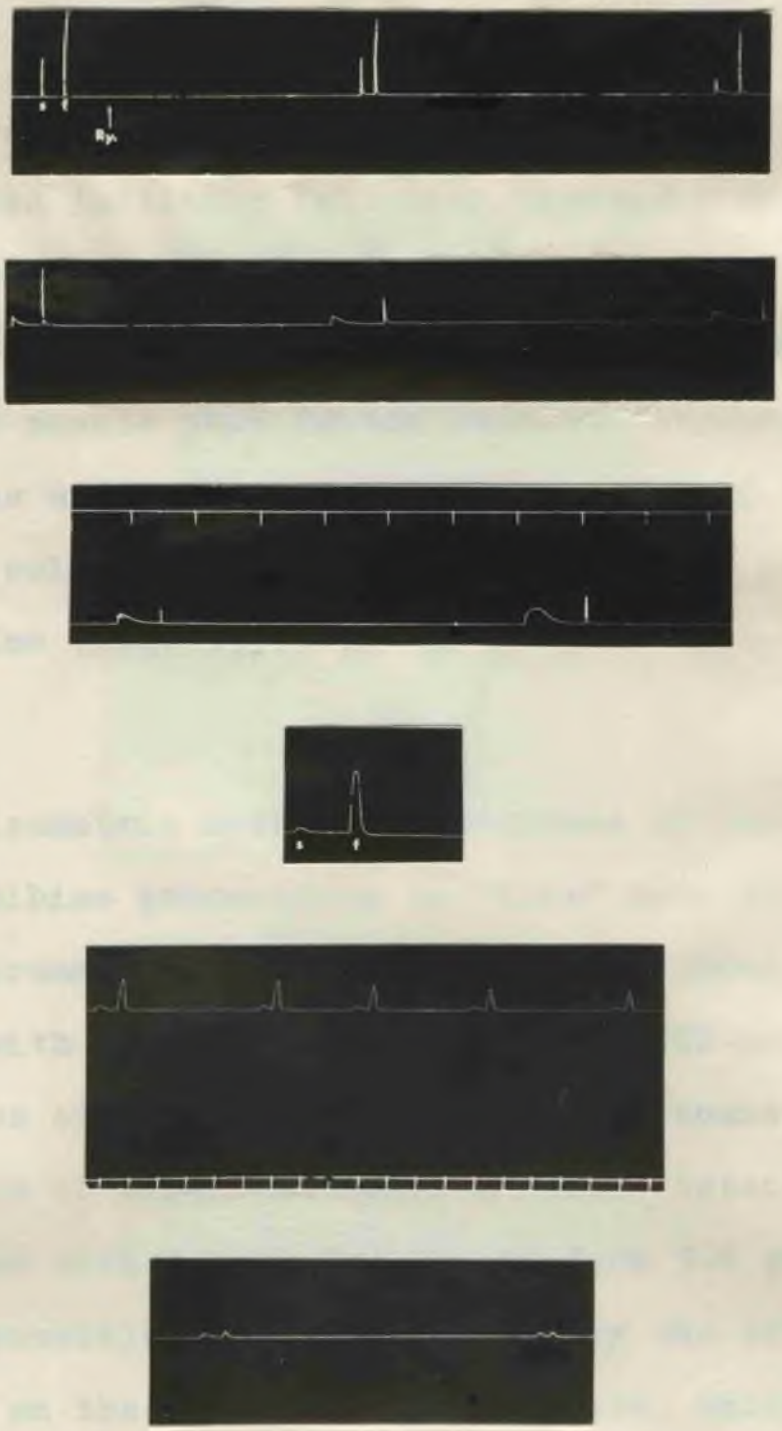


Fig. 64. Effect of ryanodine on the "fast" and "slow" mechanical responses of the locust metathoracic extensor tibiae preparation. Recordings were made with an auxotonic-lever system and kymograph. The records show pairs of responses; the first response of each pair was to "slow" axon stimulation (s) and the second response was to "fast" axon stimulation (f). Both axons were stimulated at 100 c/s. Note initial decline of both "fast" and "slow" responses and decreased rate of relaxation of both responses after treatment of the muscle with ryanodine. Note also that the relaxation of the "slow" response is initially affected to a greater extent that the relaxation of the "fast" response. The apparent recovery of both "fast" and "slow" responses seen in (d) was possibly due to summation of the prolonged but depressed, individual twitch contractions. The first pair of responses in (a) were recorded in normal locust saline. Time calibration in (c) is at 1/min and applies to records (a-d). Time calibration in (e) also at 1/min applies to records (e-f).

frequencies of stimulation an increase in the twitch tension was recorded initially following treatment of both cockroach and locust preparations with 10^{-3}M ryanodine. Hoyle (personal communication) has recorded from the locust spiracular muscle peak twitch tensions assuming tetanic proportions under these conditions. During the later stages of poisoning the twitch response was prolonged for a short time (Fig. 63).

"Slow"axon

The isometric mechanical response of the locust extensor tibiae preparation to "slow" axon stimulation by single supramaximal shocks consists of a weak twitch response with a peak tension of about 0.01 gm. At high frequencies of stimulation the twitches summate and at a frequency of about 150 c/sec a "slow" tetanic response is obtained with a peak tension of from 3-6 gm. It has not been possible to record accurately the effects of ryanodine on the "slow" twitch response, which is very weak, but the effect of the drug on the "slow" tetanic response has been determined (Figs 64 and 65) and compared with the effects of the drug on the "fast" tetanic response (Fig. 64). The rate of depression of the "slow" mechanical response was not as quick as the initial rate of depression of the "fast" response. However, when the "fast" tetanic response had been depressed to the level

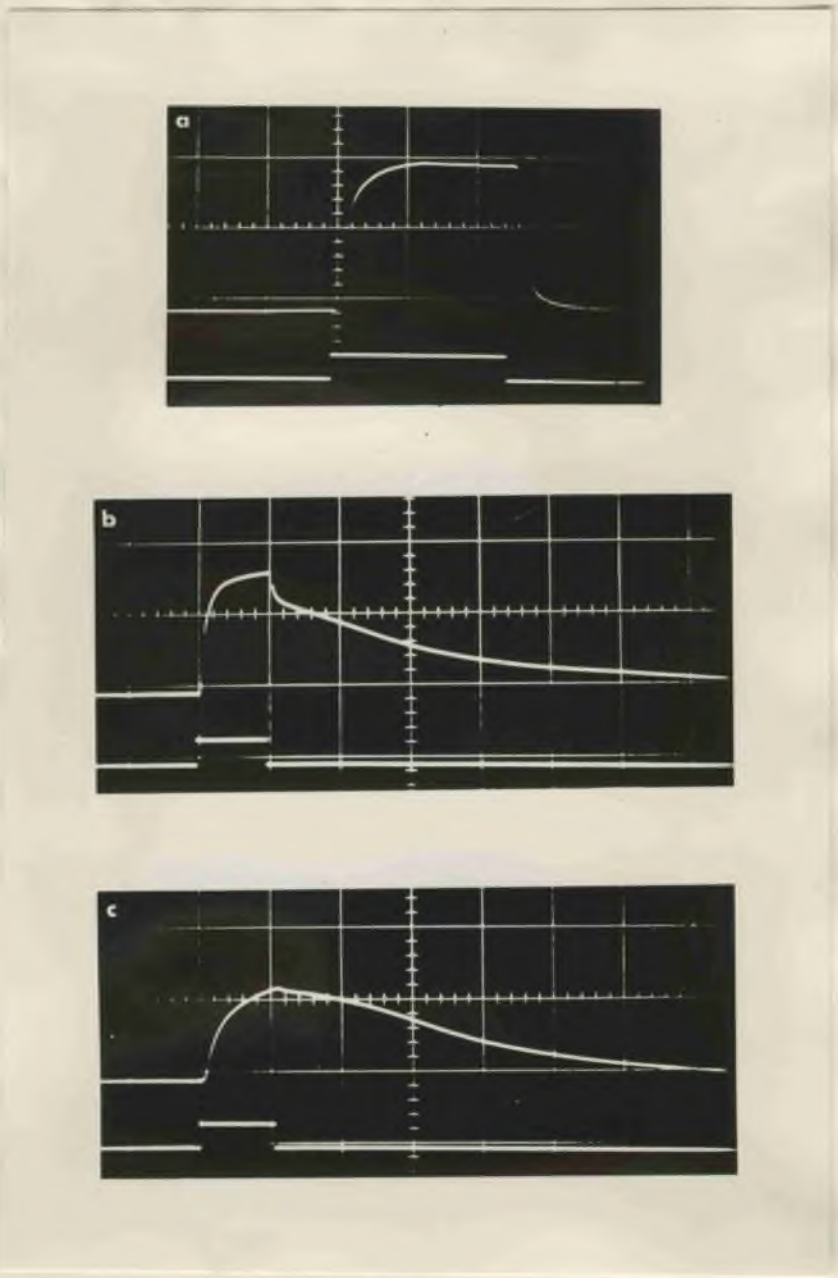


Fig. 65. Effect of 10^{-4}M ryanodine on the isometric mechanical response of the locust metathoracic extensor tibiae preparation to "slow" axon stimulation at a frequency of 50 c/s. (a) Response in normal saline. Response (b) 10 min; (c) 55 min after application of the drug. Duration of stimulation monitored in the lower trace of each record. Note in (b) reduced rate of recovery of response before any marked changes have taken place in total force developed. In record (a) each horizontal division represents 2 sec. In records (b) and (c) each horizontal division represents 5 sec. In all records each vertical division represents 0.9 gms.

of the "slow" their subsequent histories were similar (Fig. 64). Even at this late stage repeated stimulation of the "fast" axon soon resulted in fatigue of the "fast" mechanical response, whereas the "slow" response showed only slight fatigue under prolonged stimulation at any stage throughout the experiment. The rates of relaxation of both "slow" (Fig. 65) and "fast" tetanic responses were reduced in ryanodine-saline.

Effect on nerve conduction

Repetitive firing of locust muscle fibres in ryanodine is not neural in origin except on occasional instances. Fig. 59 illustrates one of the rare instances when the electrical responses of both nerve and muscle showed repetition. A single stimulus elicited two responses from the nerve followed by two muscle spikes; the latter also showed repetition. In most preparations, however, little or no increase in membrane excitability of the innervating motor nerves was recorded. One in about fifty preparations showed some form of spontaneous activity consisting of vigorous contractions of the extensor muscle. Since these contractions consisted of the co-ordinated activity of all the fibres it seems probable that the spontaneity was neural in origin.

Although the effects of ryanodine on conduction of the nerve impulse have not been studied in detail, an increase in the latent period between stimulation and the appearance of the muscle response has been recorded from some preparations. This indicates either a reduction in the velocity of conduction of the nerve impulse or an increase in synaptic delay or both, but the matter was not investigated further.

DISCUSSION

"Slow" and "fast" contractions of vertebrate skeletal muscle. It has long been known that the skeletal muscles of some vertebrates as well as some invertebrates differ widely in their speed of contraction, their ability to maintain tension and in many other properties. Sommerkamp (1928) and later Wachholder and his colleagues (Wachholder and von Ledebur, 1930, 1931; Wachholder and Nothmann, 1932) showed that certain muscles of frogs, and even certain parts of some muscles, exhibited "tonic" properties not possessed by other "non-tonic" muscles of the body. The tonic muscles responded to various stimuli with a sustained contraction. Kuffler and Williams (1953a and b) have since established that there are two functionally distinct nerve-muscle systems in the frog; the small-nerve slow-muscle system, and the large-nerve twitch-muscle system. The twitch-producing muscle fibres respond to nerve stimulation with propagated electrical responses and twitch contractions. Acetylcholine or potassium chloride contracture lasts for only a short period in these muscle fibres. The "slow" muscle fibres, on the other hand, do not give twitch contractions or propagated electrical responses, have a lower membrane excitability (Burke and Ginsbourg, 1956) and give maintained contractures in

acetylcholine or potassium chloride. The resting potentials of the "slow" fibres are less than those of the twitch or "fast" fibres. The two types of fibre also differ anatomically and histologically (Krüger, 1952). In "slow" fibres the contractile system is activated locally at numerous points around multiple small-nerve junctions which are distributed over the surface of the muscle fibre. Vertebrate twitch fibres usually receive a single motor nerve ending, although in some fibres two or three endings are present. It is the innervation pattern which enables the individual "slow" muscle fibres to shorten at multiple points along their length to overcome the shortcomings created by the absence of propagated impulses.

"Slow" muscles and muscle fibres are also found in the skeletal muscles of other vertebrates including man but they do not have analogous functional characteristics to the "slow" fibres of the frog. They all appear to give propagated electrical responses and twitch contractions which differ in duration from those of the "phasic" or "twitch" fibres. In the hind limb muscles of the cat Buller, Eccles and Eccles (1960) have found that at birth all the muscles have similar isometric twitch durations. However, after four months the muscles become divisible into two types, those with brief contractions and those with prolonged twitch contractions. The duration of the

twitch response of the "slow" muscle fibre is about three times that of the "fast" fibre, with the result that "slow" fibres show considerable summation of contractions at relatively low frequencies of stimulation. Buller and his co-workers demonstrated by nerve-cross-union experiments that if a "fast" motor axon is made to innervate a "slow" muscle, the "slow" muscle is transformed to a "fast" muscle; likewise, "slow" motoneurones convert "fast" muscles to "slow" muscles. They ruled out the possibility that the neural influence on muscle speed is exerted by nerve impulses as such and suggested instead that a substance or substances which may act by altering either the contractile properties of the muscle fibres or the duration of the active state or both is transferred across the nerve-muscle synapse. Eccles and Sherrington (1932) have shown that the tonic motoneurones of the cat have smaller diameters than the phasis motoneurones, an interesting similarity with insects. The "slow" motoneurones also have slower conduction velocities and higher thresholds and give smaller spikes.

In vertebrates, gradation of contraction is achieved in two ways; by varying the frequency of nerve impulses and by varying the number of motor units in the muscle active at any one moment. This second method of control,

often termed recruitment, is made possible by the large number of motor axons innervating most vertebrate muscles. Most insect muscles are innervated by only a small number of motor axons so that control of this type is not practicable. Insect muscles innervated by more than one "fast" motor axon may, however, have a simple type of recruitment process similar to the vertebrate plan, but the usual method of tension control in insects apparently involves variation of the rate and extent of membrane depolarisation. Similarly, activation of the "slow" fibres of frog muscle and to a lesser extent the mammalian "slow" fibres can be very finely graded by variations in the frequency of "slow" nerve discharges. "Slow" nerve fibres of both arthropods and amphibians can produce a wide range of tensions, from a scarcely perceptible contraction at low frequency, to a relatively quick and strong contraction during a rapid tetanus. It seems possible that the mammalian system has been evolved as a compromise between loss of contraction speed on the one hand and gain of maintained tension development on the other and still depends to some extent on the recruitment mechanism for tension control. It is possible that evolution of the amphibian "slow" system at the expense of the "fast" explains why this mechanism has not been adopted by the mammals, although it appears so admirably suited to the

function of general postural activity. In this respect a system such as is found in insects, that utilizes the same muscle fibres for both "fast" and "slow" contractions, appears very economical.

Dual contraction mechanisms in invertebrates. Dual contraction mechanisms in invertebrates are not restricted to the arthropod phyla but have been discovered in annelids, tunicates, molluscs and coelenterates. In a few groups the muscles are innervated by two sets of nerve fibres, "fast" and "slow". The short-fibred non-striated proboscis retractor of Phascolosoma, which is innervated by two nerve fibres, when stimulated directly shows two kinds of action potential and two kinds of contraction. Prosser and Sperelakis (1959) have shown that the individual fibres of this muscle have a dual innervation and are presumably capable of performing both types of contraction. In some invertebrate muscles the "fast" and "slow" systems are restricted to separate muscle fibres as in vertebrates. In the adductor muscle of Pecten the two sets of fibres are restricted to different parts of the muscle. Muscles such as the smooth adductors of lamellibranch molluscs maintain a tonic contraction with very little expenditure of energy. In the anterior byssus retractor muscle of Mytilus the tension persists long after stimulation has

ended and the active state has declined to zero (Abbot and Lowy, 1958). This muscle is also able to perform quicker contractions. It is not known whether or not the "slow" and "fast" systems utilize the same contractile elements.

Histological properties of slow- and fast-contracting muscle fibres. Histological differences between the "phasic" and "tonic" muscle fibres of vertebrates were suggested by Krüger (1952) who discovered that the "fast" and "slow" muscles described by the physiologist do not differ only in innervation properties. The "fast" muscles apparently consist of fibres that show a uniform distribution of the fibrils in cross-section (Fibrilenstruktur), whereas in the posture muscles the fibrils are in small groups in which the individual muscle fibres are scarcely discernible. These groups often form angular areas (Felderstruktur). Krüger's results for frog muscle have been confirmed by Gray (1958).

Gray (1957) also found that the "slow" muscle fibres of the frog have characteristic "grape" end-plates, which are morphologically distinct from the end-plates of "fast" fibres. So far, however, direct proof that the different histological arrangements are related to "slow" and "fast" fibres is lacking but would be available if individual fibres known to be "fast" and "slow" could be isolated and sectioned separately and their fibril pattern ascertained.

There are, however, conflicting opinions regarding the correlation of contraction speed with the histological features of the muscle fibres, and Walls (1960) has suggested that in certain cases the histological appearance of the fibres has been misinterpreted. Also Kuschinsky, Lüllman, Hoefke and Muscholl (1956), in an investigation of the function and histological structure of the "fast" and "slow" muscles of the rat, found no relation between the arrangement of the fibrils in the muscle cell and the function of the muscle.

Differences of colour between different muscles or between fibres of the same muscle are also found in vertebrates. In a few mammals, such as the rabbit and the guinea pig, red and white muscles occur separately but in most mammals the muscles are a mixture of red and white fibres. Ranvier (1874) showed that red fibres which are thought to contain more myoglobin than the white, have a slower, more prolonged contraction than the white fibres, but later studies have not left the situation so clear-cut. Denny-Brown (1929) believed that the red pigmentation is 'the outward sign of some function not closely related to contraction'.

The existence of different histological types of muscle fibre in insect striated muscle has been known for

some time (Tiegs, 1955). These types differ from each other in the arrangement of the myo-fibrils, the presence or absence of sarcosomes, the presence or absence of a sarcolemma, etc. In the Coleoptera, Hymenoptera and Diptera the muscles responsible for high-frequency wing movements have a fibrillar structure and contain a high density of sarcosomes, whereas the rest of the flight muscles are tubular in structure. 'A histological distinction can thus be made in these orders between phasic and tonic flight muscles' (Pringle, 1957). Histological evidence for myogenic control of contraction in the leg muscles of the cockroach has been described by Smit (1958). He examined some of the mesothoracic coxal muscles of P. americana using a variety of fixation and staining techniques, on the basis of which he divided the coxal muscles into two categories, A and B.

Type A muscles are characterized by fibres with a large central lumen and radially arranged sarcostyles situated peripherally. Type B muscles have apparently no central lumen and the sarcostyles are not arranged radially. Instead they are distributed either in arbitrary rows or scattered all over the fibre. Smit concluded that muscles 135b and 135d and e are of Type B and muscles 136 and 137 are of Type A. Smit's classification closely parallels

that proposed by Becht and Dresden (1956) on the basis of physiological differences between the different coxal muscles. However, detailed examination of Smit's results has shown them to be rather confused and in fact there appears to be little correlation between his histological data on the one hand and Becht and Dresden's physiological data on the other. For example, Smit found that the lumen and the radial arrangement of the sarcostyles of the Type A muscles were often absent. On other occasions Smit found that frozen sections of the Type A muscles were similar in appearance to those of the Type B muscles. Furthermore, Smit (personal communication) has since obtained frozen sections of muscles 135a and c which were very similar in appearance to those of muscle 135d and e. Muscles 135a and c are, according to Becht, "fast" muscles. If a relation between structure and function in these muscles does in fact exist, one would expect sections of muscles 135a and c to be similar to those of muscles 136 and 137.

Smit concluded that in frozen sections both the "fast" and the "slow" muscles are of Type A, whereas in paraffin sections the "fast" muscles are for the major part of Type A but the "slow" muscles are always of Type B. He explained these differences in terms of different reactions

with the fixation liquids. Unfortunately, he has not stated whether his frozen sections were made from fresh or from fixed material. If they were prepared from fixed material then it is difficult to explain the similarities between the frozen sections of Becht's "fast" and "slow" muscles if the muscles do respond differently to fixation.

One very significant feature of Smit's results is that he has omitted any reference to the fact that muscles 135d and e, according to Becht (1959), contain "fast" as well as "slow" parts. Perhaps he examined only the "slow" parts of these muscles.

According to Becht (1959) both extensor and flexor tibiae muscles of Periplaneta are possibly "mixed" muscles. However, histological examination of these muscles has shown that the fibres of which they are composed fall exclusively into Smit's 'A' category and not, as was expected, into his 'B' category. There is need of a thorough re-examination of the relationship between histological structure and function in the skeletal muscles of the cockroach. It is possible that an examination of these muscles, using electron-microscopic techniques, would solve many of the existing anomalies. The possibility that the small physiological differences recorded from the various coxal muscles in the present investigation are

related to micro-anatomical differences cannot be completely discarded at present.

The relationship between sarcomere length and speed of contraction. It was formerly thought that sarcomere lengths are shorter in the fastest striated muscles, but so many exceptions are known that this statement is no longer valid. A wide range of sarcomere rest lengths has been measured in insect striated muscle but the variations found appear to bear little relation to the speed of contraction of the different muscles. However, Smit (personal communication) has found that the fibres of the different coxal muscles of Periplaneta have different sarcomere rest lengths. Becht (1959) used these findings in support of his claim for three types of muscle in the coxa of the cockroach. An analogy was drawn with crustacean striated muscle where, according to Jasper and Pezard (1934), there is a wide range of sarcomere lengths in situ, and narrowness of striation appears to be correlated with high speeds of contraction. Smit (cited by Becht, 1959) concluded from his results that the "slow" coxal muscles of the cockroach have broad striations and the "fast" muscles have narrow striations. However, Pringle (1957) has concluded that in insect flight muscle the figures for sarcomere rest length in various species

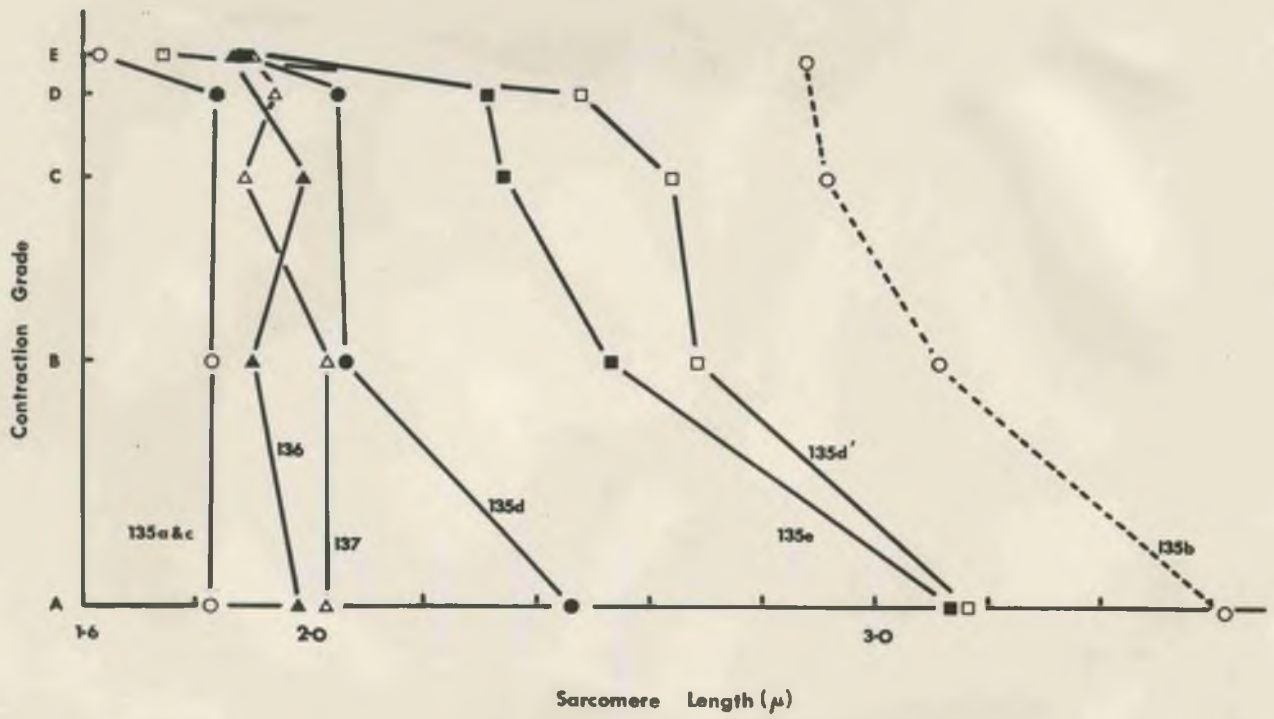


Fig. 66. Sarcomere rest lengths of the mesothoracic coxal muscles of P. americana (after Smit, personal communication). For description see text.

cannot be correlated with histological type or with the speed of contraction as judged by wing beat frequency.

The main results of Smit's investigations are summarized in Fig. 66. These results were kindly supplied by Dr Smit for publication in this thesis. Smit fixed the muscles in what he has termed different contraction grades. Contraction grade A was obtained with the trochanter held at an angle of about 120° to the coxa. With the trochanter in this position the extensor muscles were stretched to the maximum, in situ, length. Contraction grade E was obtained by fixing the muscles at minimum body length with the trochanter held at 180° to the coxa. The intermediate contraction grades B-D were obtained by fixing the muscles with the trochanter held at angles to the coxa between the two extremes described above.

In the light of Becht's conclusions on the control of muscle contraction in Periplaneta, the results summarized in Fig. 66 pose a number of questions. It is undoubtedly true that the sarcomere lengths of Becht's "fast" muscles 136, 137 and 135a and c appear to be considerably shorter than the sarcomere lengths of his "slow" muscle 135b. This is true for all the contraction grades or degrees of stretch investigated by Smit. However, according to Becht, muscle 135d' is a "fast" muscle. It follows

therefore that a narrow type of striation would be expected for this muscle as well; according to Smit this is not the case. In fact, at maximum body length the sarcomere rest length for muscle 135d' is similar to that recorded for the "slow" muscle 135b. Furthermore, a sarcomere rest length similar to that recorded for Becht's "fast" muscle 137 has been recorded for muscle 135d at minimum body length. Muscle 135d has been classified by Becht as a "slow" muscle.

The present investigations on the sarcomere rest lengths of the coxal muscles of the cockroach confirm Smit's findings that at minimum body length slight differences between the sarcomere rest lengths of the different muscles are apparent. However, these differences almost completely disappear if the sarcomere measurements are made with the different muscles fixed at equilibrium length.

Control of contraction in insect skeletal muscle. The results of the present set of experiments, in which the proposed classification of the different coxal and femoral muscles of the cockroach into "fast", "mixed" and "slow" (Wilson, 1954; Becht and Dresden, 1956; Becht, 1959) has been examined critically using the isometric recording technique, suggest that there are no significant intrinsic

physiological differences between the various muscles. The slightly different mechanical responses obtained from these muscles may be explained on the basis of differences in neuromuscular transmission rather than on any inherent variations in the contractile properties of the muscles themselves. This same reasoning applies to the thoracic muscles of the locust. A clear correlation is often seen between the number and type of axons innervating the muscles and the number and type of mechanical responses performed.

All the muscles examined receive an innervation from a motor axon of the "fast" type. Without exception, stimulation of this axon elicits a rather uniform electrical response of the "fast" type, in every muscle fibre it innervates, similar to that found in other muscles of the cockroach and locust (Hoyle, 1955a and c) and in other insect muscles. It consists of a large junctional potential evoking varying degrees of electrical response from the muscle fibre membrane. The magnitude of this electrical response varies from one muscle to the next in the same animal and more particularly between muscles of different animals. For example, the "fast" electrical response of the muscle fibres of Periplaneta was usually larger than that of either Schistocerca and Blaberus. Whether these are in fact real differences or not is

difficult to assess. It is not improbable that they are due to the arbitrary ionic composition of the salines which may not resemble exactly the composition of the body fluids.

The "fast" electrical response is always associated with what may be termed a "fast" mechanical response of the muscle. If the assumption that the muscles of the cockroach differ from each other by their intrinsic properties had been correct, then it was to be expected that stimulation of the "fast" axon or axons supplying the "fast" muscles (Becht and Dresden, 1956) would produce different isometrically recorded mechanical responses from those obtained from "slow" muscles stimulated via their "fast" motor axons. This expectation has not been realized in the present set of experiments. In every muscle or muscle group examined, the response to "fast" axon stimulation is, without exception, very similar in character. In the cockroach the response to a single stimulus consists of a twitch contraction lasting about 40-70 msec, with a rise-time to peak tension of 10-14 msec and a half-decay time of 9-20 msec. Nevertheless, the isometric twitch response of Becht and Dresden's "slowest" muscle, 135b, has a duration slightly longer than that of their fastest muscles. However, in comparing these results it must be borne in mind that recordings taken from 135b can be

obtained only with some difficulty and that Becht and Dresden failed to record kymographically any response to a single shock from this tiny muscle using their isotonic technique.

What is perhaps more significant is that the isometric twitch responses of their "mixed" muscles, groups 138 and 139c, and 135d and e and possibly the flexor and extensor tibiae muscles are usually no greater in duration than those of their "fast" muscles and may be even briefer in duration. The difference between an isometric twitch duration for muscle group 138 and 139c of about 40 msec and an isotonic twitch duration of over 1 sec is so great that a ready explanation for it is not forthcoming. It is possible that the lever system employed by Becht and Dresden did not enable them to record an accurate picture of the mechanical responses of the smaller muscles of the coxa. The system consisted of a writing pin, attached to the femur by a piece of straw, which recorded the movements of the trochanter on a horizontal kymograph. The dicondylic hinged joint connecting coxa to trochanter acted as the fulcrum of their lever.

Hill (1938) showed that for the frog sartorius muscle the velocity of shortening and the total shortening of the muscle in an isotonic contraction depend on the difference

between the external load on the muscle and the maximum force that it can develop. This relation has since been shown to apply to a number of other muscles (e.g. Wilkie, 1954), including insect muscles (Buchthal, Weis-Fogh and Rosenfalck, 1957), and there is no reason to suppose that the skeletal muscles of the cockroach are any different in this respect. Becht and Dresden made no attempt to ensure that the loads on the different coxal muscles were comparable in terms of gm/cm^2 of muscle cross-section. Therefore, even if it is assumed that the inertia of their recording device was small enough to be neglected, the results they obtained for the different muscles, which have very different cross-sectional areas, are still not strictly comparable. Furthermore, their recordings from muscle 135b, the smallest of the coxal group, were made using a writing arm three times as long as that used to obtain recordings from the other coxal muscles. It is possible that the weight of the two writing arms was the same although Becht and Dresden do not state this specifically in their report. Apart from this criticism of their technique on the basis of the relative mass of the lever system, it cannot be reasonably supposed that the inertia of the system was negligible. If the inertia of the system has to be taken into account, then it is not surprising that they obtained different types of myograms from the

different muscles. On the other hand, there is no doubt that the condition of the preparation at the time the recordings are made plays a large part in determining the characteristics of the isometric myograms obtained. Even in the present set of experiments, deterioration of the preparation resulted in an increased twitch duration as well as a fall of peak twitch tension. Therefore, it is possible that an explanation for the discrepancies in the two sets of results may be found in different conditions of the preparations at the time of recording.

The flexor tibiae muscle of the cockroach and locust and many of the larger muscles of the coxa of the cockroach are innervated by more than one motor axon of the "fast" type, each axon with its own distinct excitation threshold. By graded excitation of the motor nerve innervating these muscles it is possible to stimulate the axons in turn. Each of the "fast" axons appears to innervate a separate bundle of muscle fibres, dividing the muscle up into a number of "fast" motor units.

It has been demonstrated that in some insect muscles the "slow" mechanical response to low-frequency stimulation also consists of twitch contractions, therefore it is perhaps best to describe the responses to "fast" axon stimulation as "fast" twitch contractions. In some muscles,

e.g. muscles 136 and 137 of the cockroach, the twitch contraction appears almost all-or-none in character; the muscle is stimulated to almost maximal activity by a single shock. In other muscles, e.g. 135, 138 and 139c of the cockroach and the femoral muscles of the cockroach and locust, the maximal response is obtained only at high stimulation frequencies. In these muscles a single stimulus evokes a "fast" twitch contraction which is much smaller in magnitude than the tetanic response. It is only through twitch summation at high frequencies of stimulation that the maximum response of the muscle is achieved. These differences of mechanical responsiveness do not appear to be associated with any differences in electrical responsiveness.

That different muscles vary in their mechanical responsiveness is illustrated by the different twitch/tetanus force ratios that they possess. To explain the low twitch/tetanus ratio of the locust extensor tibiae muscle, Hoyle (1955c) suggested that the contractile material is not fully activated by a single muscle depolarisation, however complete, but that more than one depolarisation is required for full activation. The shortness of the muscle fibres and the apparent absence of any elastic component in series with the contractile elements adds some weight to this suggestion. However, Buchthal et al. (1957) suggested that

the contractile elements themselves might possess a certain amount of elasticity. If the elasticity of the contractile elements of different muscles varied to any extent then, other factors being equal, it is to be expected that variations in twitch/tetanus ratios will occur. If it is assumed that complete activation of the contractile elements of insect muscle fibres follows a single stimulus as in vertebrate muscle (Hill, 1951), then it is possible to explain the variations of twitch/tetanus force ratio in terms of differences between (a) rates of decay of active state, (b) maximal shortening velocity, and (c) compliance of the series elastic component.

If the active state of the muscle is of short duration, relaxation begins before the contractile elements have had time to completely stretch the series elastic components. Unfortunately, very few measurements of active state duration in insect muscle have been made. Pringle (1954, 1957) published an active state curve for the tymbal muscle of the cicada. In this muscle the duration of the active state was relatively short, which could possibly account for the low twitch/tetanus ratio recorded from it. A longer active state duration has been calculated for the locust flight muscle by Buchthal et al. (1957).

In vertebrates, where activation of the muscle is

all-or-none, during the early part of the twitch the muscle is fully active and behaves as though it were tetanised; hence the early part of the twitch curve coincides with that of tetanus. The point where the twitch and tetanic curves separate indicates the beginning of the decline of the active state (Macpherson and Wilkie, 1954). If a second shock is applied so that the plateau of full activity is maintained it is possible, by comparison, to arrive at an approximate value for active state duration without resorting to involved tetanic stimulation techniques. This method was applied to the extensor tibiae muscles of Schistocerca and Blaberus. Bearing in mind that the fibres of these muscles are not stimulated simultaneously, approximate values for active state duration may be obtained. The locust muscle with its very low twitch/tetanus force ratio of about 1/10 (Hoyle, 1957) has an active state duration for the twitch of about 21 msec (mean of 23 readings), whereas the cockroach muscle with its slightly higher twitch/tetanus force ratio of about 1/5 has an active state duration for the twitch of about 10.6 msec (mean of 27 readings). Obviously there is little relation between the calculated durations for active state and the twitch/tetanus force ratios of the muscles. If the ratio were dependent on the duration of the active state, then the value for locust muscle should be lower than that of the

cockroach muscle. Obviously some other factor is responsible here.

Unfortunately, neither the maximal shortening velocity nor the series elastic component of these muscles has been calculated. The cockroach coxal muscles 135a, b, c, d and e have been shown to contain more lipids and to possess a stronger reducing capacity for methylene blue than muscles 136 and 137 (Smit, 1958). Furthermore, histochemical investigation of these two sets of muscles has shown that there is more lipase activity in muscles 135b, d and e than in muscles 136 and 137 (George and Bhakthan, 1961). The presence of lipids in conjunction with the contractile proteins could increase the compliance of the muscle fibres and thereby explain the lower twitch/tetanus ratios recorded from muscles 135a, b, c, d and e than from muscles 136 and 137. In this respect it is possible that the very low twitch/tetanus force ratios recorded from some of the preparations of muscle 135b were not due to a deterioration of the preparation as was suggested earlier but were the result of a relatively more compliant elastic component in these muscles. However, this would not explain the relatively high twitch/tetanus force ratios recorded from other preparations of muscle 135b unless, as intimated by George and Bhakthan (1961), the lipid content of muscle 135b varies from animal to animal.

It is hoped that in the near future it will be possible to examine in detail the mechanical properties of at least some of these insect muscles using a light isotonic lever, modified by the author from a design by Wilkie (1956).

A comparison between the mechanical performances of the muscles studied in the present investigations shows how remarkably uniform insect skeletal muscles are in this respect. In terms of unit cross-sectional area, the maximum active tensions developed by every muscle at maximum body length are very similar. All the muscles produce maximum tensions of about 1000 gm cm^{-2}. Hoyle (1955c) quoted a figure of 1000 gm cm^{-2} for the locust extensor tibiae muscle; Wood (1957) quoted a figure of 800 gm cm^{-2} for the flexor tibiae muscle of Carausius.

Most of the muscles examined are relatively inextensible with the passive tension rising steeply at lengths greater than the minimum body length. In all these muscles the isometric tension is maximal at about maximum body length.

Differences in duration of the isometric twitch contractions of the various muscles examined within any one animal were small. However, the twitch durations of muscles of different animals often show considerable differences. This is especially evident if the twitch responses of

cockroach and locust muscle are compared. On the other hand, the isometric twitch contraction of Blaberus muscle differs in duration from that of Periplaneta by only a very small amount. It is possible to correlate the differences between the responses of the cockroach and locust with differences in active state duration, and with differences in duration of the electrical response. The duration of the "fast" electrical response of Periplaneta muscle fibres is almost half that of locust muscle fibres (see also Hoyle, 1955a), whereas action potentials of durations only slightly longer than those recorded from the muscle fibres of Periplaneta have been recorded from the muscle fibres of Blaberus. If there is a causal relationship between the electrical and mechanical events it follows that an increase in the duration of the electrical response should lead to an increase in the duration of the mechanical response. Experiments with ryanodine have shown that prolongation of the electrical response, which occurs soon after treatment with the drug, is associated with prolongation of the mechanical response.

One of the most constant features of the "fast" mechanical response of these insect muscles is its marked fatiguability. The mechanical response to "fast" axon stimulation at tetanic frequencies is maintained for only a

very short time before it declines to zero. In some muscles, e.g. muscles 136 and 137 of the cockroach, fatigue of the "fast" response occurs at relatively low stimulation frequencies. These muscles, which are white in colour, are only sparsely supplied with sarcosomes, whereas muscles 135a and c, which are red in colour and fatigue more slowly, are richly supplied with sarcosomes (Smit, personal communication). In dipteran muscles the sarcosomes have been shown to be mitochondria containing enzymes for oxidative phosphorylation (Watanabe and Williams, 1951). In the Odonata the presence of sarcosomes has been correlated with need for a high sustained rate of metabolism.

George and Bhakthan (1961) suggested that the high concentration of lipase in, and the high fat content of, the cockroach coxal muscles 135b, d and e are indicative of more fat being split for energy utilization. This might explain why the maximal mechanical response of these muscles is slightly less fatiguable than the maximal response of muscles 136 and 137. It is interesting to note that the red skeletal muscles of vertebrates, which fatigue very slowly, take up fat stain more readily than the white muscles.

If a causal relationship exists between the electrical and mechanical responses of these insect muscles, then it is possible that fatigue of the mechanical response would

be associated with depression of the electrical response. This phenomenon has been recorded from a few muscles but on other occasions the electrical response appeared only slightly reduced while the mechanical response had already fatigued to a very low level. However, an exact determination of the correlation between the electrical and mechanical events of muscular activity in insects is difficult to achieve with present techniques. Attempts so far have been restricted to comparing the mechanical response of a complete muscle with the electrical response of a single fibre of that muscle. Until the two events have been compared in a single muscle fibre preparation, any correlation between them should be viewed with extreme caution.

The cockroach muscle groups 136 and 137, 135a and c, and 139a and b and 140 and the locust muscles are innervated by axons of the "fast" type only. The other coxal muscles of the cockroach, the femoral muscles of the cockroach and the other thoracic muscles of the locust that were examined are innervated by one or more "slow" axons together with one or more "fast" axons. Since many of the fibres of the muscles innervated by both "slow" and "fast" motor axons are dually innervated it seems possible that they are capable of performing both "slow" and "fast" contractions. Stimulation of the "slow" axon evokes electrical responses

described as junctional potentials, often showing considerable facilitation with repetitive activation.

"Slow" systems have been previously demonstrated in the extensor tibiae muscle of Periplaneta (Pringle, 1939), Schistocerca and Locusta (Hoyle, 1955c) and in the flexor tibiae muscle of Carausius (Wood, 1957). In the coxal and femoral muscles of the cockroach the electrical responses evoked through stimulation of the "slow" axon are similar to the S_{1b} responses of the locust (Hoyle, 1955c). In some of the thoracic muscles of Schistocerca and Locusta, "slow" electrical responses not unlike Hoyle's S_{1a} potentials have been recorded from a few fibres. These consist of slow depolarisations of the muscle fibre membrane which reach little more than 1 mV in magnitude. Responses of the S_{1b} type are post-synaptic potentials which vary in magnitude between 2-30 mV. The larger responses sometimes elicit small local membrane responses which propagate decrementally. The smaller responses show marked facilitation with repetition and all the S_{1b} responses, except the largest, summate at high frequencies of stimulation. The "slow" electrical responses of the muscle fibres are always associated with characteristic mechanical responses, which at low frequencies of stimulation are so small in most of the muscles that they are barely recordable, but which at higher frequencies of

stimulation summate to give a smooth response showing little sign of fatigue. In the locust metathoracic extensor tibiae muscle, stimulation of the "slow" motor axon with a single supra-threshold shock evokes a minute twitch contraction. The "slow" twitch contraction appears to be a minute replica of the "fast" twitch. A "slow" twitch response contraction has been observed in, but not recorded from, some of the coxal and femoral muscles of the cockroach. In the locust it is best recorded with the muscle stretched to maximal body length.

At high frequencies of stimulation the "slow" twitches summate and fuse to give a slow, smooth contraction. In the present experiments the maximum peak "slow" tension recorded from any one muscle was lower than the maximum peak "fast" tension of that muscle but could be maintained for long periods without any signs of fatigue. In a few preparations of the locust extensor tibiae muscle slight fatigue of the "slow" response occurred initially; the tension was then maintained at the new, lower, peak level.

Stimulation of a "slow" motor axon always evokes a mechanical response of the "slow" type. On the other hand, it is not possible to evoke a mechanical response of the "slow" type from muscles which are innervated solely by motor axons of the "fast" type.

The results of the present investigations do not support the suggestion (Wilson, 1954; Becht, 1959) that in some of the coxal and femoral muscles of the cockroach the "slow" and "fast" systems are restricted to specialized muscle fibres. Wilson's observations on the flexor tibiae preparation of the cockroach were correct in so far as he obtained "fast" electrical responses from some fibres and "slow" electrical responses from others. However, if he had attempted to stimulate for these responses through the motor nerve innervating this muscle, he would probably have discovered that a proportion of his "slow" muscle fibres give both "fast" and "slow" responses.

The functions of the "slow" and "fast" mechanical responses in the locust have been studied by Hoyle (1957). He suggested that the "fast" response of the metathoracic extensor tibiae muscle was employed for jumping and that other less vigorous movements were achieved through the "slow" system. Since responses to "slow" axon stimulation are graded events, a fine central control of frequency of "slow" impulses could result in either a very delicate or a very strong contraction, as required.

In the locust there is a second "slow" motor axon which is thought to be responsible for very slow movements and for the maintenance of tonus (Hoyle, 1955c). The occurrence of a similar type of axon innervating the muscles

of the cockroach has not been demonstrated, although it is possible that more than one "slow" axon innervates some of the muscles of this animal. Furthermore, although only one "fast" and one or possibly two "slow" responses have been recorded from the extensor tibiae preparation of Blaberus, histological examination of this muscle has shown it to be innervated by at least seven axons. It is not impossible therefore that the responses of this muscle are more complicated than suggested by the results presented in this thesis. On the other hand, in those muscles which are known to receive only one "slow" axon it is possible that maintenance of tonus and also slow movements are performed by the same system.

Intracellular recordings from single muscle fibres of the locust S. gregaria (del Castillo, Hoyle and Machne, 1953; Cerf, Grundfest, Hoyle and McCann, 1959; Hill and Usherwood, 1961) have shown that the properties of the electrically-excitable activity are similar to those of vertebrate all-or-none decrementlessly propagated responses except for the small size of the maximal potentials and the decremental propagation which is associated with graded responsiveness. The small size and local nature of both "fast" and "slow" electrical responses of insect muscle fibres are offset by the nature of the distributed end-plate

innervation which ensures a uniform depolarisation of the muscle fibre. Multiple endings in single muscle fibres were first described in insects by Foettiger (1880). Marcu (1929) found that the fibres of the thoracic muscle of Geotrupes and Musca had a multi-terminal innervation. Weiant, cited by Hoyle (1957), has described endings at intervals of about 40μ in the leg muscles of Periplaneta. Smit (personal communication) has described multiple endings on the thoracic muscles of this insect and Hoyle (1955b) has described a similar situation for the leg muscles of Locusta. In some insect muscles, however, a uni-junctional relationship between synapsing peripheral nerve endings and single muscle fibres has been described (Edwards, Ruska and de Harven, 1958a and b). Edwards (1959) forwarded electron-microscopic evidence for a multi-terminal innervation of cockroach abdominal muscle and Smit (personal communication) has evidence for a similar type of innervation for some of the coxal muscles of this insect.

It is concluded that in many insect muscles the same muscle fibres are utilized for both "slow" and "fast" contractions but the question of how a single muscle fibre can contract in two different ways is left mainly unanswered. It has often been suggested that the "slow" and "fast" contractions of dually-innervated muscle fibres may occur

in separated cytoplasmic divisions of the muscle cell; "fast" contractions are supposed to occur in the fibrils and the "slow" contractions in the non-fibrillar sarcoplasm (Bozler, 1930; Hoyle, 1957). An alternative suggestion restricts the two types of mechanical response to the same contractile substrate.

Perhaps the greatest barriers, which exist at present, to further understanding of the differences between "fast" and "slow" contractions in insect muscle are the complete lack of any information on the nature of the transmitter agents or agent involved in neuromuscular-synaptic transmission and on the mechanism coupling excitation to contraction. Acetylcholine (ACh) has been tested on the insect peripheral and central nervous systems with little success. Although this compound is found in considerable quantities in different parts of the insect body and despite the fact that Harlow (1958) found that ACh produced a tetanic contraction of the intact leg of the locust, it is generally concluded that neuromuscular and perhaps central transmission in insects is not cholinergic (e.g. Twarog and Roeder, 1957; Hill and Usherwood, 1961). Davy (1960) has suggested that an o-dihydroxyindolalkylamine may have an excitatory role in the insect peripheral nervous system and that its action may be inhibited by 5-hydroxytryptamine

(5-HT). 5-HT and a number of related compounds do in fact block transmission in locust and cockroach peripheral nerve-muscle systems (Hill and Usherwood, 1961) but so does the o-dihydroxyindolalkylamine that Davy (1960) has isolated from the cockroach accessory organs (Usherwood, unpublished). It is possible, however, that the highly specific action of 5-HT on the insect peripheral synapse is due to competition by 5-HT with a structurally similar transmitter agent for receptor sites on the post-synaptic membrane.

Bay, Goodall and Szent-Gyorgyi (1953) and Csapo and Suzuli (1957) proposed a hypothesis to link contraction in vertebrate skeletal muscle to the electric field which is set up by the propagated action potential. This hypothesis, sometimes termed the 'window-field hypothesis' has been disputed by Sten-Knudsen (1954) and, because of the absence of propagated responses in most normal insect and crustacean muscles, cannot in any case be applied to the arthropods. One possibility that has been tentatively suggested (Fatt and Katz, 1953) is that contraction in crustacean muscle starts at a particular level of membrane potential. This hypothesis is in line with the currently accepted hypothesis of excitation in smooth muscle and in the "slow" muscle system of the frog. Indirect support for a hypothesis of this nature for insect muscle has been obtained

pharmacologically. Hill and Usherwood (1961) found that although 5-HT blocked neuromuscular transmission in insects it did not affect the electrical excitability of the muscle-fibre membrane. After treatment with 5-HT, stimulation of the "fast" motor axon evoked neither electrical nor mechanical responses from the muscle fibre. However, if the muscle fibre membrane was then stimulated directly with depolarising current pulses the fibre contracted. It appears that 5-HT inhibits the mechanical response of insect skeletal muscle by virtue of its action on the electrically-inexcitable response of the muscle-fibre membrane.

Although there appears to be a relationship between the electrical and mechanical responses of insect muscle, this relationship is sometimes not very apparent in crustacean muscle. For example, in the dually-innervated closer muscles of the brachyuran Randallia ornata and the anomuran Blepharipoda occidentalis, stimulation of the "slow" closer axon at low frequencies results in a smooth tetanic response, yet stimulation of the "fast" axon under the same conditions produces no response at all. Microelectrode studies have demonstrated that the accompanying potentials are very small on "slow" and quite large on "fast" axon stimulation (Wiersma and van Harreveld, 1938; Hoyle and Wiersma, 1958). Since all the muscle fibres

are innervated by both axons the "slow" response cannot result from the contractions of specialized muscle fibres. It is possible, however, that this 'paradox state' is not normal for these muscles. It is significant that Hoyle and Wiersma (1958) found that at the start of their experiments with Randallia the contraction in response to "fast" axon stimulation was larger than the response to the "slow" axon at the same frequency. As an alternative to Fatt and Katz's hypothesis, Hoyle and Wiersma (1958) suggested that the crustacean excitatory transmitter agents could possibly be affecting the coupling mechanism in one of three different ways. Firstly, these substances could cross the muscle fibre membrane and act directly on the coupling process. Secondly, they could cause an increased permeability to a specific ion which in turn affects the coupling mechanism. Thirdly, the transmitter substances could initiate the release of a substance which in turn diffuses inwards and affects the coupling mechanism. They suggest further that the electrically-excitable response, when it occurs, 'boosts' the excitation of the coupling mechanism. A further hypothesis might be postulated for insect muscles. It is possible that in insects the "fast" and "slow" transmitter agents are one and the same substance released in different quantities at the two types of nerve ending. The larger post-synaptic responses recorded to

"fast" axon stimulation could be correlated with the greater quantities of transmitter agent released by the "fast" pre-synaptic regions. A direct coupling between excitation and contraction would mean a larger mechanical response to stimulation of the "fast" axon. It is interesting to note that treatment of insect muscle fibres with ryanodine results in an increased electrical response which is associated with an increased mechanical response. This phenomenon could be explained by a 'direct-coupling' hypothesis. Furthermore, it is possible that there is not only a link between the magnitudes of the electrical and mechanical responses but that the temporal characteristics of the two processes are also linked in some manner. This would account for the slightly slower relaxation of the "slow" mechanical response and for the increased duration of both "fast" and "slow" mechanical responses following treatment with ryanodine.

As an alternative to the hypothesis that the "slow" and "fast" responses of insect muscle differ only in degree, it is possible that the transmitter agents liberated at the two sets of nerve endings are in fact different chemical compounds, and that their actions on the contractile process are different. The transmitters may excite the contractile process with the formation of linkages in a sliding-filament

acto-myosin system, with the linkages formed to "slow" axon stimulation differing from those formed to "fast" axon stimulation. A slower making and breaking of the "slow" linkages could account for the slower relaxation and rate-of-rise of the "slow" mechanical response. A mechanism of this type has been postulated for the anterior byssus retractor muscle of Mytilus (Lowy and Millman, 1959) to account for the phasic and tonic responses recorded from this muscle. Lowy and Millman suggested that the "tonic" linkages may be associated with a paramyosin filament system.

The advantages to an animal of having muscles capable of two types of mechanical responsiveness are obvious. The "tonic" response which appears to be widespread throughout the animal kingdom is important for maintenance of muscular tone and possibly for the slower movements of the animal. Obviously a fatiguable "fast" system with its high energy requirements would be of little value for performing these functions. In vertebrates the large number of motor units present in the skeletal muscles has made possible the evolution of specialized "slow" muscle fibres. In insects, however, where the number of motor units in a single muscle is very small and where economy of space is highly important, the same muscle fibres are often used for both "fast" and "slow" activity. Due to the graded-electrical responsiveness

of insect muscle a large variety of tensions may be developed by a single muscle by varying the frequency of stimulation. Further graduation of contraction is achieved by virtue of the graded-mechanical responsiveness apparently found in many insect muscles. It is true that some insect muscle fibres are innervated by a "slow" axon only and are capable presumably of performing only "slow" mechanical responses. The electrical properties of these fibres do not differ apparently in any way from those of fibres innervated by a "fast" axon although it is possible that the "slow" fibres may have structural peculiarities as adaptations to a "tonic" function. The present investigations, however, have not revealed any structural peculiarities.

The action of ryanodine. Edwards et al. (1948) concluded from their experiments on the skeletal muscle of the cockroach that ryanodine inhibits the mechanical activity of the muscle but does not have any effect on neuromuscular transmission or on excitation and conduction of either nerve or muscle. In the present set of experiments it has been demonstrated that the drug not only interferes with the contraction mechanism but also has marked effects on the electrical properties of insect skeletal muscle. In many experiments the effects of

ryanodine on the "fast" and "slow" mechanical responses to nerve stimulation were recorded before any changes in the electrical events were recorded. From these results it appears that the drug is acting independently on the two processes, at least during the initial stages of poisoning. Edwards et al. (1948) suggested that ryanodine inhibits the mechanical responses of insect skeletal muscle by its action on the phosphagen-system supplying energy for contraction. The increased oxygen output of insect muscle observed during the preliminary stages of poisoning (Hassett, 1948; Roeder et al., 1948) added support to the suggestion of some form of metabolic disturbance. However, it is possible that the drug either partially or completely uncouples the link connecting the process of excitation and contraction.

The reduced rate of recovery of the locust isometric "fast" twitch response recorded during the later stages of ryanodine poisoning was similar to that obtained by Blum et al. (1957) for ryanodinised frog sartorius muscle. In both the cockroach and the locust preparations prolongation of the twitch resulted in the appearance of a contractural response due to a summation of successive twitch contractions at relatively low frequencies of stimulation. The manner in which ryanodine affects the recovery phase of

the twitch response is difficult to explain. If it is assumed that relaxation is a purely passive process then some physical action on the muscle must be postulated. On the other hand, if it is assumed that relaxation is an active process, the action of the drug could be through some form of metabolic disturbance. Alternatively it may be related to the reduced rate of membrane repolarisation following the muscle action potential. This would require that the rate of relaxation of the mechanical response is dependent upon the rate of membrane repolarisation, i.e. a causal relationship would hold.

Both "fast" and "slow" mechanical responses of the locust muscle in high drug concentrations are initially increased in magnitude if the preparation is stimulated intermittently. Under these conditions mechanical inhibition does not occur until a late stage. With short time intervals between successive stimuli the main action of the drug is an inhibition. The different results show that the inhibitory action of the drug is enhanced in some manner by stimulation of the muscle fibre. The drug may enter more readily the active than the resting fibre. The increased mechanical response at low stimulation frequencies is correlated possibly with the increased magnitude and duration of the electrical response which occurs at the same time.

The effects of ryanodine on the electrical activity of insect muscle did not show as much variation as the effects of the drug on the mechanical activity. In many experiments an increase in magnitude and duration of both "slow" and "fast" electrical responses was recorded initially with the formation of a marked plateau of repolarisation. Narahashi and Yamasaki (1960) found that the negative after-potential of the cockroach giant axon is increased both in magnitude and duration following treatment with DDT and that there is a brief period during which repetitive responses may be induced by a single shock. Although the action of ryanodine on the electrical properties of insect nerve has not been studied in detail its action on the electrical properties of insect muscle fibres is very similar to the action of DDT on the nerves. It has been suggested that under the influence of DDT the ionic mechanism responsible for the production of nerve action potentials with long-lasting plateaus of repolarisation differs from the ionic mechanism responsible for the normal nerve action potential of the insect. Narahashi and Yamasaki (1960) suggested that the plateau results from a partial suppression and delay in the rise of the potassium conductance causing a decreased rate of repolarisation of the membrane; the magnitude and duration of the plateau would depend on the

degree of potassium conductance inactivation. In terms of the ionic hypothesis (Hodgkin and Huxley, 1952) it is possible to explain graded responsiveness as demonstrated by insect muscle fibres on the basis of a high potassium conductance either at rest or at an early stage during the depolarisation phase (Werman et al., 1961). A partial suppression and delay in the rise of the potassium conductance would result in much larger depolarisations with the possible appearance of all-or-none responses. A reduction in the potassium conductance would lead to a prolongation of the action potential, through repression of repolarisation, with the formation of a plateau following the spike.

During the preliminary stages of ryanodine poisoning the "fast" action potentials are potentiated but do not show an increased duration. During this period positive undershoots frequently follow the spike responses and hypopolarise the membrane by as much as 15 mV. It is possible that during this brief period the potassium conductance is very high, which would explain the undershoot, and that the potentiated spike results from an increased sodium conductance.

The manifestation of repetitive activity during ryanodine poisoning may be explained on the basis of a

maintained high sodium conductance and a maintained low potassium conductance. With repeated stimulation of the membrane it has been demonstrated that the plateau following each response is greatly reduced or even abolished, and that the duration of the primary spike is considerably diminished. It seems possible that under these conditions the potassium conductance is maintained at a relatively high level. This would lead to a much faster repolarisation of the membrane.

Narahashi (1960) found that the negative after-potential of the cockroach giant axon, induced by treatment with isotonic barium, was also reduced by repetitive stimulation. He suggested that the potassium conductance during the falling phase of the after potential is increased slightly with each impulse.

Werman et al. (1961) suggested that Ba^{++} not only reduces the resting membrane conductance of arthropod muscle fibres but also reduces sodium inactivation. Changes in resting conductance were indicated by the increased membrane resistance following Ba^{++} treatment and the loss of rectification of the membrane. In the present experiments ryanodine undoubtedly caused an increase in resting membrane resistance, at least initially. However, preliminary experiments with radioactive tracers indicate

that there is very little change in the magnitude of either the sodium or the potassium flux of the resting membrane of locust muscle fibres soaked in ryanodine (Dr Ahmad, personal communication).

The results of the present experiments fail to confirm Becht's conclusions that in ryanodine the "fast" mechanical response of insect muscle is inhibited before the "slow". It is concluded that the effects of ryanodine on the "fast" and "slow" contractions of insect muscle are correlated with the different electrical changes which characterize the various stages of poisoning.

The results presented in this thesis mainly support the view (Hoyle, 1957) that control of contraction of insect skeletal muscle is principally nervous in origin and not myogenic as suggested by Wilson (1954), Ewer (1957) and Becht and Dresden (1958). It is concluded that the neuromuscular mechanisms of the muscles studied in the course of these investigations are essentially similar to those described for the locust (Hoyle, 1955c) and the stick insect Carausius (Wood, 1958) and that the different mechanical responses recorded from the different muscles can be explained mainly on the basis of differences in their innervation properties.

SUMMARY

1. The anatomy and innervation of the mesothoracic coxal and femoral muscles of the cockroaches, Periplaneta americana and Blaberus giganteus, have been described. The innervation of the metathoracic femoral muscles and the anatomy and innervation of some of the thoracic muscles of the locusts, Schistocerca gregaria and Locusta migratoria, have been studied.

2. Histological examination of the sarcomere lengths of the muscle fibres of the coxal muscles of Periplaneta and Blaberus has shown that the sarcomere rest lengths of the various muscles are not significantly different and that it is not possible to correlate the small differences that have been recorded with differences in the physiology of these muscles.

3. The mechanical and electrical responses of the various muscles and muscle groups of the coxal and femoral segments of the mesothoracic legs of Periplaneta and Blaberus have been examined simultaneously. The results of recordings made from the metathoracic femoral muscles of the locust have been compared with those obtained from the cockroach. Mechanical recordings were made

isometrically with the coxo-trochanteral and femoro-tibial joints disarticulated. The electrical responses of the muscle fibres were examined with the aid of glass capillary intracellular micro-electrodes.

4. The electrical events of neuromuscular transmission in some of the thoracic muscles of the locusts, Schistocerca and Locusta have been described. The mechanical responses of these muscles to reflex and indirect electrical stimulation have been recorded visually.

5. Every muscle or muscle group is innervated by a "fast" motor axon. This elicits a uniform type of electrical response which is associated with a uniform type of mechanical response. The "fast" electrical responses, which are markedly refractory, are large post-synaptic potentials which evoke varying degrees of electrical response from the muscle fibre membrane. In the muscles of Blaberus and Periplaneta the mechanical responses to low-frequency "fast" axon stimulation consist of twitch contractions lasting 40-70 msec with rise-times to peak tension of 10-16 msec and half-decay times of 9-21 msec. In the femoral muscles of the locust the "fast" isometric twitch contraction has a rise-time of about 50 msec and a half-decay time of about 60 msec.

6. At high frequencies of stimulation the "fast" axon evokes a tetanic response which fatigues quickly. The cockroach coxal muscles 136 and 137 fatigue much faster than the rest of the extensor muscles of the trochanter.

7. Tension-length relationships, similar to those established by previous investigators for other insect muscles, have been established for the femoral muscles of the locust and cockroach. In every muscle the maximum peak twitch tension was recorded when the muscle was held at its maximum body length. Estimated values for peak tetanic tension at maximum body length are similar for all the muscles studied, i.e. about 1000 gm cm^{-2}.

8. The peak twitch tensions per unit cross-sectional area of muscle recorded from the different muscles at maximum body length were not the same. In a few muscles, i.e. those with high twitch/tetanus force ratios, the peak twitch tension closely approached the peak tetanic tension. In other muscles, i.e. those with low twitch/tetanus force ratios, the peak twitch tension was much lower than the peak tetanic tension.

9. Some of the muscles receive also an innervation from one or more "slow" motor axons. The mechanical responses to low-frequency stimulation of the "slow" axon consist

of small twitch-like contractions which appear as minute replicas of the "fast" twitch in the locust extensor tibiae muscle. The "slow" twitches summate at higher frequencies of stimulation to give a smooth response showing little signs of fatigue. The "slow" tetanic response is characterized by a slow rate-of-rise of tension and usually a slow rate of relaxation.

10. The electrical responses to "slow" axon stimulation are post-synaptic potentials of various heights. The smaller potentials facilitate during repetition.

11. The action of the alkaloid ryanodine on the "fast" and "slow" electrical and mechanical responses of locust and cockroach skeletal muscles and on the electrical properties of the excitable membrane of the locust muscle fibre has been examined.

12. In ryanodine-saline the resting membrane resistance, which increased initially up to 50% above normal, later declined to a value well below normal. Values for effective resting membrane resistance of normal Schistocerca muscle fibres ranged between 150-800 K ohms., i.e. slightly higher than those recorded for other insects.

13. The changes of length constant and time constant during ryanodine treatment closely paralleled the changes of membrane resistance.

14. The normally graded electrically-excitable-responsiveness of Schistocerca muscle fibres was sometimes converted initially to all-or-none responsiveness by ryanodine. Later stages of poisoning were often accompanied by a return to graded responsiveness. These changes were produced only with ryanodine concentrations exceeding 10^{-7}M.

15. The electrical excitability of the fibre membrane was raised during the early stages of poisoning, resulting in the appearance of repetitive spike responses to either direct or indirect stimulation. The repetitive responses were mainly myogenic in origin although spike repetition was recorded from the motor nerves of one or two preparations. Spontaneous repetitive discharges recorded from a few preparations were neurogenic in origin. Later stages of poisoning were characterized by a reduced membrane excitability.

16. Small changes of resting potential occurred in ryanodine. Some muscle fibres showed initially a decreased resting potential in ryanodine. In other fibres addition of

the drug resulted initially in a slight hyperpolarisation of the membrane. During the final stages of poisoning the resting potential of all fibres fell considerably.

17. The electrical response of the extensor muscle to "fast" axon stimulation was potentiated. The falling phase of the action potential was lengthened with drug concentrations exceeding 10^{-7}M. Prolonged after-discharges often appeared on the repolarisation plateau formed. Later stages of poisoning were accompanied first by a fall in the magnitude and duration of the spike component and later by loss of the spike and decline of the post-synaptic response.

18. The "slow" post-synaptic potential was initially enhanced and prolonged in ryanodine-saline. If the enhanced response exceeded the depolarisation threshold for the fibre, large spikes, sometimes all-or-none in character, appeared.

19. In terms of the ionic theory the electrical changes occurring following ryanodine treatment may result from a diminished resting potassium conductance. It is possible that this conductance change is reversed during the later stages of poisoning.

20. When the "fast" motor axon was stimulated at a rate of

1 c/sec the "fast" mechanical response of the extensor muscle declined progressively during the early stages of poisoning. When the "fast" axon was stimulated intermittently an increased mechanical response was recorded for a short period after application of the drug. These changes often took place before any changes in the electrical response of the muscle were apparent and were accompanied by a small contractural response. The contracture resulted from summation of successive twitch responses due to a marked reduction in the rate of relaxation of the twitch contraction. In many preparations repetitive mechanical activity occurred. During the later stages of poisoning the mechanical response declined to zero.

21. The observation that ryanodine inhibits muscles innervated by only "fast" axons faster than it inhibits muscles innervated by "fast" and "slow" axons has not been confirmed in the present investigations. It is suggested that ryanodine affects the mechanical responsiveness of insect muscle mainly by virtue of its action on the neuromuscular transmission mechanisms.

22. The results of these investigations are discussed in relation to previous work on these and other insect muscles. It is concluded that the mechanical responses

of the various muscles examined in these investigations are not significantly different when account is taken of the fact that some receive an innervation from "slow" as well as from "fast" motor axons and that some receive a single and others a dual or multiple innervation, i.e. the different mechanical responses recorded from these muscles appear to result from differences in neuromuscular transmission properties.

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