PHYSIOLOGY AND ULTRASTRUCTURE OF PHASIC AND TONIC SKELETAL MUSCLE FIBRES IN THE LOCUST, SCHISTOCERCA GREGARIA

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SUMMARY

Insect muscle fibres can be classed as either phasic or tonic according to their response to potassium depolarization. The phasic fibres contract only transiently during prolonged potassium depolarization, whereas the tonic fibres give a sustained contracture.

The extensor tibiae muscle in the metathoracic leg of the locust contains both tonic (T/et) and phasic (P/et) fibres; the electrical, mechanical and ultrastructural properties of these fibres have been compared with those of phasic fibres from the retractor unguis muscle (P/ru) in the same leg. A broad correlation has been established between the mechanical response and the amount of sarcoplasmic reticulum (SR) in the fibres.

At maximal body length the rise time to peak twitch tension for the T/et fibres was found to be 790 ± 60 ms, for the P/et fibres 59 ± 2.5 ms and for the P/ru fibres, 30 ± 1.1 ms. The half-decay times for the isometric twitch contractions were 2950 ± 88 ms for the T/et fibres, 119 ± 4.2 ms for the P/et, and 35 ± 2.3 ms for the P/ru. The P/et and P/ru gave brief isometric contractures during potassium depolarization; under the same treatment the T/et fibres remained contracted throughout the treatment period.

The major structural differences between the 3 types lies in the SR. Expressed as percentages of total fibre volume, the SR represents in the T/et 11%, in the P/et 6.8%, and in the P/ru 19%. The surface area of the SR, in terms of μm²/μm³ of fibre volume is 1.0 ± 0.1 in the T/et, 2.9 ± 0.2 in the P/et and 11.9 ± 1.0 in the P/ru. Microtubules, often associated with elements of the SR, are sparsely distributed amongst the contractile elements in the T/et fibres. All 3 muscle types have a well developed T-system which forms dyadic associations with the SR. Larger-diameter Z-invaginations which conduct tracheoles into the muscles also give rise to 'longitudinal T-tubules', particularly in the T/et fibres. Dyads arise by association of cisternae of the SR: (i) with T-tubules sensu strictu, (ii) with Z-invaginations and T-tubule-like extensions from them, and (iii) directly with the plasma membrane at the surface of the fibre.

INTRODUCTION

Physiologists have long recognized the existence of vertebrate skeletal muscle fibres with differing contraction speeds (Kruger, 1929). For example, amphibian skeletal muscle fibres fall into 2 major categories: those which give a brief mechanical response, or twitch contraction, to a single nerve impulse and those which give a measurable contraction only during repetitive stimulation. The former have, on occasion, been called 'twitch' fibres whilst the term 'slow' has been applied to the latter. From the comparative standpoint, this terminology is unsatisfactory. For example, mammalian 'twitch' fibres are called either 'fast' or 'slow' according to the temporal characteristics of their twitch contractions, while in arthropods some muscle fibres,
by virtue of their graded responsiveness and polynervous innervation, can undergo both ‘fast’ and ‘slow’ contractions.

The main differences between amphibian twitch and slow fibres lie in their response to potassium depolarization, the multiterminal and polynervous innervation of the slow fibres and the absence of propagated action potentials in the slow fibres. In arthropods, the skeletal muscle fibres are always multiterminaly and frequently polynervously innervated and only rarely produce all-or-none action potentials (Atwood, 1967; Usherwood, 1967). However, the fibres of arthropod muscles, like those of amphibian muscles, do fall into 2 major categories according to their responsiveness to potassium. During sustained potassium depolarization one class of fibres undergoes a rapid transient contraction whilst the other undergoes a prolonged contracture which may last for the duration of the potassium depolarization. There would appear to be some justification, therefore, for calling the former phasic and the latter tonic. Since a measurable contraction (like a very slow twitch) can be recorded from some of the fibres which give prolonged potassium contractures the terms tonic and phasic seem much more suitable and less confusing than the terms ‘slow’ and ‘fast’, and ‘tonic’ and ‘twitch’.

Amongst invertebrate muscles, fibres with structural properties similar to those of the amphibian ‘slow’ fibres have been found and some arthropod skeletal muscles contain a mixture of tonic and phasic fibres (Hoyle, 1961, 1966a, b; Jahromi & Atwood, 1967, 1969; Cochrane, Elder & Usherwood, 1969; Anderson, Cochrane, Elder, Josephson & Usherwood, 1970). For example, the abdominal extensor muscle of the crayfish contains phasic and tonic fibres; the most clear-cut morphological difference between the 2 types of fibre was found in the arrangement of the contractile proteins. In the myofibrils of the phasic fibres each thick filament has an orbital array of 6 thin filaments (ratio of thick:thin about 1:3), whereas in the tonic fibres there are 9–12 orbital thin filaments to each thick filament (thick:thin about 1:5) (Jahromi & Atwood, 1967).

Evidence for the occurrence of tonic fibres in insect muscles was first obtained by Hoyle (1961). He found that the second thoracic spiracle muscle of the locust *Schistocerca gregaria* gave a prolonged sustained contracture (only 15% down after 20 h) during treatment with locust saline containing 150 mM potassium. The fibres of this muscle give only weak twitch contractions in response to electrical stimulation and are either electrically inexcitable or only poorly electrically excitable. More recently, electrically inexcitable fibres with similar structural properties to crustacean tonic fibres have been found in the retraction muscles of the air-guide of the giant water bug, *Lethocerus* (Walco & Burrows, 1969). Tonic fibres also occur in locust leg muscle (Hoyle, 1966a, b; Usherwood, 1967; Cochrane et al. 1969); in the metathoracic extensor tibiae muscle they are found in a discrete bundle at the proximal end of the femur. In the present study we have compared some of the physiological and ultrastructural properties of this bundle of tonic fibres with those of phasic fibres from the extensor tibiae muscle and from the femoral part of the retractor unguis muscle in the same leg. Brief accounts of this work have already been published (Cochrane et al. 1969; Anderson et al. 1970).
MATERIALS AND METHODS

Preparations were taken from muscles in the femur of the metathoracic leg of the locust *Schistocerca gregaria*. Tonic and phasic fibres were obtained from the large extensor tibiae muscle found in the dorsal region of the femoral segment. This muscle contains about 3000 fibres (Hoyle, 1955) which are arranged in bundles and insert diagonally on an apodeme running down the centre of the femur (Fig. 1). Most of the fibres in this muscle are phasically responsive but at the proximal end there is a small discrete bundle of tonic fibres (Fig. 1), innervated by a slow excitatory neuron and an inhibitory neuron (Usherwood & Grundfest, 1965). The small excitatory neuron also innervates some of the phasic fibres of the extensor tibiae muscle, most of these fibres being located at the proximal end of the muscle adjacent to the bundle of tonic fibres. All of the phasic fibres are innervated by a large ('fast') excitatory neuron. Preparations were taken from the region innervated only by this latter neuron. Other preparations were obtained from the tonic bundle and from the retractor unguis muscle in the femur. The latter is a fine narrow muscle, lying sandwiched between, but quite separate.

![Diagram of the dissected metathoracic femur](image)

Fig. 1. Diagram of the dissected metathoracic femur of the locust leg to show the location of the muscles used in the present study. The flexor muscles have been reflected to reveal the extensor tibiae and retractor unguis muscles. The innervation of each muscle is indicated: *fe*, fast excitatory; *i*, inhibitory; *se*, slow excitatory.
from, the large extensor tibiae and flexor tibiae muscles in the femur. It is inserted on a fine apodeme through which it operates the tarsal claw. It is composed of about seventeen phasic fibres running the full length of the muscle and is innervated by 2 large ('fast') motoneurons (Fig. 1). Preparations were taken at random along the whole length of this muscle.

All muscle preparations were first isolated in locust saline (Usherwood & Grundfest, 1965) and then fixed at a constant length after a 3–6 h period of equilibration. Specimens were fixed in cold 3 % glutaraldehyde in a 50/50 mixture of locust saline and 3 % sucrose buffered with 0.1 M phosphate at pH 6.8 for 40 min; washed in buffered sucrose/saline for 1 h; postfixed in cold 1 % osmium tetroxide (Zetterqvist, based on locust saline) at pH 6.8 for 40 min; dehydrated in ethanol and propylene oxide and embedded in Araldite. 0.5-nm sections for phase-contrast microscopy were stained with 1 % KMnO₄; thin sections were stained with uranyl acetate and lead citrate and examined with AEI EM 6 and EM 6B electron microscopes.

Stereometric analysis of various structural components of these muscle fibres was made using the combined point count and intersect incidence method of Freere & Weibel (1967). A sampling grid with 84 lines and 168 points was made on cellulose acetate sheet and superimposed on selected areas of electron micrographs. For analysis of the sarcoplasmic reticulum (SR) and myofibrillar volumes and the SR surface areas, prints of transverse sections magnified ×50,000 were found to be most suitable. From such prints only the central regions of the A-bands were selected, to minimize possible variations in measured parameters along the sarcomere length.

The mechanical responses of the muscle fibre preparations were investigated by using either isolated muscles (retractor unguis) or isolated bundles of muscle fibres (extensor tibiae). These isolated preparations were equilibrated for 3 h in locust saline before any recordings were made. The muscle fibres were stretched to maximal body length and stimulated neurally, using either paired silver/silver chloride electrodes or a suction electrode. The mechanical responses were recorded by attaching the preparations to the shaft of a Grass force-displacement transducer with Terylene thread. The output of the transducer was amplified and displayed graphically on a Devices pen-recorder.

RESULTS

Mechanical responses

The response of the retractor unguis muscle during potassium depolarization is illustrated in Fig. 2B. Similar responses were obtained from the phasic fibres of the extensor tibiae muscle. In Fig. 2A, the response of an isolated preparation of tonic fibres from the extensor tibiae muscle during treatment with potassium is illustrated. The main difference between the tonic and phasic fibres in their response to potassium lies, by definition, in the time course of the contraction, that of the tonic fibres being extremely prolonged. Twitch/tetanic contractions are recorded from the phasic fibres of the extensor tibiae muscle during stimulation of either the large or the small excitatory axons to this muscle. Although the responses are quantitatively different they have the same temporal characteristics (Usherwood & Grundfest, 1965). When these axons are stimulated at high frequencies (c. 100 Hz) slowly fatiguing tetanic contractions are obtained from the phasic fibres of the extensor tibiae muscle. Stimulation of the large excitatory axons which innervate the retractor unguis muscle also evokes twitch contractions from this muscle.

The retractor unguis muscle is structurally and functionally differentiated into 2 parts. One contains exclusively red fibres and is innervated by one of the excitatory axons, while the other contains white fibres and is innervated by the other excitatory axon. Although the electrical responses of the red and white fibres to neural stimulation are identical, the white fibres fatigue more rapidly during high-frequency
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stimulation (Usherwood, 1967; Usherwood & Machili, 1968). Whether the differences in rates of fatigue reflect differences in synaptic physiology remains to be established. The mechanical and electrical responses of the phasic fibres of the extensor tibiae muscle and the retractor unguis muscle are summarized in Table 1.

![Figure 2](image.png)

Fig. 2. Potassium contractures of (a) tonic fibres from extensor tibiae muscle and (b) phasic fibres of retractor unguis muscle. Note prolonged time course of contracture of tonic fibres compared with phasic fibres.

<table>
<thead>
<tr>
<th>Fibre type</th>
<th>Innervation</th>
<th>Electrical responses</th>
<th>Mechanical responses</th>
<th>Rise time, ms</th>
<th>Relaxation time, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tonic/extensor tibiae</td>
<td>1 small excitatory neuron</td>
<td>EPSP*</td>
<td></td>
<td>790 ± 60†</td>
<td>2950 ± 88</td>
</tr>
<tr>
<td></td>
<td>1 inhibitory neuron</td>
<td>IPSP†</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Phasic/extensor tibiae</td>
<td>1 large excitatory neuron§</td>
<td>EPSP + large electrically excited response</td>
<td></td>
<td>59 ± 2.5</td>
<td>119 ± 4.2</td>
</tr>
<tr>
<td>Phasic/retractor unguis</td>
<td>2 large excitatory neurons</td>
<td>EPSP + large electrically excited response</td>
<td></td>
<td>30 ± 1.1</td>
<td>35 ± 2.3</td>
</tr>
</tbody>
</table>

* Excitatory post-synaptic potential.
† Mechanical responses expressed as mean of 10 preparations ± s.d.
‡ Inhibitory post-synaptic potential.
§ Some of the phasic extensor tibiae muscle fibres are innervated by a small excitatory neuron as well as a large excitatory neuron. Nevertheless, the time course of the mechanical responses of these fibres and their ultrastructure are identical with those of the mononeuronally-innervated phasic extensor tibiae fibres.
The tonic fibres of the extensor tibiae muscle have very different mechanical and, to a lesser extent, electrical responses from the phasic fibres of this muscle. A single supramaximal stimulus applied to the excitatory axon innervating the tonic fibres evokes a small 'slow' twitch contraction. These fibres are also innervated by an inhibitory axon and stimulation of this axon alone either relaxes 'resting' tonic fibres or evokes no perceptible mechanical response (Usherwood & Grundfest, 1965; Usherwood, 1968; Usherwood & Runion, 1970). However, when the inhibitory and excitatory axons are excited simultaneously the amplitude and time course of the slow twitch are greatly reduced. The mechanical and electrical responses of the tonic fibres are summarized in Table 1.

Ultrastructure

The 3 sets of fibres will be referred to by the following abbreviations: extensor tibiae phasic fibres (P/et); extensor tibiae tonic fibres (T/et); and retractor unguis phasic fibres (P/ru).

Table 2. Stereometric properties of structural components of muscle fibres

<table>
<thead>
<tr>
<th>Fibre type</th>
<th>Thick filaments</th>
<th>Sarcoplasmic reticulum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length, μm</td>
<td>Diameter, nm</td>
</tr>
<tr>
<td>Tonic/ext. tibiae</td>
<td>5.2</td>
<td>19</td>
</tr>
<tr>
<td>Phasic/ext. tibiae</td>
<td>5.5</td>
<td>21</td>
</tr>
<tr>
<td>Phasic/retractor unguis</td>
<td>3.9</td>
<td>18</td>
</tr>
</tbody>
</table>

* Values for SR volume and surface area which were published in an abstract (Cochrane et al. 1969) differ significantly from these given above and were subsequently found to be erroneous.

Apart from differences in the amount of SR the P/et, T/et and P/ru fibres have a similar structural organization. The myofibrils are generally straplike, although in transverse sections of the T/et they tend to be very poorly defined, due to the paucity of interfibrillar space and SR in these fibres (Fig. 3). The sarcomeres are usually in register but the pattern is frequently disrupted by misalignment of the Z-bands (Figs. 5, 6, 14). Under the conditions of fixation, with the muscles held at a constant length, the sarcomeres exhibit broad A- and I-bands with poorly defined boundaries. Thick filament lengths were measured on sections cut with the knife edge parallel to the filaments and are given in Table 2. Some variability, however, was encountered because of the ill-defined A-band margins. An H-band is not readily apparent in longitudinal sections but can be identified in transverse sections by the absence of thin filaments from the filament array (Figs. 4, 7, 8). No M-band was present in any of the muscle fibres. In transverse sections the filament array corresponds in the P/et and P/ru to that described for insect visceral muscle by Smith, Gupta & Smith (1966). The thick filaments are of constant diameter throughout their length and are in a
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standard hexagonal arrangement. In regions where the thick and thin filaments overlap each thick filament is surrounded, in the P/et and P/ru, by an orbit of 10–12 thin filaments; the ratio of thin to thick filaments is about 5:1 (the apparent discrepancy between the number of orbital filaments and their ratios may be due to thick filaments at the edge of the fibrils having incomplete orbitals (Fig. 9)). In the T/et the orbital array of the thin filaments is less clearly defined and the ratio is higher (Fig. 10; Table 2).

In all 3 types of fibre the transverse tubule system (TTS) is formed mainly by open-ended tubular invaginations of the cell membrane which pass transversely across the fibres at the region of overlap between the thick and thin filaments, midway between the Z-line and centre of the A-band (the A-I region) (Fig. 6). It is augmented by tubules, also open-ended, which originate as side-branches of large sarcolemmal invaginations (often containing tracheoles) which enter the fibre at the Z-disk level (Fig. 11). The tubules arising from these sarcolemmal invaginations may run longitudinally in the fibres (Fig. 12). Peachey (1968) refers to these respectively as 'A' tubules and 'Z' tubules. Both sets of tubules form dyadic junctions with the SR in the interfibrillar spaces, usually opposite the A-I region (Brandt, Reuben, Girardier & Grundfest, 1965; Cochrane & Elder, 1967). Occasionally a very elongated dyad or a series of smaller dyads was found stretching across almost the whole length of the sarcomere from Z-disk to Z-disk. A third type of dyad is formed where the SR comes in contact with the outermost surface membrane. These 'surface dyads' (Fig. 7) are also found opposite the A-I region.

In the P/et and P/ru the SR forms a dense meshwork of branching and anastomosing tubules surrounding the myofibrils and stretching from end to end of the sarcomeres (Fig. 13). There are usually one or two layers of tubules in the interfibrillar space in the P/et (Fig. 4) and two to three layers in the P/ru (Fig. 7), except at the end of the sarcomeres where the SR is reduced to a few tubular elements which appear to link the SR of adjacent sarcomeres. In the T/et the SR is reduced to isolated tubules in the interfibrillar space, which is itself greatly reduced so that, particularly in transverse sections, the myofibrillar pattern is obscured and the contractile filaments occur in large undifferentiated groups (Fig. 3). In all 3 types of fibre, at the A-I region of the sarcomere, the tubules tend to run at right angles to the sarcomere axis. Here they merge together to form a broad cisternal plaque which is clamped against the incoming T-system to form a dyad (Figs. 13–15). The tubules forming the cisternal plaque are clearly continuous with the tubules of the SR proper on either side of it (Fig. 14). In the dyadic regions the TTS component may also be expanded into a broad plaque up to 600 nm in diameter (Fig. 13).

Since it is not yet clear whether either the surface area of the SR or the contained volume is the important feature in relation to its capacity to bind calcium ions (Ebashi & Endo, 1968), we have estimated both these parameters by stereometric analysis, and have obtained the volume of the SR as a percentage of the total fibre volume and an absolute value for the surface area of the membrane enclosing the cisternae in terms of \( \mu m^2/\mu m^3 \) of fibril. In quantifying the SR, counts were made on the grid of the incidence of point hits on SR cisternae (P∕SN) and the intersect frequency of the grid-
lines on SR cisternal membranes \((N_t)\). On the basis that the areas of tissue components in the micrographs are in the proportions of their volumes in the tissue, the volume of SR \((V_{SR})\) as a percentage of the total fibre volume is given by the formula:

\[
V_{SR} = \frac{P_{SR}}{P_t} \times 100,
\]

where \(P_t\) is the total number of points (168) on the grid. By taking the final print magnification into account an absolute value can be put on the surface area \((S_{SR})\) of the SR cisternae in terms of \(\mu m^2\) of cisternal membrane per \(\mu m^3\) of tissue, according to the formula:

\[
S_{SR} = \frac{2N_e}{84Z^2},
\]

where \(Z\) = the length of one grid line at the magnification of the particular micrograph (Freere & Weibel, 1967). The values obtained from these calculations are given in Table 2.

Microtubules have been noted in both longitudinal and transverse sections of the tonic fibres, associated with elements of the SR (Fig. 10), and in all 3 fibre types vesicles similar to those figured by Ashhurst (1967) have frequently been noted associated with the Z-disks.

**DISCUSSION**

While visual inspection of micrographs allows us to conclude that there is a marked difference in the quantity of SR present between the tonic and phasic fibres, we found it necessary to make stereometric measurements of the SR of these fibres for 2 reasons. Firstly, the results (21 measurements from 1 animal) in a preliminary note (Cochrane *et al.* 1969) were more variable and the differences less marked (probably due to lack of expertise in isolating the tonic fibres) than in the present results (35 measurements from 6 animals). Secondly, quantification of the SR is a prerequisite of studies of the Ca\(^{2+}\)-binding capacity of the various fibre types which we have planned for the future. The measurements made, however, are open to a number of criticisms. Firstly, from Fig. 7, it can be seen that in the P/ru fibres in particular the quantity of SR varies markedly according to the position along the sarcomere at which measurements are made. Secondly, measurements made towards the margins of the A-band regions would include TTS elements and this would markedly affect the SR values for the tonic fibres in particular, since it is not always possible to distinguish the T-tubules from elements of the SR. Thirdly, the form of the SR changes abruptly from tubules in the mid A-band region, to cisternal plaques in the vicinity of the TTS towards the A-band margins. For these three reasons, we have limited our measurements to mid A-band regions; fields exhibiting dyads were rejected. As a result, our extrapolated estimates of SR quantity for the whole fibres are probably too high, particularly in the phasic retractor unguis fibres. A fourth criticism is that the degree of contraction of the muscle fibres at the time of, and during, fixation will affect the
estimates of total SR. The tonic fibres, unlike the phasic fibres which always showed H- and I-bands, were mostly contracted despite the standard procedure of fixing both types of muscle fibres in situ, and this could lead to an overestimate of the amount of SR in the tonic fibres. Fifthly, the diameters of the SR tubules are approximately the same as the thickness of the sections from which the measurements were made. While the errors implicit in making measurements under these circumstances have been well documented (Weibel, 1969), the dimensions of the SR vesicles were similar in all 3 muscle types examined and the errors will be less serious on a comparative basis than in absolute terms. Despite these criticisms it is felt that the stereometric measurements that we have made are valuable as a first approximation and until more refined methods become available.

Recent reviews (e.g. Peachey, 1968; Hess, 1970) have discussed the various features which they consider to typify tonic (slow) fibres. Notable amongst these (Hess, 1970) are: (1) the fibrils of phasic (twitch) fibres are more or less regularly separated from each other and equal in size, whereas the fibrils of the tonic (slow) fibres are not; (2) SR is relatively abundant in the phasic fibres but relatively sparsely distributed in the tonic fibres; (3) the TTS is a consistent characteristic of phasic fibres but is virtually absent or consists only of aberrant elements in the tonic fibres; (4) the Z-line is jagged in the tonic fibres, but in the phasic fibres is relatively straight; (5) there is no M-line in the tonic fibres (except in avian tonic fibres).

A number of authors have noted the correlation in invertebrate muscles between time course of isometric twitch contractions and sarcomere length, and this is particularly so in crustacean muscles (Hoyle, 1969). A similar tendency was noted in insect muscles by Jahromi & Atwood (1969); it applies also to the phasic muscles of the present study (Tables 1, 2). It is significant, however, that all the fibres so far considered which fit this relationship have been phasic and that neither the tonic fibres described by Jahromi & Atwood (1969) nor the presently figured extensor tibiae tonic fibres fit the relationship.

The number of actin filaments associated with each myosin filament appears to be particularly variable in the tonic fibres. Several factors could account for the configurations found in the present study. As noted above, the tonic fibres are probably under tension for long periods and were at least partially contracted in most of our preparations. It is likely therefore that the configurations with the highest actin to myosin ratios (Fig. 3) were probably caused by double overlap of the thin filaments. Such configurations have been noted by other workers in insect muscle (e.g. Spiro & Hagopian, 1967). While the tonic fibres do not exhibit supercontraction of the type described by Osborne (1967), Leyton & Ullrick (1970) and others, they are probably capable of the type of shortening described by Hagopian (1970) in which the myosin filaments can penetrate to a limited extent into the Z-disk structure; and as a consequence the extent of interdigitation of the thin filaments in the mid-sarcomere is thereby increased. Other reasons for the high actin/myosin ratios seen in transverse sections passing through the margins of A-band regions are the variable myosin filament length and lack of register of the myosin filaments (Fig. 8). This is readily recognizable by the fewer, and irregularly spaced, thick filaments. It has been noted
also in crustacean muscles and examined in detail by Franzini-Armstrong (1970).
However, in some electron micrographs of tonic extensor tibiae muscle fibres of the
locust there is an obvious H-band (Fig. 8), yet actin/myosin ratios in excess of 5:1
have been noted in areas not adjacent to I-band regions (Fig. 10). These areas are
characterized by less regular arrangement of the myosin filaments and the frequent
occurrence of more than one thin filament between adjacent thick filaments.
Such regions would not of course be subject to the double overlap of thin
filaments, which probably explains the very high (c. 8:1) ratios obtained in fields such
as that of Fig. 3, taken from a more contracted specimen. They could, however, be
explained on the basis of the evident longitudinal displacement of individual myosin
filaments.
Spiro & Hagopian (1967) have shown that there is a clear relationship between the
length and diameter of the thick filaments and the ratio of thick to thin filaments; in
general, the length of the thick filaments is proportional to their diameter and to the
number of thin filaments with which they are associated. This presumably indicates
a difference in the packing and stagger of the myosin monomers (Spiro & Hagopian,
1967). The tonic extensor tibiae fibres of the present study have a thin-to-thick fila-
ment ratio (> 5:1) at least as high as that of the phasic fibres (5:1) of the same
muscle, but the A-band length of the tonic fibres at 5.2 μm is less than that of the
phasic fibres (5.5 μm). This probably does not constitute a valid exception to the
trend noted above because many of the thin filaments of the tonic fibre are packed
together in groups and as such are not associated with thick filaments. It is assumed
that where additional thin filaments are present at any location along the length of
adjacent thick filaments, only some of the thin filaments are required to accommodate
all the available myosin bridges. A more striking arrangement of myosin ribbons and
filaments associated with a large number of thin filaments has recently been reported
in vertebrate smooth muscle (Lowy & Small, 1970; Rice, Moses, McManus &
Brady, 1970). Presumably in the vertebrate smooth muscle and possibly in the locust
tonic extensor tibiae there may be some exchange of thin filaments between the thick
filaments along their length if all thin filaments are to be capable of some cross-bridge
attachment with the thick filaments.
The form of the junction between the SR and the T-system tubules or clefts is
clearly the dyad typical of most other insect muscles (Smith, 1966). Occasional
triads have been noted (Fig. 13) and the association of TTS elements with higher
numbers of SR cisternae in arthropod muscles has been noted by other workers
(Fahrenbach, 1964).
Longitudinal sections in a plane tangential to the surface of the fibrils in the
phasic fibres frequently show a central T-tube element flanked by cisternal plaques of
SR (Fig. 14). Pasquali-Ronchetti (1970) has suggested that similar, apparently triadic,
configurations in another insect muscle represent the functional linking of mor-
phologically separate regions of SR by T-tube elements. However, in the present
study sections of muscle cut longitudinally which show the association of TTS and
SR in cross-section (Fig. 15) reveal that the normal configuration is a dyad with the
C-shaped cisternal plaque of the SR folded around the T-tube. Where the SR and
TTS lie in the plane of section the pseudo-triad appearance may therefore be an artifact caused by the thinness of the section. This interpretation would seem to apply also to some of Pasqualli-Ronchetti's (1970) figures. Certainly in the phasic muscles of the present study and probably in the tonic fibres also, the SR forms a continuous system throughout the sarcomere and probably also across the Z-line regions between sarcomeres.

Despite configurational differences a well developed TTS is present in all 3 types of the muscle fibre examined in this study. Thus apart from the differences in dimensions of sarcomeres and A-bands, and in filament ratios noted above, the most striking structural difference between these muscle fibres lies in the different degrees of development of the SR. This might result in a critical difference in the rate of release and uptake of calcium and contribute to the slower rate of contraction and relaxation of the tonic fibres.

Autoradiographic methods (Winegrad, 1968) and spectrophotometric studies by Jobsis & O'Connor (1966) and Ridgeway & Ashley (1967) have provided confirmation of the earlier concept that in phasic striated muscle fibres, contraction occurs after the release of calcium ions from the SR, relaxation being achieved by the re-sequestration of calcium by the membranes of the SR. In the much slower acting vertebrate smooth muscle which contains little or no SR, the precise role of calcium in contraction has not yet been elucidated (Somlyo & Somlyo, 1968). Aidley (1965) demonstrated that the potassium contracture of the locust mesothoracic extensor tibiae muscle is dependent upon the presence of external calcium. In the tonic fibres in the metathoracic extensor tibiae muscle of this insect it is possible that calcium reaches the contractile elements from the extracellular fluid by moving inwards across the surface membrane during excitation. Peachey (1961) suggested that contraction of the narrow strap-like striated fibres of Amphioxus is initiated by calcium influx.

Bárány (1967) has found a difference, up to 200-fold, in ATPase activity amongst a variety of vertebrate muscles and the interesting experiments of Costantin, Podolsky & Tice (1967) with skinned frog tonic fibres activated by applied calcium solutions suggest that the slow mechanical responses of these fibres are due to some property of the mechano-chemical coupling system rather than the excitation-contraction coupling pathway. Therefore before attempting to examine the kinetics of calcium exchange across the membrane of the SR in the tonic and phasic muscle fibres of the locust leg, it would be worth while to investigate the mechanical properties of the fibres. It is possible that their different mechanical responses also result from differences in mechano-chemical coupling, rather than excitation-contraction coupling.

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REFERENCES


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ABBREVIATIONS ON PLATES

A-I actin and myosin overlap
bI basal lamina

d dyad
g glycogen
H H-band
I I-band
mit mitochondria
mt microtubule

sd surface dyad
sr sarcoplasmic reticulum
T T-tube
tr tracheole
tri triad
Z Z-line
Zi Z-region invagination
Zt T-tubule-like branch from Z-region invagination
Fig. 3. Transverse section of part of a tonic extensor tibiae fibre showing the myofilaments not separated into discrete fibrils and the sparse SR tubules. The high actin: myosin ratio in this field is probably due to double overlap of the thin filaments. \( \times 22100 \).

Fig. 4. Transverse section of part of a phasic extensor tibiae fibre showing well developed SR enveloping discrete fibrils. An element of the TTS and a plaque from the SR together form a dyad. \( \times 42900 \).
Phasic and tonic muscles in the locust
Fig. 5. Longitudinal section from a tonic extensor tibiae fibre showing the contracted state characteristic of most preparations of this fibre type. No I-band is present. A few dyads are present. Although Z-line material is divided into discrete areas, the separation of the myofilaments into fibrils is poor and few SR tubules are present. × 8200.

Fig. 6. Longitudinal section of a phasic extensor tibiae fibre showing the gross misalignment of the sarcomeres frequently encountered in these muscles. Numerous dyads are located in the A-I regions. Mitochondria are usually located in pairs in the interfibrillar spaces flanking the Z-lines. × 8100.
Phasic and tonic muscles in the locust
Fig. 7. Transverse section of parts of 2 phasic retractor unguis fibres. In this fibre type the strap-like fibrils are invested in abundant SR tubules, particularly at the H-band regions. In the upper fibre several TTS invaginations form dyads with the SR at the A-I region; in the lower fibre a surface dyad is formed between the SR and the plasma membrane at the A-I region. × 26400.
Phasic and tonic muscles in the locust
Fig. 8. Transverse section of part of a tonic extensor tibiae fibre. Because of the marked longitudinal misalignment of the filaments composing the sarcomere from which this field was taken, all bands are represented in one field. Branches of the large diameter Z-region invaginations are also shown. Poor myosin filament register is shown. × 34 800.

Fig. 9. Transverse section of a small portion of a retractor unguis fibre showing the well ordered filament array typical of this type of fibre. The actin orbits around individual myosin filaments are frequently incomplete along the fibril margins. Several dyads are also shown. × 71 300.

Fig. 10. Transverse section of a small portion of a tonic extensor tibiae fibre showing the frequent occurrence of additional actin filaments in the filament array. Also present are a T-tube making a dyad with an element of the sparse SR, and several microtubules running in the poorly defined interfibrillar space. × 43 600.
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Fig. 11. Transverse section of a small portion of a phasic extensor tibiae fibre. A tracheole is shown within a branch of a large diameter Z-region invagination. A plaque of the SR forms a dyad-like association with the latter invagination and a T-tube-like branch from the invagination runs transversely to form a further dyad. A third dyad, probably formed between the SR and a T-tube proper, is also present. × 51,400.

Fig. 12. Longitudinal section, one sarcomere length from a tonic extensor tibiae fibre. Two Z-invaginations are seen, from each of which 'longitudinal T-tube' branches extend. One of these forms a dyad with the SR. × 21,600.

Fig. 13. Longitudinal section from a phasic extensor tibiae fibre. The plane of section shows the SR en face and the branching and anastomosing tubules are seen to form a perforated curtain investing the fibrils. The T-tubes appear very electron-dense and include a number of disk-like expansions. A rare triadic association of a central T-tube element sandwiched between 2 SR plaques is also figured. × 17,100.

Fig. 14. Longitudinal section from a phasic extensor tibiae fibre. The section shows the pseudo-triadic appearance of a T-tube flanked by the margins of an SR plaque (see Discussion). Z-line dislocation and poor thick filament register are also shown. × 25,200.

Fig. 15. Dyad from phasic extensor tibiae fibre. A flattened T-tube element is enclosed by a C-shaped plaque of SR, the lumen of the latter being clearly continuous with that of SR tubules in the adjacent interfibrillar space. × 32,000.