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The Fine Structure of the Jumping Muscle

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THE JUMPING MECHANISM OF *XENOPSYLLA CHEOPIS* II. THE FINE STRUCTURE OF THE JUMPING MUSCLE

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[Plates 37 and 38]

The ultrastructure of the trochanteral depressor muscle of the oriental rat flea is described. It is shown to be similar to that of the tubular leg muscles of other insects except in the volume and arrangement of the sarcoplasmic reticulum. The sarcoplasmic reticulum occupies approximately 18% of the volume of the muscle fibres. It is in three configurations: a regular array of parallel tubules opposite the A-band, a collar of sacculi involved in the formation of the dyads at the edge of the A-band and a loosely woven arrangement of tubules around the I-band. This tripartite arrangement of the sarcoplasmic reticulum and its large surface area is discussed in relation to the action of the muscle as the main propulsive muscle in the jump of the flea.

Introduction

The remarkable jumping ability of the oriental rat flea *Xenopsylla cheopis* (Roths.) is aided by the retention and modification of certain skeletal features normally associated with flight in insects, in particular the resilin-containing wing hinge ligament (Rothschild, Schlein, Parker & Sternberg 1972). The trochanteral depressor is the principal muscle involved in the jump, bringing about the descent of the trochanter and the femur and the straightening of the upper part of the leg. The muscle originates in the tergum and inserts at the tip of a long tendon which runs down through the coxa to the anterior rim of the trochanter. Tergotrochanteral muscles are used for jumping by insects of several orders including the Hemiptera, Diptera and Coleoptera. In the Dictyoptera and Orthoptera the tergotrochanteral muscles are used in both walking and flying and have the ultrastructure of flight muscles (Tiegs 1955; Wilson 1961; Jahromi & Atwood 1969). In view of the uncertainty that exists about the ancestry of the Aphaniptera and in view of the fact that many fleas have skeletal characteristics associated with flight, it was considered worthwhile to investigate whether the ultrastructure of the jumping muscle resembled that of the insects which use it in flight or that of the insects which use it for jumping or in fact whether it shows features unique to its functioning in the flea.

Materials and methods

The fleas were killed and immediately dissected in glutaraldehyde in sodium cacodylate buffer plus 5% sucrose at pH 7.3 and 4 °C. The surrounding muscles were cut away from the trochanteral depressor muscle which was left attached to the coxa. The presence of the

coxae enabled the preparations to be more easily seen with the naked eye and made orientation of the muscle less difficult. The muscles were left in the fixative for 2 h at 4 °C and washed in the buffer for a further 2 h. They were then postfixed in 1% osmium tetroxide in veronal–acetate buffer for 2 h and dehydrated in graded alcohols. They were embedded in Spurrs resin. Thin sections were cut on a Reichert Om U2 ultramicrotome and collected on uncoated grids. The sections were stained on the grids with uranyl acetate and lead citrate and viewed with a Siemens Elmiskop 102 microscope. A point counting technique (Hally 1964; Weibel, Kistler & Scherle 1966; Weibel 1972*a*) was used for the morphometric analysis of the electron micrographs. Morphometric analysis of muscle is complicated by the extremely high degree of organization of its fine structure. The sections used for the point counting were therefore cut slightly obliquely to minimize any anisotropy in them (Wiebel 1972*b*). The electronmicrographs were examined at a final magnification of × 24000 and the test system was a square lattice of 100 points, 1.5 cm apart. The measurements of dimensions were carried out on transverse and longitudinal sections. Sections were considered to be truly transverse when the orbitals of I-filaments around the A-filaments showed up distinctly. They were considered to be truly longitudinal when an A-filament could be followed for its whole length in the section. The ratio of I-filaments to A-filaments was calculated as the mean of ten different ratios from two areas in transverse sections of five fibres.

Results

The fibres of the flea trochanteral depressor muscle are roughly circular in cross section with a diameter of 20–25 μm (figure 1, plate 37). The fibre nuclei are situated centrally and form a discontinuous axial core along the length of each fibre. The contractile material is arranged in lamellae radiating out from the central core. The lamellae are 0.5–0.9 μm wide and are separated by double or triple layered sheets of sarcoplasmic reticulum (s.r.) (figure 2, plate 37). Division of the lamellae towards the periphery of the fibre results in no point within them being more than 0.4 μm from vesicles of the s.r. Mitochondria lie between the lamellae at the level of the I-band and Z-line. In places they extend across the whole radius of the fibre and may be up to 15 μm in length. Their diameter as seen in longitudinal and transverse sections seldom exceeds 1 μm. Mitochondria are also located in the central core of the fibres between the nuclei (figure 3, plate 34).

Transverse sections through the A-band of the lamellae show an array of thick (A) and thin (I) filaments. The A-filaments are usually arranged linearly and more or less hexagonally

Description of plate 37

Figure 1. Transverse section through a fibre of the trochanteral depressor muscle of *Xenopsylla*. Note the central nucleus and the radial lamellae. (Magn. × 6000.)

Figure 2. Transverse section through part of the periphery of a fibre. Tubules of the T-system enter the fibre from the plasmalemma and dyads are formed along their length. (Magn. × 40000.)

Figure 3. Longitudinal section through part of the central core of a fibre. Mitochondria are situated in the core and between the lamellae at the level of the I-bands. (Magn. × 35000.)

Figure 4. Longitudinal section showing a dyad situated between a nucleus and the contractile material. (Magn. × 90000.)

Inset. A glancing section through a dyad showing the electron dense blocks on the sarcoplasmic reticulum membrane (large arrows) and fine ridges in the membrane of the T-system (small arrows). (Magn. × 110000.)

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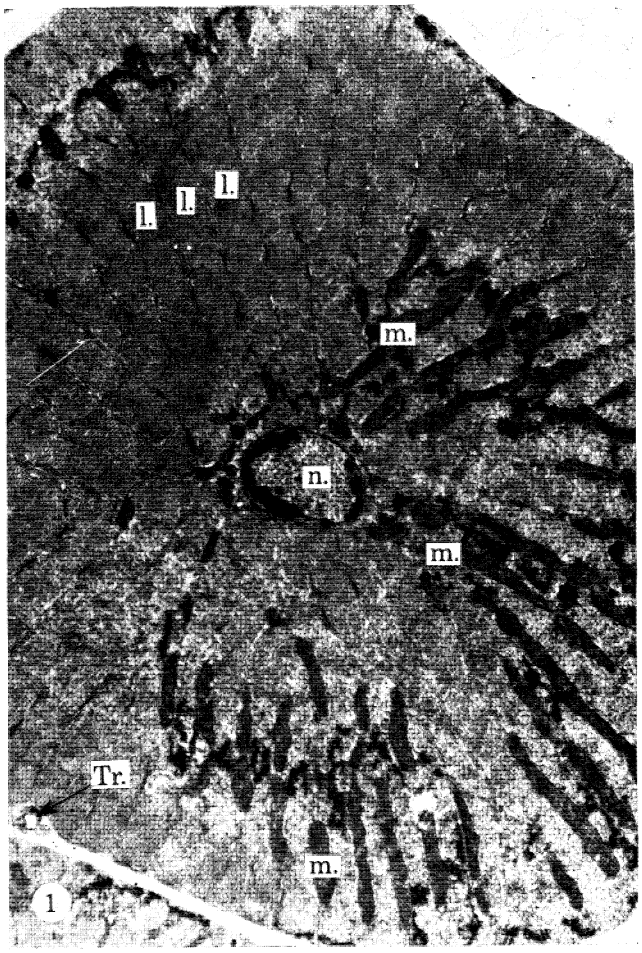

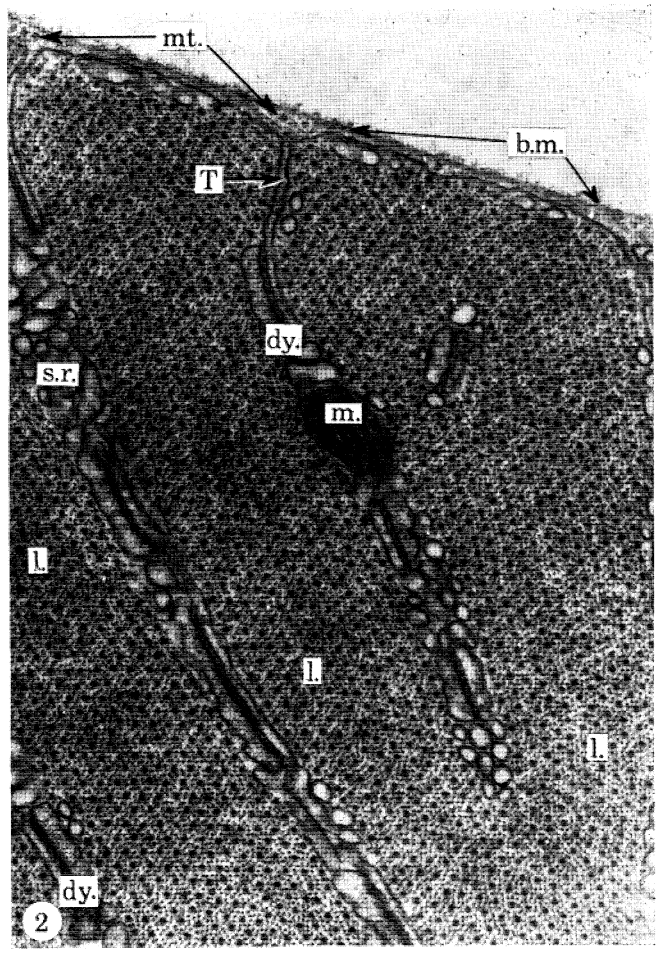

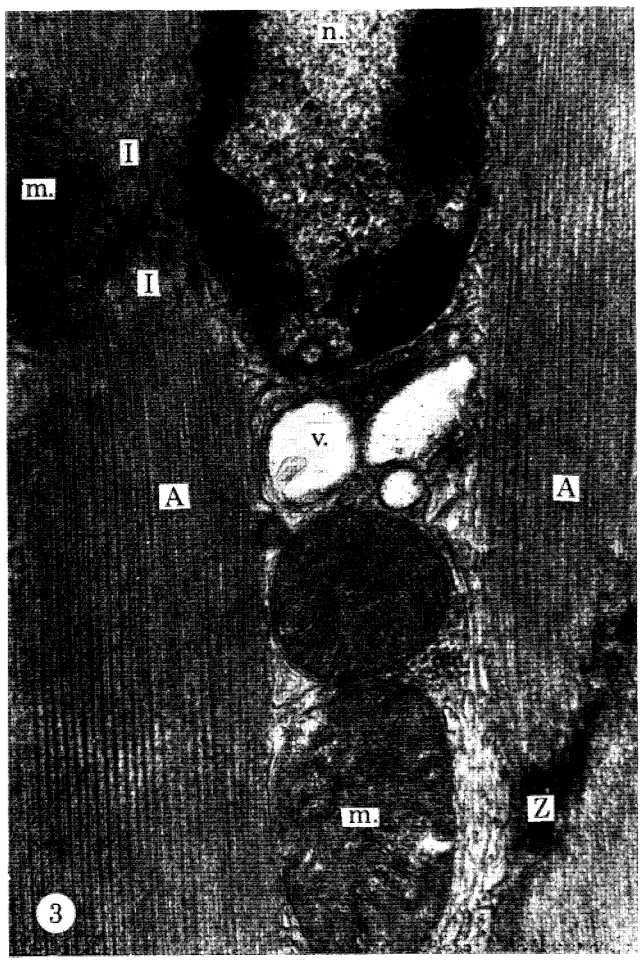

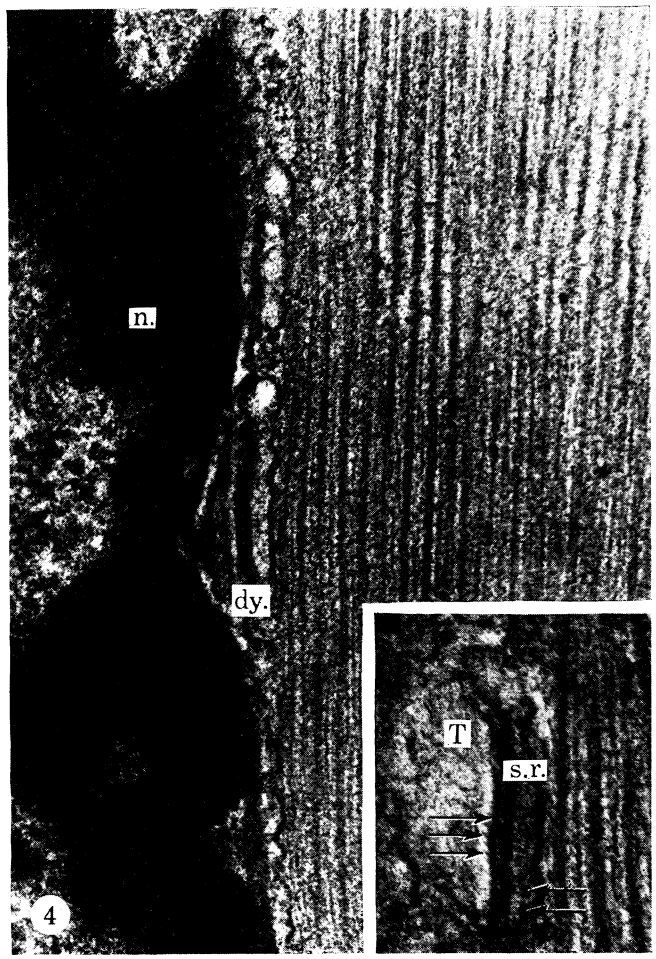

FIGURES 1–4. For description see opposite.

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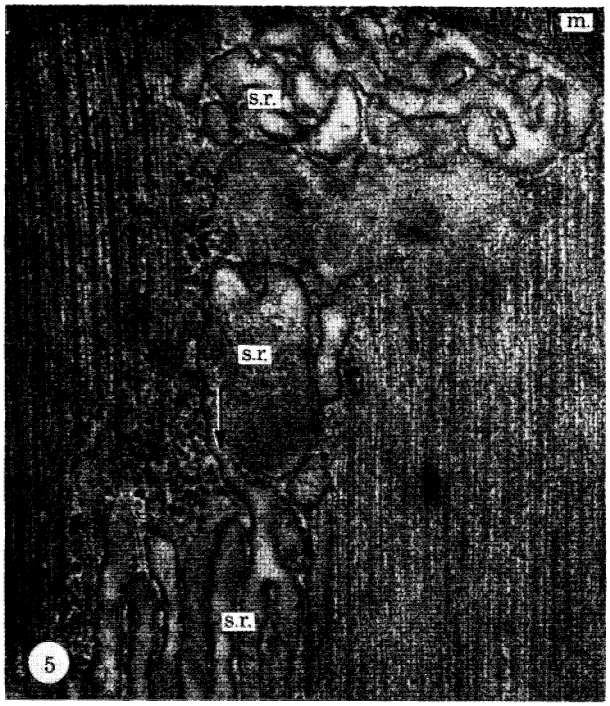

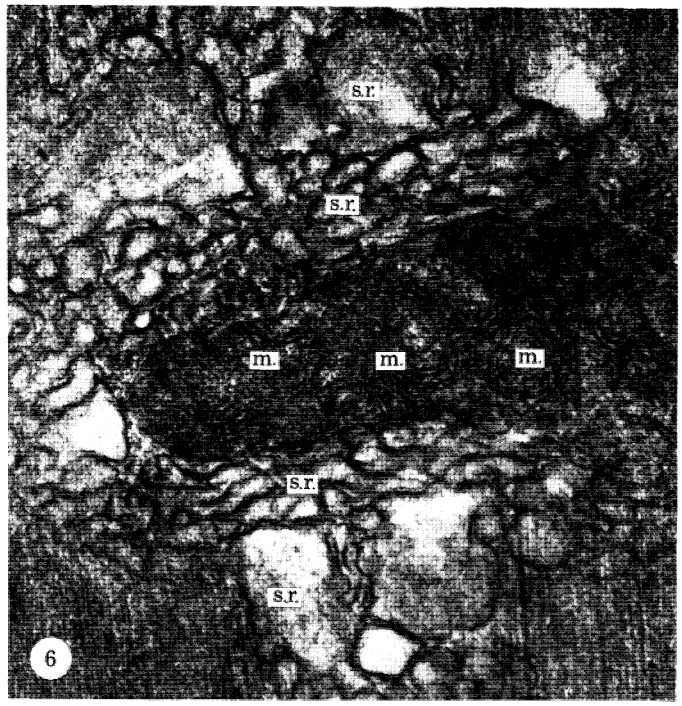

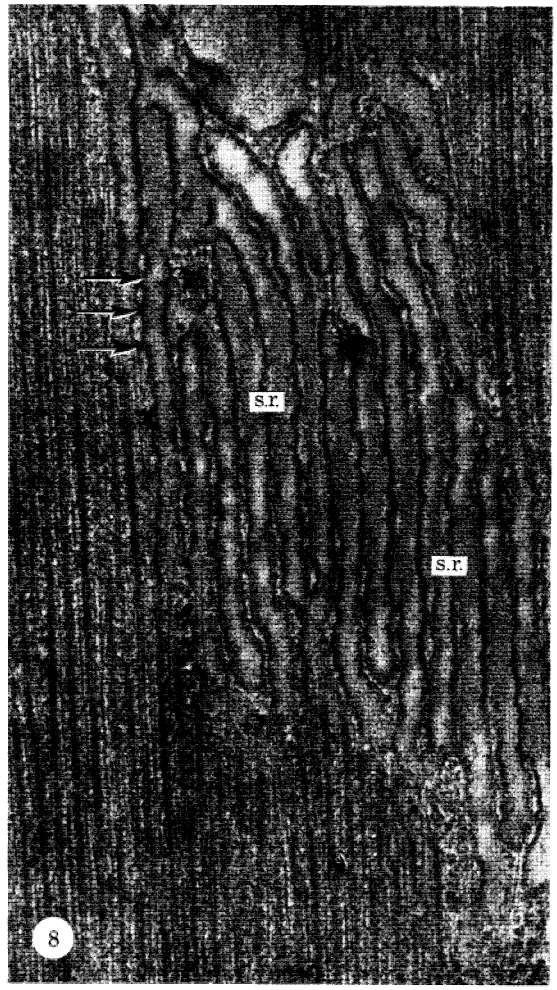

FIGURES 5–8. For description see opposite.

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(figure 2). There are normally between nine and eighteen rows making up the width of one lamella. The A-filaments are 18–20 nm in diameter and their centre to centre spacing in the hexagonal lattice is 50–55 nm. Orbitals of 9–13 I-filaments surround each A-filament. The I-filaments are 5–6 nm in diameter and the ratio of I-filaments to A-filaments is about 5.47:1. This is necessarily an approximate figure because occasional I-filaments are difficult to resolve when they lie obliquely in the plane of the section or lie close to a neighbouring filament.

Transverse sections through the periphery of the fibres show elements of the transverse tubular system (T-system) 25–40 nm wide, threading into the fibre from the sarcolemma and passing between the lamellae (figure 2). Dyadic contacts with elements of the sarcoplasmic reticulum are present along most of the length of the T-system tubules. Dyads are also formed between elements of the s.r. and the plasmalemma. At the dyads the s.r. is in the form of flattened sacs up to 700 nm broad and 30–50 nm wide. The contents of these sacs are more electron dense than the contents of the rest of the s.r. Blocks of electron dense material about 15 nm in diameter pass from the membranes of the s.r. saccules towards the membranes of the T-system without making contact with them. The junctional gap between the two elements of the dyad is 10–14 nm wide and the blocks are about 15 nm apart. Outside the dyads the s.r. in transverse section appears to be in the form of rounded vesicles, 25–70 nm in diameter, showing occasional lateral continuity (figure 2). Each fibre is bounded by a basement membrane 20–30 nm wide. Between the basement membrane and the plasmalemma microtubules lie parallel to the long axis of the fibre (figure 2). Tracheoles pass over the surface of the fibres but do not penetrate them (figure 1).

In longitudinal section the trochanteral depressor muscle fibres show the familiar interdigitating array of thick and thin filaments. A-bands, I-bands and Z-lines are present but there are no M-lines. The Z-lines are not straight but tend to be slightly wavy or zig-zag in form (figure 3). The Z-line width is consequently difficult to measure accurately but is in the range 90–120 nm. In the fibres examined here the sarcomeres are contracted but a short I-band is still present (figure 3). The I-filament length is 1.7–1.8 μm. The A-filaments are 3.5–3.6 μm long and occupy most of the sarcomere length.

Where the plane of sectioning has passed between two lamellae the arrangement of the membrane systems is clearly seen (figures 5–8, plate 38). Most of the A-band area of the lamellae is covered with a sheet of tubules of the s.r. The tubules, which are 30–60 nm in diameter, lie parallel with each other and with the longitudinal axis of the fibre. In places the tubules fork (figure 7), and there are occasional lateral connections between them (figure 8). Towards the edge of the A-band the tubules run into sac-like expansions of the s.r., the contents

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Description of plate 38

Figure 5. A longitudinal section which has glanced between two lamellae and shows parts of the three configurations of the sarcoplasmic reticulum. For details see text. Note the continuity between the saccules and the longitudinal tubules (arrow). (Magn. × 80000.)

Figure 6. Longitudinal section through the sarcoplasmic reticulum at the level of the I-band. The fibre is contracted so the sarcoplasmic reticulum at this level is compressed. (Magn. × 80000.)

Figure 7. Longitudinal section through the sarcoplasmic reticulum showing the continuity between the saccules and the longitudinal tubules (arrows). (Magn. × 80000.)

Figure 8. Longitudinal section through the sarcoplasmic reticulum at the level of the A-band. Note the lateral connexions between the longitudinal tubules (arrows). (Magn. × 80000.)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

of which are more electron dense than those of the tubular elements (figures 5, 7). These saccules whose transverse appearance was described above are involved in the formation of dyads. Between the s.r. saccules of adjoining sarcomeres there is a third type of s.r. which like the first is in the form of tubules but differs from it in that the tubules do not lie parallel with each other but interloop and interconnect in an apparently unorganized pattern (figures 5, 6). This segment of the sarcoplasmic reticulum partly surrounds the mitochondria which lie along the Z-line. The mitochondria which lie between the nuclei in the central core of the fibre share the position with vesicles of variable dimension and unknown origin (figure 3). In the inset of figure 4 the section has glanced obliquely through a dyad. It can be seen that the blocks of electron dense material which are attached to the membrane of the sarcoplasmic reticulum are circular in cross section (large arrows). Fine electron dense ridges can be distinguished on the face of the T-tubule membrane which opposes the s.r. membrane (fine arrows).

The results of the morphometric analysis of the trochanteral depressor muscle carried out on random oblique sections of the fibre are shown in table 1. Perhaps more informative than the volume of the sarcoplasmic reticulum is an indication of its surface area in square micrometres per cubic micrometre of myofibre (its surface density). It can be shown that if, for simplicity one takes the s.r. to be all in the form of smooth surfaced open ended cylinders the surface density can be calculated from the formula

$$\frac{S_{\text{s.r.}}}{V_{\text{m}}} = \frac{V_{\text{s.r.}}}{100}\,\frac{2\pi rL}{\pi r^2 L},$$

where $S_{\text{s.r.}}$ is the surface area of s.r., $V_{\text{s.r.}}$ the volume fraction of s.r., V_m the unit reference volume of muscle, r the radius of s.r. tubule, and L the unit length of s.r. tubule.

The diameter of the s.r tubules is 45.0 ± 11.1 nm ($n = 26$) which gives a surface density of 16.02 μm^2 per μm^3 of muscle. This calculation ignores the fact that part of the sarcoplasmic reticulum is in the form of saccules rather than tubules but the figure of 16.02 $\mu m^2/\mu m^3$ is more likely to be an underestimation rather than an overestimation because closed ends to the tubules are common and because the surfaces of the tubules are slightly wrinkled and not completely smooth.

TABLE 1. THE VOLUME FRACTION OF THE COMPONENTS OF THE FLEA TROCHANTERAL DEPRESSOR MUSCLE FIBRES EXPRESSED AS A PERCENTAGE OF THE FIBRE VOLUME: THE NUCLEI ARE OMITTED BECAUSE OF SAMPLING PROBLEMS

fibre component	no. of points counted	percentage of total
lamellae	1798	69.39
mitochondria	250	9.65
sarcoplasmic reticulum	467	18.02
T-system	27	1.04
sarcoplasm	49	1.89
total	2591	99.99

DISCUSSION

The basic arrangement of the fibres of the trochanteral depressor muscle with the contractile material organized into radial lamellae is typical of most insect leg muscles (Tiegs 1955). The presence of a central core of nuclei has resulted in thist ype of muscle being termed tubular

(Pringle 1957, 1972; Chapman 1969; Matsuda 1970). Apart from those muscles in cockroaches and locusts which are also part of the flight system, all the leg muscles examined to date have an I-filament to A-filament ratio between 5:1 (*Calliphora* tergotrochanteral muscle, Auber 1967) and 6:1 (cockroach coxotrochanteral muscle, Jahromi & Atwood 1969). Hoyle (1967) describes a ratio of 4:1 in the locust jumping muscle but tracings from the published electronmicrograph show a ratio of 5.44:1. The ratio of 5.47:1 for the flea muscle therefore falls into the normal range for insect leg muscles. The sarcomere lengths found in leg muscles from other insects range from 2–3 μm (resting length in *Musca*, Pasquali-Ronchetti 1970) to 5–8 μm (cockroach stretched femoral muscle, Hagopian 1966) but are typically 4–5 μm. The minimum contracted length for the flea jumping muscle sarcomeres (Z-line width + A-filament length) will be about 3.6 μm while the maximum extended length (Z-line width + A-filament length + I-filament length × 2) will be about 7.1 μm. These dimensions fall within the range found for other insects.

The arrangement of the mitochondria also does not differ from that described in other insect leg muscles, apart from those involved in flight. In *Xenopsylla* the mitochondria occupy about 9.7 % of the volume of the fibres of the jumping muscle. To the author's knowledge no accurate measurement has been made of the volume fraction of the mitochondria in other insect leg muscles although a figure of < 10 % has been given for the trochanter extensor of the cockroach (Jahromi & Atwood 1969). In the same insect another leg muscle which is also used for flight has a mitochondrial volume of 35 % of the fibre volume. These figures compared with the figures of 25–40 % and 40 % for the flight muscles of *Sarcophaga* (Tribe & Ashhurst 1972) and *Aeschna* (Smith 1961) respectively.

The membrane systems

The only insect leg muscle membrane systems which have been described in any detail are those of a cockroach *Leucophaea* (Hagopian & Spiro 1967), a fly *Musca* (Pasqualli-Ronchetti 1969) and several Hemiptera (Cullen 1971). The sarcoplasmic reticulum in the cockroach is a fenestrated sheet but in the fly and in the Hemiptera it is a system of interconnecting tubules as in *Xenopsylla*. However, the fly differs from the flea in that *all* the sarcoplasmic reticulum outside the dyads is in the form of tubules lying roughly parallel with each other and running along the longitudinal axis of the fibre, and the diameter of the tubules (50–80 nm) is greater than in the flea. In the Hemiptera, where the sarcoplasmic reticulum is not so regularly organized, and in *Leucophaea*, the sarcoplasmic reticulum resembles that of *Xenopsylla* in having sac-like expansions which are involved in the formation of dyads and which have more electron-dense contents than the rest of the reticulum. In *Musca* similar electron-dense saccular dilatations project longitudinally from the transverse tubules of the T-system. The sarcoplasmic reticulum does not pass over the T-system tubules which therefore divide it into discrete segments. In *Xenopsylla* the s.r. tubules run across the T-tubules so that the whole s.r. system is interconnected.

The presence of three distinct configurations of s.r. as exists in the rat flea jumping muscle has not been observed in other insect muscles. It is likely that the conspicuous difference between the highly organized arrangement of the s.r. in the A-band region and the unorganized arrangement around the I-band is related to differences in internal forces experienced during contraction of the fibre. Except in those rare muscles which undergo supercontraction (Crossley 1967; Osborne 1967; Rice 1970) the A-band maintains its length during normal contraction, whereas the I-band shortens, and if the dyadic segment of the s.r. maintains its position at the edge of

the A-band the structures around the I-band must become compressed. The more flexible arrangement of anastomizing interlinked tubules of the s.r. in this region will be more suited to absorb the compression.

The trochanteral depressor muscle of *Xenopsylla* contains more sarcoplasmic reticulum than any other insect muscle which has been examined by electron microscopy. The only known arthropod muscle which contains more s.r. is the fast-acting antennal remotor muscle of the lobster (Rosenbluth 1969). The s.r. is believed to be involved in the excitation–contraction–relaxation cycle of muscle, both vertebrate and invertebrate (Smith 1966; Ebashi, Endo & Ohtsuki 1969) and there is good evidence that in general there is a positive correlation between the amount of sarcoplasmic reticulum in a muscle fibre and its speed of contraction (Franzini-Armstrong 1973). Cochrane, Elder & Usherwood (1969) estimated the amount of sarcoplasmic reticulum in two phasic leg muscles and one tonic leg muscle of the locust. They found that the two phasic muscles had s.r. surface areas of 14.4 and 11.3 $\mu m^2/\mu m^3$ and times to peak twitch tension of 30 and 56 ms respectively, whereas the tonic fibres had a s.r. surface area of 5.5 $\mu m^2/\mu m^3$ and a time to peak twitch tension of 810 ms. Cochrane *et al.* pointed out that the differences in contraction speed could be related to the differences in the amount of s.r. although there is also the possibility that the contractile components in the two types of fibre have different properties. The figure for s.r. surface area of 16.02 $\mu m^2/\mu m^3$ obtained from *Xenopsylla* is in the same range as that of the phasic jumping muscle of the locust. This suggests that the relatively large volume and surface area of the s.r. in the flea trochanteral depressor muscle is a specialization for the rapid contraction involved in the insect's jump.

I should like to thank Dr Miriam Rothschild for first suggesting that I examine the flea's jumping muscle and Dr Yosef Schlein for dissecting, fixing and embedding the material. I should also like to thank Professor J. N. Walton for allowing me to use the facilities of the Muscular Dystrophy Research Laboratories in Newcastle upon Tyne.

Note added in proof 26 *September* 1975. My attention has recently been drawn to a fuller account of the work of Cochrane, Elder & Usherwood (1972) on the physiology and ultrastructure of the leg muscles of the locust (*J. Cell Sci.* **10**, 419–441). Their corrected figures for the s.r. volume include a figure of 19% of the fibre volume in the phasic retractor unguis muscle which is slightly higher than the figure recorded here for the flea trochanteral depressor muscle.

References

Auber, J. 1967 Distribution of the two kinds of myofilaments in insect muscle. *Am. Zool.* **7**, 451–456.

Chapman, R. F. 1969 *The insects: structure and function*. English Universities Press.

Cochrane, D. G., Elder, H. Y. & Usherwood, P. N. R. 1969 Electrical, mechanical and ultrastructural properties of tonic and phasic muscle fibres in the locust (*Schistocerca gregaria*). *J. Physiol., Lond.* **200**, 68–69*P*.

Crossley, A. C. 1967 The fine structure and mechanism of breakdown of larval inter-segmental muscle in the blowfly *Calliphora erythrocephala*. *J. Insect Physiol.* **14**, 1389–1407.

Cullen, M. J. 1971 A comparative study of the anatomical basis of flight in Hemiptera. D.Phil. thesis. Oxford University.

Ebashi, S., Endo, M. & Ohtsuki, I. 1969 Control of muscle contraction. *Quart. Rev. Biophys.* **2**, 351–384.

Franzini-Armstrong, C. 1973 Membranous systems in muscle fibers. In *The structure and function of muscle* (ed. G. H. Bourne), 2nd edn, vol. II, pp. 531–619. New York & London: Academic Press.

Hagopian, M. 1966 The myofilament arrangement in the femoral muscle of the cockroach *Leucophaea maderae*. *J. Cell Biol.* **28**, 545–562.

Hagopian, M. & Spiro, D. 1962 The sarcoplasmic reticulum and its association with the T-system in an insect. *J. Cell Biol.* **32**, 535–545.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Hally, A. D. 1967 A counting method for measuring volumes of tissue components in microscopical sections. *Q. J. microsc. Sci.* **105**, 503–517.
Hoyle, G. 1967 Diversity of striated muscle. *Am. Zool.* **7**, 435–449.
Jahromi, S. S. & Atwood, H. L. 1969 Structural features of muscle fibres in the cockroach leg. *J. Insect Physiol.* **15**, 2255–2262.
Matsuda, R. 1970 Morphology and evolution of the insect thorax. *Memoirs ent. Soc. Can.* no. 76.
Osborne, M. P. 1967 Supercontraction in the muscles of the blowfly larva: an ultrastructural study. *J. Insect Physiol.* **13**, 1471–1482.
Pasqualli-Ronchetti, I. 1969 The organisation of the sarcoplasmic reticulum and T-system in the femoral muscle of the housefly, *Musca domestica*. *J. Cell Biol.* **40**, 269–273.
Pasqualli-Ronchetti, I. 1970 The ultrastructural organisation of femoral muscles in *Musca domestica* (Diptera). *Tissue and Cell* **2**, 339–354.
Pringle, J. W. S. 1957 *Insect Flight*. London: Cambridge University Press.
Pringle, J. W. S. 1972 *Arthropod muscle*. In *The structure and function of muscle* (ed. G. H. Bourne), 2nd edn, vol. 1, pp. 491–541. New York and London: Academic Press.
Rice, M. J. 1970 Supercontracting and non-supercontracting visceral muscles in the tsetse fly, *Glossina austeni*. *J. Insect Physiol.* **16**, 1109–1123.
Rosenbluth, J. 1969 Sarcoplasmic reticulum of an unusually fast-acting crustacean muscle. *J. Cell Biol.* **42**, 534–547.
Rothschild, M., Schlein, Y., Parker, K. & Sternberg, S. 1972 Jump of the oriental rat flea *Xenopsylla cheopis* (Roths.) *Nature, Lond.* **239**, 45–48.
Smith, D. S. 1961 The organisation of the flight muscle in a dragonfly, *Aeschna* sp. *J. biophys. biochem. Cytol.* **11**, 119–145.
Smith, D. S. 1966 The organisation and function of the sarcoplasmic reticulum and T-system of muscle cells. *Prog. biophys. molec. Biol.* **16**, 107–143.
Tiegs, O. W. 1955 The flight muscles of insects – their anatomy and histology: with some observations on the structure of striated muscle in general. *Phil. Trans. R. Soc. Lond.* B, **238**, 221–348.
Tribe, M. A. & Ashhurst, D. E. 1972 Biochemical and structural variations in the flight muscle mitochondria of ageing blowflies, *Calliphora erythrocephala*. *J. Cell. Sci.* **10**, 443–469.
Weibel, E. R., Kistler, G. S. & Scherle, W. F. 1966 Practical stereological methods for morphometric cytology. *J. Cell Biol.* **30**, 23–36.
Weibel, E. R. 1972*a* The value of stereology in analysing structure and function of cells and organs. *J. Microsc.* **95**, 3–14.
Weibel, E. R. 1972*b* A stereological method for estimating volume and surface of sarcoplasmic reticulum. *J. Microsc.* **95**, 229–242.
Wilson, D. M. 1961 The central nervous control of flight in a locust. *J. exp. Biol.* **38**, 471–490.

Abbreviations used on figures

A	A-band	n.	nucleus
b.m.	basement membrane	s.r.	sarcoplasmic reticulum
dy.	dyad	T	T-system tubule
I	I-band	Tr.	tracheole
l.	lamella	v.	vesicle
m.	mitochondrion	Z	Z-line
mt.	microtubule		

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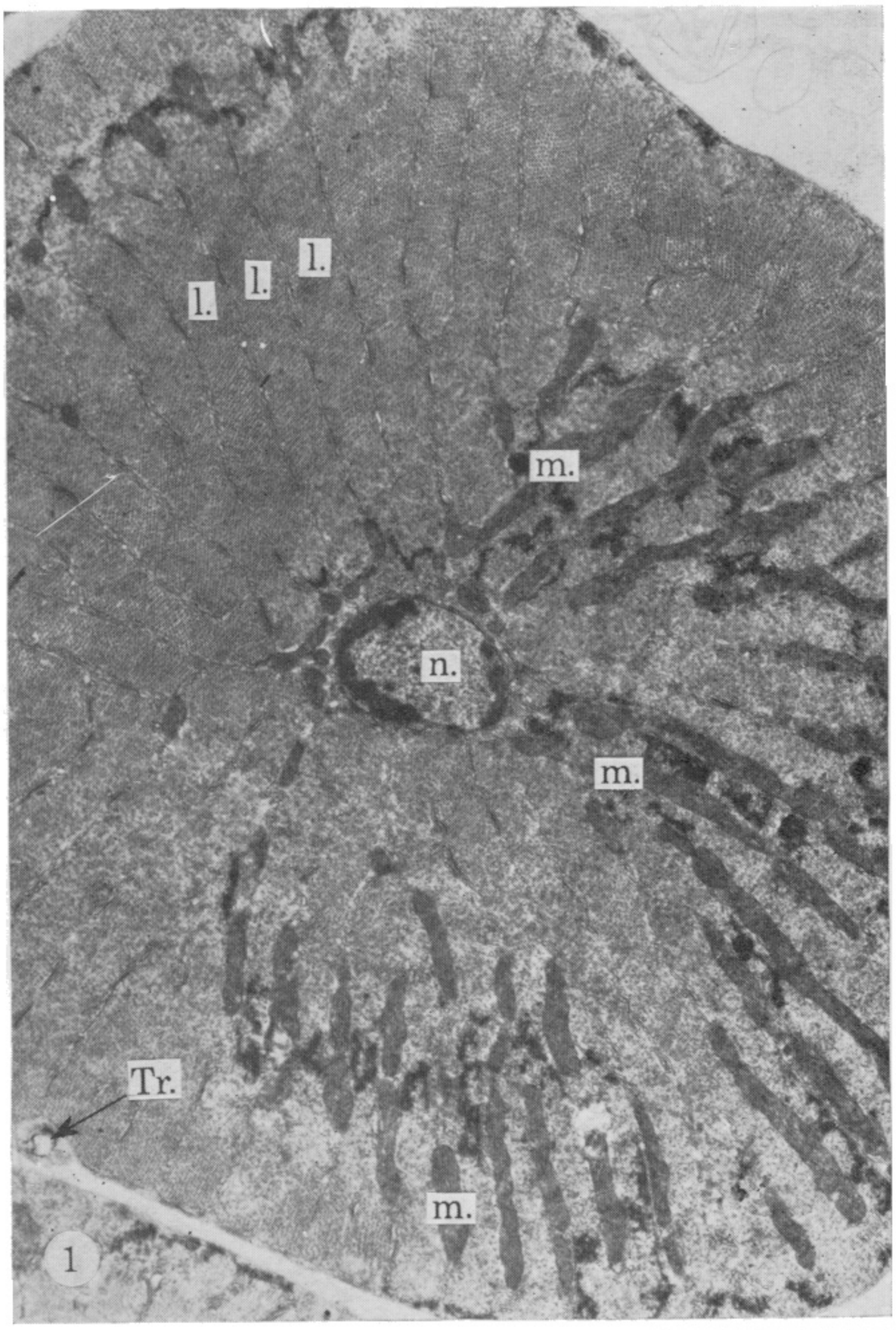
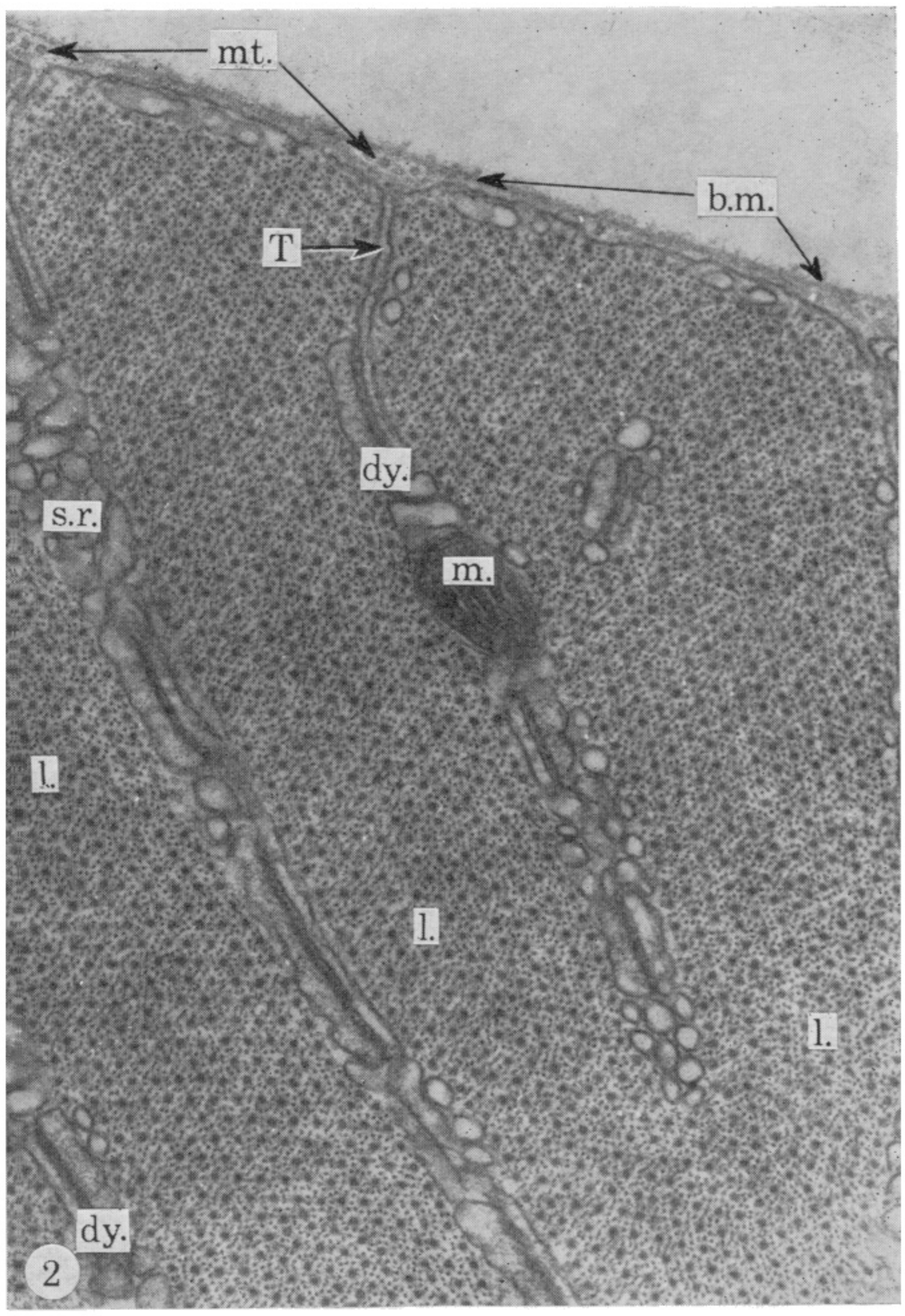
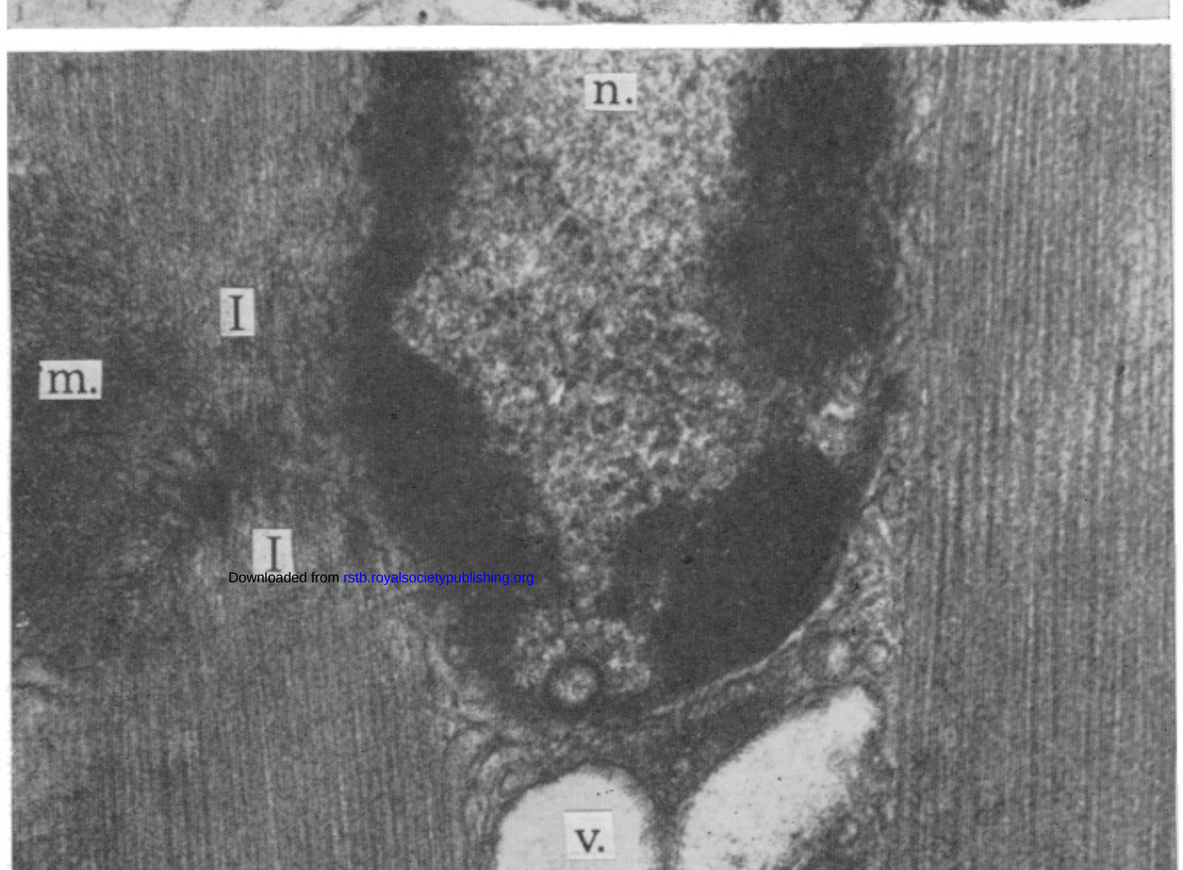
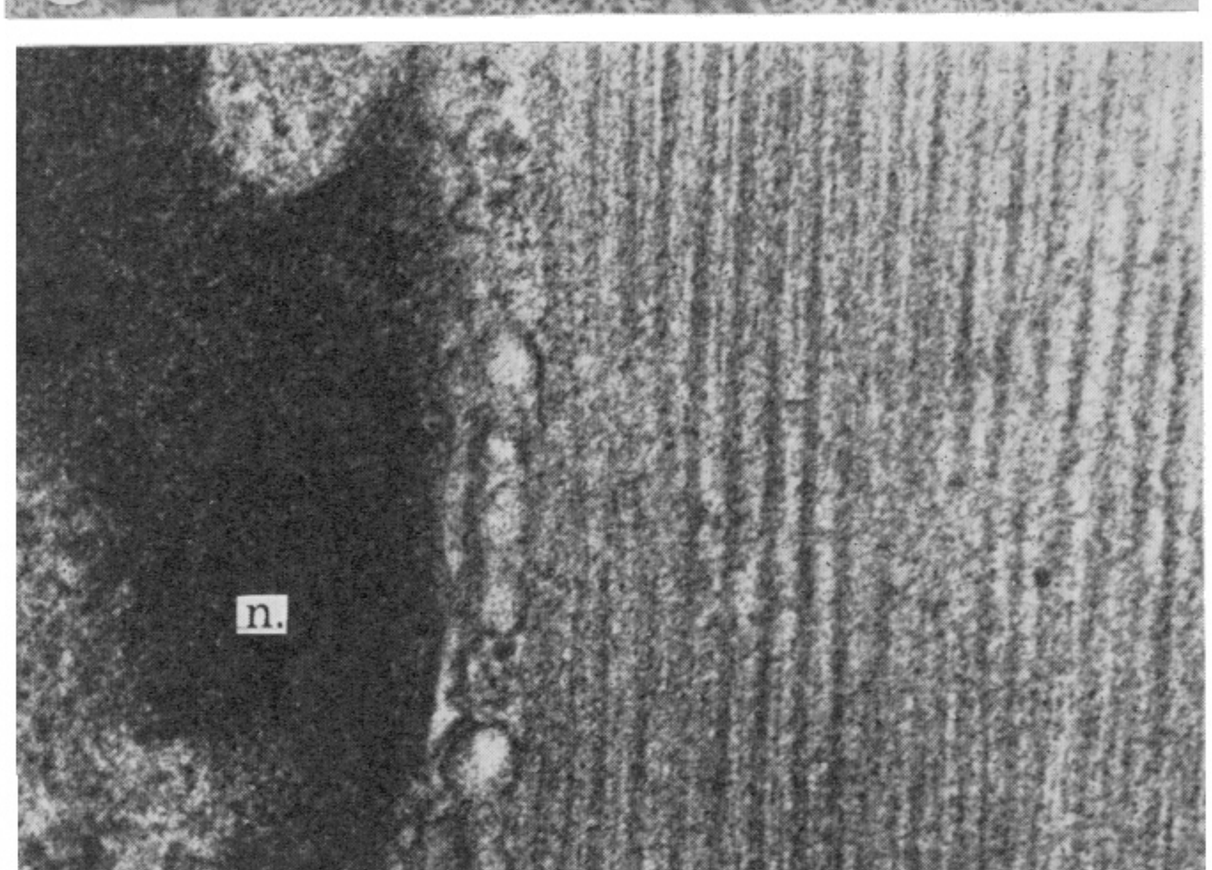
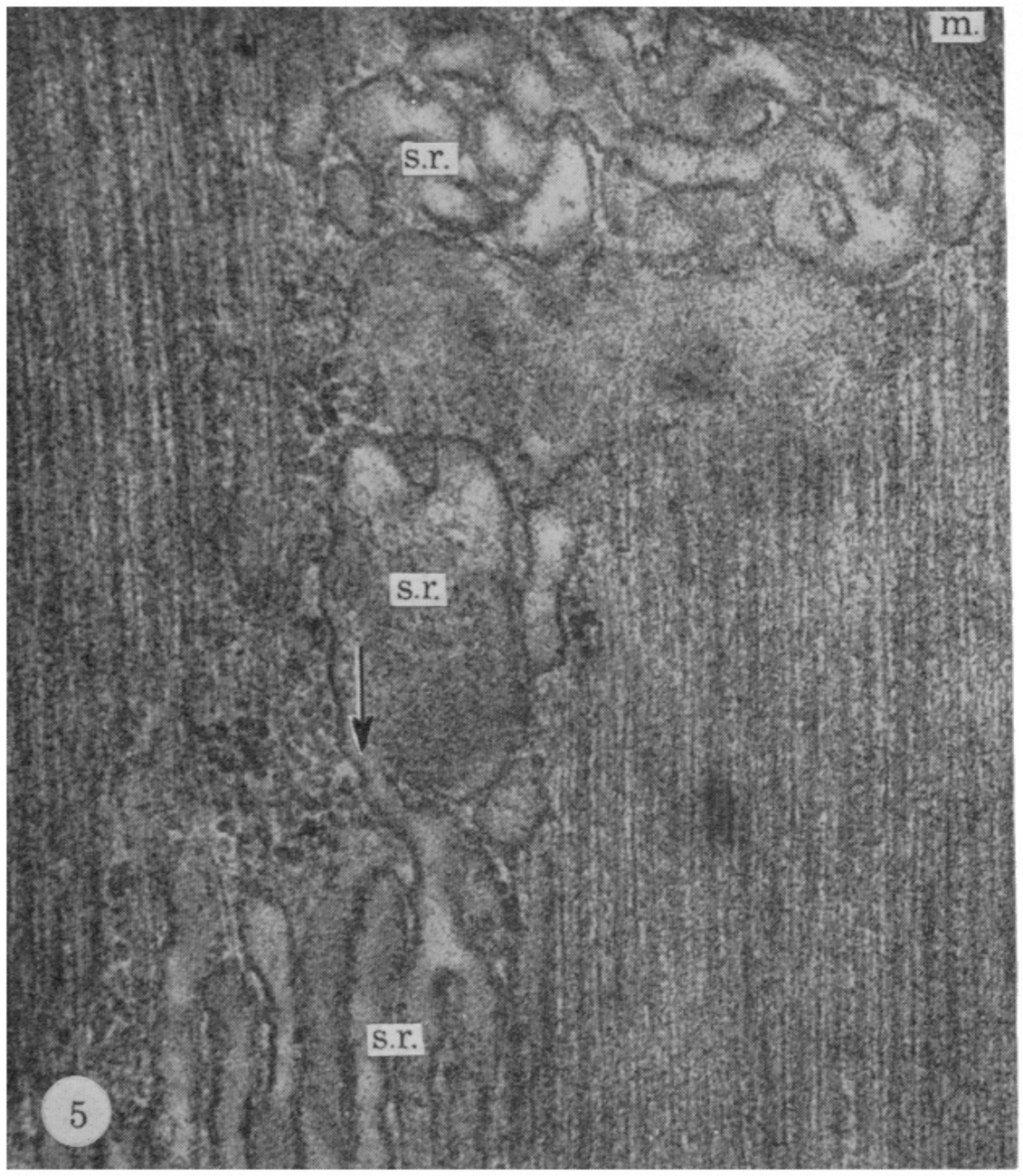








FIGURES 1–4. For description see opposite.



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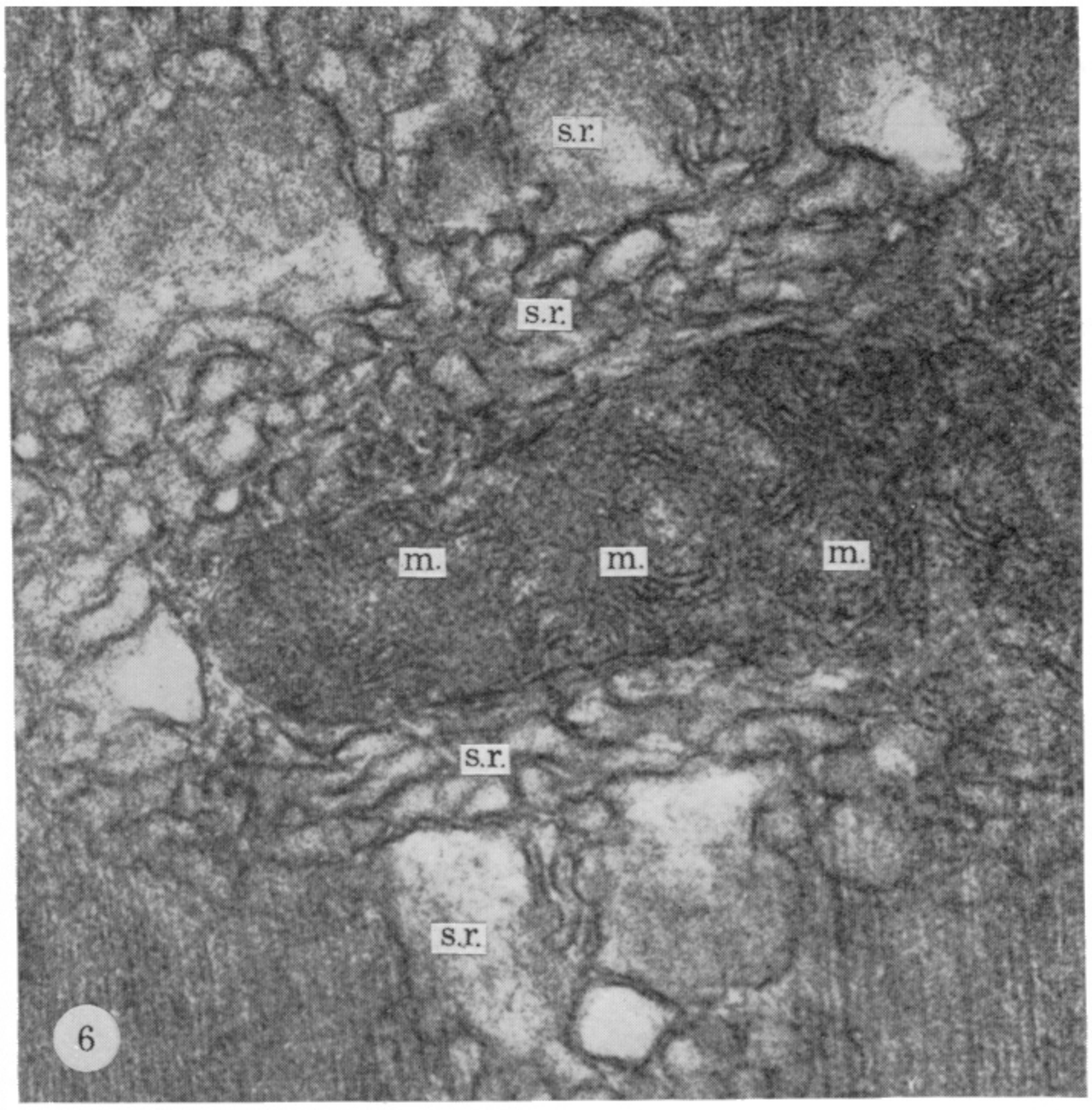

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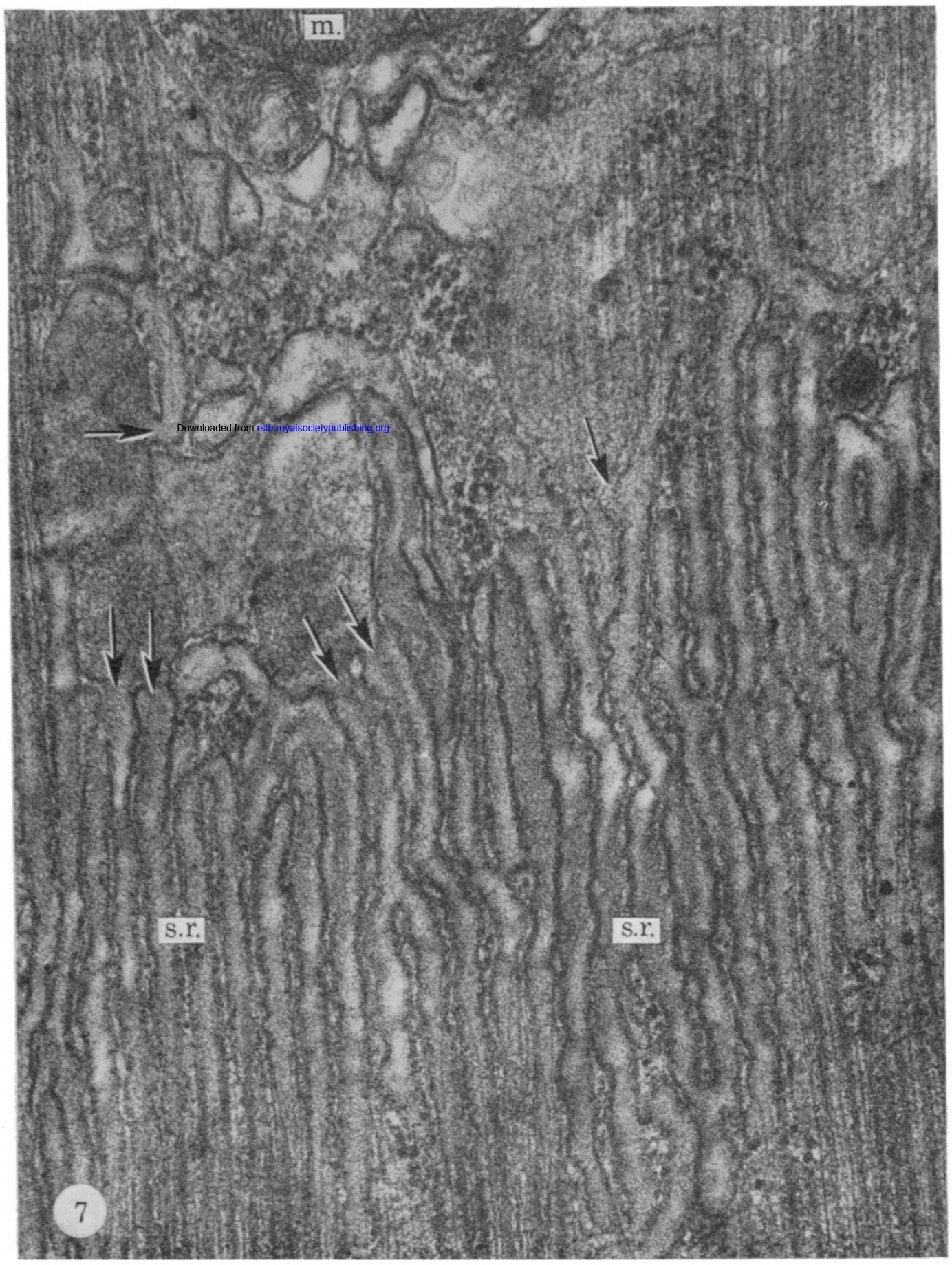
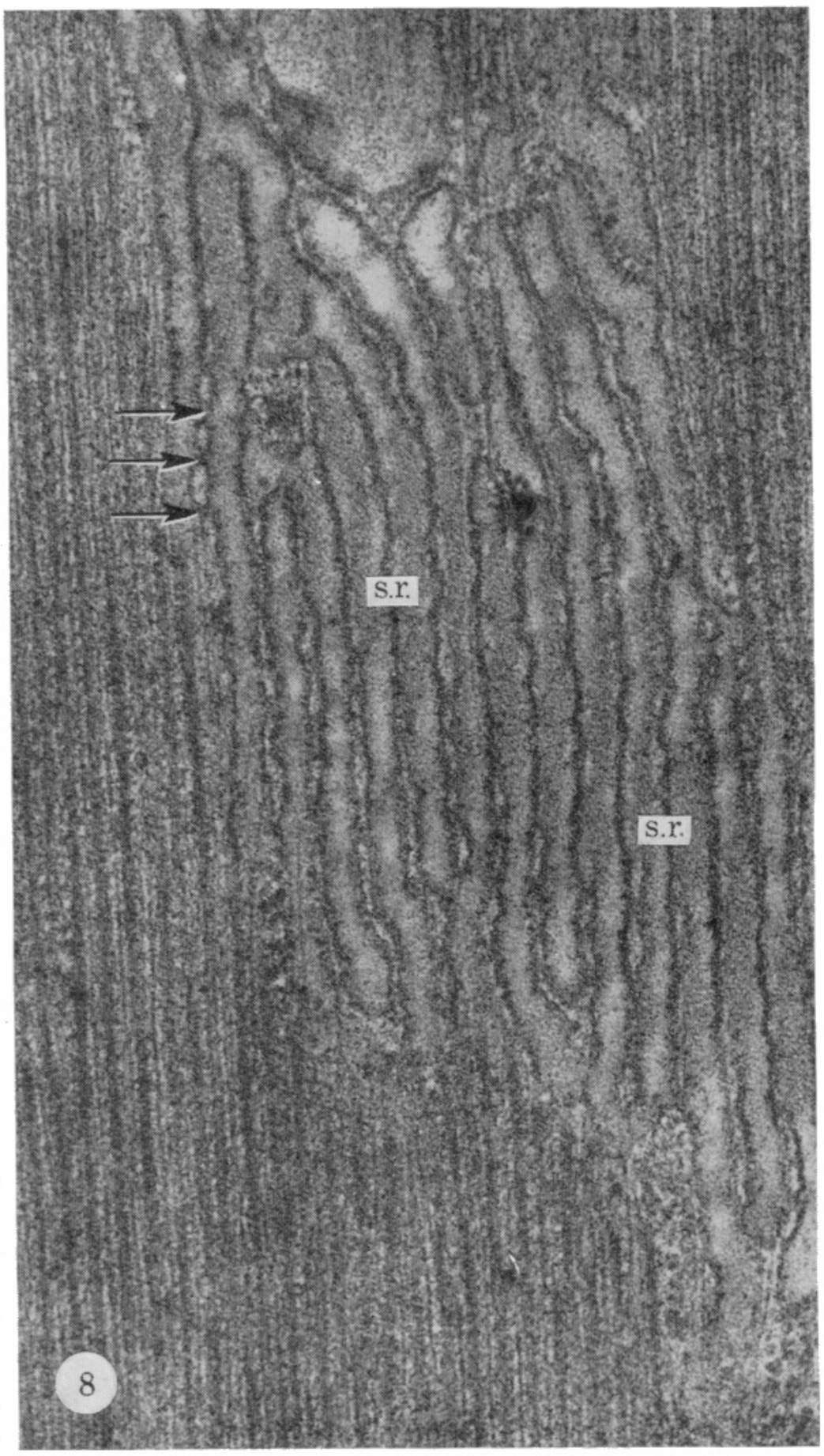


7



8

FIGURES 5–8. For description see opposite.