THE MUSCULATURE OF *PERIPATUS* AND ITS INNERVATION

By G. HOYLE AND MELISSA WILLIAMS
Department of Biology, University of Oregon, Eugene, OR, 97403, *U.S.A.*

(*Communicated by J. Z. Young, F.R.S. – Received* 4 *January* 1979)

[Plates 1–24]

CONTENTS

The musculature of the Onychophoran *Peripatus dominicae*, its ultrastructure and details of innervation are described. Significant differences were noted between its gross anatomy and that reported in previous accounts, notably in the presence of inner circular body wall muscle and a prominent, functionally significant, levator of the leg. The former is important in regard to the evolutionary position of the Onychophora while the latter helps us to understand the control of walking in a lobopodial leg, and therefore the evolution of arthropod locomotion, which was the focus of our interest.

Individual muscle fibres are either directly or indirectly attached to the body wall by collagen. There is a small degree of branching of fibres, with or without anastomosis, near their insertions, but most are as long as the muscle of which they are part, and are unbranched except for an occasional thin arm, emerging at an angle, that becomes invaded by collagen fibres and inserts in the skin. Diameters of muscle fibres vary from 1 to 45 μm. They are invaginated by two separate systems of unique wide (0.3 μm) tubules, longitudinal and radial. These are lined with similar material to that forming the basement material of the sarcolemma, and also contain fine strands with collagen-type cross-banding that connect to collagen bundles outside the fibres. In addition there are narrow tubules of ordinary T-tubule diameter. Both wide and narrow tubules make contacts with sarcoplasmic reticulum cysternae.

Dense Z bodies are attached to both kinds of wide tubule, to the inside of the sarcolemma, and are scattered, without any obvious array, in the sarcoplasm. Thin myofilaments emerge from the Z bodies parallel to the fibre axis. Thick filaments occur in clusters with a loosely hexagonal array, but without any regular relation to thin ones: relatively few orbits of thin around thick filaments were seen in many muscle fibres regardless of fibre length and conditions during fixation.

A unique innervation pattern was found, consisting of a combination of muscle arm to nerve contacts, which appear to be the commonest, and nerve on muscle fibre synapses. At least 13 motor axons were found to supply each small muscle or cluster of muscle fibres in a large muscle. Each muscle arm simultaneously makes synaptic contact with 3 to 7 axons. Nerve on muscle junctions contain from 1 to 8 axons, each making synaptic contacts. The details of the postsynaptic endplate-specializations resemble those seen in mammalian endplates and are markedly different from both arthropod and annelidan neuromuscular synapses.

INTRODUCTION

General accounts of the body musculature of members of the group of animals termed Onychophora have been given by Snodgrass (1938), Manton (1973) and Birket-Smith (1974). These descriptions have been extensively utilized, particularly by Manton (1950, 1966, 1973, 1977) in comparative studies of locomotion, one one hand, and considerations of evolution and taxonomic classification on the other. There has, however, been no study on the neuromuscular physiology of this evolutionarily important organism until recently when the species *Peripatus dominicae* obtained from the rain forest of Puerto Rico was studied by Hoyle & del Castillo (1979).

In the course of dissecting this *Peripatus* species in order to make neuromuscular transmission experiments, some discrepancies were noted both between the Snodgrass, Manton and Birket-Smith accounts, and also between these and what was actually found in *P. dominicae.*

The Snodgrass account was based upon *Peripatoides novae zealandiae*, that of Manton on *Peripatopsis sp.* and that of Birket-Smith on the same species used in the present work as well as *Peripatopsis capensi.* The latter worker had available only material preserved in 70% alcohol some 70 years previously but the drawings indicate clearly that the features to which attention will be drawn in the present paper were seen, although their consequences not fully realised.

Some of the discrepancies cannot be ascribed to species differences. For example, the leg of *P. dominicae* contains a large, fan-shaped muscle extending from the tip of the foot to the dorsal longitudinal muscle that can easily be shown experimentally to be a levator of the leg. No such muscle is mentioned in either the Snodgrass or the Manton accounts. The muscle is clearly illustrated in figures 2 and 3 of Birket-Smith (1974), where it is termed the retractor of the lobopod and assigned the number 21, extending the sequence used by Snodgrass.

Accordingly, a description of the anatomy of the musculature of *P. dominicae* is presented here, to serve as a basis for following papers on the physiology of the muscles and their neural control. Two main purposes were borne in mind. One was determining the extent to which the onychophoran leg provides a model system from which an understanding of the fundamental aspects of arthropod locomotion might be achieved. The other was to obtain a new perspective on the extensive discussions that have arisen regarding the possible phylogenetic position of Onychophora from the viewpoint of a specialist on arthropod muscles and their neural control.

No published description of the fine structure of the muscles of an Onychophoran was available, although it has been observed by F. W. Schürmann (personal communication) in connection with studies on the nerve cord (Schürmann and Sandeman 1976; Schürmann 1978*a*) and has been mentioned in an abstract (Saita & Camatini 1976). It was desirable to know this and also basic details of innervation, to complement physiological studies. The taxonomic position of *Peripatus* is uncertain and the subject of intensive debate. One view, dating from Sedgwick (1888; 1909), and still commonly held by writers of Zoology texts, regards *Peripatus* as a living fossil or 'missing link' between annelids and arthropods (see, for example, Buchsbaum, 1976). Researchers often preface their studies on Onychophorans by saying that 'they occupy a unique phyletic position between the annelids and arthropods' (Schürmann & Sandeman 1976). Their proposed phylogenetic status among leading evolutionists ranges widely. At one end of the spectrum they are regarded as members of a phylum Articulata that includes annelids, crustaceans and insects (Beklemischev 1969). Manton (1977) wishes to associate them only with myriapods and insects in a proposed new taxon of phylum rank, the Uniramia, that is supposed to be widely separated from crustaceans on one hand and from annelids on the other. At the other extreme Sharov (1965*a*, *b*; 1966) denies that Onychophorans are arthropods as do some specialists in fields outside taxonomy. Neville (1975), after a detailed comparison of cuticle structures, concluded that the Onychophora should not be regarded as arthropods. Locke & Huie (1977) recently found that the Golgi complex of a wide variety of arthropod species including arachnids, crustaceans and insects, showed characteristic complex beads when stained with bismuth. Golgi complexes of the Onychophoran *Epiperipatus* did not show them.

The anatomy and physiology of the neuromuscular apparatuses of diverse arthropods is now well-known (see reviews by Smith 1970; Elder 1975; Atwood 1976; Huddart 1975). There is less extensive coverage of annelids, but examples of the major classes, polychaetes (Rosenbluth 1968), oligochaetes (Rosenbluth 1972) and hirudineans (Tulsi & Rosenbluth 1971) have been examined and show an homogeneity of fine structure that is distinctive for the phylum.

Finally, onychophoran neuromuscular junctions, which have never been described, were included in the study. This knowledge was needed both to serve as a basis for physiological and pharmacological studies and to round out the material for comparative phylogenetic considerations. There is an extremely close similarity between all aspects of innervation; gross anatomy, innervation patterns, neuromuscular physiology and pharmacology, of insects and crustaceans. Their common features are markedly different in some respects from those of annelids. It might be expected that a knowledge of these features in Onychophora would help to clarify their relationships, or throw some light on the evolution of their presumed relatives. At least, in such a controversial situation it is desirable that the opinion of nerve/muscle specialists be added to the balance.

MATERIALS AND METHODS

Peripatus dominicae juanensis Clark (1913) (figure 1†) were collected above 2000 ft (610 m) in the rain forests of Puerto Rico. This species is viviparous: females are larger than males, a mature one being almost 10 cm long during walking and 4.5 cm long when contracted, with 31 pairs of legs. The legs are about 1.2 mm thick and 1.5–2.0 mm long. They were kept until needed in a moist terrarium in an air-conditioned room at about 22 °C and fed on termite larvae. They were examined after live dissection and by light and electron microscopy.

Specimens were fixed by rapidly filling the body cavity with cold, fresh 3 % formaldehyde/3 % glutaraldehyde buffered in 0.1 M sodium cacodylate through a syringe until extended to about average body length, followed by immersion in the fluid overnight. Selected parts were dissected out in a fume hood and placed in fresh fixative, washed in 0.1 M sodium cacodylate buffered at pH 7.3, post-fixed by standard procedure in osmium tetroxide, dehydrated and embedded in Epon/Araldite and sectioned with an ultramicrotome. Selected regions of the musculature were serially sectioned.

MUSCULATURE

The various muscles will be first described individually. Then general features that are peculiar to onychophoran muscles will be outlined. Finally, a comparison of the present findings with the descriptions of Snodgrass (1938), Manton (1973) and Birket-Smith (1974) will be made. The numbering used by Snodgrass will be retained, except for muscles for which his account is not acceptable but this is arbitrary and not convenient to follow. So we shall use an anatomical order, with abbreviations.

Muscles of the body wall

The principal body wall muscles may be observed in any transverse section but can be clearly understood only by extensive dissection, as is beautifully brought out in figure 2 of Birket-Smith (1974). They are interlaced with both masses of collagen fibres and fine muscle processes and there is considerable interweaving of component fibres at zones of overlap.

(*a*) *Outer circular* (*OC*-13)

The entire body is surrounded by the outermost layer, comprising thin (5–15 μm) outer circular muscle fibres one layer deep lying just below the skin of cuticle plus collagen feltwork. The fibres are generally well separated from each other (figure 2*a*). They run into the legs where these occur, terminating on them.

(*b*) *Outer oblique* (*OObl*-10) *and inner oblique* (*IObl*-9)

Immediately inside OC is a layer of fairly thin (2–10 μm) parallel fibres about 4 deep. In the relaxed animal these fibres point backwards at an angle of about 35° with respect to the midline. Therefore, in the dorsal midline these fibres cross each other, at the same time making numerous collagenous connections. As they pass, they cross over and under (figure 2*b*) so that the outer oblique muscle (OObl) of one half becomes the inner oblique (IObl) of the other half. There are many collagen strands at the points of crossing over, which are responsible for the so-called white line (Birkett-Smith 1974). This structure makes it difficult to ascertain the

† Figure *n* (*n* = 1–24) appears on plate *n*.

fate of individual fibres, but it is evident that although some do terminate at the midlines many continue after crossing over. The two layers cross again in the ventral midline, where similar conditions prevail.

In addition to these major cross-over points, broad bands of oblique muscle also cross each other near the bases of the feet, as clearly figured by Snodgrass (1938). Undoubtedly this weaving serves to strengthen the body wall at these critical places from the point of view of stress, but it is somewhat surprising to see such a high degree of complexity in an otherwise primitive muscular system.

(*c*) *Dorsal longitudinal* (*left and right DL*-1)

On the dorsal surface in the midline lies a pair of stout parallel bundles of dorsal longitudinal muscles (left and right DL) running the entire length of the body. Each half has a flattened oval shape in cross section (figure 3) and contains about 350 muscle fibres. The ones on the outside are of much smaller diameter than those on the inside, which range up to 45 μm; this feature is present in all the longitudinal muscles. Collagen strands bind the muscle fibres to each other and to the body wall after passing through OObl and OC. Dissection of individual muscle fibres proved to be difficult, even after soaking in collagenase, but lengths up to 3 cm were obtained and the impression was gained that they may be as long as the whole muscle.

DESCRIPTION OF PLATES 1, 2 AND 4

FIGURE 1. *Peripatus dominicae* head, leg and foot viewed by scanning electron microscope. Top: Head, with left antenna. The mouth and jaws are fully protruded. The left eye is at the base of the antenna, and just below it the motile 'nozzle' of the glue gun. From this, long thin strands of a very sticky, quickly-drying glue are projected anteriorly for distances up to 2½ ft (0.75 m) when the animal is disturbed. Lower left: Leg in the levated position, with the foot also raised (leg was 1.7 mm long). Lower right: View of the foot in the retracted position. Cone-shaped bases of unique tactile sense organs fold over the claw.

FIGURE 2. Photomicrographs of outer and inner circular, and oblique muscle sheets of the body wall at the dorsal mid-line. Ostia are from the haemocoel into the pericardium, exactly above the heart, one at each fold in the cuticle in the centre of the so-called 'dorsal white line'. (A) Viewed from above after removal of the cuticle and collagen layer (= skin), anterior above. Note the outer circular muscle fibres and the over-and-under crossing of oblique muscles. (B) Viewed from inside after removal of the dorsal longitudinal muscles. Portions of a few LL fibres remain. Note the giant inner circular fibres. Some of these were dissected intact around almost the entire circumference of the body. (Magn. × 50.)

FIGURE 4. Scanning electron microscope pictures of thick transverse slices through *P. dominicae*.

(*a*) View from left and above, of a complete body segment slit open dorsally. The floor of the pericardium, heart and dorsal longitudinal muscles were removed, also the connective tissue lining and the ventral longitudinal muscle, of which a few fibres remain. The line denotes the position of the ventral midline. To its right may be seen ostia leading into the haemocoel above the feet. Parallel lines are formed by giant inner circular muscle fibres, that traverse the whole section, and emphasised by collagen between them. These muscle fibres are all about 40 μm thick. (Magn. × 15.)

(*b*) Slice viewed from the front. The right leg (at left) is cut close to the border of the leg, just posterior to the outside attachment of the septal muscle (S – see D). The left leg (at right) was cut across its anterior border. Landmarks include the heart (H), the salivary glands (SG) and nerve cord (NC). Note that the right lateral longitudinal muscle (LL) is whole in this region. The more anterior slice through the right leg passes through the levator of the leg (Le) which passes through LL. (Magn. × 18.2.)

(*c*) Enlarged view of the right leg. The retractor of the leg is clearly seen passing on the inside of LL, and twisting through 90° to pass down the posterior border of the leg. (Magn. × 48.)

(*d*) Enlarged view of the left leg. The levator is seen at right, forming the outer margin of the outer hemocoelic space. The anterior depressor forms the floor of the inner anterior hemocoelic chamber. The S-shaped septal muscle divides the leg. At the top may be seen the giant inner circular muscle fibres, and two ostia. (Magn. × 48.)

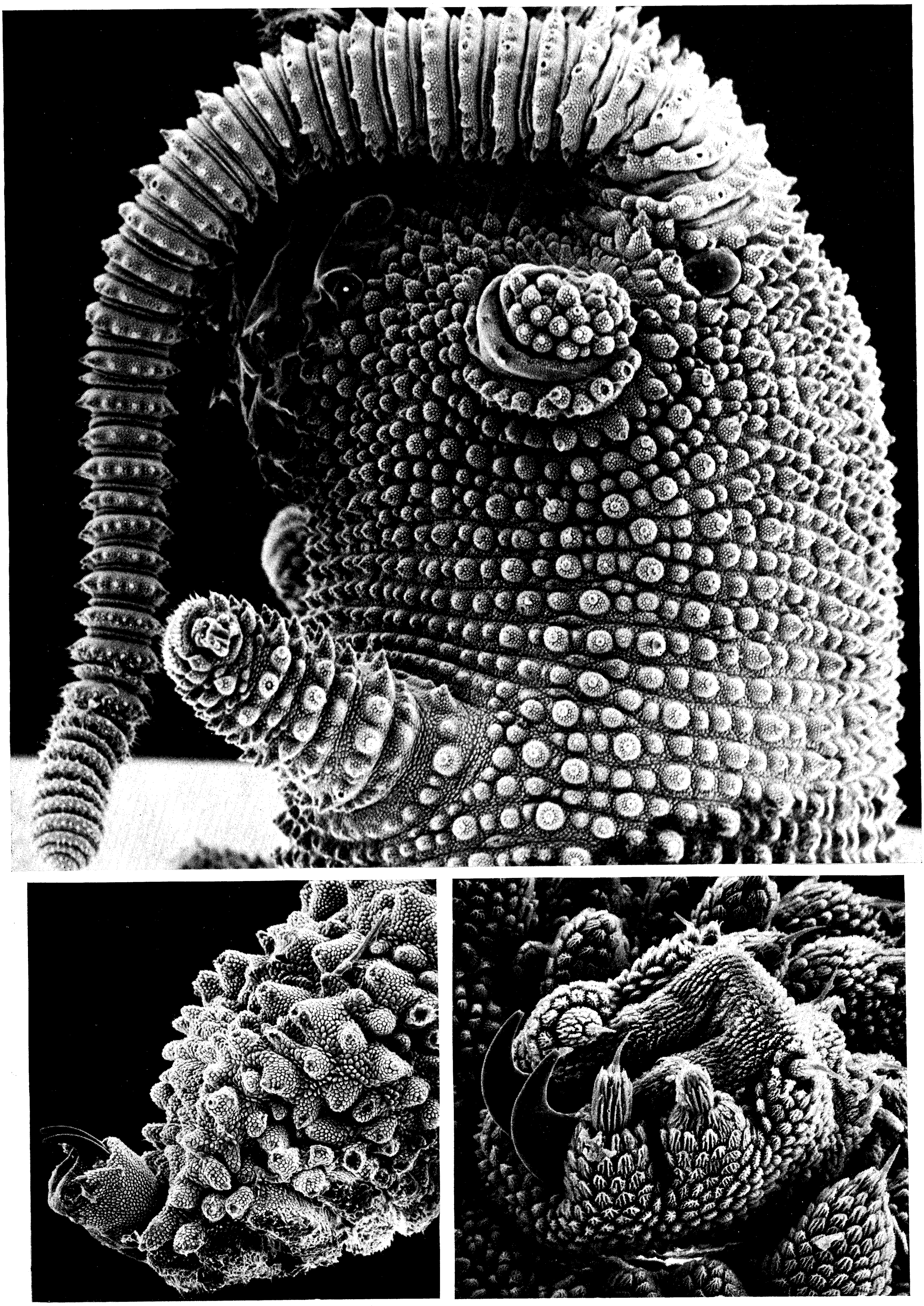

FIGURE 1. For description see p. 486.

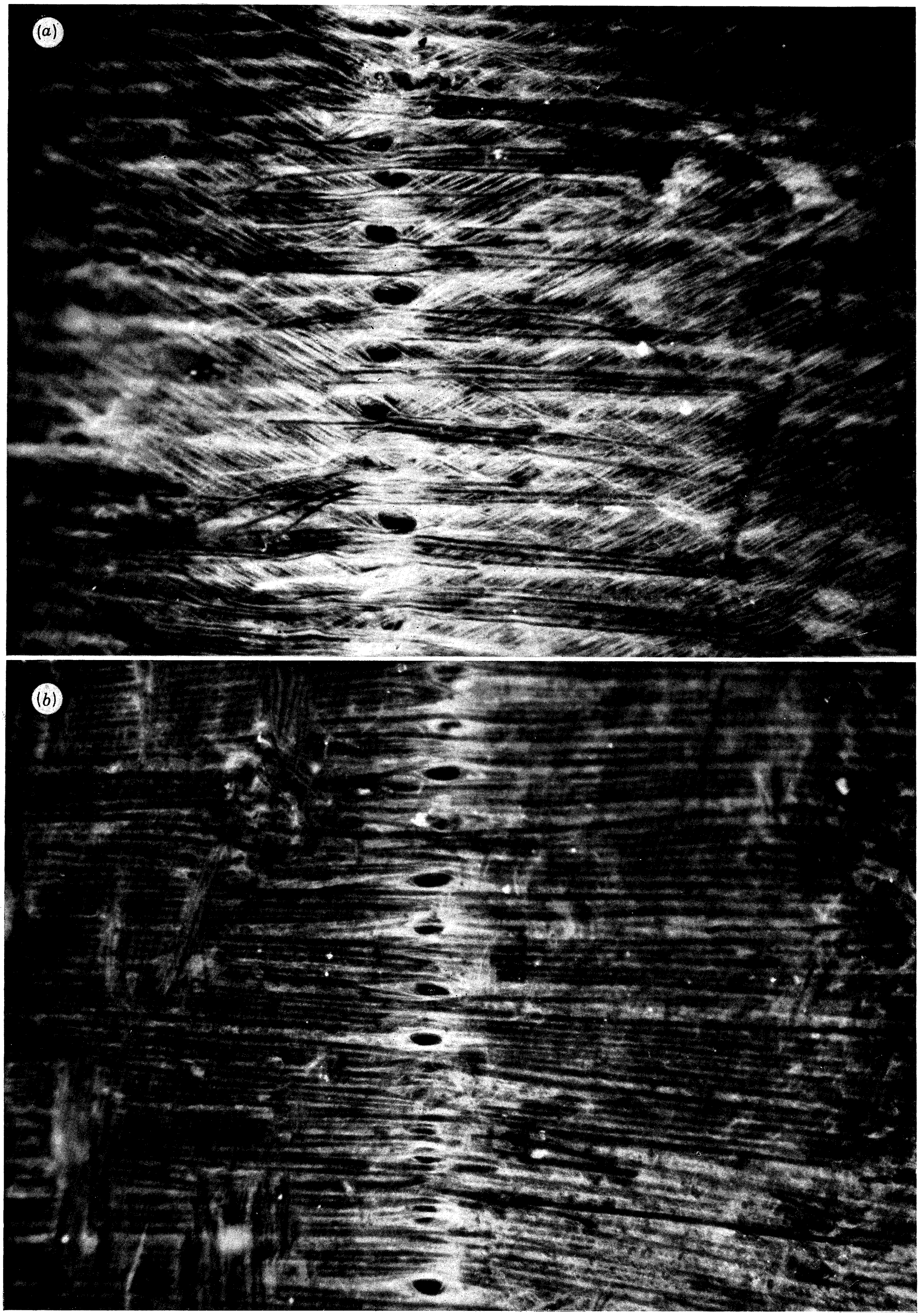

FIGURE 2. For description see p. 486.

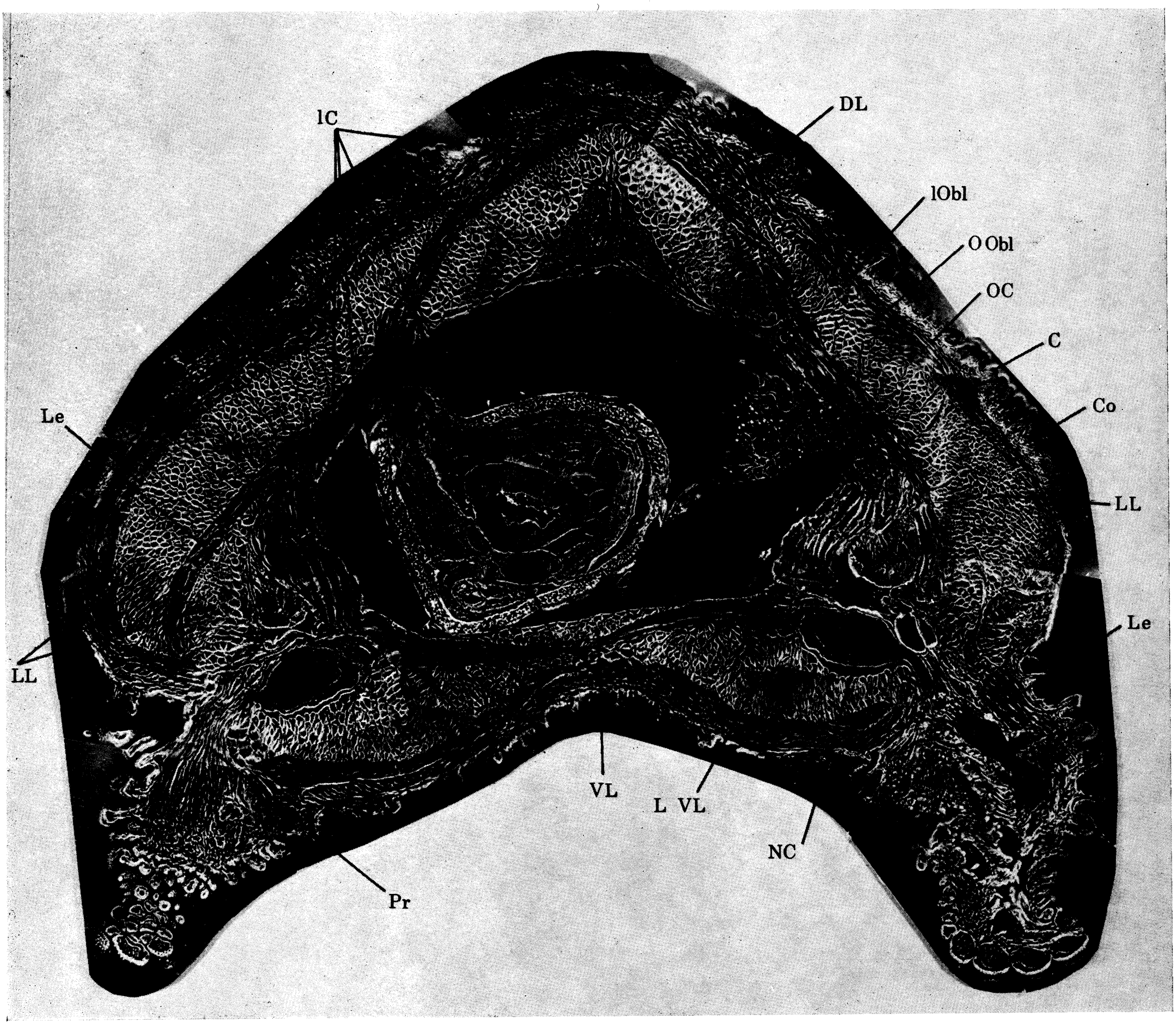

FIGURE 3. Montage phase-contrast light micrograph of transverse section of *Peripatus dominicae*, through a pair of legs with the image adjusted to emphasise outlines of individual muscle fibres. The left leg section passed through its anterior wall and shows the promotor muscle (see also figures 4 and 5). The right leg section passed just posterior to its centre, and shows the levator muscle, the outer and inner cavities and the septal muscle between them. Cu, cuticle; Co, collagen meshwork; OC, outer circular muscle; OObl, outer oblique muscle; IObl, inner oblique muscle; DL, dorsal longitudinal muscle; LL, lateral longitudinal muscle; VL, ventral longitudinal muscle; L-VL, latero-ventral longitudinal muscle; IC, inner circular muscle (g.C – giant circular fibres); Le, levator of the leg; Pr, promotor of the leg. Width = 5 mm.

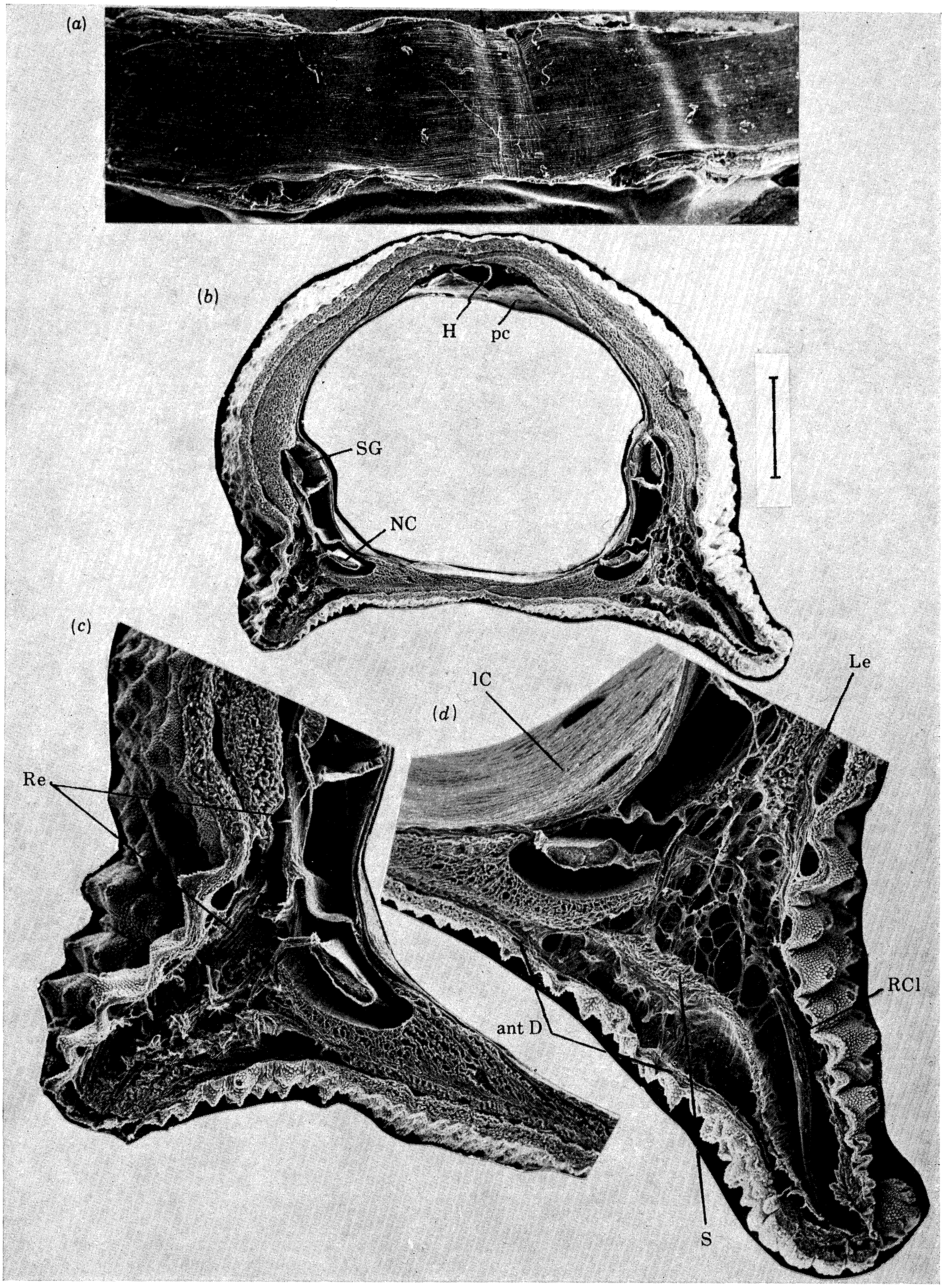

FIGURE 4. For description see p. 486. (Scale bar ≡ 1 mm.)

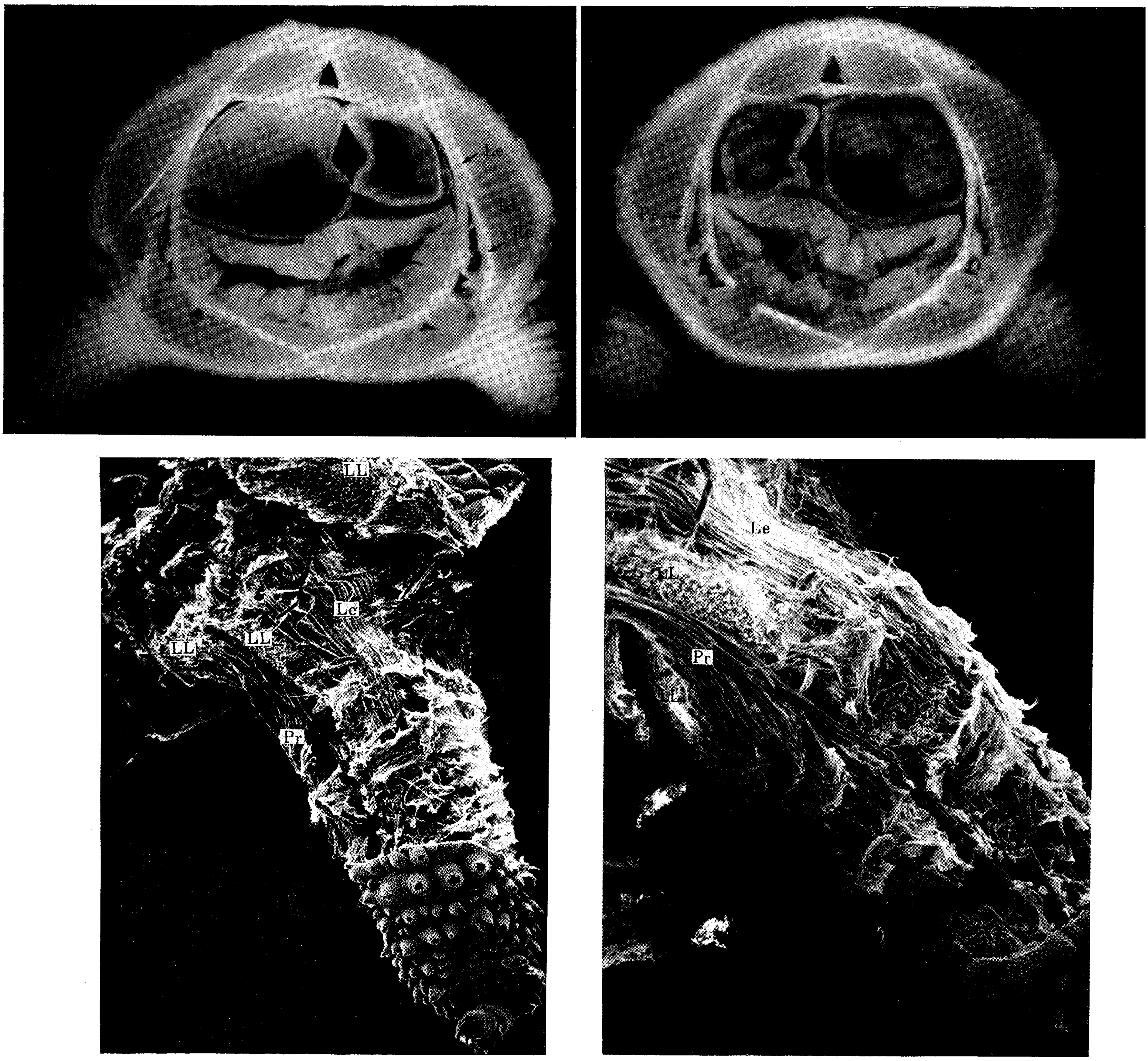

FIGURE 5. Locations and pathways of levator and promotor of the leg. *Top:* views of both sides of a slice of *Peripatus* to show the pathway of the levator of the leg through the lateral longitudinal (LL) muscle. *Left:* View from behind. At left the section passes through the posterior region of the levator (Le) which is fan-shaped and passes into the outer border of the leg just anterior to the cut surface. Also seen is the retractor of the leg (Re) at the region where it turns through 90° as it enters the posterior margin of the foot. On the right the cut is a little more posterior, so shows less of the levator and more of the retractor. (Magn. × 16.) *Right:* View from in front. The cut was anterior to the levator, which does not show. Instead the promotor of the leg (Pr) can be seen clearly on both sides. It is beginning to turn, after passing through a few LL fibres, on the right. (Magn. × 16.)

Below: Scanning electron micrograph pictures of a left leg and body wall. *Left:* View from the outside after cutting away a major, outer portion and a minor, inner portion of the lateral longitudinal muscle (LL). The levator (Le) is clearly seen and also the promotor (Pr) with a glimpse of the remotor (Re). (Magn. × 25.) *Right:* View from the front of the leg at higher magnification, showing the 90° turn of Pr. (Magn. × 46.)

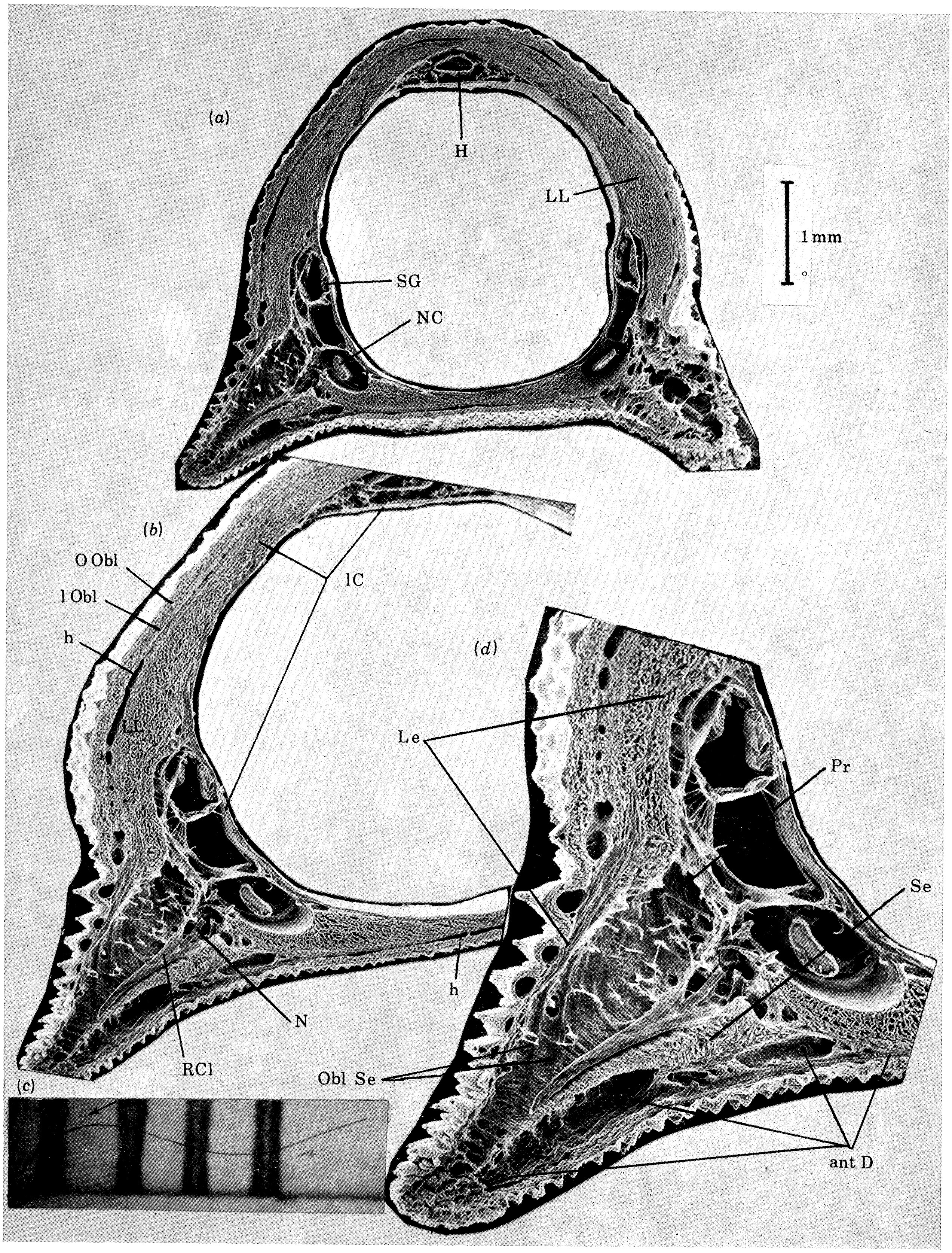

FIGURE 6. For description see p. 487.

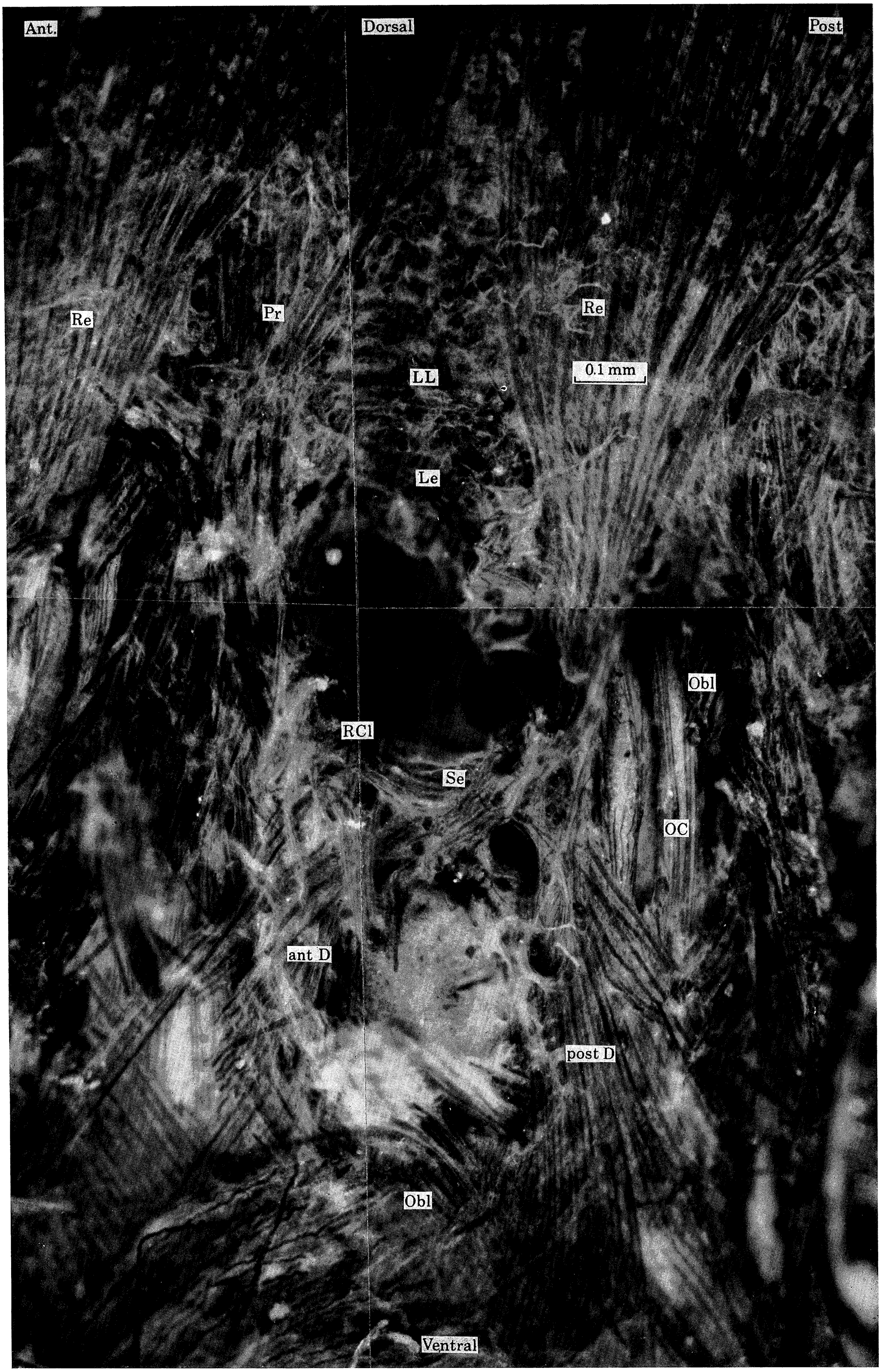

FIGURE 7. For description see p. 487.

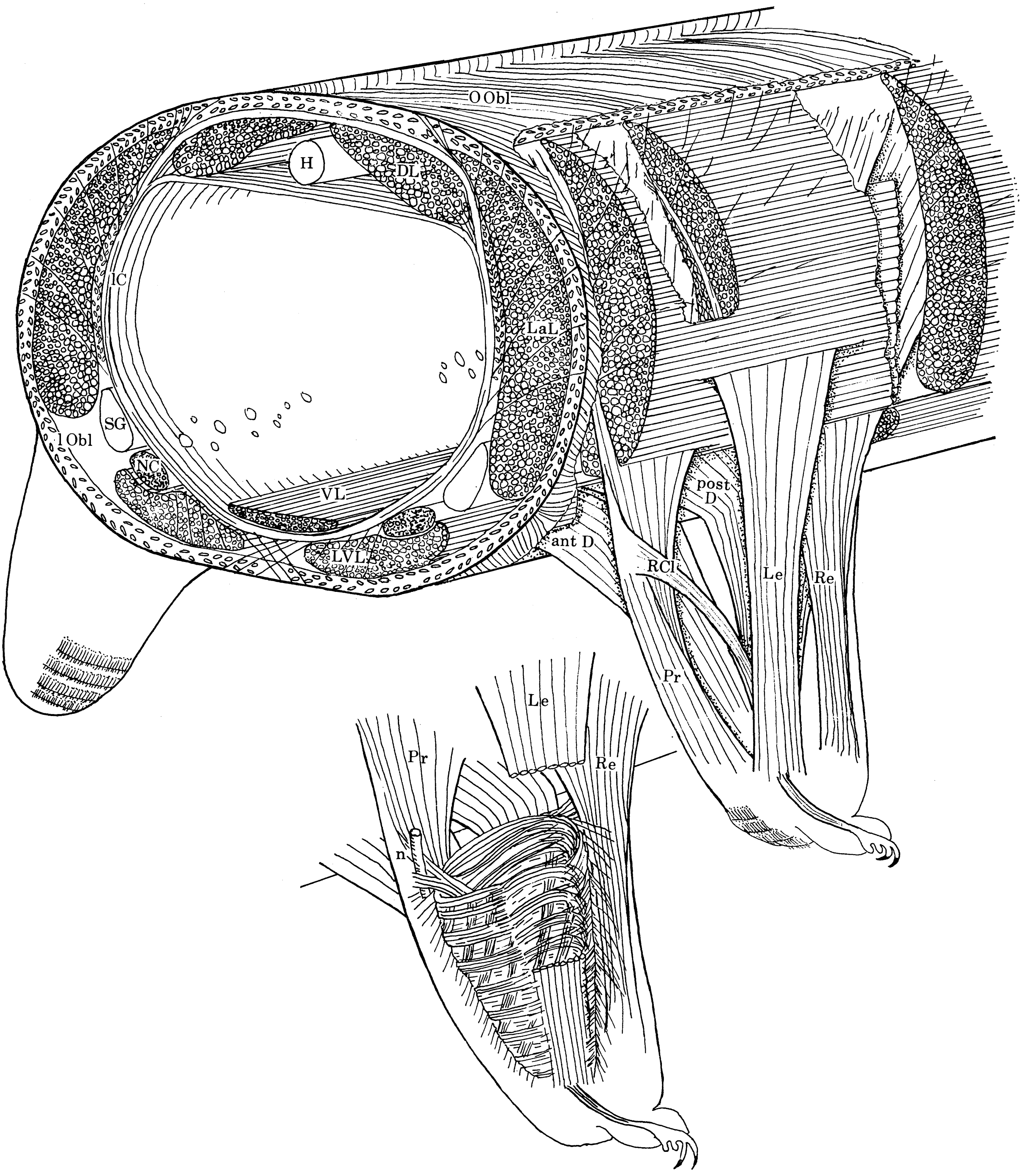

FIGURE 8. Body wall and leg muscles. *Top:* Three dimensional drawing of the principal body-wall and leg muscles of *P. dominicae*. Both the levator of the leg (Le) and the promotor of the leg (Pr) pass through the lateral longitudinal (LL). Other abbreviations are as in fig. 2 – also see table 1. The septal muscle has been omitted. *Lower:* Diagram of the complex septal muscle of a left foot viewed from the outside. Horseshoe-shaped muscle fibres attach to the anterior margin of the levator (shown partly cut away) and curl back to attach to its posterior margin (there are no circular fibres). Anteroposterior fibres attach just outside the promotor, and to several posterior sites. Bundles of oblique fibres reinforce the semicircular ones.

The DLs are separated from each other in the midline by a gap of about 0.1 mm. Through this gap ostia (figure 2) occur at regular intervals corresponding to each fold in the body wall. Blood can pass through these into the pericardium. The heart (H) lies immediately below the ventral openings of the ostia.

(*d*) *Lateral longitudinal* (*left and right LL*-4)

By far the largest muscle masses in the body are the left and right lateral longitudinal muscles (figure 3), extending the whole length of the body. They are located immediately below the inner oblique layer. In the Snodgrass account the LLs were termed laterodorsal longitudinal muscles and divided into internal and external (nos 4 and 5). The basis for this division was Snodgrass' observation that the promotor of the leg (no. 7) passes through LL. This is not the only split in LL, however, since the levator of the leg also passes through it (figure 4). In regions between the legs there is no trace of a split so the numerical division of LL indicated by Snodgrass is not called for and only the number 4 will be used by us for each whole LL complex. Birket-Smith (1974) also considered there to be but a single muscle.

In thick sections LL has a partitioned or radially fasciculated appearance. This is due to the passage through it of anchoring strands associated with inner circular muscle fibres. These start out as muscle fibre branches that become progressively permeated with collagen strands until they are purely collagenous.

(*e*) *Latero-ventral longitudinal* (*left and right L-VL*-6)

On the ventral surface, on either side of the midline, with a separation of about 0.2 mm, are two large muscle masses running the whole length of the body, the latero-ventral longitudinals (figure 2). These have a complex shape on their outer margins to accommodate the channels containing the nerve cords (NC's). In the Manton (1973, figure 3) drawing of the muscles of *Peripatopsis* the L-VLs are referred to as part of a mass that she termed ventral muscle. Also shown in her drawing as dividing the mass into three sections are bands designated left and

---

Description of plates 6 and 7

Figure 6. Leg and body wall muscles (see also Figure 4). (*a*) SEM photograph of a slice of *Peripatus* body wall made through the middle of the left leg and viewed from behind. (*b*) Shows the left leg and body wall at higher magnification. The inner circular muscle can be seen particularly clearly. Its large fibres pass dorsal to the dorsal longitudinal muscles and its small ones under the pericardium. (*c*) Part of a single inner circular fibre that was dissected out starting at the right midline, passing dorsally over the pericardium and down the left side. A single major branch from the fibre passed across LL (arrow). (*d*) Shows the foot at yet higher magnification. The levator of the leg (Le) is also clearly seen, and there are glimpses of the promotor (Pr) and the anterior depressor (ant D). The attachment of the retractor of the claw (RCl) is seen, and the major sensory nerve branch (N – labelled in B). The intra-leg septal muscle (Se) was cut through at its thickest point where it comprises antero-posterior and horseshoe-shaped fibres. It also contains some oblique fibres (Obl Se). Note the locations of hemocoelic spaces (h).

Figure 7. Photomicrograph of dissection of the musculature of a right leg viewed from the inside. To avoid distortion the leg was fitted into a hole in the block to which the body wall was pinned. The 90° rotation of the retractor (Re) and the promotor (Pr) are readily seen. A few lateral longitudinal (LL) fibres were removed (cut ends at left – *) to expose the promotor. Between Pr and Re the levator (Le) emerges from behind LL. The septal muscle which attaches just posterior to L was cut out (**). Also to be seen are the anterior depressor (ant D), the posterior depressor (post D), the septal muscle, outer circular (OC) and oblique (Obl) and the attachment of the retractor of the claw (ReC). Also figures 8 and 24. Bar represents 0.1 mm.

right deep dorsoventral muscles. These are shown attaching to the ventral cuticle in the midline, and therefore dividing and passing through the oblique muscle mid-ventrally. The photomicrograph (figure 2) shows that this does not happen in *P. dominicae*. A simple blood space occurs in the midline, crossed only by collagen fibres.

Latero-ventral muscle fibres, like DLs, are of much smaller diameter on the outside than on the inside, where they range from 25 to 45 μm in a mature adult female.

(*f*) *Ventral longitudinal* (*VL*-2)

The body cavity of *P. dominicae* is lined by a continuous membranous sheet of connective tissue. On the ventral surface near the midline this sheet is split into dorsal and ventral layers between which is located a thin bundle of longitudinal muscle fibres (figure 3) that extends the whole length of the animal. This is the ventral longitudinal muscle, whose component fibres are in the same size range as the other longitudinal muscles.

(*g*) *Inner circular* (*IC*-3)

Giant circular fibres. These comprise a single layer of large parallel (30–45 μm) muscle fibres (figures 2 and 4*b*), but unless the animal is fully stretched the giant circular fibres come to lie on top of each other in the folded sheet, as in figure 2. Removal of the lining of the body cavity reveals a bi-layered sheet of muscle fibres running at right angles to the antero-posterior axis. The fibres of the inner layer are conspicuously thicker than those of the outside layer. It was determined by dissection of single fibres of the inside layer that they are to be regarded as circular. They will be called giant circular fibres. Single fibres were dissected out, starting in the lateral wall, both in the dorsal and ventral directions. Passing ventrally, many of the fibres were found to pass across the midline, travelling under VL, around to the opposite side. Others terminate just before the midline, breaking into finer branches that become collagenous. At the level of the pericardium the inner thick fibres cross over the thin outer ones, passing dorsally on the outside of the DLs, at least as far as ostia in the dorsal midline. There, some of them terminate, in a collagenous matrix. At least one third of them, as shown clearly in figure 2*b*, cross the midline dorsally. Thus, some inner giant fibres pass across the ventral midline and some cross the dorsal midline. Although no single thick fibre was traced entirely around the body, it seemed quite possible that some could link up with each other and thus be really circular. A few had a side branch or two that linked with a neighbouring fibre, but true anastomoses were rare. Manton (1973) illustrated for *Macroperipatus* anastomosis of giant fibres around the large ostia that lead into the cavity above a foot. Birket-Smith (1974) by contrast, found those fibres around the ostia to be unbranched, and so did we. Most of the giant circular fibres dissected out in *P. dominicae* had a single fine branch (see figure 8) which passed through a major longitudinal muscle. Such branches terminated in a tuft of collagen fibres running to the cuticle and were clearly anchoring the fibre. Some examples of side branches may be seen in figure 10. The side branches of inner circular giant muscle fibres that pass through LL are clearly responsible for the impression gained by earlier investigators, from Sedgwick (1888) on, so that there are dorso-ventral sheets. Because of these branches, local activation of the giant circular fibres could, in principle, cause discrete dorso-ventral contractions, but the muscles are undoubtedly basically circular.

Small circular fibres. Immediately peripheral to the giant circular fibres is a layer of much thinner circular fibres. These follow the giants dorsally as far as the floor of the pericardium.

There, some cross the giants (which pass outside the DLs) to enter and cross the pericardial floor, as reported by Birket-Smith (1974). These circular fibres are the sole contributors to the pericardial floor musculature. The others pass dorsally to attach to the dorso-lateral body wall.

Vascular channels in body muscle

Manton (1973, figure 3) shows a vascular channel passing between the outer oblique muscle sheet and the thick collagenous connective tissue layer of the skin. In *P. dominicae* the major body-wall vascular channel lies between the oblique muscle sheets (figures 4 and 6*a*, *b*). Tiny channels lead from this major space to the cysternae under the papillae. The collagen layer is linked closely to the outer oblique muscle, by radial collagen bundles, so there is very little space there.

Lack of segmentation of body muscle

None of the body wall muscles shows the slightest indication of segmentation. There are no septa, nor even any emphasis at positions indicated by the limb repetitions such as might have been expected, of muscle fibre origins being emphasised serially with the same periodicity. Serially repeating muscles of the legs, to be described below, overlap each other before making attachments laterally on the body walls.

Muscles operating the leg

The stepping pattern of the onychophoran leg has never been minutely described although there are detailed accounts of overall walking (Manton, 1950; see also Gray 1968). Manton (1973) and also Birket-Smith (1974) followed Snodgrass (1938) in a simple designation of the muscles operating the leg that does not satisfactorily explain stepping. In this account there is a pair of promotors, dorsal and ventral, opposed by a pair of remotors, also dorsal and ventral. A step is executed by the leg first being levated, then swung forwards. Next it is depressed against the substrate, then power-stroked by remotion.

In the present account the five major muscles operating the step: a protractor, a retractor, a levator and two depressors, anterior and posterior will be described individually. The first three are attached high up on the body at the level of the overlap between LL and DL. In labelling these muscles a major departure is made from the descriptive terms of Snodgrass (1938), adopted by Manton (1973) and Birket-Smith (1974). The Snodgrass terms are not appropriate to describe stepping, probably because Snodgrass did not discern the very important levator of the leg. This will be described first. The maximal diameters of all leg muscle fibres range from 10 to 20 μm, tapering at their ends. They are markedly thinner than either the innermost fibres of longitudinal muscles or the giant inner circular fibres.

(*a*) *Levator* (*Le*-15)

In *P. dominicae* a fan-shaped muscle runs down the outer margin of the leg, tapering and terminating peripherally near the foot (figures 5, 6, 7). It is the only muscle involved in stepping that is not located outside the principal cavity of the leg, of which it forms the outer wall. This cavity occupies most of the space inside the leg near the body, but is reduced in the middle of the leg by the presence of a septal muscle that essentially divides the leg into outer and inner cavities. The other four stepping muscles run in sub-divisions of the inner cavity.

The levator is a stout muscle so it is surprising that Snodgrass missed describing it. He does appear to have seen a part of it within the leg, where he designates a muscle number 15 described as 'peripheral muscles of basal part of leg'. Although in two drawings of legs (Snodgrass 1938; figures 31*g* and 33*c*) he showed the levator clearly extending well onto the side of the body, the muscle is not included in his major drawing of body wall muscles (Snodgrass 1938, figure 3). This muscle, like the promotor, passes through the lateral longitudinal, but more laterally than Pr (figure 5). Its pathway through LL is shown in figures 5 and 6 (see also the three-dimensional drawing, figure 8). It lies mid-way between the promotor and the remotor and its basal attachments overlap with those of these muscles. In the present account the levator has been assigned the number 5, which Snodgrass (1938) used for 'peripheral muscles of basal part of leg', that were probably a part of the levator of the leg.

In none of Manton's drawings is the levator shown, nor are any muscles passing through LL. In Manton (1973, figure 3*a*), the remotor is drawn passing to the outer margin and there taking the place of the levator. This must be incorrect regardless of possible species differences.

(*b*) *Promotor* (*Pr*-7)

The promotor (Pr = dorsal promotor, muscle 7 of Snodgrass) is also attached at the level of overlap between DL and LL. It passes between these muscles, then passes through LL, but not as far as the levator. It does not cause a clean split in LL since the anteriormost fibres pass through LL more laterally than do the posterior ones. The reason for this is that the promotor turns at right angles to be body axis before attaching to the anterior margin of the leg (figures 6 and 7).

Pr passes anterior to the internal muscular basket formed by the septal muscle and associated muscle strands so can only be seen from the inside after removing the septal complex.

(*c*) *Remotor* (*Re*-8)

The remotor is an almost exact posterior counterpart of the promotor except that it has a broader, markedly fan-shaped root and comprises at least twice as many muscle fibres. It passes ventralwards between DL and LL and then across the inner face of LL before turning at right angles as it enters the leg (figures 4*c* and 7) to run down its posterior wall. Along the body wall the two fan-shaped muscles, the remotor of one leg and the next succeeding promotor overlap each other.

(*d*) *Depressors: Anterior depressor – ant D*-11 = *ventral promotor of Snodgrass. Posterior depressor – post D*-12 = *ventral remotor of Snodgrass*

There are two depressor muscles, anterior and posterior, lying close together, with their inner margins aligned with the axis of the leg (figure 7). There is a high degree of overlap of ant D of one leg by the next succeeding post D as viewed from the outside. In a relaxed *Peripatus* each depressor brings the whole leg sharply downwards. In an extended animal the bases of these muscles are greatly extended, emphasising their fan-shape, and their angles of attachment to the leg change markedly. Therefore in the extended animal as well as providing depressive force ant D promotes the leg and post D remotes it.

(*e*) *Retractor of the claw* (*RCl*-19)

In the centre of the principal cavity of the leg (the outer) there runs a 1.5 to 2 mm long narrow muscle that tapers progressively as it runs outwards, eventually being about one-third its thickness near the proximal insertion. Serial sections of the muscle show that the tapering occurs in individual muscle fibres. There is a small degree of branching near the apodeme, but no anastomosis. The fibre runs directly into the two-pronged claw via a very short apodeme. Manton (1973, figure 3) shows this muscle as attaching to the dorsal margin of the leg and terms it as a levator of the leg. A weak contraction of RCl leads to a levator movement; strong contraction pulls the whole foot into the tip of the leg (figure 1). This action, as well as the anatomy, support Snodgrass' designation of the muscle as a retractor.

Snodgrass also shows a synergist situated in the inner cavity. There is no trace of a second retractor in *P. dominicae*. Manton shows two other fine muscles attached to the retractor tendon, neither of which could be found in *P. dominicae*, in agreement with Birket-Smith (1974). Manton also shows the retractor as travelling in the middle of the leg and terminating somewhere above LL on its inner surface. In *P. dominicae* the retractor always bends forwards, forks around a stout anterior nerve and then terminates just above the anterior wall of the foot lateral to the promotor. Its morphology and location are clearly shown in figures 5 and 6. Muscle fibre insertions from the inner fork are generally attached to the outer margin of Pr.

Passing through the fork is a stout nerve (see figure 6*c*) that crosses the anterior wall of the foot to innervate the large sensory pad.

Manton (1973) also illustrates a fine (depressor) muscle attached to the ventral margin of the foot, for which no counterpart occurs in *P. dominicae*.

(*f*) *Septal muscle* (*Se*-16)

Snodgrass and Manton both described a thick muscle (16) termed the anteroposterior septal muscle of the leg. This is actually a complex of many small muscle bundles and a few sheets, some of which are oblique, others almost in the longitudinal axis of the leg. There is an anteroposterior orientation but the whole is horseshoe-shaped in cross-section. The form of Se may be discerned from figures 4 and 6: a three-dimensional drawing is presented in figure 8. A majority of Se fibres attach just anterior to the levator, pass around the principal chamber and return to attach to the outer leg wall just posterior to the levator. The short anteroposteriorals run from the outside of the promotor to the inside of the posterior depressor, making a slight S shape. The horseshoe-shaped fibres form, and surround, a large cavity on the outside of the leg that contains the excretory organ and the retractor of the claw. The S-shaped fibres line an inner cavity and carve this into three spaces, each containing a leg muscle, either the retractor or one of the depressors. When this strangely-shaped complex muscle mass contracts, it cannot do much more than increase the stiffness of the leg, which probably is its function.

(*g*) *Other leg muscles*

Manton (1973) figures a thick layer of 'superficial oblique muscles' completely surrounding the leg. In *P. dominicae*, as can be seen from figures 4 and 6, there are no such layers. There are a few external fibres that are continuations of outer circular muscle of the body wall extending along the leg. There is a single layer of small circular fibres immediately under the skin of the foot, but there are very few oblique fibres. The oblique muscle sheets of the body wall pass across the base of the leg and do not extend over it.

Operation of the leg

A summary diagram of the body wall and foot-moving muscles of *P. dominicae* is shown in figure 8. It is proposed, on the basis of the musculature described above, that *Peripatus* stepping is achieved in just about the simplest possible manner. It thus comes close to the elemental pattern envisaged as the basis for evolution of the arthropod leg (Hoyle 1976).There is a single levator, a single promotor and a single remotor. The only complexity is in the double depressor. This certainly offers a mechanical advantage over a single depressor, provided each part, anterior and posterior, can be operated independently in association with the swing and power strokes. *Peripatus* can walk equally easily forwards or backwards and so may use the posterior depressor only in forwards walking and the anterior depressor in backwards walking.

The separation of the depressors means that the power each one provides to locomotion in the appropriate direction increases with increasing length of the animal. This could be a principal reason why Onychophora elongate so much while walking.

Operation of the foot

When the leg is raised the foot is always levated and generally also retracted. This is related, perhaps, to the location of the proximal attachment of the retractor of the claw, on the outer margin of the promotor. When the levator contracts the retractor must be stretched. This

---

Description of plates 10–12

Figure 10. Attachments of muscle fibres to each other and to the skin. (*a*) V-LL muscle has a partitioned appearance in this phase contrast micrograph due to muscle arms from the giant inner circular muscle fibres (top, right) passing through it towards the cuticle. (Magn. × 60.) (*b*) Higher power view of a portion of (*a*), showing collagen strands connected to muscle arms passing across the oblique body wall muscle (Magn. × 100.) (*c*) Another region, at still higher magnification, in which fine collagen connecting strands are especially clear (Magn. × 145.) (*d*) Portion of LL showing long, fine straight muscle branches (larger arrows) passing between fibres. These originate on the innermost muscle fibres and pass between muscle fibres, in nearly straight lines, towards the skin. Note the wide separation between fibres and the occurrence between, and curving round, of fine strands (smaller arrows): these are muscle arms that make synaptic contacts with motor nerves (see p. 500). (Magn. × 250.) (*e*) Origin of a long muscle branch. The central branch is seen originating from the muscle fibre at lower left. Such branches occur on innermost fibres only, where these abut an antagonist muscle (in the case thin fibres of the inner circular muscle). (Magn. × 800.) (*f*) Portion of skin, to which the collagen strands are passing. Radially aligned collagen is from muscle fibre branches. (Magn. × 3000.)

Figure 11. *Peripatus* collagen. (*a*) Phase contrast photomicrograph of a piece of skin cut transversely to the body axis. The entire thickness, except for outer cuticle and epidermis, comprises bundles of collagen fibres about 12 layers deep, that cross each other in an irregular manner (see also figure10*f*). Thinner bundles of collagen pass radially between the thicker bundles: these are attached to muscle fibres on the inside and cuticle on the outside. (Magn. × 500.) (*b*, *b′*) Phase contrast micrographs of successive sections through a lateral longitudinal muscle near the head end. Comparison emphasises the thick collagen layer between muscle fibres, the loose, wavy nature of the collagen bundles, and their primary circular orientation, i.e. at right angles to the muscle axis. Note the anastomosis of one large fibre. (Magn. × 300.) (*c*) Electron micrograph of patch of intermuscle fibre collagen emphasises its loose, wavy interlaced nature. (Magn. × 32000.)

Figure 12. General structure of *P. dominicae* muscle fibre. Part of large 30 μm diameter LL fibre seen in transverse section. Shown are radial wide tubules (RWT) of different lengths cut longitudinally, and longitudinal wide tubules (LWT) cut transversely. Also of note are dense Z bodies (Z) attached to the tubules, sparse sarcoplasmic reticulum (SR) and cisternal elements (C) of sarcoplasmic reticulum contacting or surrounding tubules. A few bits of the meandering narrow tubules (NT) may also be seen. (Magn. × 21200.) *Lower right:* High-power view of myofilaments, which do not interdigitate fully, with a dense Z body (Z). (Magn. × 90000.)

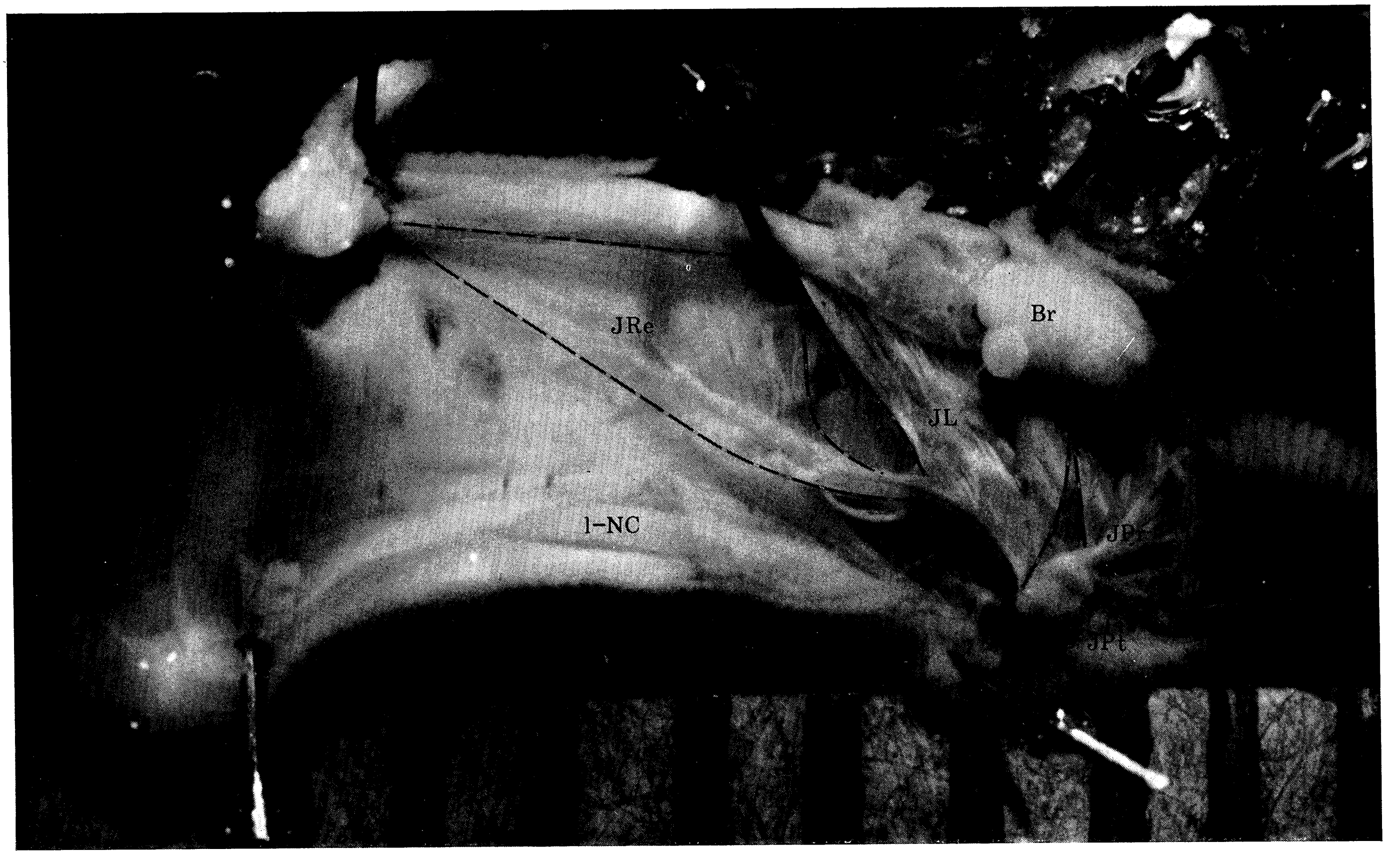

FIGURE 9. Jaw muscles of *P. dominicae*. Left head region following simple longitudinal bisection and removal of gut, salivary gland and left glue gun. The left half of the brain, with nerves going to the jaw muscles, is intact. There are four jaw muscles: a *protractor* (Pr); a *retractor* (Re) that provides the power stroke: its base is between the two pins, and which curls around the glue gun (removed) to attach to the ventro-lateral border of the jaw apodeme; a *lateral* (La); and a *protruder* (Pt). (Scale in millimetres.)

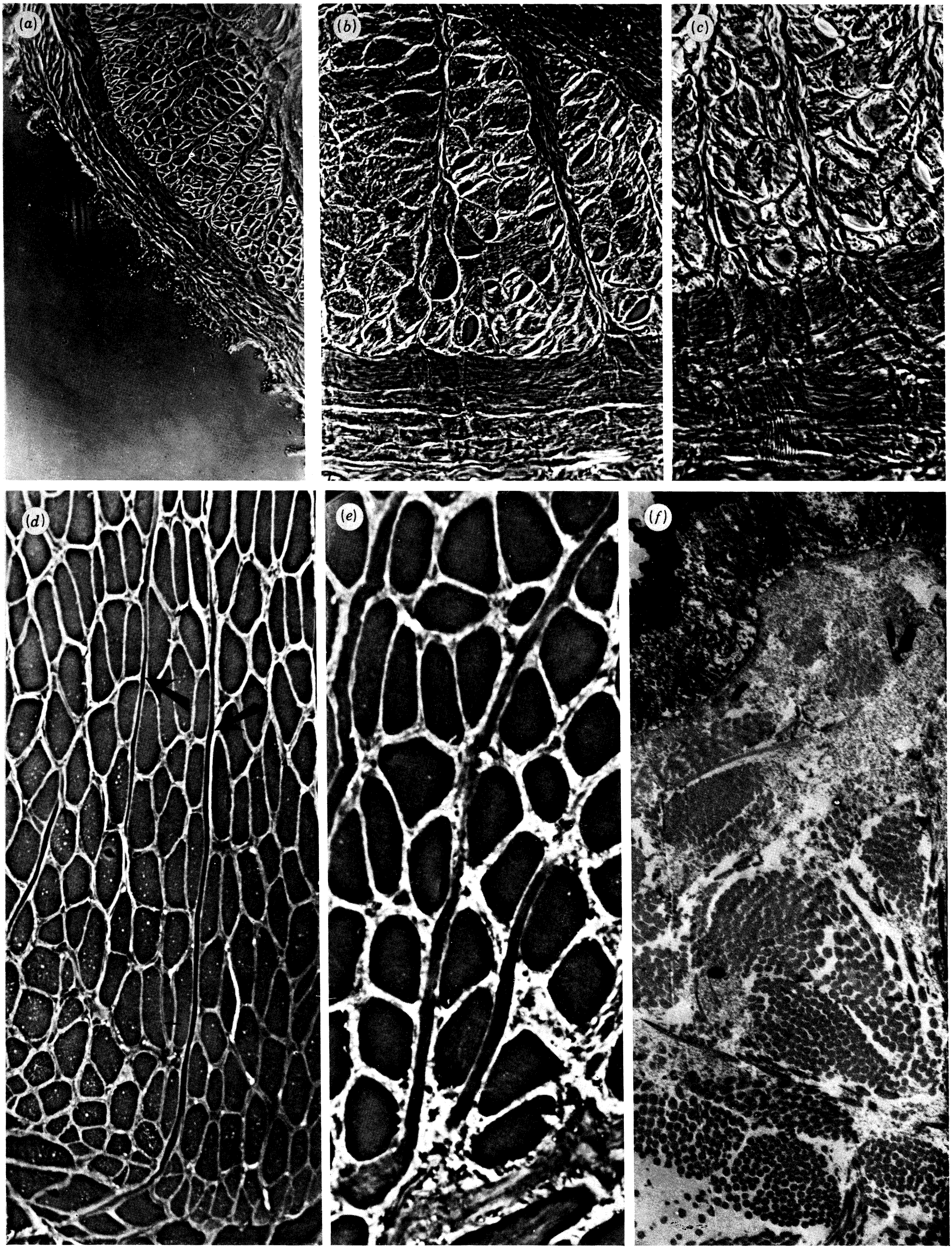

FIGURE 10. For description see p. 492.

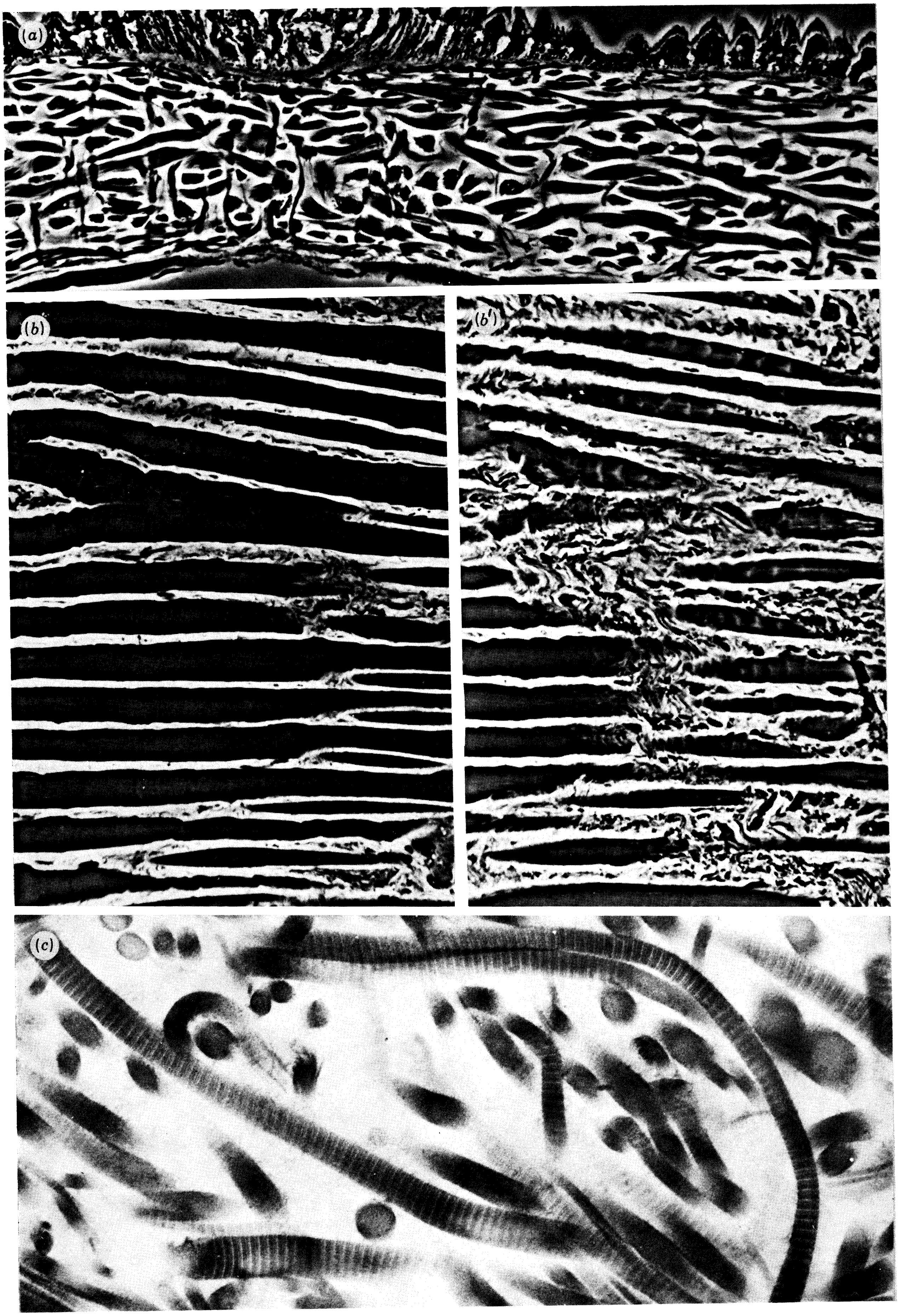

FIGURE 11. For description see p. 492.

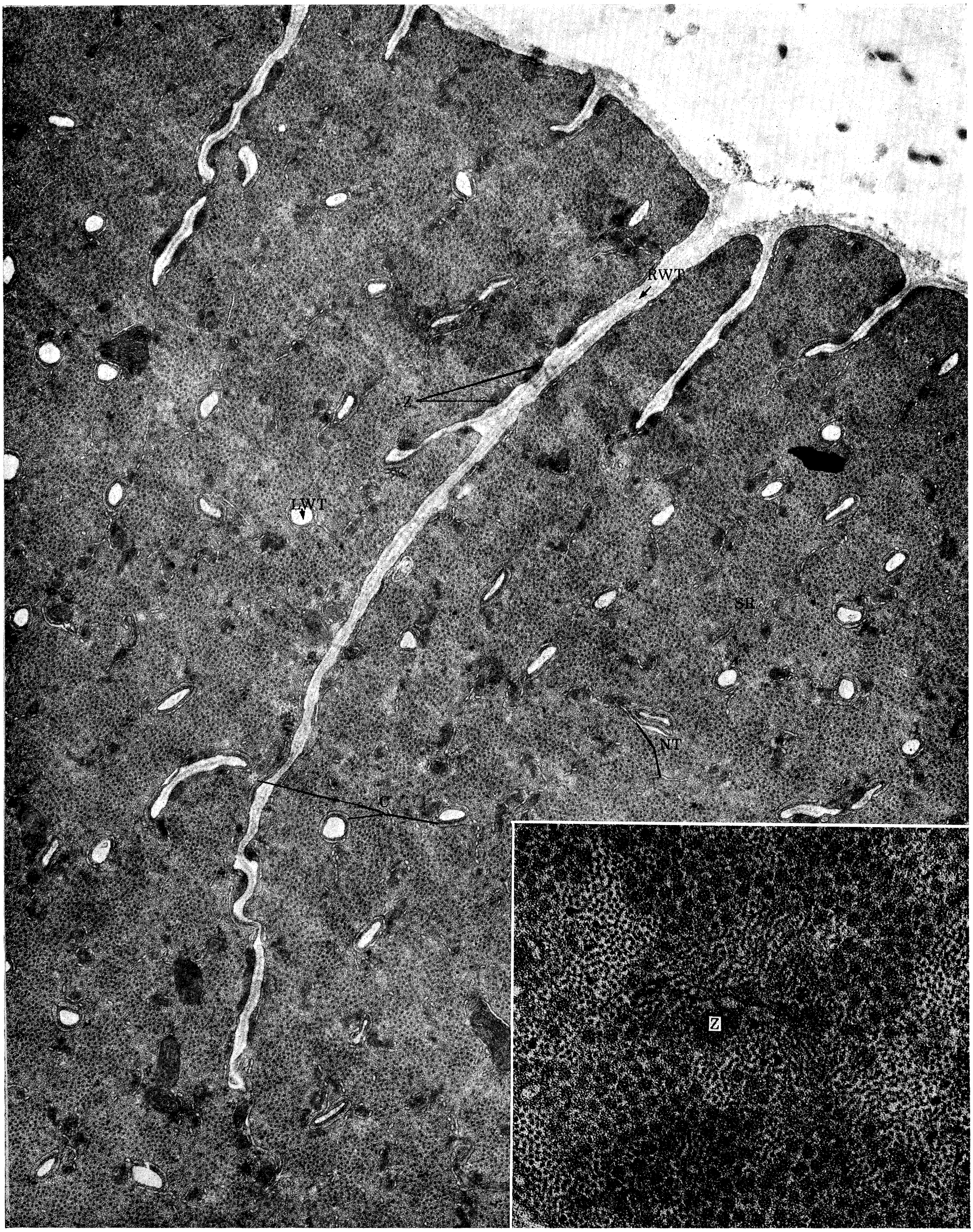

FIGURE 12. For description see p. 492.

Figure 13. For description see p. 493.

FIGURE 14. For description see p. 493.

FIGURE 15. For description see p. 493.

Figure 16. For description see p. 493.

action is functional because it automatically protects the foot during stepping in earth and vegetation. Manton (1973) points out that the clawed foot is not placed on the ground when *Peripatus* walks on a smooth surface. It is, however, planted down on a rough one and kept extended, during the power stroke. Extension and depression of the foot in *P. dominicae* can be produced only by hydrostatic pressure.

Muscles operating the jaw

Snodgrass (1938, figure 21, F) presented a drawing of the jaw and jaw muscles of *Peripatoides novae-zealandiae* that is labelled a dorsal view. This is clearly an error in the legend since the jaws are shown horizontally, pointing inwards. It is not possible to imaging how the jaw can work from this drawing or how the muscles are attached, likewise with the drawing of the jaw muscle in the account by Pflugfelder (1968). Manton (1977, figure 3) gives a simplified drawing that is both at odds with the Snodgrass drawing and also fails to convey a functional understanding.

A photograph of the anterior half of a *P. dominicae* with only the gut and glue gun apparatus removed is shown in figure 9. There are four distinct and separately innervated muscle flags, two anterior, one lateral and one large posterior one.

(*a*) *Protractor* (*JPr*)

Half of the first flag, the protractor of the jaw (JPr), is attached to the snout.

(*b*) *Protruder* (*JPt*)

While JPr can pull the jaw forwards preparatory to a bite it cannot get the jaw out of the mouth. This is the task of the protruder of the jaw (JPt) attached to the ventral border of the inner lip.

DESCRIPTION OF PLATES 13–16

FIGURE 13. General structure of *P. dominicae* muscle fibres (lateral longitudinal – LL), viewed in longitudinal section. Longitudinal wide tubules (LWT) are seen, cut longitudinally, including one originating in the upper section, where fibre thickness is increasing, scattered dense (Z) bodies, myofilaments and a neuromuscular junction between a single motor axon and the lower fibre. The wide space between the fibres, containing collagen, is characteristic of all *Peripatus* muscles. (Magn. × 10000.)

FIGURE 14. Termination of a muscle fibre (LL). *P. dominicae* muscle fibres terminate in a number of small processes, not laterally aligned, such as the one shown here at the anterior end of a LL fibre. Terminations are characterized by funnel-shaped openings into longitudinal wide tubules. Fine collagen strands enter these tubules along with sarcolemma basement material. A number of mitochondria and accretions of glycogen occur in and near the processes. (Magn. × 18500.)

FIGURE 15. Details of longitudinal wide tubules, a narrow tubule, and longitudinal dense Z bodies. *Left:* Longitudinal section of LL muscle fibre at high magnification showing details of longitudinal wide tubules (LWT), ordinary dense Z bodies (Z) and long Z bodies attached to the muscle plasma membrane both at the periphery and the invaginated LWTs. The section also shows the paucity of thin/thick filament interdigitation. (Magn. × 25700.) *Right:* Portion of another fibre to show non-membrane bound space containing glycogen, and a narrow tubule running longitudinally. (Magn. × 15000.)

FIGURE 16. Attachment of collagen to a muscle fibre along its length. Collagen runs between muscle fibres in parallel, at right angles and obliquely. At intervals bundles of collagen strands dip into muscle fibres and attach to them (see figure 12*b*). This electron micrograph shows such a point. Note the numerous terminal dense Z bodies. The sarcolemmal basement material is very thick at the point of attachment, with fine collagen strands embedded in it. (Magn. × 20000.)

(*c*) *Retractor* (*JRe*)

The power stroke is provided by the large retractor of the jaw (JRe). This large but thin sheet of muscle, with fibres up to 7 mm long and 10 to 40 μm diameter, is attached to the dorsal wall of the body between DL and LL. To get to the midline the retractor fibres must curl round the large glue gland, which they do, bringing them below the jaw apodeme. Thus they attach from below and to the sides, not from above.

(*d*) *Lateral* (*JL*)

Opening of the jaws to get round a piece of food is the task of the lateral jaw muscle (JL).

The position of JRe is such that a strong contraction in it must exert pressure on the glue gland. Since the glue gun can squirt glue up to $2\frac{1}{2}$ ft (0.75 m), a mechanism is needed to raise pressure in the gland. There seems to be no way the body wall muscles can do this satisfactorily and there are no muscles intrinsic to the gland, so it is possible that JRe is involved in the ejaculation.

General form of Peripatus muscles

At a low magnification the larger *Peripatus* muscles appear to be subdivided into fascicles (figures 2 and 10). At higher magnifications the apparent partitions were found to be formed by clusters of small branches from nearby, usually antagonistic muscles, passing through, with a central one always heading towards the skin. Serial sections showed that similar pathways tend to be used by successive attachments, but there actually are small differences that have a cumulative effect so that after a few millimetres a different detailed pattern is observed: there is not true fasciculation.

In addition, the large longitudinal muscles have a secondary fascicular appearance that derives from a tendency for clusters of 30 to 50 adjacent fibres to run closely parallel to each other but at an angle with respect to neighbouring bundles of similar size. At the contact faces there is generally more collagen and other matrix material, as well as the collagen-secreting cells. But when a bundle was followed in serial sections for 3 mm or more, the original outlines could no longer be clearly discerned. Fibres are added, lost or exchanged between 'bundles'. Again, there is no fasciculation.

Forms of individual muscle fibres

A conspicuous feature of all the major muscles as viewed in cross sections (figure 3) is that the diameters of fibres located near the outside are smaller than those on the inside. The explanation for this is that the outside fibres are attached to the body wall not only at their ends, but also at several places along their length. To make these attachments they are both invaded by collagen bundles and split into smaller fragments that eventually taper abruptly, often becoming wavy first (see figure 11*b*). At the same time, the smaller branches and the sites where these merge with the main body of the fibre, become inundated with collagen. The collagen bundles form complex, but relatively orderly, arrays that in turn attach to the cuticle.

Muscle fibres deep in the body wall do not pass to the outside to make attachments. Instead, they send off small branches laterally, at right angles to their main axis, that weave between their neighbours in the direction of the cuticle. The inner, thicker fibres are in turn attached to their neighbours by circular collagen bundles that weave between fibres, and by lateral fine muscle arms that make their way directly to the outside.

The extensive invasion of muscle fibres along their length by collagen means that muscle fibres do not have the usual neat spindle or tube shapes. Instead, they are alternately thick and thin, have pieces gouged out or make lumpy side connections. Quite apart from this, many muscle fibres have sections that are extremely convoluted. This was true even of longitudinal fibres fixed at an extended length, and when neighbouring fibres were obviously stretched. These regions are considered to be close to fibre terminations, in most cases. But in others, it was found that on following the wavy portion in serial sections, it turned at right angles and there enlarged into a muscle fibre of a major antagonist muscle. Some longitudinal fibres continued, after thickening, as circular fibres and vice versa. Because of the large number of inelastic collagen attachments to every muscle fibre there is no functional conflict in this arrangement. Both circular and longitudinal fibres give off lateral branches for attachment. Possibly the aberrant fibres are developed from incipient branches as these pass through their antagonists.

Anastomosis

In a drawing of *Peripatopsis* (Manton 1973, figure 4*a*) the two inner giant (circular) muscle fibres surrounding an ostium were shown making anastomosis around the opening. In all body wall muscles of *P. dominicae* some examples of anastomosis were seen (figure 11*b*) but it is not a common occurrence. Most longitudinal fibres dissected out were discrete and parallel over a few millimetres. Giant inner circular fibres were dissected out over lengths up to 7 mm without an anastomosis, though with a branch or two. In the claw retractor, branching of fibres is common at each end, but does not occur in the middle. The jaw fibres branch only near their body wall attachments, not along their length or near the apodeme.

Anastomosis was most frequently encountered in fibres running parallel with, and close to, the cuticle, in association with extensive interweaving between the muscle fibres of collagen that holds the body muscle together and attaches them to the cuticle.

Contacts between muscle fibres

Although adjacent muscle fibres are widely separated along most of their length by collagen, they make occasional contacts with each other. The contact is made by gradual approximation, not by an arm, although small projections tend to be made by both fibres. One of these contacts will be illustrated in the following section on fine structure.

Inter-muscle fibre collagen

All *P. dominicae* muscle fibres are well separated from each other along most of their length. The space between, a 5 to 50 μm surround, is largely occupied by collagen strands ranging in thickness from only a few nm to as much as 5 μm. There is a great deal of undulation and also some branching and fusing of collagen strands. Their predominant axis runs at right angles to the muscle fibres, about 75 % running this way. About 20 % run along the fibre axis and the other 5 % run obliquely. These percentages change abruptly near fibre attachments. Collagen strands run into muscle fibres at some places along their length, becoming abundant at points where fibres split or branch, and at terminal attachments. It is difficult to dissect out single muscle fibres because they are welded tightly together by the collagen meshwork.

Attachments of muscles

(*a*) *Cuticular attachments*

It is well known that there is a lot of collagen in the 'skin' of *Peripatus*. Immediately beneath the cuticle of the entire skin of *Peripatus* lies a thick layer (about 120 μm) containing a complex array of bundles of collagen fibres (figure 11*a*). The collagen layer was mistaken for muscle by early investigators and was still so labelled in figure 16 of volume 6 of Grasse's *Traité de Zoologie* (by Cuénot 1949). Hepburn and Hefron (1976) stated that collagen fibres in *P. moseleyi* are orthogonally arranged. In *P. dominicae* skin, collagen occurs in bundles stacked up to fourteen deep. We went to considerable trouble to make serial sections of this to see if we could detect any pattern to it, but failed to find any. Possibly there is a lattice arrangement, for some cross at right angles, but they often run nearly parallel and cross each other only at intervals equal to an average of about four collagen bundle thicknesses, in transverse sections. Extensibility seems to depend on the collagen being loosely folded.

The relation of the collagen layer to the muscle layers is the main point of interest. Birket-Smith (1974) was surprised at how readily (in 70-year-old preserved material) the 'skin', comprising cuticle and collagen, could be peeled off. This point is of considerable interest in relation to the question of muscle fibre attachments so we pursued it by making serial sections that were examined first by phase contrast light microscopy, then electron microscopy. These studies showed that collagen bundles of the skin can generally be traced into muscle fibres, and vice versa. However, the elements that do the linking are always very thin (figure 11*a*). The links are, somewhat surprisingly, all radial, not oblique. The diameters of the component collagen strands of the links are much less than those of the bundles in the skin, as if they are intended to break easily.

Links to the cuticle other than those made at the ends of a fibre are made at many points along its length for fibres situated close to and parallel with the cuticle. These consist of fine strands of collagen that cross the muscle fibres at right angles to their long axis. They indent into the fibre where fine strands of collagen turn into the wide tubules and also into the sarcolemmal complex. Body wall muscle fibres also send off fine arms, at right angles to their long axes, extending towards the skin (figure 11*d*, *e*). Entirely muscular at first, they become progressively more collagenous before running into the skin as solely collagen.

(*b*) *Apodemal* (*tendon*) *attachments*

Only muscle fibres operating the claws of the feet and the jaws have an apodeme. This is formed of inelastic sclerotised cuticle into which muscle fibres run directly without the interposition of collagen.

FINE STRUCTURE OF MUSCLE FIBRES

Most of the major muscles described above were prepared for electron microscopy and subjected to preliminary examination. Whilst the diameters of the fibres examined differed a great deal, from only 1 to 45 μm, and the functions of the muscles ranged from phasic to prolonged tonic, no significant differences were detected in the fine structure. Accordingly, all will be treated together, the source of each micrograph being stated.

Some features not previously, to our knowledge, found in any other kind of muscle were encountered, notably two sets of wide invaginated tubules and a nearly random array of thin and thick filament clusters.

Wide invaginated tubules

The most conspicuous feature of *Peripatus* muscle fibres, and one that is without precedent in other systems, is the occurrence of an extensive system of wide invaginated tubules (figures 12 and 13). These occur in all types of *Peripatus* muscle, regardless of the diameter or function. Wide tubules occur in about equal distribution except in the cores of the larger fibres, where they are less frequent. There are two entirely independent systems of wide tubules: longitudinal and radial. Extensive examination failed to locate any fusions or even close contacts between them. The radial system has very little in the way of a longitudinal component so there is little obliquity and no spiralling, and the longitudinal wide tubules only travel parallel to the fibre axis. Each system will be described separately.

(*a*) *Longitudinal*

Longitudinal wide tubules originate mainly at the extreme ends of a fibre and also along its length at places where an increase in diameter occurs. They rarely start in the main body of a fibre. Near the terminations some are irregular in shape, or branch, but in the body of the fibre they are straight. They do not run into each other, being fairly evenly spaced; excluding the core area they have an average separation of 0.93 ± 0.29 μm s.d., range 0.4–1.5 μm. A large, 40 μm diameter fibre has a total of about 150 wide tubules which may extend along its whole length.

Near their terminations, and for several hundreds of microns inwards, the longitudinal wide tubules contain granular material similar to that present in the outer layer of the sarcolemma (figures 14 and 15). In this matrix near the terminations, are embedded many fine collagen strands. Farther into the body of the fibre the collagen strands are absent and eventually the granular material also. Funnel-shaped openings to the LWTs occur at the terminals (figure 14), filled with the granular material.

The mean diameter of the lumens of LWTs, determined in the mid region of a 20 μm diameter fibre, was 0.13 μm ± 0.047 μm in the range 0.07–0.27 μm, ten times the width of vertebrate T tubules.

(*b*) *Radial*

Radial wide tubules (figure 12) occur all along the thick portions of fibres, being sparse at the ends. In large circular body-wall fibres they are separated by intervals of 0.7 to 3.5 μm, with a mean of 1.7 μm s.d. ± 0.7 μm s.d. A radial tubule may have a wavy path, with a few short branches, but more often is straight, with a wide range in lengths, from only 0.5 to 20 μm. The lengths are adjusted so that there is a fairly even separation of the tubules, with one or two extending to the centre of the fibre. Radial tubules are lined with the same granular material that forms the outer layer of the sarcolemma as far as their inner termination.

Dense Z bodies

The thin filaments are attached to dense bodies, which we shall refer to as Z bodies since they clearly serve the same basic purpose as Z-line material in striated muscle. Commonly about 70 nm in diameter, they are generally 3 to 5 times longer than they are wide, with the long axis parallel to that of the fibre. However, some are forked and much longer ones also occur, attached to longitudinal wide tubules. Along the radial wide tubules, are Z bodies whose long axis is radial. About 50 % of the surface of radial wide tubules has Z bodies attached compared with 20 % of the surface of longitudinal wide tubules in the main body of the fibre. Near the ends of a fibre almost the entire tubule is covered with Z bodies (figure 14).

In the body of a fibre, Z bodies are scattered about without any regular order (figure 13) although there is some tendency for them to form staggered arrays (see also Saita and Camatini 1966) as in obliquely striated muscle (see, for example, Rosenbluth 1965*a*). However, a cluster of myofilaments with an array of Z bodies aligned in one direction is likely to abut one in which they are arranged oppositely. The fine structure of Z bodies is obscure. The lines formed by thin filaments continue into and across Z bodies which may therefore be composed of cementing material between otherwise continuous or overlapping thin filaments. Z bodies also occur in small non-staggered clusters, with about 120 (2 × 60) thin filaments attached to each central one. An average of 54 dense bodies per 10 μm^2 was counted in body wall muscle.

Z bodies near the ends have thin filaments going in only the direction of the body of the fibre. This is also true of dense bodies attached to the margins of radial wide tubules that face the long axis of the fibre. Those on the lateral margins can be regarded as having double (left and right-handed) sets of thin filaments (figure 15*a*). Very long Z bodies attached to longitudinal wide tubules have thin filaments that emerge laterally and turn in one direction or the other before running along the fibre axis.

Many Z bodies are located at the periphery just below the sarcolemma, apparently being attached there. As with thin filaments attached to longitudinal tubules, these must turn to pass longitudinally, or be obliquely attached except at the outer fringes. The fine structure of Z bodies is obscure. The lines formed by thin filaments continue into and across Z bodies, which may therefore be composed of cementing material between otherwise continuous or overlapping filaments.

Narrow (*E- or T-type*) *tubules*

Narrow (E) tubules with a lumen of less than 0.02 μm, entering from the periphery along the entire length of a fibre, are also present, though sparse (figures 12 and 15*b*). There are more of these than occur in slow snake muscle (Hoyle *et al.* 1966) but fewer than in slow arthropod fibres. They enter obliquely as well as radially, and their pathways are radial, spiral and longitudinal. Some originate from wide tubules. E tubules are not lined.

Sarcoplasmic reticulum

A small amount of perforated, single-layer sarcoplasmic reticulum of conventional appearance is scattered throughout the *Peripatus* muscle fibres. Intrafibre organization is lacking: the contractile material of a fibre is not subdivided into fibrils (Felderstruktur).

Sarcoplasmic reticulum contacts the wide tubules at several points along their length, in forms ranging from a small area to a complete surround (figure 12). Most of the contacting SR lacks perforations, being in the form of cisternae filled with a fine granular material. The gap between cisternae and wide tubules is about 10 nm, and it is bridged by small feet, forming a structure resembling that of typical invertebrate dyads (Hoyle 1965; Franzini-Armstrong 1973).

Contacts between the narrow tubules and SR also occur, at irregular intervals. These are of conventional invertebrate (dyadic) type, although some 'triads', in which the E tubule is central between two cisternae, occur and there are also places where the cysternal element completely surrounds the narrow tubule.

Mitochondria

Small mitochondria, 0.1 to 3.0 µm long, are scattered throughout the fibre with concentrations at the periphery just below the sarcolemma. They are also concentrated at the bases of muscle arms (see p. 502) and near muscle on nerve synapses.

Nuclei

Nuclei are generally fairly large, up to 30 × 15 × 15 µm. They occur within the main body of the fibre but are more often peripheral. Smaller fibres have regions where the entire cross section is occupied by a nucleus so that force must be transmitted via the sarcolemma.

Intramuscle fibre spaces

Running longitudinally in some of the muscle fibres are a few long spaces not bound by membrane, containing particles of glycogen. These spaces tend to be located near longitudinal narrow tubules (figure 15*b*).

Lateral muscle fibre attachments

The transition from the fine radial collagen strands to muscle fibre is associated with an increase in both the number and thickness of the collagen strands and the accretion of an amorphous ground substance that is probably identical with that forming the outer part of the sarcolemma (figure 16). In the latter there appear mitochondria, dense Z bodies and wide tubules. The dense bodies are elongated in the long axis; thin myofilaments arise out of them in clusters of 50 to 100.

Myofilaments

Thin filaments of *Peripatus* muscle are of conventional 7 nm diameter. Thick ones are uniformly 23 nm, i.e., thicker than vertebrate and insect, though like those of many crustaceans. They are quite unlike the very thick (32 nm at the centre), highly tapered filaments of annelids. There can be little or no tapering of thick filaments, since in all sections they have the same diameter. They are collected together in clusters of about a hundred, spaced about 300 nm from neighbouring clusters. There is a basically hexagonal array, but with many irregularities. Thick filaments are not well aligned: they are long, of the order of a few micrometres, but we were not able to determine even whether or not they are of consistent length. In almost all of our micrographs there is little overlap of thin and thick filaments. Overlap occurred mainly at the region of first abutment, and rarely did it take the form of regular orbital arrays. Such orbits as did occur had 10 thin filaments around each thick one, which would indicate a 4:1 ratio if it occurred throughout a muscle. Estimates of total numbers of thin and thick filaments showed that this is about the overall ratio.

The heterogeneity of locations of dense bodies clearly determines the lack of order in the relationship between thick and thin filaments. Dense bodies lying along wide tubules, particularly the longitudinal ones, determine new organizational patterns of myofilaments.

Contacts between muscle fibres

P. dominicae muscle fibres branch near their bases and the outside, thinner fibres of longitudinal muscle branch along their entire length, as well as making an occasional anastomosis. Because of this these fibres have cytoplasmic continuity with neighbours. More centrally-placed fibres that do not have such connections make occasional contacts with each other via projections. Their membranes are brought into close apposition but there is no fusion. One such contact is illustrated in figure 17. One (donating) fibre makes a small projection, whose surface has several small invaginations. The other (receiving) fibre has a similarly folded surface at the contact region. The plasma membranes are separated only by shared sarcolemmal basement material at this point. Dense material occurs just beneath the plasma membranes in the contact zone.

MYO-NEURAL JUNCTIONS

Two kinds of neuromuscular relations were found: the commonest was a novel kind of muscle arm (figures 18–20) that extends to the motor nerve and there makes synaptic contacts. A second, less frequently encountered kind was a conventional nerve branch terminating on muscle fibre, but with an unique fine structure of the subsynaptic region. There are also intermediate structures in which a muscle fibre has made small projections at intervals along its length extending towards the nearest motor nerve. The latter has made branches extending towards the muscle fibre so that the two meet *en passant*. Some of these contacts are very long synapses whilst in others the nerve tip is engulfed by the muscle arm. When the muscle fibre is very close to a nerve branch the arm is short, but when the nearest motor nerve branch is some distance away from the muscle it must extend a considerable distance, although because of fairly extensive nerve branching, muscle arms are seldom more than about 0.5 mm long. They are never straight, as muscle fibres attachment branches always are, forcing adjacent muscle fibres to line up and giving the appearance of fasciculation. Instead they curl around the muscle fibres to reach the nerve. Each muscle arm has a large club-shaped expansion close to the nerve that will be termed the head region, in and on which synapses occur.

Muscle arm on nerve synapses

(*a*) *Head region*

A major *Peripatus* motor nerve branch contains from 10–24 motor axons, a small one 8–13. Along the lengths of these branches *within the muscles* are located sites where muscle arms contact the axons. There are several arms, generally 3–7, from different muscle fibres, at each major

---

DESCRIPTION OF PLATES 17–20

FIGURE 17. *Above:* Contact between muscle fibres. Transverse section through two LL fibres of smaller than average size, at a point of contact between them. The apposed plasma membranes are separated by a 90 nm gap. Dense material lines the inside of the many membrane folds near the contact. (Magn. × 5500.) *Below:* A large LL muscle fibre with a specialised region at lower left that is of the kind associated with an incipient motor nerve contact, for comparison with the muscle/muscle contact. Note the concentration of mitochondria. (Magn. × 2900.)

FIGURE 18. Phase contrast micrographs of transverse sections of LL. The wide spaces between muscle fibres almost all contain one or two strands, sometimes convoluted, of various thicknesses. These are extensions of muscle fibres passing towards a motor nerve branch, where they will make synaptic contacts. (Magn. × 1000.) The details cannot be resolved at this magnification: see figures 19–22.

FIGURE 19. Transverse section through a bundle of lateral longitudinal muscle fibres with a motor nerve bundle at a branch point. Roots of muscle arms are seen arising from five muscle fibres (1–5). Heads of four muscle arms (i–iv) are making synaptic contacts with motor axons. (Magn. × 2480.)

FIGURE 20. Higher magnification view of the synaptic regions of figure 19. (i) and (iii) are muscle arm heads of the light type; (ii) and (iv) are of the dark type. (i) and (iv) synapsing with 3 axons, (ii) with 5, (iii) with 6. Also note the trachea (Tr) and the muscle arm (MA). (Magn. × 7180.)

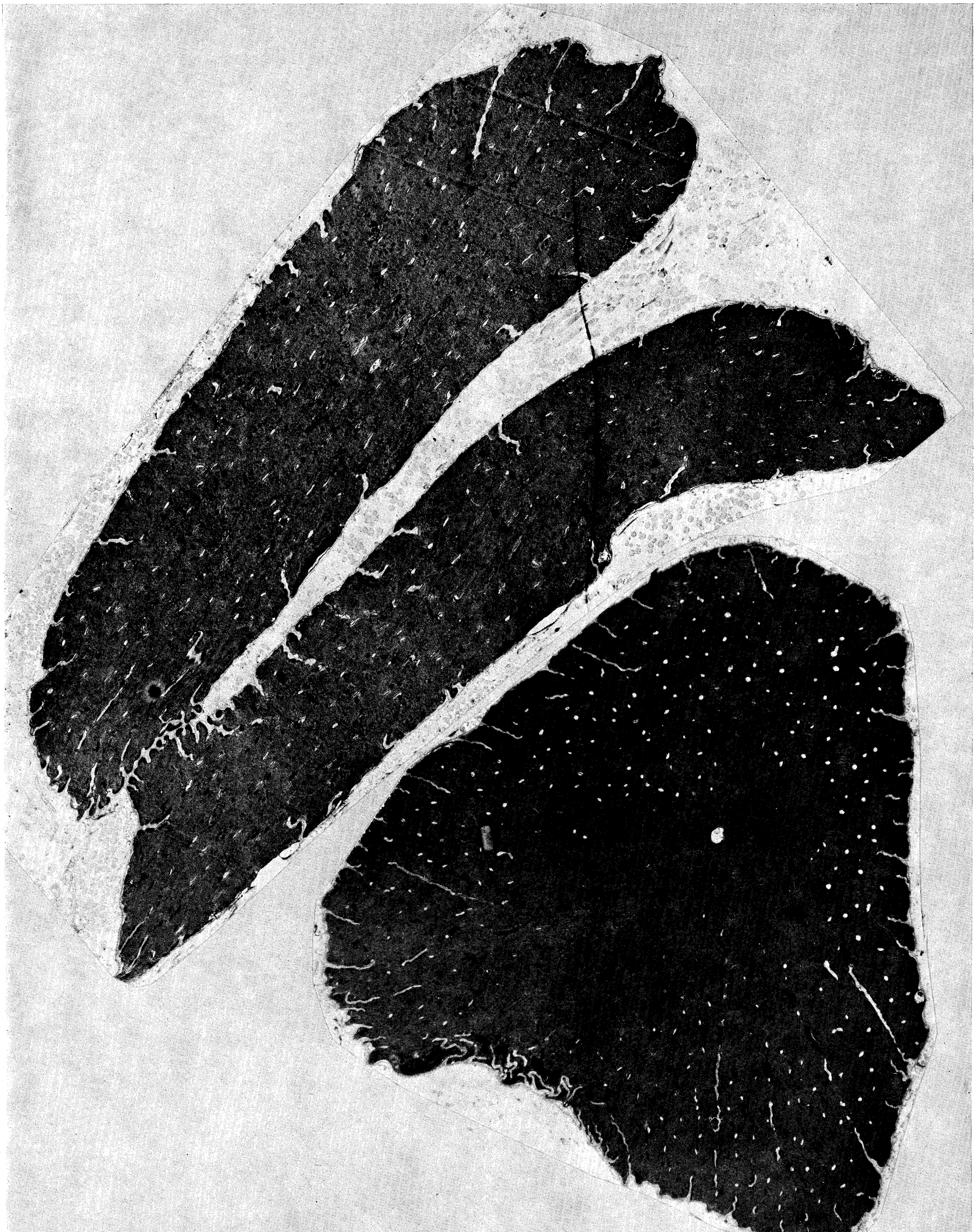

FIGURE 17. For description see p. 500.

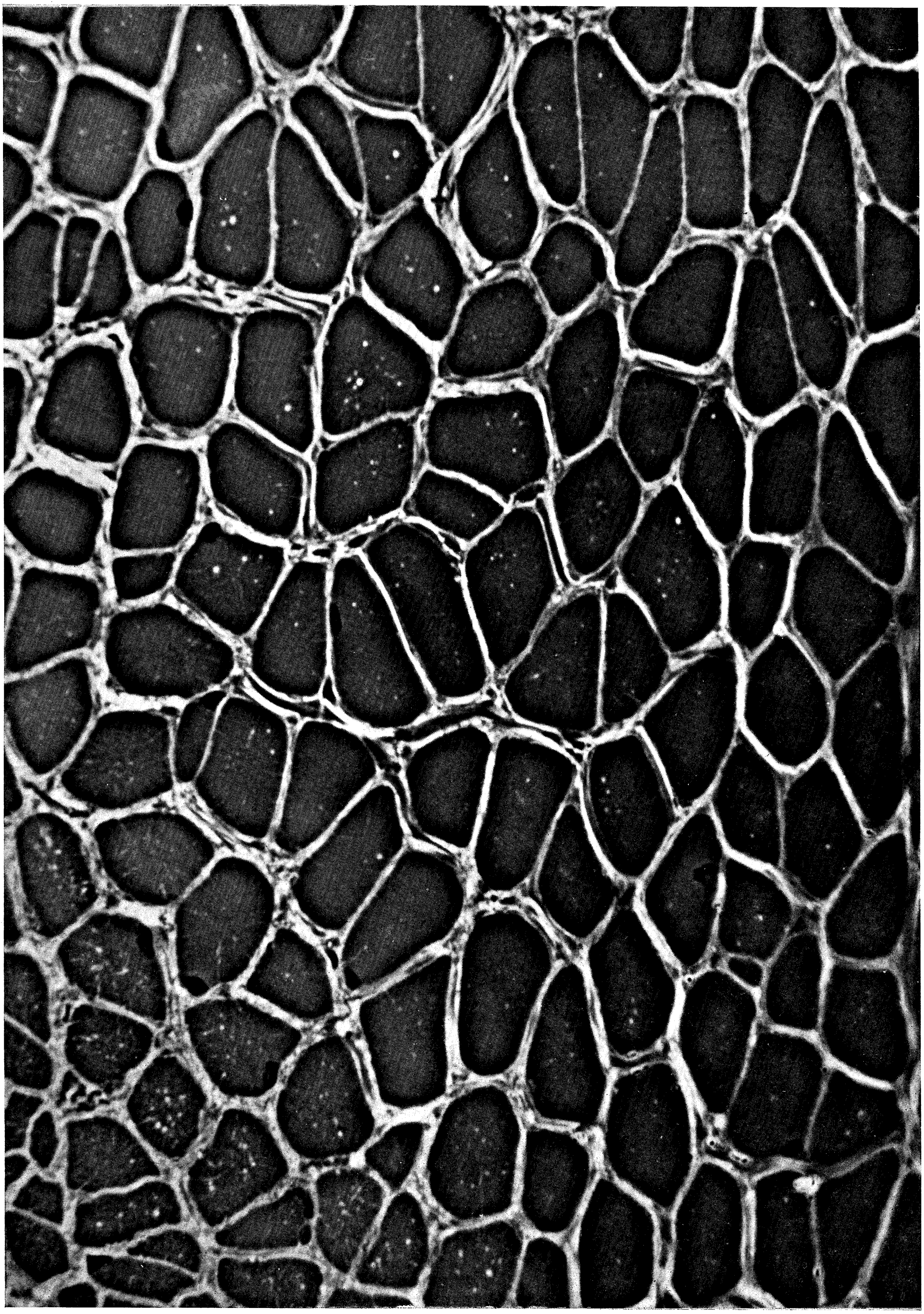

FIGURE 18. For description see p. 500.

FIGURE 19. For description see p. 500.

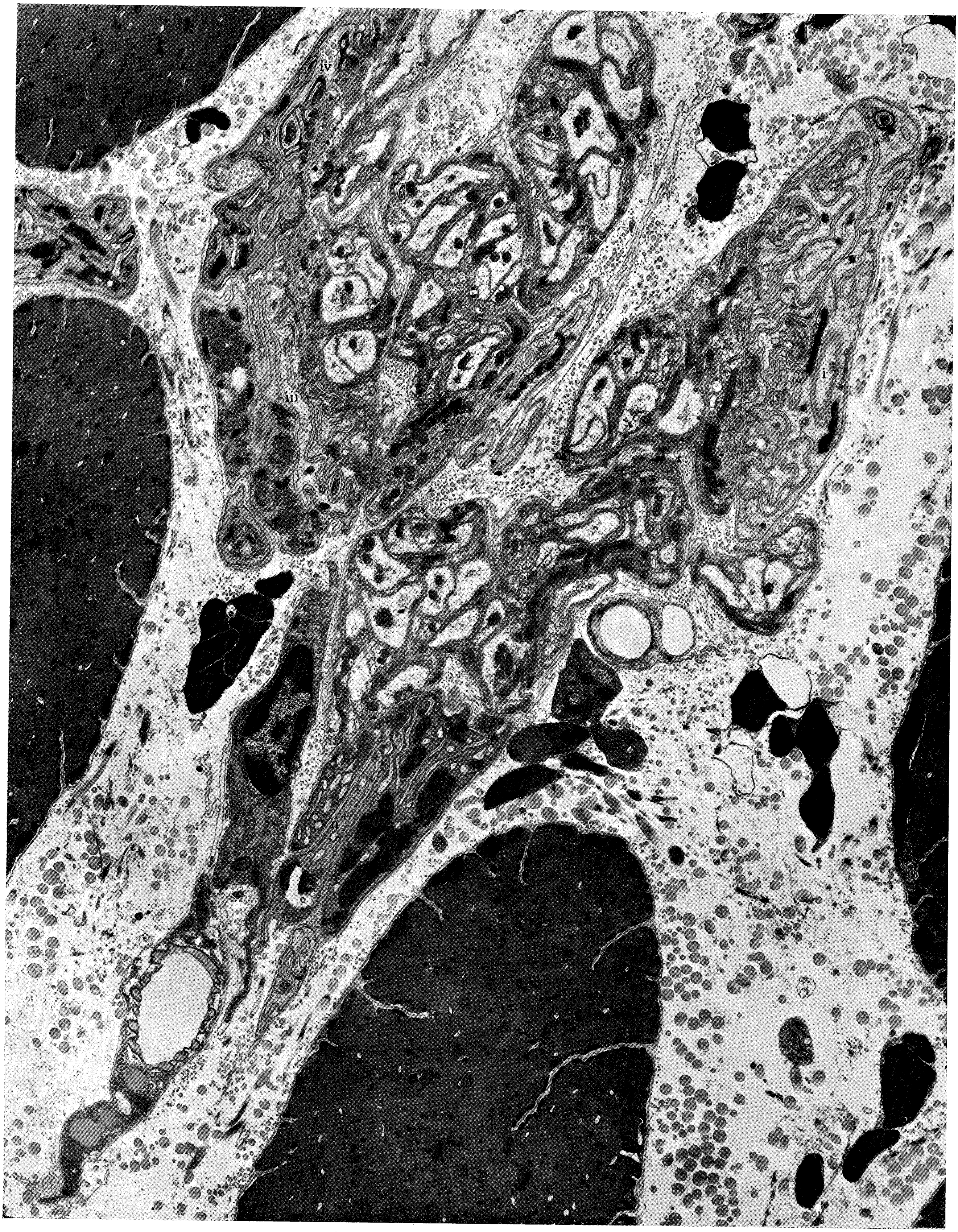

FIGURE 20. For description see p. 500.

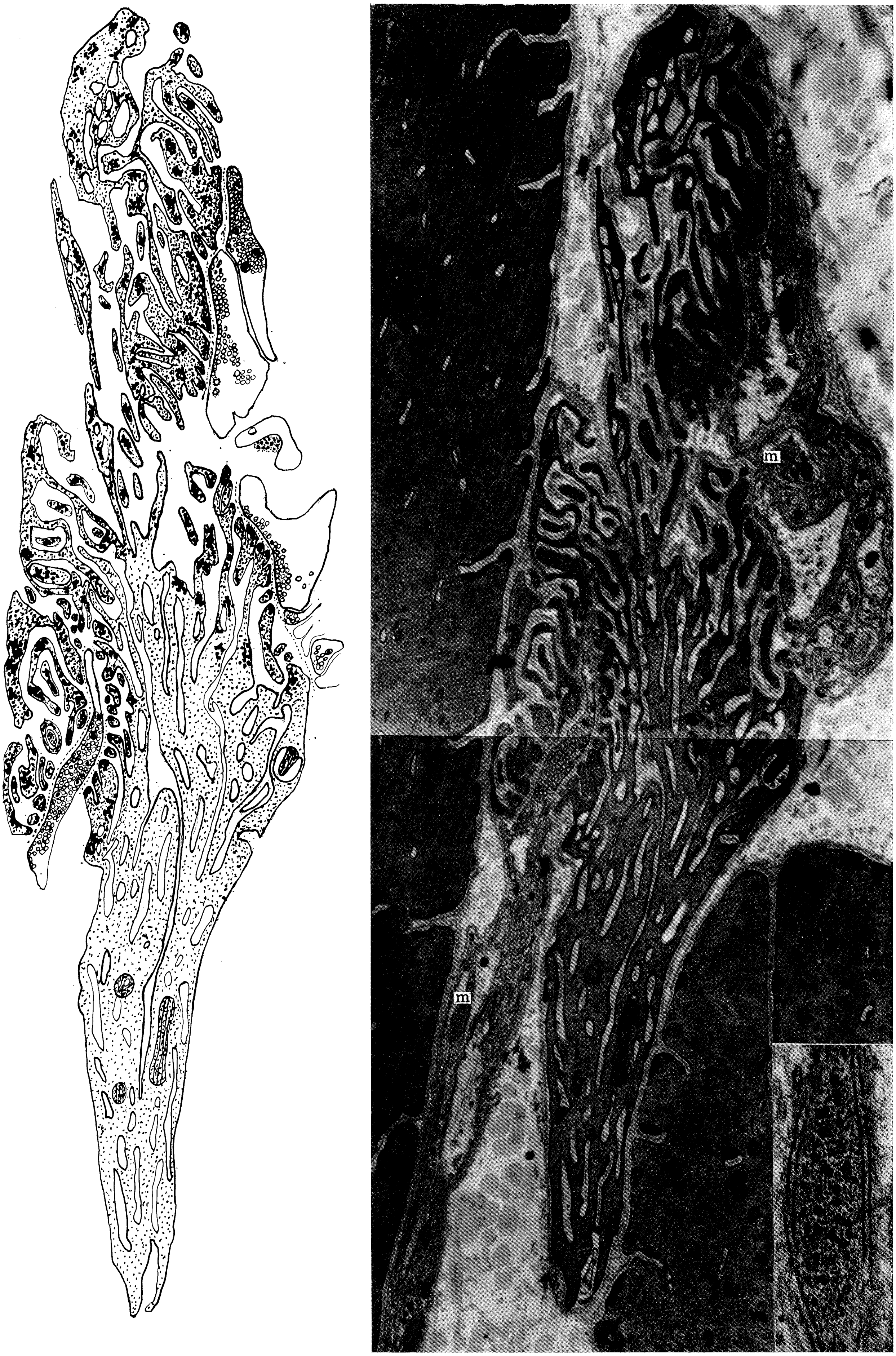

FIGURE 21. For description see p. 501.

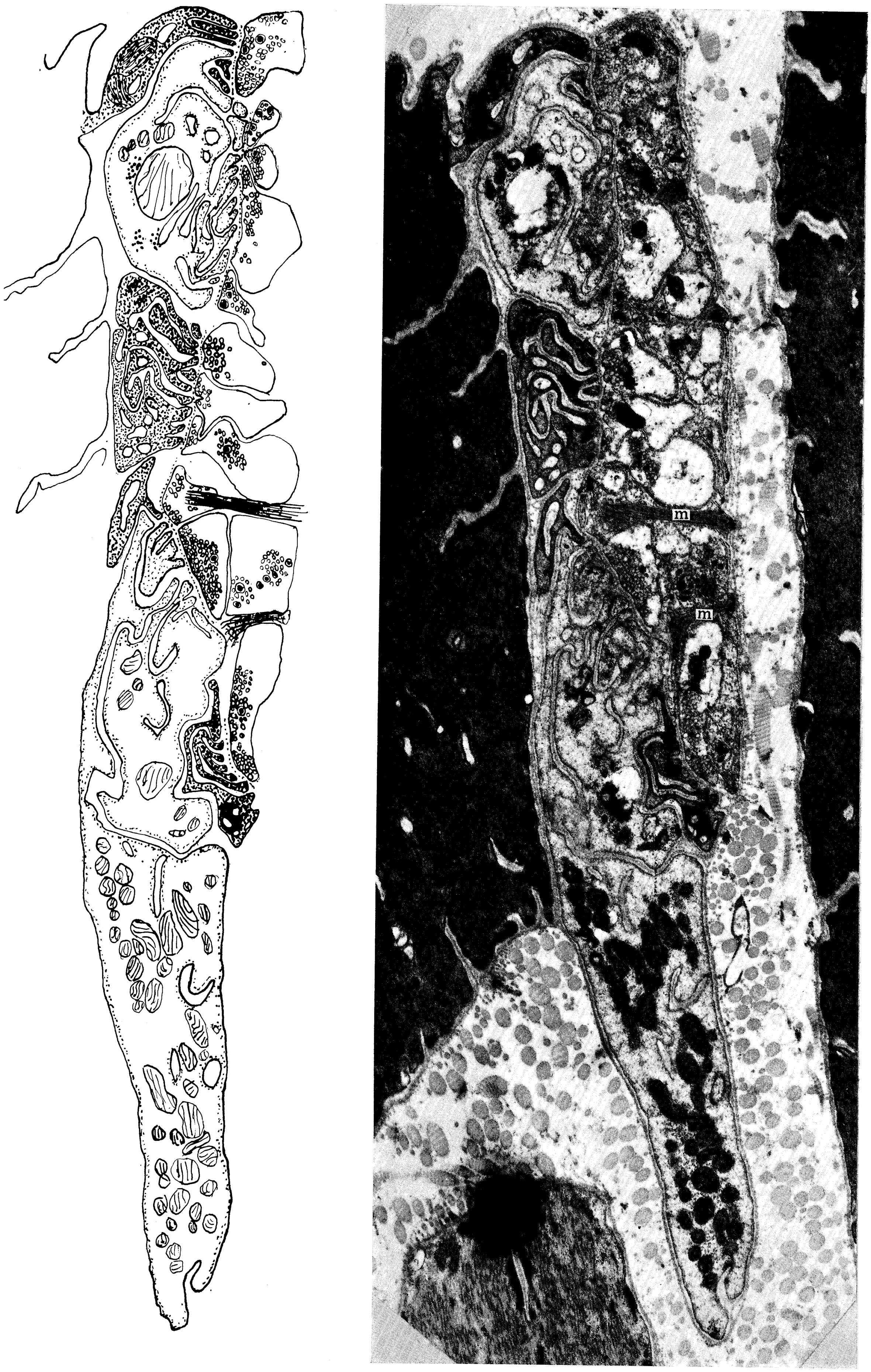

FIGURE 22. For description see p. 501.

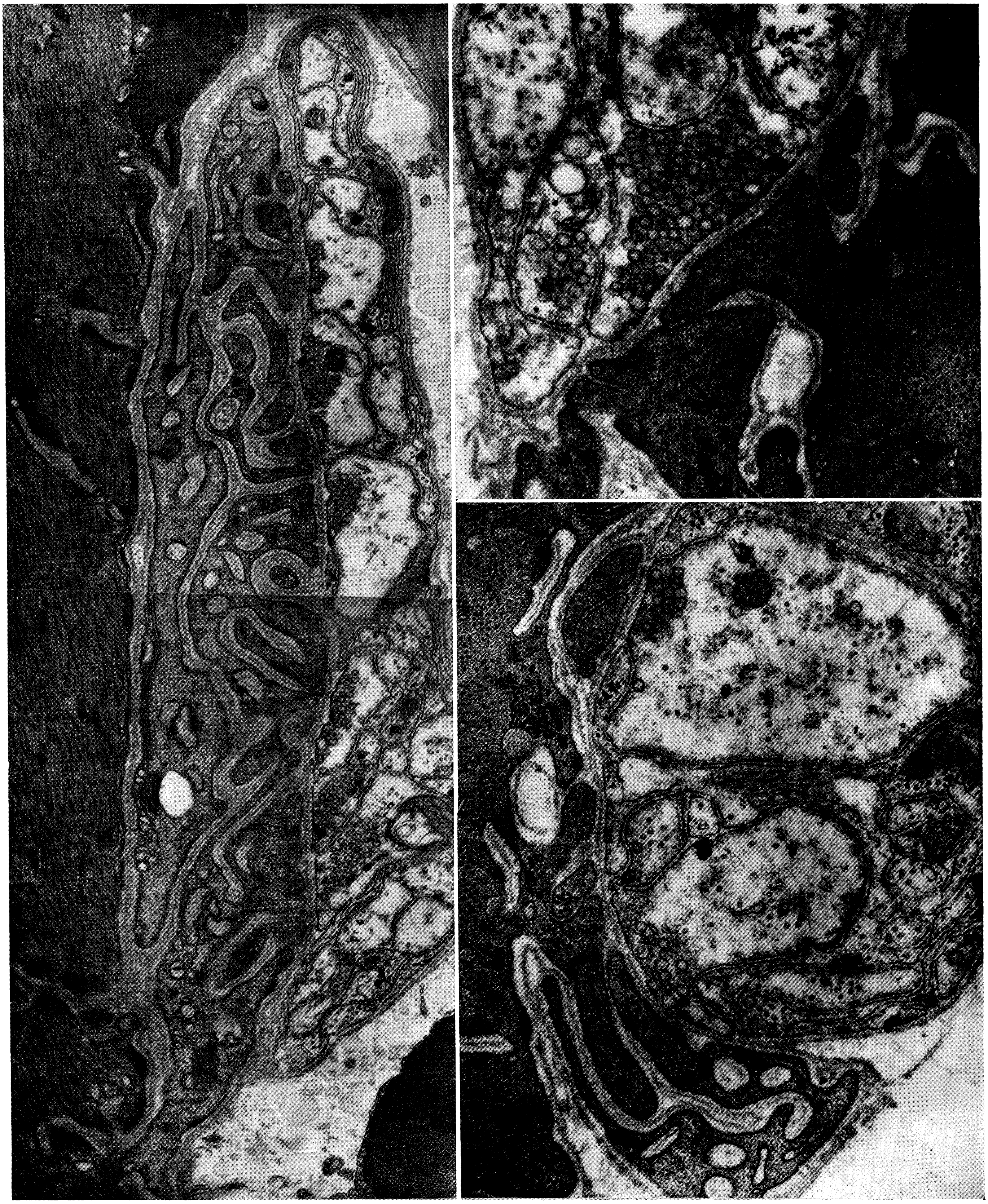

FIGURE 23. For description see p. 501.

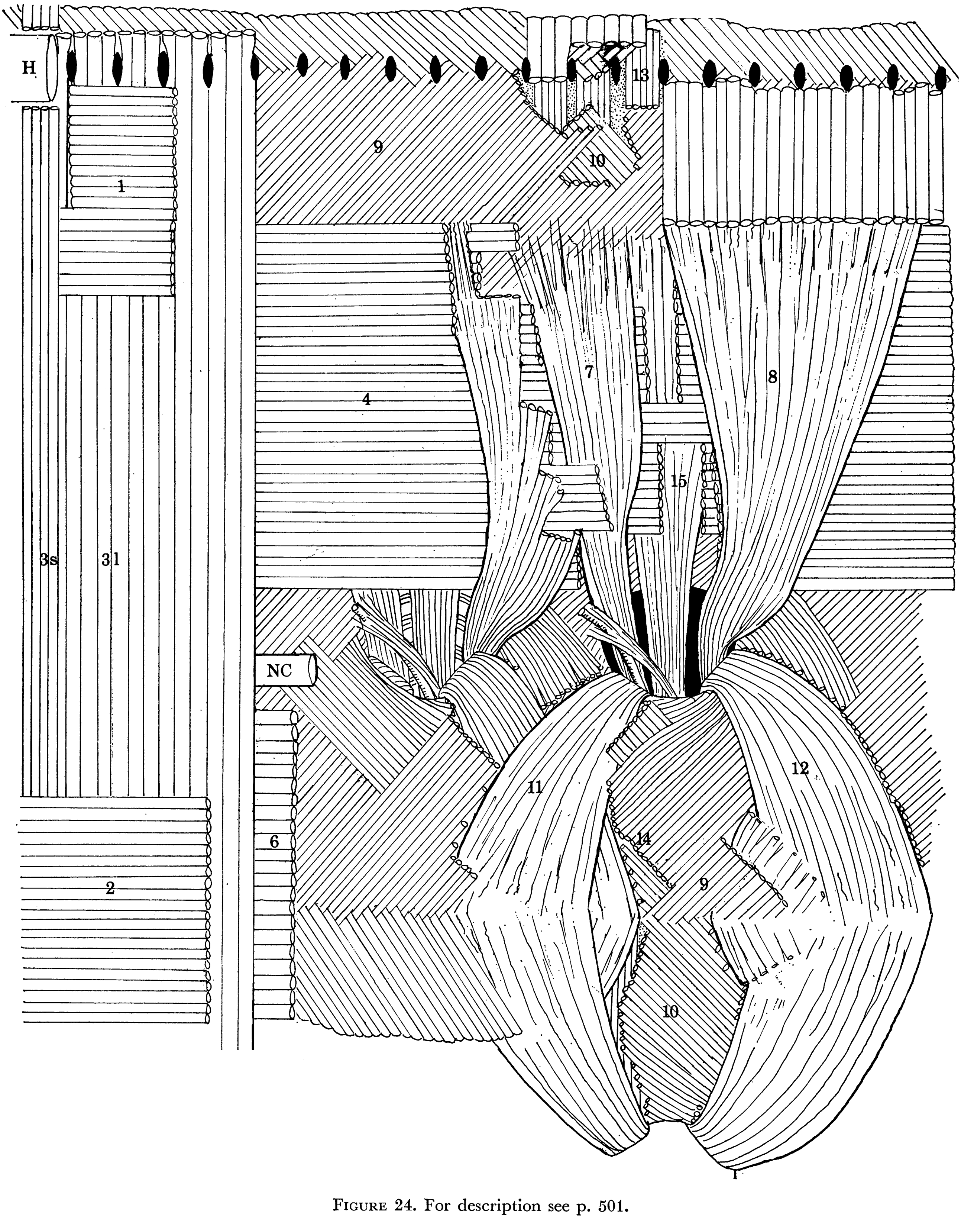

FIGURE 24. For description see p. 501.

site, though single arm contacts also occur. Points of branching of motor nerves are favoured points for these contacts. Each arm expands into a head section that is much larger than the diameter of the arm. The head has a complex basic structure consisting of numerous infoldings, together with some invaginated wide tubules. The head itself may be single or divided into two or three parts if near a branch point of the nerve (figure 21), in which case one head will be on the major nerve and the other(s) on branch(es). A wide variety of forms was seen: the shape of the head is apparently dependent upon a variety of local factors, notably the details of the nerve branching near the contact, and the presence of heads from other muscle fibres competing for axon contacts.

The reader may have noticed that in figure 11*d*, which shows a transverse section through a part of LL, almost all of the wide spaces between muscle fibres contain one or more thin strands. These are the muscle arms *en route* to motor nerve branches. Every light micrograph of 5 μm thickness shows muscle arm branches in the majority of spaces between muscle fibres.

At places where a few arms arrive at about the same point on a nerve branch, since the heads are almost as large as the nerve, they tend to almost completely surround it (figure 19). The shapes of the heads are always complex with intertwined branches competing to make contacts with the axons.

(*b*) *Presynaptic terminals*

Each muscle arm head makes three, four, five, six or seven synapses with different motor axons (figures 20, 21, 22). Similar numbers of different junctional potentials have been identified at innervation sites by intracellular recording (Hoyle & del Castillo 1979). These synapses contain accumulations of round, clear vesicles in the size range 48–70 nm, mean 56 ± 1.6 nm. Some contain also a few large dense-core vesicles and fewer still contain a large number of dense-core vesicles in addition to clear ones. Omega shapes and coated vesicles were also seen,

Description of plates 21–24

Figure 21. Single, complex muscle arm head making synaptic contacts with five axons. This head derived from a more distant muscle fibre than the two shown and met a small branch *en route* to innervating muscle fibres. The two regions labelled m are fine strands of muscle, the one at right being attached to the motor nerve. (Magn. × 11000.) *Lower right:* Enlarged view of muscle strand passing through nerve seen at lower left. (Magn. × 62000.)

Figure 22. Combined muscle on nerve and nerve on muscle junctions. Two light-type heads of muscle arms contact a motor nerve branch that is making synaptic contact with the muscle fibre at left (dark subsynaptic regions). Two fine branches of muscle (m) are associated with the nerve. (Magn. × 11000.)

Figure 23. Examples of nerve on muscle fibre synapses. *Left:* Multiple axon nerve on muscle fibre junction with six vesicle-containing motor axons synapsing side-by-side on a typical end-plate, with highly complex subsynaptic folding, attached to the fibre by a small pillar (arrow). (Magn. × 22500.) *Right: Top:* Simpler synapse with minimal sub-synaptic folding. (Magn. × 55000.) *Lower:* Synapse having intermediate complexity. (Magn. × 36900.)

Figure 24. Principal muscles of the body wall and feet of *Peripatus dominicae*, inside view, anterior to the left. This drawing follows the style of Fig. 30 of Snodgrass (1938) and retains his numbering of muscles as far as possible. It is intended to correct the error caused by his missing the functionally important levator of the leg (numbered 15). H is the heart and NC the nerve cord. 11 and 12 are the anterior and posterior depressors of the leg respectively, previously the ventral promotor and ventral remotor. 3s and 31 are small and large muscle fibres of the inner circular muscle (previously dorsoventral lateral). 7 is the promotor of the foot (previously the dorsal promotor) and 8 the remotor (previously the dorsal remotor). The regularly spaced black ovals are ostia into the pericardium in the dorsal midline. The point of crossing over of inner oblique fibres (9) near the bottom of the figure denotes the ventral midline. Also see table 1, p. 505.

and an occasional presynaptic dense body around which clear vesicles were clustered, but no membrane thickenings.

(*c*) *Postsynaptic structure*

The postsynaptic region is distinguished by multiple infoldings, with complex branching. The infoldings have the same dimensions as wide tubules and are similarly lined with material that is indistinguishable from that of the outer part of the sarcolemma. Two types were discernible in the electron microscope: clear and dense. The forms of the two were similar; the difference being in the amount of granular material, which was sparse in the former, dense in the latter. The dense ones also tended to contain more and larger mitochondria and more glycogen particles.

(*d*) *Synaptic cleft*

No matter where the synaptic contact occurs there is always a large gap of almost 100 nm between the outer pre- and post-synaptic membranes. In between these is a uniform layer of dense material. This type of contact is closely similar to its counterparts in vertebrate neuromuscular junctions and quite unlike those of either annelids or of arthropods. The gap in arthropods is only one-quarter that of *Peripatus* and clear. There is little or no folding in the postsynaptic structure. Annelids possess two types of neuromuscular junction; one with a narrow 15 nm gap, the other with a wide 90 nm gap and a granular layer as in *Peripatus*, but only the latter has a complex subsynaptic structure.

(*e*) *Muscle arm details*

Muscle arms of various thicknesses were encountered, from as little as 0.2–12 μm, though most had both wide and narrow regions. The arm may start out from a region containing a large nucleus and be as thick at its base as the muscle fibre, before narrowing to a micron or two. From one to a few large nuclei are usually situated in the arms, giving rise to complex shapes. A single motor nerve branch supplies a cluster of 20–40 muscle fibres, running near the centre of the cluster parallel to the fibres. At a site at which a muscle arm occurs four or five others are likely to occur also, including ones from muscle fibres most distant from the nerve. These arms are always pointed inwards towards the nerve even though they will have to pass around several other muscle fibres to reach the nerve.

From such a point we made serial sections in order to determine the frequency with which a given muscle fibre gave off muscle arms. We sectioned about 350 μm before a fibre that had produced one gave off another. No nerve on muscle fibre synapses occurred in the gap. We do not have sufficient data to ascertain the frequencies of occurrence of either muscle arms or nerve on muscle fibre synapses, but both were fewer than we expected to find in view of the appearance of large miniature junctional potentials with every microelectrode insertion (del Castillo and Hoyle, in preparation).

Nerve on muscle fibre synapses

Examples of nerve on muscle fibre synapses are shown in figure 23. A nerve on muscle fibre synapse is one in which the motor nerve has sent an axon or multiple axon branches to contact a muscle fibre at points which have developed a specialised subsynaptic apparatus. A sharp distinction between nerve on muscle and muscle arm synapses is not possible. There is a continuous gradation which depends on the distance between the muscle and nerve fibres. The closer a muscle fibre is to a motor nerve branch the shorter the length of muscle arm required to reach the nerve. Commonly there is at least some extension, which we shall call a pillar, of the muscle fibre towards the approaching nerve. Proximity of motor axons to a muscle fibre is no guarantee that synapses will occur. The two can run in parallel, not more than a micron apart, without any trace of synaptic specialisation arising on the muscle over a few hundred μm. When the specialisation does occur it is obvious, with an extensive series of infoldings of the muscle fibre membrane, lined by dense material. The motor axon or axons lie across these stretches of specialised muscle fibre, with the characteristic synaptic gap material between. Pillars range widely in size, from 400 nm to 3–4 μm: they may be stubby, with broad or narrow bases expanding into broad contact regions. Accretions of presynaptic vesicles occur opposite the pillars. Presumed functional synaptic contacts ranged in size from patches of only 0.2 μm diameter to plates up to 6 × 10 μm.

Contractile material never extends into either muscle arms or pillars. The farther the point of contact is from the muscle fibre surface, the greater the extent of the elaboration of the post-synaptic plate. The larger post-synaptic plates contain highly complex labyrinths of wide tubules and mitochondria. There is a tendency in some of the larger plates for the WT's to run parallel to each other, perpendicular to the muscle fibre surface. These WT's extend into the fibre for various depths, some not at all.

Motor nerves tend to send out branches along muscle arms; likewise a muscle fibre tends to extend along an innervating nerve branch.

The number of axons in nerve on muscle fibre synapses was from 1–8, with 3 the commonest. Multiple axon synaptic sites on single muscle pillars or pillarless patches were brought about by a shift from fasces to fascia, the axons coming to lie side by side along the muscle fibre axis.

Nerve on muscle fibre synapses did not occur in much of the material we examined and we concluded, tentatively, that muscle arm on nerve synapses predominate. Two extreme types of ending were recognised in a continuous spectrum, on the basis of vesicle content. One contains a preponderance of clear vesicles, the other a preponderance of dense core vesicles.

(*a*) *Clear vesicle terminals*

Synaptic contacts for terminals containing only clear vesicles, or clear plus only a few dense vesicles, had concentrations of vesicles of conventional kinds, sometimes with a presynaptic density central to the cluster. The dense-core vesicles were always located away from these clusters.

(*b*) *Endings containing mainly dense-core vesicles*

The nerve on muscle fibre ending that occurs in figure 13 contains many dense-core vesicles (d.c.vs) although there are also many clear vesicles in it. In a few terminal nerve branches d.c.vs were present in dense clusters, and some were found in which there were alternate clusters of d.c.vs and clear vesicles. The meaning of this is obscure at this time. No difference was seen in the synaptic gap opposite d.c.v. clusters compared with sites where there were only clear vesicles. There was always a thick layer of fine granules in the gap. In annelids, a distinctive type of gap, that is not granule-filled and only 15 nm wide occurs where d.c.vs are predominant presynaptically (Rosenbluth 1972).

En passant neuromuscular contacts

These are quite common for motor nerve branches that meet a muscle arm *en route* towards muscle fibres they will innervate. The muscle arm continues into a head section on a nerve in spite of the *en passant* contact being synaptic in nature. Whilst a few of these contacts had a compact structure like that of the head region, most consisted of a long (10–30 μm) parallel juxtaposition with little postsynaptic structure. Clusters of synaptic vesicles occur at intervals along these contacts, and the gap is of characteristic synaptic appearance along its entire length. The axon and muscle arm diameters were quite similar, generally 1–2 μm, along the lengths of these contacts.

Some of the *en passant* contacts were bizarre, with large muscle arm portions of complex shape. These might have resulted from the tendency of a muscle arm to develop several heads under the influence of both major and minor nerve branches.

Muscle branches in nerve

Most of the nerve branches encountered in muscles had fine muscle strands running through them. These comprised 12–30 thick filaments surrounded by inter-digitating thin ones. They may have been branches from muscle fibres passing through en route to the skin for anchoring. They must help to hold synaptic contacts in place during large movements of the body wall. No synaptic contacts occur on them even though they pass close to motor axons.

Discussion

In gross structure the muscles of *Peripatus dominicae*, as encountered in the present studies, closely resemble those of other onychophoran species as described by Snodgrass (1938 – *Peripatoides*), Manton (1973 – *Peripatopsis*) and Birket-Smith (1974 – *P. dominicae, Peripatopsis*). However, there are some differences in the descriptions that are significant either for phylogenetic assessment or for understanding *Peripatus* walking. The disparities seem likely to be due to incomplete dissections by earlier investigators rather than to genuine species differences. A summary drawing of the musculature of the body and legs is shown in figure 24. This follows the style and numbering system of Snodgrass' (1938) summary drawing to permit a direct comparison with the other accounts. The differences may also be discerned from the muscle designations of the various authors listed in table 1. They are as follows: Muscle 19*a*, designated the retractor of the claw by Snodgrass, but a levator by Manton (1973) was determined definitely to be a retractor in *P. dominicae*. Manton's drawing for *Peripatoides* shows this muscle attached to the upper margin of the foot. Muscle 19*b* of her drawing, listed as an accessory retractor of the claw is definitely not present in *P. dominicae*. This could be a species difference, but may have been a dissection artifact, since such a muscle would have to cross the tough septal muscle (16). Muscle 5 of the present work, the levator of the foot, was not described by either Snodgrass or Manton but is of major significance. Muscle 4, in the present account, is the whole lateral longitudinal. In *P. dominicae* two leg muscles, the levator and the promotor, pass through it in different planes. Birket-Smith (1974) found the levator and assigned it a new number, 21, but termed it the retractor of the leg. If hydrostatic pressure is low it will indeed cause retraction, but when it is high, and especially if the depressors are relaxed, it is strictly a levator.

TABLE 1. MUSCLES OF ONYCHOPHORANS

muscle number (Snodgrass 1938)	*Peripatoides novae-zealandiae* (Snodgrass 1938)	*Peripatopsis spp.* (Manton 1973)	*Peripatopsis capensis* and *Peripatus dominicae* (Birket-Smith 1974)	*Peripatus dominicae* (present paper)	abbreviation in illustrations (figures 3–8)
1	dorsal longitudinal	dorsal longitudinal	internal dorso-longitudinal	dorsal longitudinal	(left and right) DL
2	ventral longitudinal	ventral longitudinal	internal ventro-longitudinal	ventral longitudinal	VL
3	dorsoventral lateral	deep dorso-ventral	internal coarse dorso-ventral internal fine dorso-ventral	inner giant circular inner small circular	IC (l) IC (s)
4	internal laterodorsal longitudinal	lateral	laterodorsal longitudinal	lateral longitudinal	(left and right) LL
5	external laterodorsal longitudinal	longitudinal	external laterodorsal longitudinal		
6	lateroventral longitudinal	ventral longitudinal	lateroventral longitudinal	latero-ventral longitudinal	(left and right) L-VL
7	dorsal promotor of leg	dorsal promotor of leg	dorsal promotor of lobopod	promotor of leg	Pr
8	dorsal remotor of leg	dorsal remotor of leg	dorsal remotor of lobopod	remotor of leg	Re
9	internal oblique	inner oblique	internal oblique	inner oblique	IObl
10	external oblique	outer oblique	external oblique	outer oblique	OObl
11	ventral promotor of leg	ventral promotor of leg	ventral promotor of lobopod	anterior depressor	ant D-11
12	ventral remotor of leg	ventral remotor of leg	ventral remotor of lobopod	posterior depressor	post D-12
13	circular	circular	external circular	outer circular	OC
14	tranverse, of leg base	tranverse, of leg base	tranverse lobopod sheet	transverse, of leg base	—
14*a*		not named, but as 14 attaching to outside of leg base	not present	not present	—
14*b*	—	not named, but dorso-ventral attaching to inside of leg base	not present	(part of 14)	—
15	peripheral basal leg	peripheral basal leg	see 17	levator of leg	Le
16	anteroposterior septal of leg	transverse	anteroposterior lobopod sheet	septal	Se
17	flexor of leg	—	flexor of lobopod	see 11 and 12, depressors of leg	D-11, 12
17*a*	flexor of distal leg rings	depressor of foot	protruder of foot pads	no clear muscle seen	—
18	circular of foot	—	protractor of claws	circular of foot	—
19	retractor of claw	levator of foot	lifter of claws	retractor of claw	RCl
19*a*	accessory retractor of claw	accessory levator of foot	not present	not present	—
20	—	—	depressor of claws	—	—
21	—	—	retractor of lobopod	(see levator of leg)	—

In commenting on muscle 15 of Snodgrass, Birket-Smith (1974) wrote: 'Snodgrass uses this designation for what in the present species (*P. dominicae*) appear as distinctly different muscles, viz...17 and the distal part of 8 and probably also including m 21'. Birket-Smith dropped the number 15 from his account and added the number 21 for the 'retractor'. We consider that the outer Snodgrass muscle 15 is the same as the Birket-Smith 21 and that this is functionally the levator of the leg, which in the present account is designated number 15.

Finally, and of significance from the point of view of assessing the evolutionary position of the Onychophora, is the muscle we have labelled inner circular. In his account of the development of *Peripatus* Sedgwick (1888 – see also Anderson 1973) figured an 'oblique' muscle in an early embryonic stage, running from the lateral to the midventral body wall. This was termed the dorsoventral lateral (number 3) by Snodgrass and deep dorsoventral by Manton. Both of these authors showed left and right muscles inserting just above the LLs and passing to the ventral cuticle just lateral to the midline. Birket-Smith (1974) recognised that there are thick inner and thin outer parallel fibres as sub-components of muscle 3 and wrote that they are 'attached mid-dorsally and mid-ventrally to the integument (to the so-called white lines). In each side they form a nearly semi-circular arch'. Thus this author realised that the thick fibres do not attach above LL and at least extend to the dorsal midline, but thought they terminate there. In fact, as figure 2*b* of the present paper shows, these fibres pass across the midline dorsally. In addition, the same fibres cross the midline ventrally (figure 3) showing that muscle 3, of which they are the major component, is *circular muscle*.

In a personal letter to us, Dr Manton wrote that in a photograph (figure 3) that we had sent to her, muscle sheet 3 was simply 'collapsed outwards against the body wall'. However, this condition was exactly similar in living *P. dominicae*. As mentioned in the text, component single thick muscle fibres could readily be dissected out in *P. dominicae* and they were found to be continuous from the lateral walls across the midline ventrally under VL as well as dorsally behind the DL's.

One final comment will be made on Manton's account. Wells (1966) wrote in *The lower animals:* 'Examination of the muscles of the legs of *Peripatus* reveals no mechanism for extending the leg. The leg is blown out by hydrostatic pressure.' Manton (1973) by contrast wrote that 'During normal walking...contraction of the oblique muscles in the superficial cylinder and of the strong transverse muscle (i.e., the septal muscle) can alter the proportions of the leg without any change in its internal volume'.

We agree with Wells, that the septal muscle can only act to cause extension indirectly, via hydrostatic force. The skin of *Peripatus* comprises mainly collagen, The remarkable ability to elongate is associated principally with the highly folded nature of the skin. But the animal can also greatly alter its shape laterally, and it is extraordinarily soft and supple. This is due to the undulation of collagen in the skin rather than to a lazy-fingers criss-cross as is the case with several worm-like invertebrates. The major orientation of collagen in and around all of the muscles is at right angles to the fibre axis with a strong component also in parallel. Together these form a very thick collagen cement. *Peripatus* muscles can function only because this collagen is arranged in loose, very wavy bundles: straightening of the waves permits stretching, bending and lengthening. If the collagen were arranged in the parallel straight filaments that are common in other animals, *Peripatus* would be a stiff, rigid animal.

The fine structure of *Peripatus* muscle fibres is clearly different in several respects from that of any other muscles so far described. The basic filament type is the same as that of a striated fibre, but with an almost total lack of transverse organization. This is because the material to which the thin filaments are attached is in the form of small dense Z bodies that are unevenly scattered. Some are situated in oblique rows in the middle of the contractile material, some are attached to one or the other set of transverse wide tubules and others are attached to the inside of the muscle fibre membrane.

The two wide tubule systems are without precedent, comparable ones not having been described in any other animal. While the radial ones are not unlike some of the smaller clefts that invaginate many arthropod muscle fibres, the longitudinal ones certainly have no equivalent. Since they undoubtedly have contacts with the SR that are similar to those between E tubules and SR, they could serve to transfer excitation into the fibres as do the narrower E or T tubules. They could serve for the exchange of dissolved gaseous material and metabolites, etc. An undoubted function for the longitudinal wide tubules is structural, since they provide pathways into the contractile machinery for collagen fibres that directly or indirectly attach the fibres to the skin. Both sets of tubules are also anchoring points for dense bodies and hence for thin filaments.

It is of interest that *Peripatus* muscle fibres have membrane invaginations of conventional T tubule dimensions in addition to the wide ones, that clearly make conventional contacts with sarcoplasmic reticulum. They often derive from wide tubules and probably do not serve a separate functional role. Between them the two sets of tubules provide an extensive system for activation of the contractile machinery.

The paucity of overlap of thin and thick filaments suggests that *Peripatus* muscles are not required to develop large forces. With prolonged excitation it may be that more overlap and activation occur: thick filaments that have no attachments to thin ones when a muscle is at average length, acquiring them when the muscle is shortened or stretched. The very loose structural arrangement is conducive to a very wide range of length changes as in smooth muscle.

Although *Peripatus* has a tracheal system, the tracheoles, unlike those of insects, do not penetrate into the muscle fibres. Hence gaseous exchange must be via the haemolymph.

Peripatus muscle is clearly markedly different in fine structure from the cross-striated fibres of arthropods. Also, it is at least as different from the highly structured obliquely striated fibres of annelids. Its thick filaments are much different from the cigar-shaped filaments, with very thick central regions, found in the latter. We conclude, on the basis of muscle structure, that *Peripatus* is definitely not a half-way stage between annelids and arthropods and seems unlikely to be closely related to either phylogenetically. Ultrastructural details of neuromuscular junctions of *Peripatus* are markedly different from any so far described in arthropods. They do resemble neuromuscular synapses of annelids in regard to the similar wide, granule-filled, synaptic cleft as noted also by Schürmann (1978*b*). Curiously, they bear a close resemblence to mammalian neuromuscular junctions in fine structure, as perceived earlier by Birket-Smith (1974) from light micrographs.

The muscle arms of *Peripatus* on which the majority of the motor synapses occur are not entirely unique because muscle to motor nerve synaptic contacts occur in nematodes (de Bell *et al.* 1965). However, in nematodes they travel to the nerve cord from non-contractile portions of peculiar, long muscle fibres. In transverse section at low magnification, *Peripatus* muscle bears a close resemblance to a vertebrate striated muscle. The muscle arms of *Peripatus* occur repeatedly, along the length of the muscle fibre, at intervals of about 350 μm. Muscle pillars occur in some insect muscles (Hamori, 1963) but these are very short compared with fibre diameter and occur all along the length of a muscle fibre at intervals of only 50–60 μm, to contact a close, parallel axon. They hardly represent long-range probes seeking out the nerves. Somewhat more similar muscle arms to those seen in *Peripatus* occur in a Polyclad flatworm (Chien and Koopowitz 1972), but these authors questioned whether the synapses they saw represented true neuromuscular junctions. There can be no doubt in *Peripatus* that muscle arm on nerve contacts serve the purpose of neuromuscular synaptic transmission. In the same muscle fibres, the nearest nerve on muscle synapse, if any, was over 400 μm distant from the arm. Intracellular recordings (del Castillo and Hoyle, in preparation) showed large spontaneous miniature junctional potentials at almost all recording sites, so probably both types of junction are active and functionally similar.

In addition to the muscle arms that make synaptic contacts with motor neurons there are muscle branches that serve to bind together muscle fibres and also to connect muscles to the skin. The former may be termed synaptic muscle arms to distinguish them from the latter structural muscle arms. Synaptic muscle arms contain no myofilaments whereas structural muscle arms contain interdigitating thin and thick filaments, at least in regions close to their origins. Structural arms range in thickness from a few hundred nm to about 10 μm. The thinner arms pass between muscle fibres or into nerve branches. The thicker ones head straight towards the nearest skin, although giving off smaller branches between muscle fibres. As they move towards their terminations all muscle arms become partly, and finally entirely, collagenous.

Structural muscle arms could be present in other soft-bodied animals, though we know of no descriptions of ones similar to those found in *Peripatus*. It seems reasonable to suggest that the structural muscle arms are the basic feature. Given that many fine branches come off muscle fibres for structural purposes it is likely that some of them would come into contact with motor nerve branches. The development of muscle arm on nerve branches in *Peripatus* can then be regarded as a secondary, not a primary feature, as it appears to be in nematodes.

There remain to be considered the phylogenetic relationships of the Onychophora. The present studies have shown conclusively that they have not only evolved a unique gross morphology of the musculature, but also unparalleled fine structural features of both muscle fibres and synapses. We see no similarities, only differences, in all these aspects, with the corresponding features of arthropods on one hand (insects, arachnids, and crustaceans are virtually identical in this regard) and annelids on the other. It is not easy to imagine how a common ancestor could have given rise to both the Onychophoran features and those of Hexapoda, and at least as difficult to imaging them either diverging into, or deriving from, those of annelids. They should be treated as a unique phylum.

Whatever their evolutionary relationships though, the onychophoran leg, as an example of a simple lobopodium, can serve as a useful living model for the kind of appendage from which the arthropod leg evolved. The finding, of the present study, that they have a simple set of leg movers, with single levator, promotor and remotor and a pair of depressor muscles, means that the basic movements executed in stepping by all arthropods, regardless of leg segment complexity, are present in almost the simplest form possible in *Peripatus*.

This research was supported by National Science Foundation Research Grant No. BNS 0075-463 to G. Hoyle

The collection and maintenance of *Peripatus* was aided by USPHS research grant NS 07464 to Dr José del Castillo. We thank Dr del Castillo for helpful criticisms of the manuscript. We are especially indebted to Mr Faustino McKenzie and the staff of the Institute of Neurobiology, San Juan, Puerto Rico.

We are grateful to Dr S. M. Manton, F.R.S., for drawing our attention to the work of Birket-Smith.

REFERENCES

Anderson, D. T. 1973 *Embryology and phylogeny of annelids and arthropods*, Pergamon, Oxford.

Atwood, H. L. 1972 Crustacean muscle. In *The Structure and function of muscle*, vol. 1, 2nd edn. (ed. G. H. Bourne), pp. 422–489. New York, London: Academic Press.

Beklemishev, W. N. 1969 *Principles of comparative anatomy of invertebrates*. Vol. 1. *Promorphology*. University of Chicago Press.

de Bell, J. T., del Castillo, J. & Sanchez, V. 1963 Electrophysiology of the somatic muscle cells of *Ascaris lumbricoides*. *J. cell. comp. Physiol.* **2**, 159–177.

Birkett-Smith, S. J. R. 1974 The anatomy of the body wall of Onychophora. *Zool. Jb. Anat.* **93**, 123–154.

Buchsbaum, R. 1976 *Animals without backbones*, 2nd edn. University of Chicago Press.

Chien, P. & Koopowitz, H. 1972 The ultrastructure of neuromuscular systems in *Notoplana acticola*, a free-living Polyclad flatworm. *Z. Zellforsch. mikrosk. Anat.* **133**, 277–288.

Cuénot, L. 1949 Les Onychophores. In *Traité de Zoologie* (ed. Grassé), vol. VI, pp. 3–37. Paris.

Elder, H. Y. 1975 Muscle structure. Chapter 1 in *Insect Muscle*, (ed. P. N. R. Usherwood), pp. 1–14. New York, London: Academic Press.

Franzini-Armstrong, C. 1973 Membranous systems in muscle fibres. In *Structure and function of muscle*, vol. 2 (ed. G. H. Bourne), pp. 532–619. New York, London: Academic Press.

Gray, J. 1968 *Animal Locomotion*. Chapter 12 by S. M. Manton (pp. 333–376). New York: W. W. Norton.

Hamori, J. 1963 Electron-microscope studies on neuromuscular junctions of endplate type in insects. *Acta biol. hung.* **14**, 231–245.

Hepburn, H. R. & Heffron, J. J. A. 1976 On the skeletal collagen of an Onychophoran, *Peripatopsis mosleyi*. *Cytobiology*, **12**, 481–486.

Hoyle, G. 1965 Nature of the excitatory sarcoplasmic reticular junction. *Science*, *N.Y.* **144**, 70–72.

Hoyle, G. 1976 Arthropod walking. In *Neural control of locomotion*. (ed. Herman, Grillner, Stein & Stuart), pp. 137–79. New York: Plenum Press.

Hoyle, G. & del Castillo, J. 1979 Neuromuscular transmission in *Peripatus*. *J. exp. Biol.* **83**, 13–29.

Hoyle, G., McNeill, P. A. & Walcott, B. 1966 Nature of invaginating tubules in Felderstruktur muscle fibres in the garter snake. *J. Cell Biol.* **30**, 197–201.

Huddart, H. 1975 *The comparative structure and function of muscle*. Oxford: Pergamon.

Krishnan, G. 1970 Chemical nature of the cuticle and its mode of hardening in *Eoperipatus weldoni*. *Acta histochem.* **37**, 1–17.

Locke, M & Huie, P. 1977 Bismuth staining of Golgi complex is a characteristic arthropod feature lacking in *Peripatus*. *Nature*, *Lond.* **270**, 341–343.

Manton, S. M. 1950 The evolution of arthropodan locomotory mechanisms. 1. The locomotion of *Peripatus*. *J. Linn. Soc. Zool.* **41**, 529–570.

Manton, S. M. 1966 The evolution of arthropod locomotory mechanisms. 9. Functional requirements and body design in Symphyla and Pauropoda and the relationships between Myriapoda and Pterygote insects. *J. Linn. Zool.* **46**, 103–141.

Manton, S. M. 1973 The evolution of arthropodan locomotory mechanisms. 2. Habits, morphology and evolution of the Uniramia (Onychophora, Myriapods, Hexapoda) and comparisons with the Arachnida, together with a functional review of uniramian musculature. *J. Linn. Soc.* (*Zool.*) **53**, 257–375.

Manton, S. M. 1977 *The arthropods*. Oxford University Press.

Neville, A. C. 1975 *Biology of the arthropod cuticle*. New York, Berlin: Springer-Verlag.

Pflugfelder, O. 1968 *Onychophora*. Stuttgart: Gustav Fischer.

Rosenbluth, J. 1965*a* Ultrastructural organization of obliquely striated muscle fibres in *Ascaris lumbricoides*. *J. Cell. Biol.* **25**, 495–515.

Rosenbluth, J. 1965*b* Ultrastructure of somatic muscle cells in *Ascaris lumbricoides*. II. Intermuscular junctions, neuromuscular junctions, and glycogen stores. *J. Cell Biol.* **26**, 579–591.

Rosenbluth, J. 1968 Obliquely striated muscle. IV. Sarcoplasmic reticulum, contractile apparatus, and endomysium of the body muscle of a Polychaete, *Glycera*, in relation to its speed, *J. Cell. Biol.* **36**, 245–259.

Rosenbluth, J. 1972 Myoneural junctions of two ultrastructurally distinct types in earthworm body muscle. *J. Cell Biol.* **54**, 566–579.

Saita, A. & Camatini, M. 1976 Body muscle cells of *Peripatopsis*: a new contractile model. *J. Cell Biol.* **70**, Abs. 68.

Schürmann, F. W. 1978*a* A note on the structure of synapses in the ventral nerve cord of the Onychophoran *Peripatoides leuckarti*. *Cell Tiss. Res.* **186**, 527–534.

Schürmann, F. W. 1978*b* On the ultrastructure of nerve-muscle contacts in Onychophora. *Experientia*. **34**, 779

Schürmann, F. W. & Sandeman, D. C. 1976 Giant fibres in the ventral nerve cord of *Peripatoides leuckarti* (Onychophora). *Naturwissenschaften*. **63**, 580–581.

Sedgwick, A. 1888 The development of the Cape species of *Peripatus*. Part 4. *Q. Jl Microsc. Sci.* **18**, 373–398.

Sedgwick, A. 1909 *A student's textbook of zoology*. London: Allen.

Sharov, A. G. 1965*a* Origin and main stages of arthropod evolution. I. From annelids to arthropods. *Zool. Zh. Ukr.* **44**, 803–817.

Sharov, A. G. 1965*b* Origin and main stages of arthropod evolution. II. Origin and phylogenetic relationships and principal groups of arthropods. *Zool. Zh. Ukr.* **44**, 963–979.

Sharov, A. G. 1966 *Basic arthropodan stock with special reference to insects*. Pergamon, Oxford.

Smith, D. 1972 *Muscle*. New York, London: Academic Press.

Snodgrass, R. E. 1938 *The evolution of Annelida, Onychophora and Arthropoda*. *Smithson. misc. Collns*. **97**, 1–59.

Tiegs, O. W. 1947 The development affinities of the Pauropoda, based on a study of *Pauropus sylvaticus*. *Q. Jl Microsc. Sci.* **88**, 165–336.

Tulsi, R. S. & Rosenbluth, J. 1971 Neuromuscular junctions on the muscle cells in the central nervous system of the leech *Hirudo medicinalis*. *J. comp. Neurol.* **141**, 1–16.

Wells, M. 1968 *Lower animals*. New York, Toronto: McGraw-Hill.

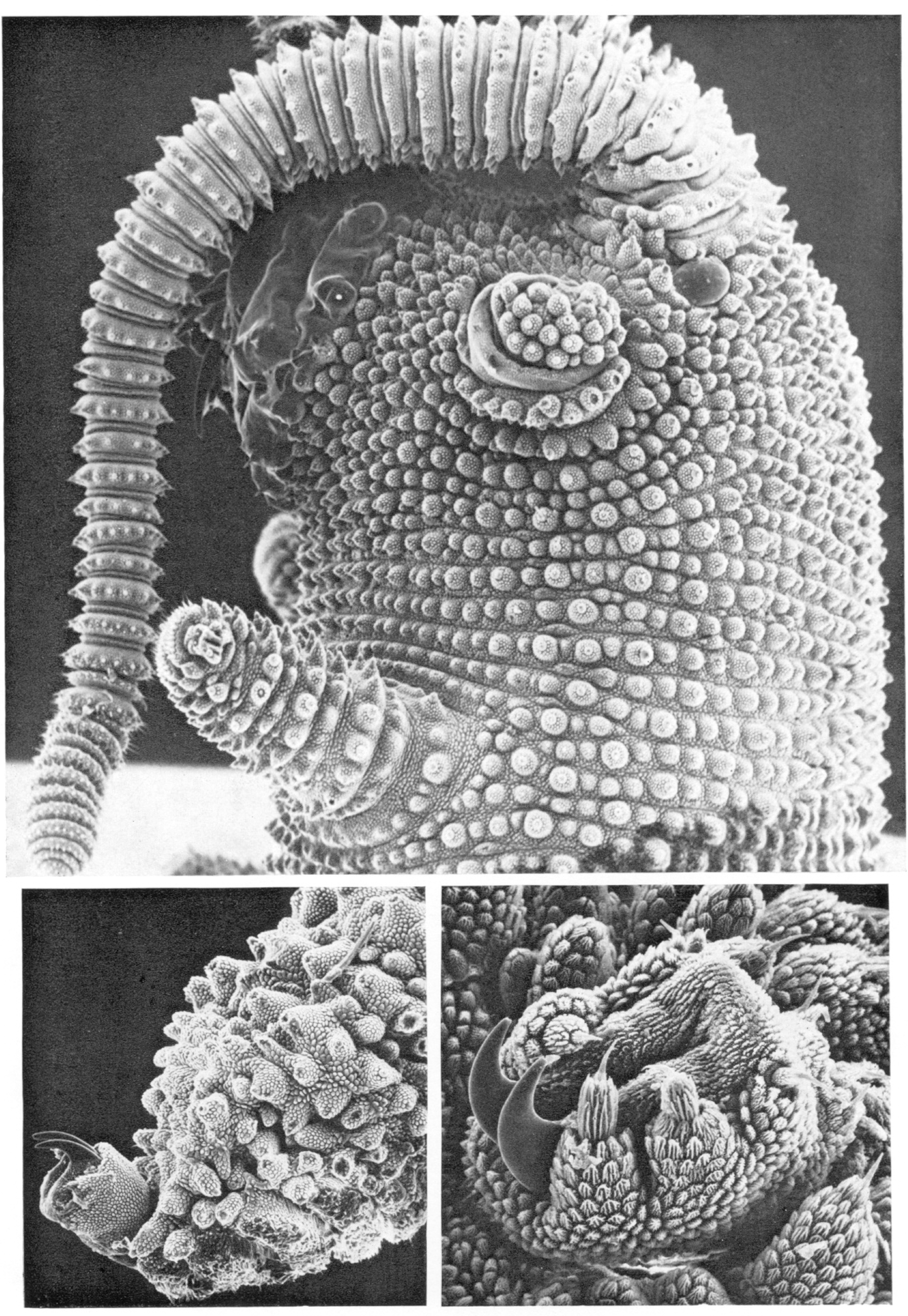

FIGURE 1. For description see p. 486.

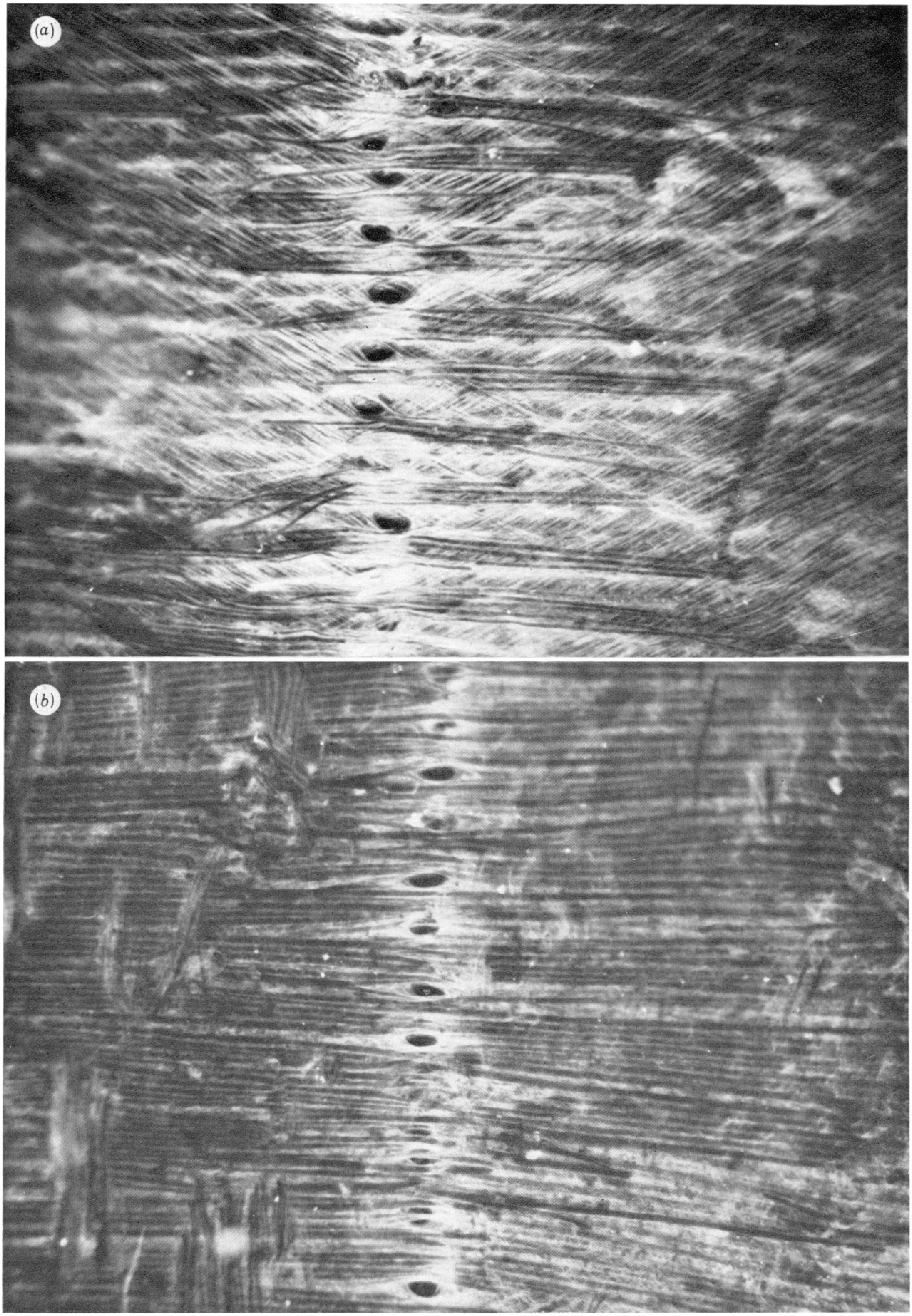

FIGURE 2. For description see p. 486.

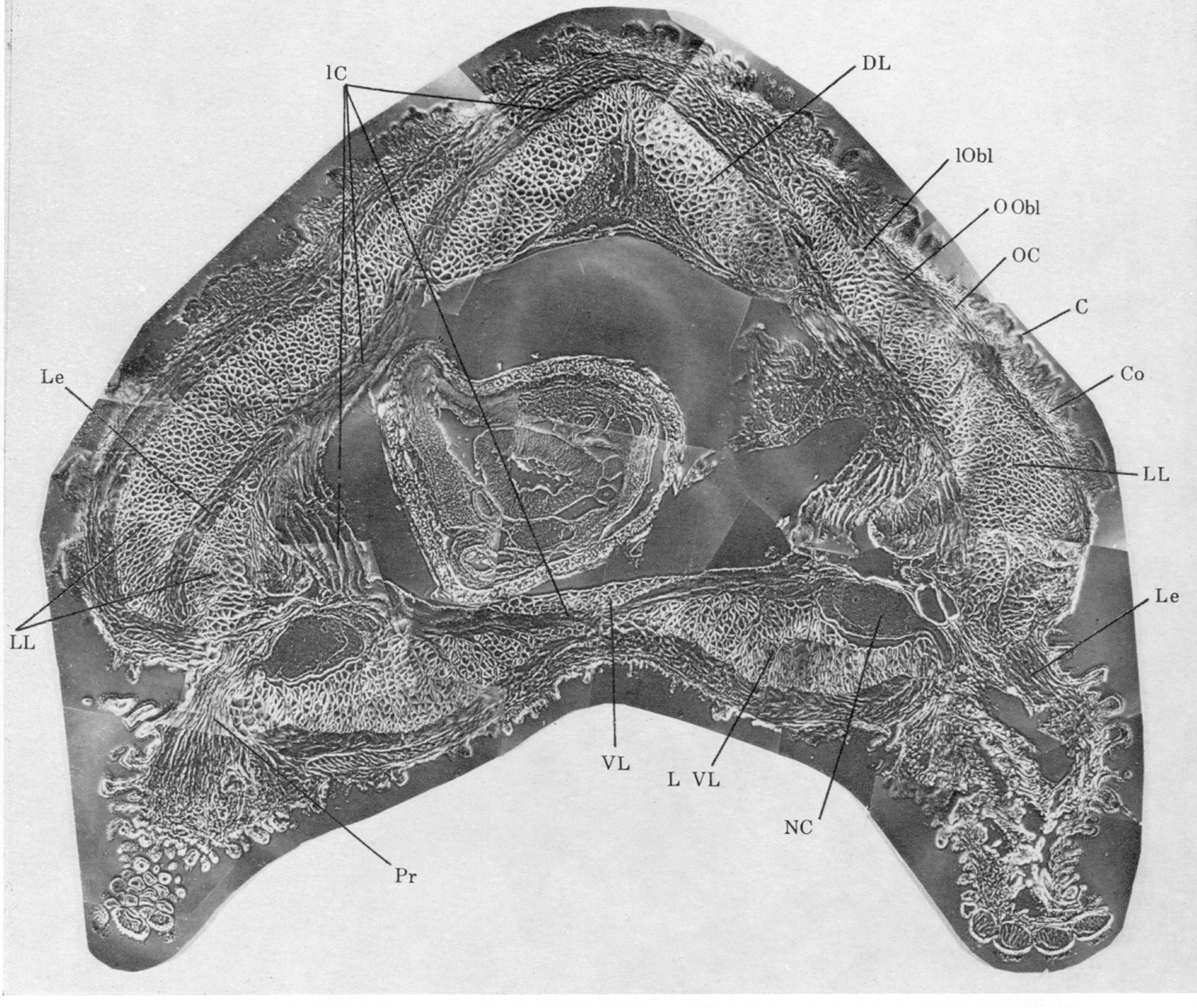

Figure 3. Montage phase-contrast light micrograph of transverse section of *Peripatus dominicae*, through a pair of legs with the image adjusted to emphasise outlines of individual muscle fibres. The left leg section passed through its anterior wall and shows the promotor muscle (see also figures 4 and 5). The right leg section passed just posterior to its centre, and shows the levator muscle, the outer and inner cavities and the septal muscle between them. Cu, cuticle; Co, collagen meshwork; OC, outer circular muscle; OObl, outer oblique muscle; IObl, inner oblique muscle; DL, dorsal longitudinal muscle; LL, lateral longitudinal muscle; VL, ventral longitudinal muscle; L-VL, latero-ventral longitudinal muscle; IC, inner circular muscle (g.C – giant circular fibres); Le, levator of the leg; Pr, promotor of the leg. Width = 5 mm.

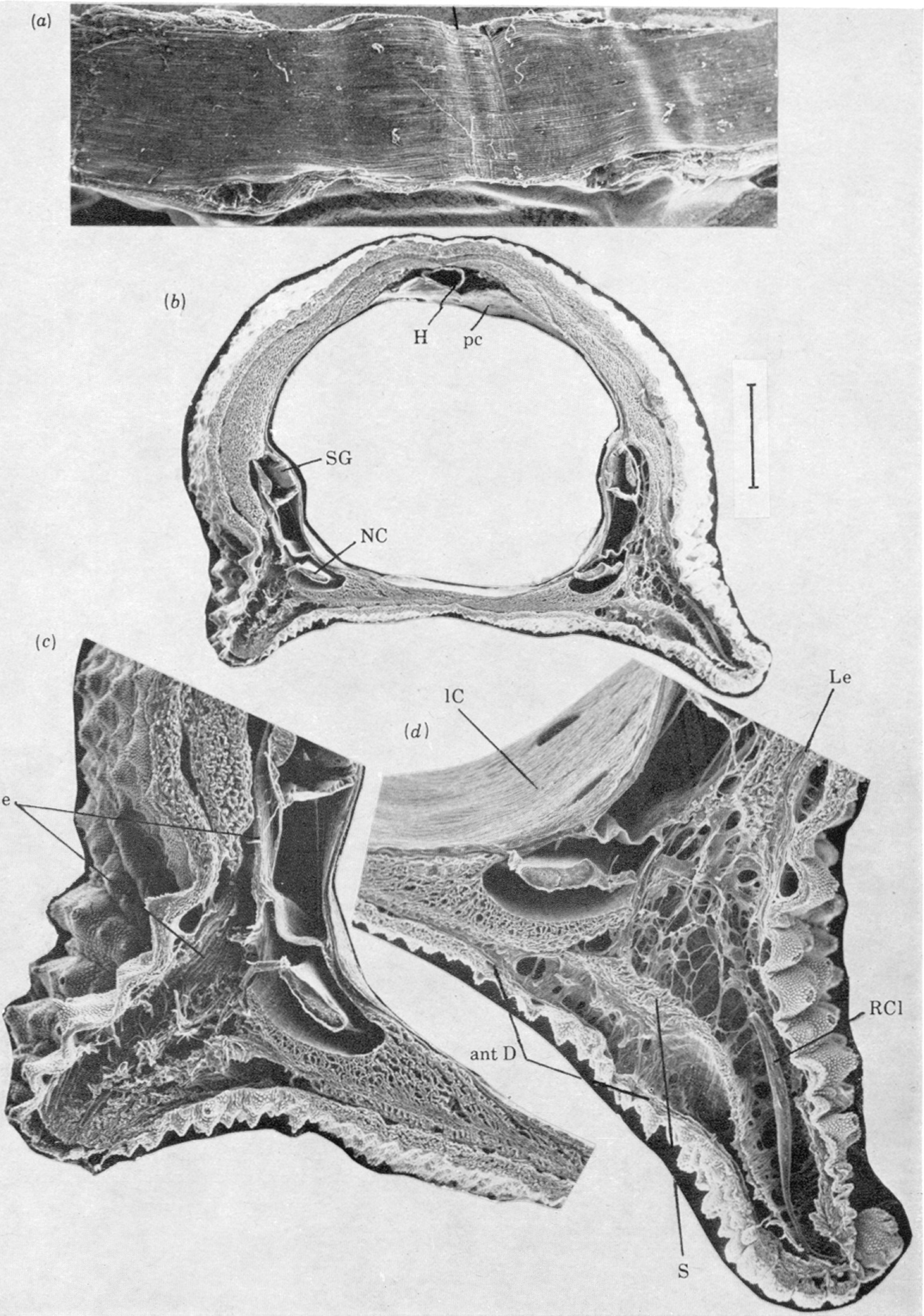

FIGURE 4. For description see p. 486. (Scale bar ≡ 1 mm.)

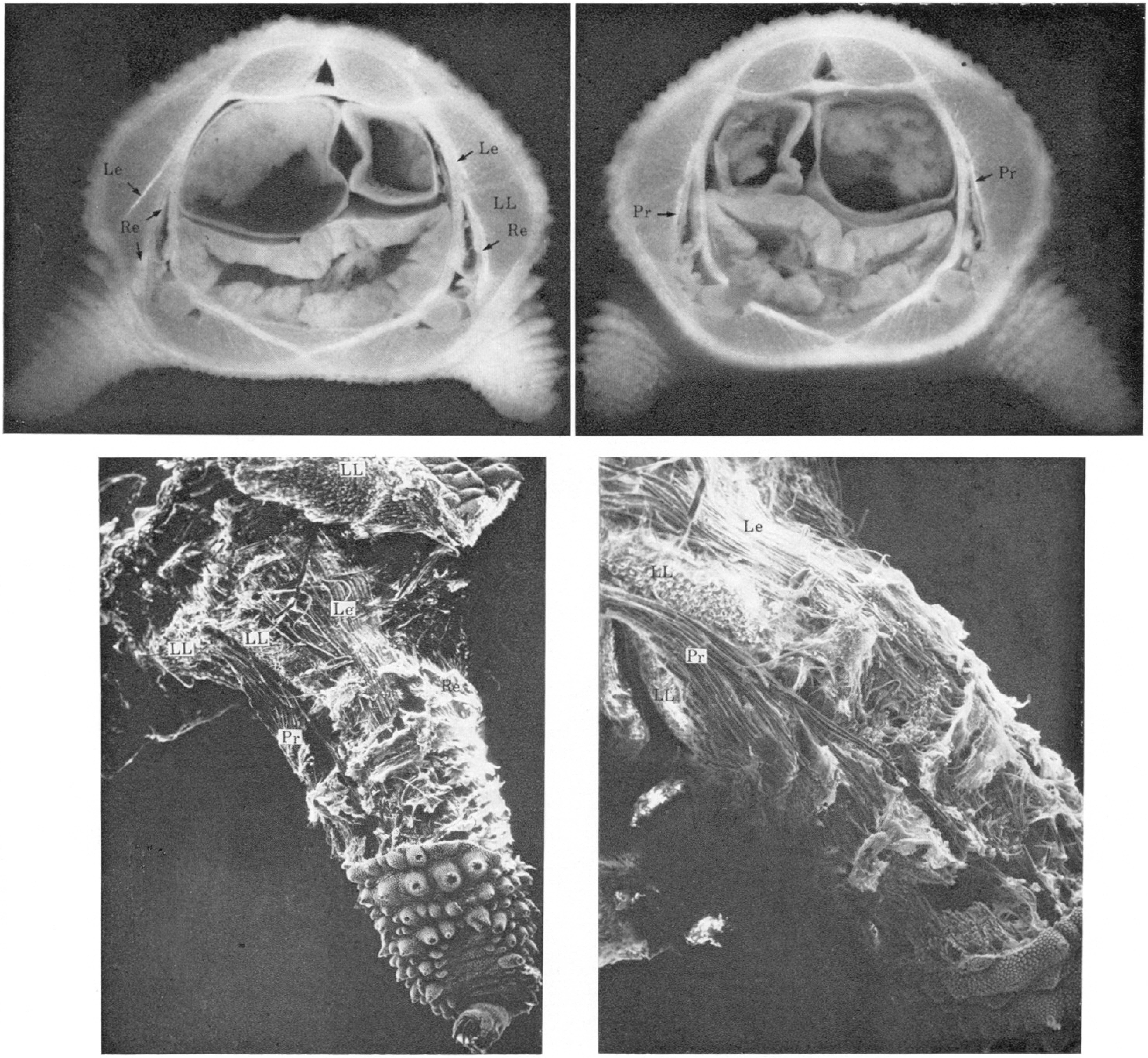

FIGURE 5. Locations and pathways of levator and promotor of the leg. *Top:* views of both sides of a slice of *Peripatus* to show the pathway of the levator of the leg through the lateral longitudinal (LL) muscle. *Left:* View from behind. At left the section passes through the posterior region of the levator (Le) which is fan-shaped and passes into the outer border of the leg just anterior to the cut surface. Also seen is the retractor of the leg (Re) at the region where it turns through 90° as it enters the posterior margin of the foot. On the right the cut is a little more posterior, so shows less of the levator and more of the retractor. (Magn. × 16.) *Right:* View from in front. The cut was anterior to the levator, which does not show. Instead the promotor of the leg (Pr) can be seen clearly on both sides. It is beginning to turn, after passing through a few LL fibres, on the right. (Magn. × 16.)

Below: Scanning electron micrograph pictures of a left leg and body wall. *Left:* View from the outside after cutting away a major, outer portion and a minor, inner portion of the lateral longitudinal muscle (LL). The levator (Le) is clearly seen and also the promotor (Pr) with a glimpse of the remotor (Re). (Magn. × 25.) *Right:* View from the front of the leg at higher magnification, showing the 90° turn of Pr. (Magn. × 46.)

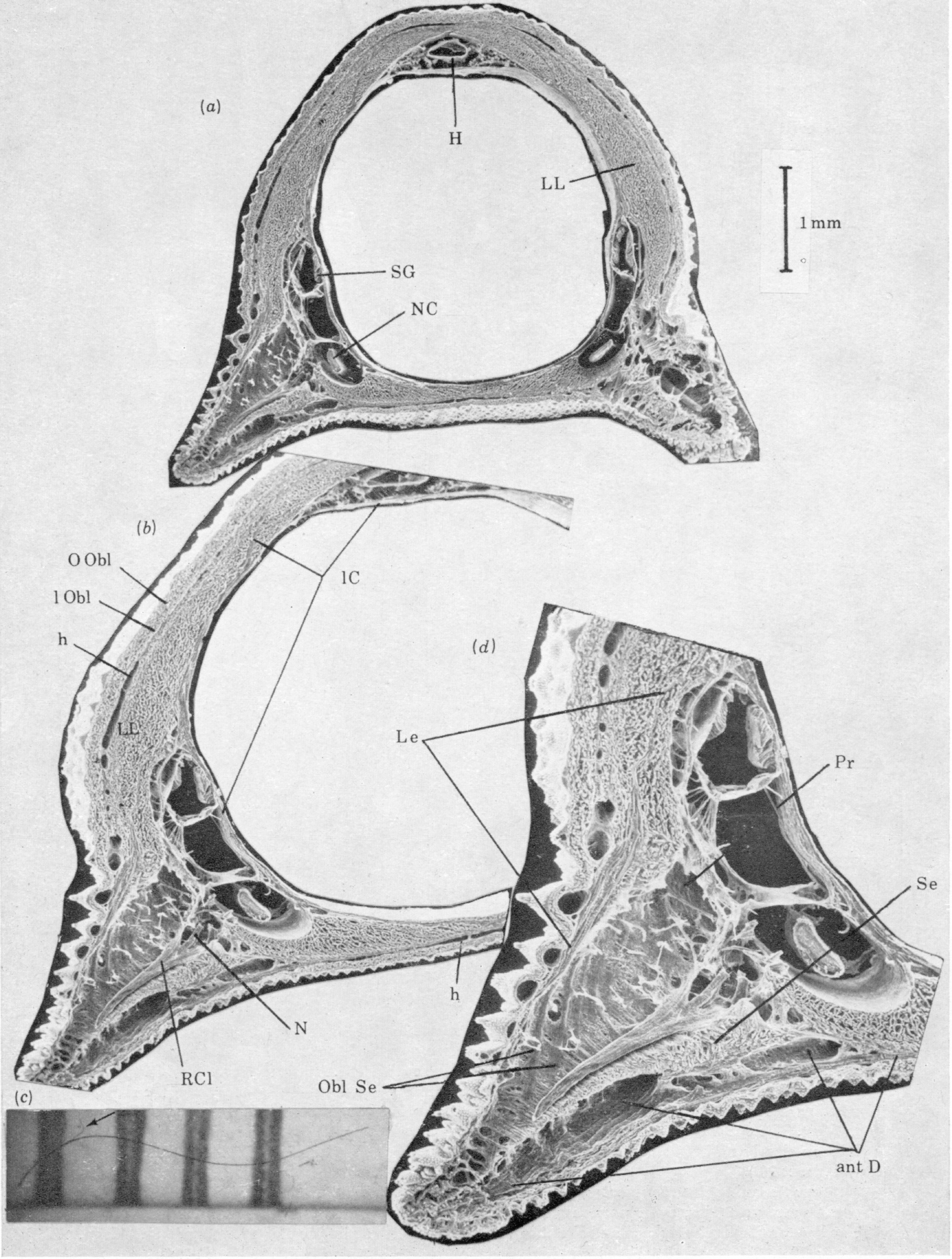

FIGURE 6. For description see p. 487.

Figure 7. For description see p. 487.

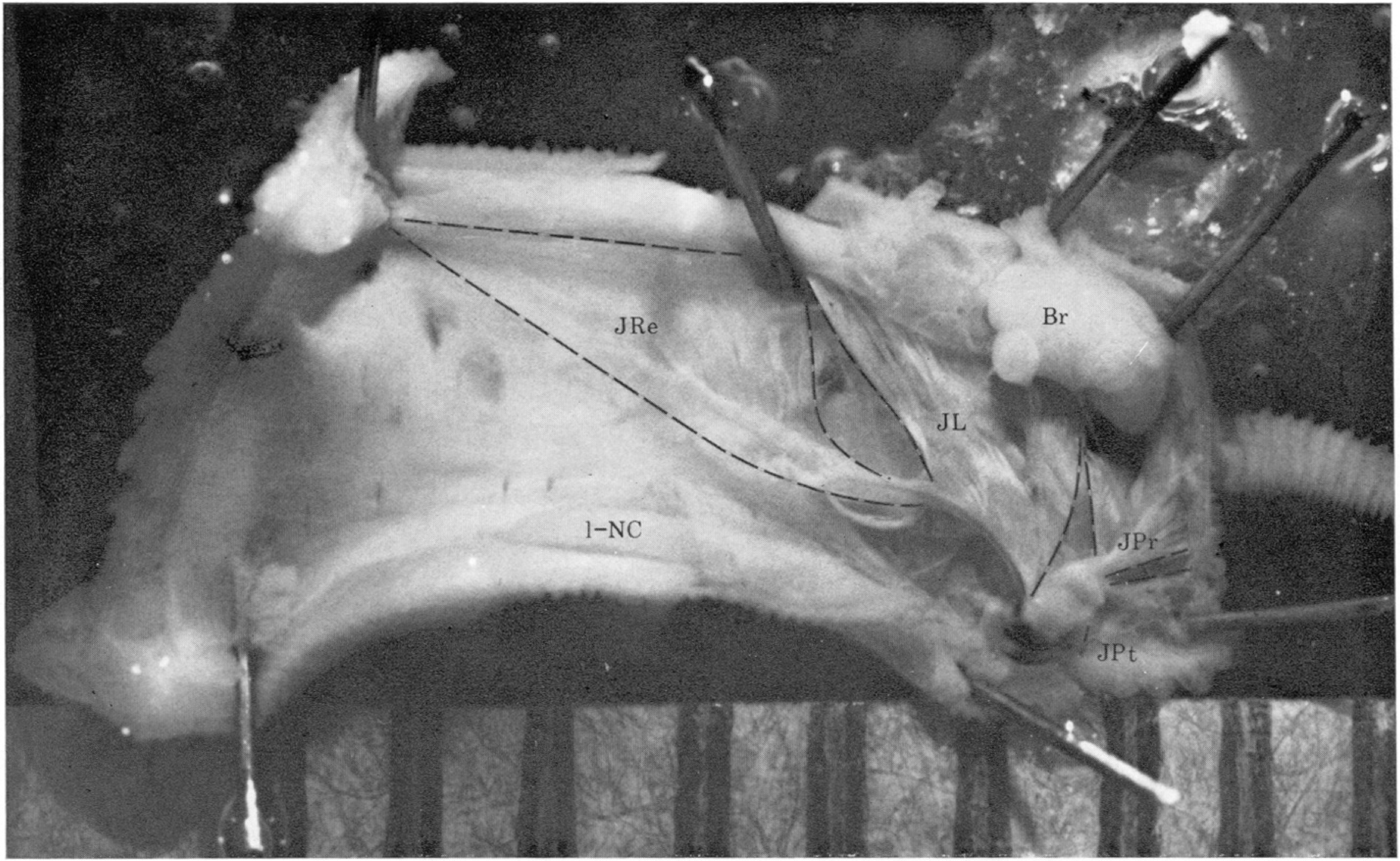

FIGURE 9. Jaw muscles of *P. dominicae*. Left head region following simple longitudinal bisection and removal of gut, salivary gland and left glue gun. The left half of the brain, with nerves going to the jaw muscles, is intact. There are four jaw muscles: a *protractor* (Pr); a *retractor* (Re) that provides the power stroke: its base is between the two pins, and which curls around the glue gun (removed) to attach to the ventro-lateral border of the jaw apodeme; a *lateral* (La); and a *protruder* (Pt). (Scale in millimetres.)

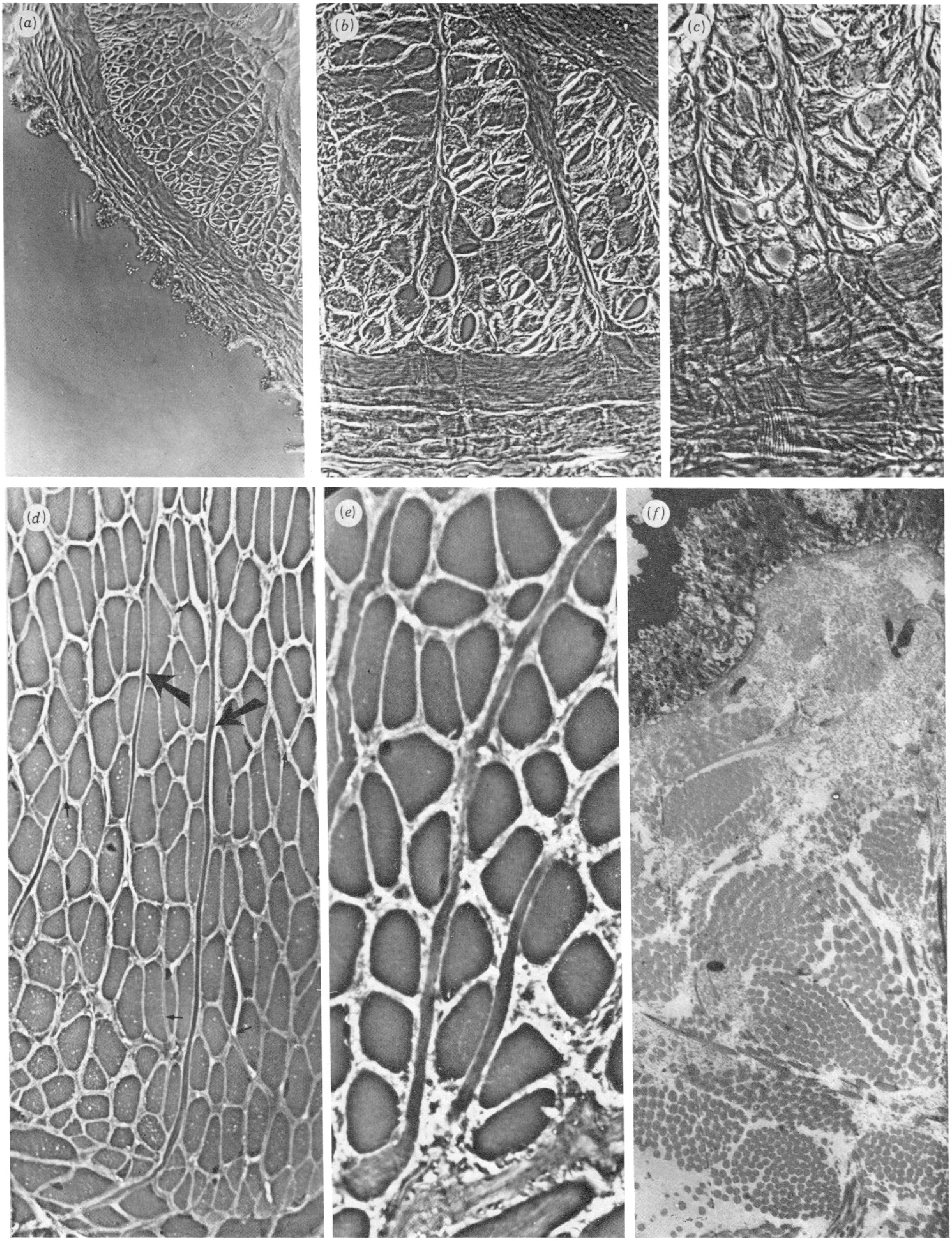

FIGURE 10. For description see p. 492.

FIGURE 11. For description see p. 492.

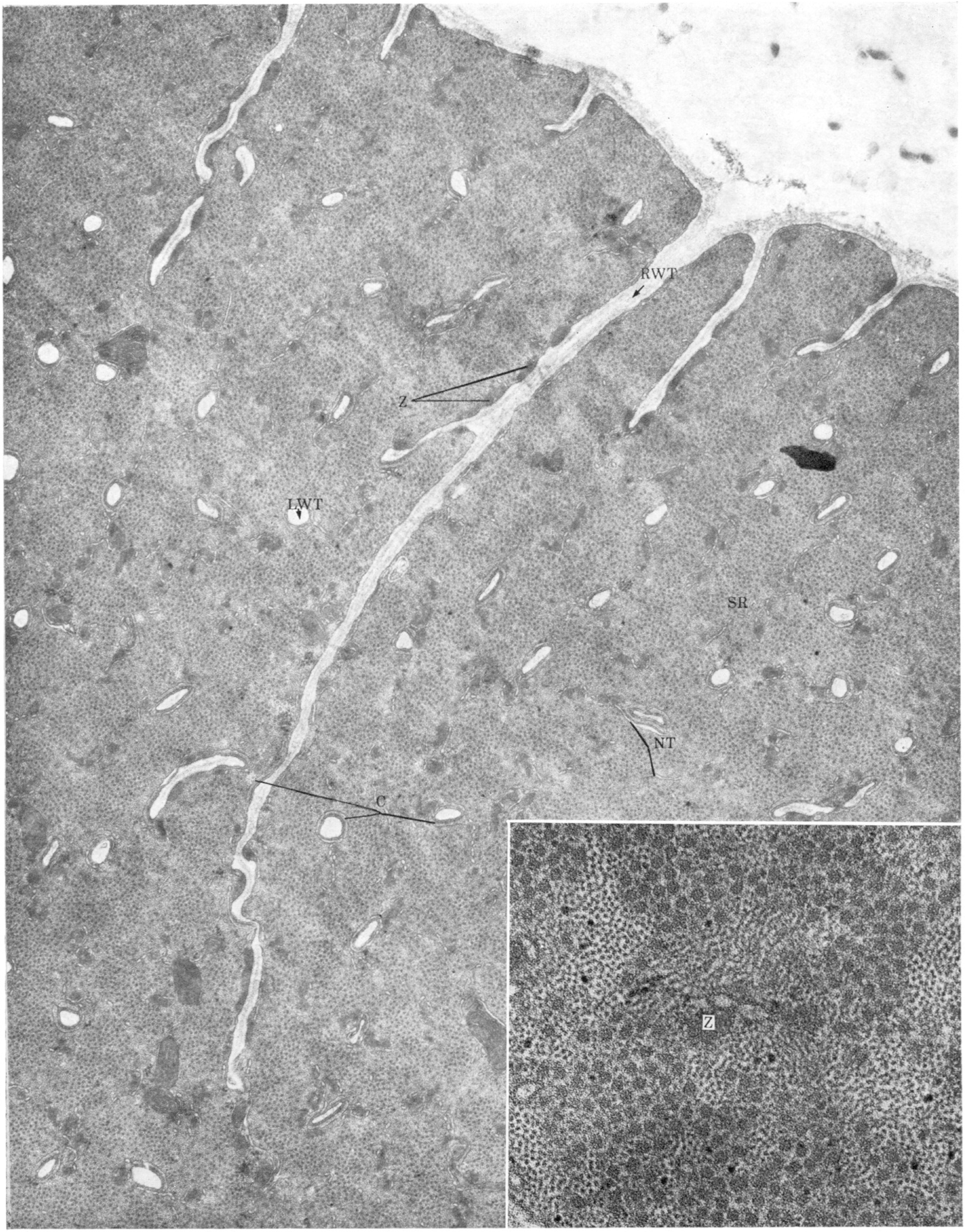

FIGURE 12. For description see p. 492.

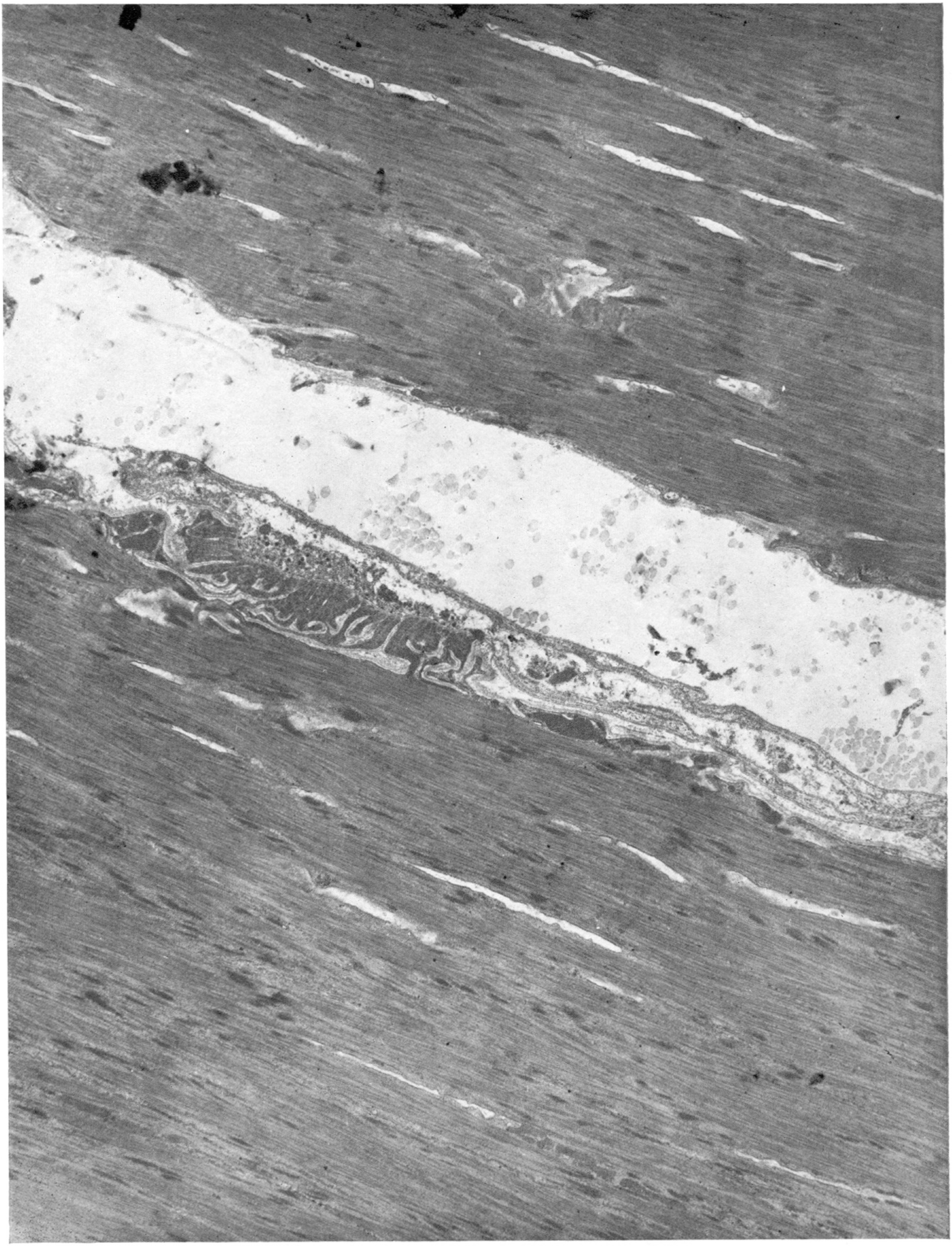

FIGURE 13. For description see p. 493.

FIGURE 14. For description see p. 493.

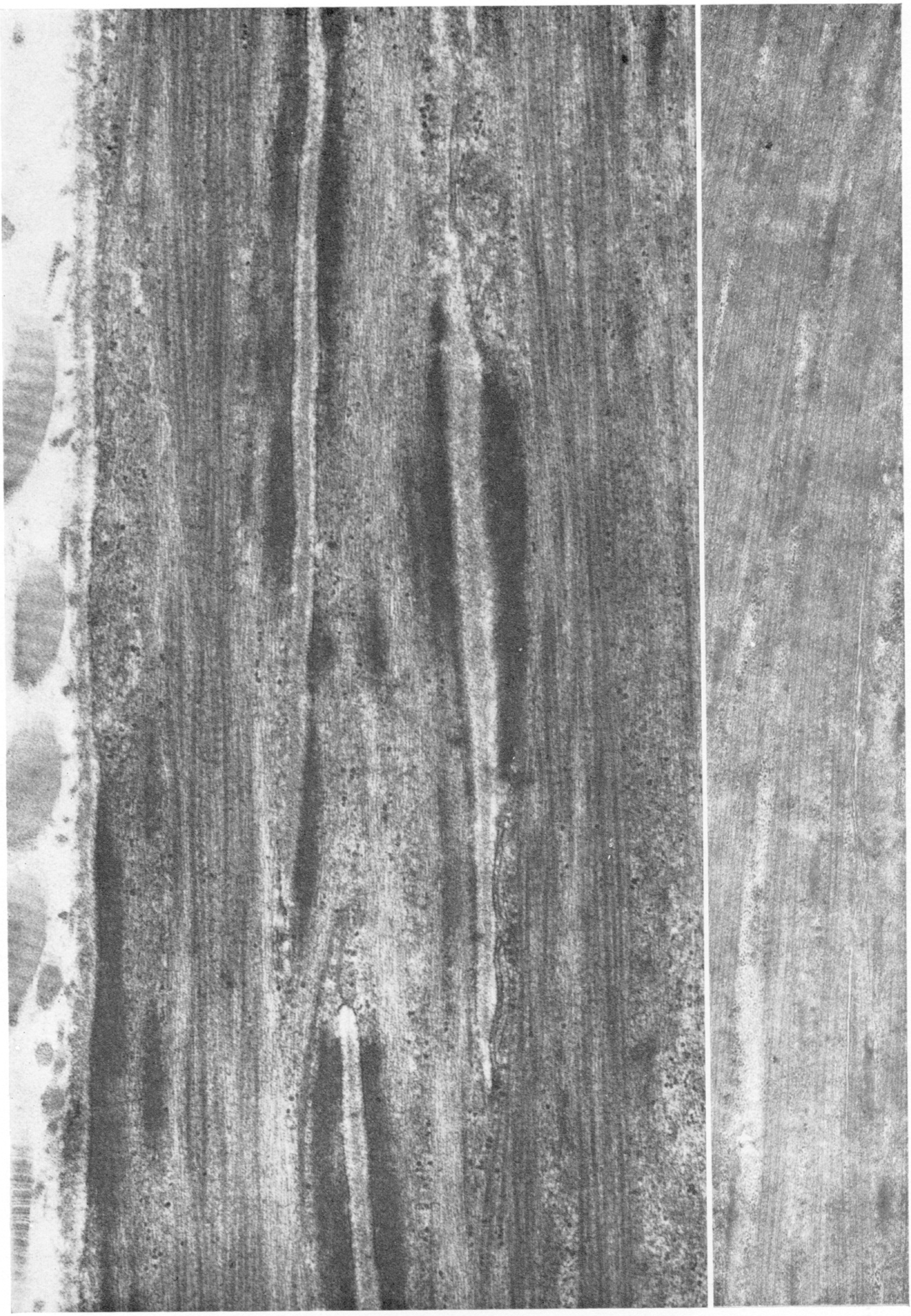

FIGURE 15. For description see p. 493.

FIGURE 16. For description see p. 493.

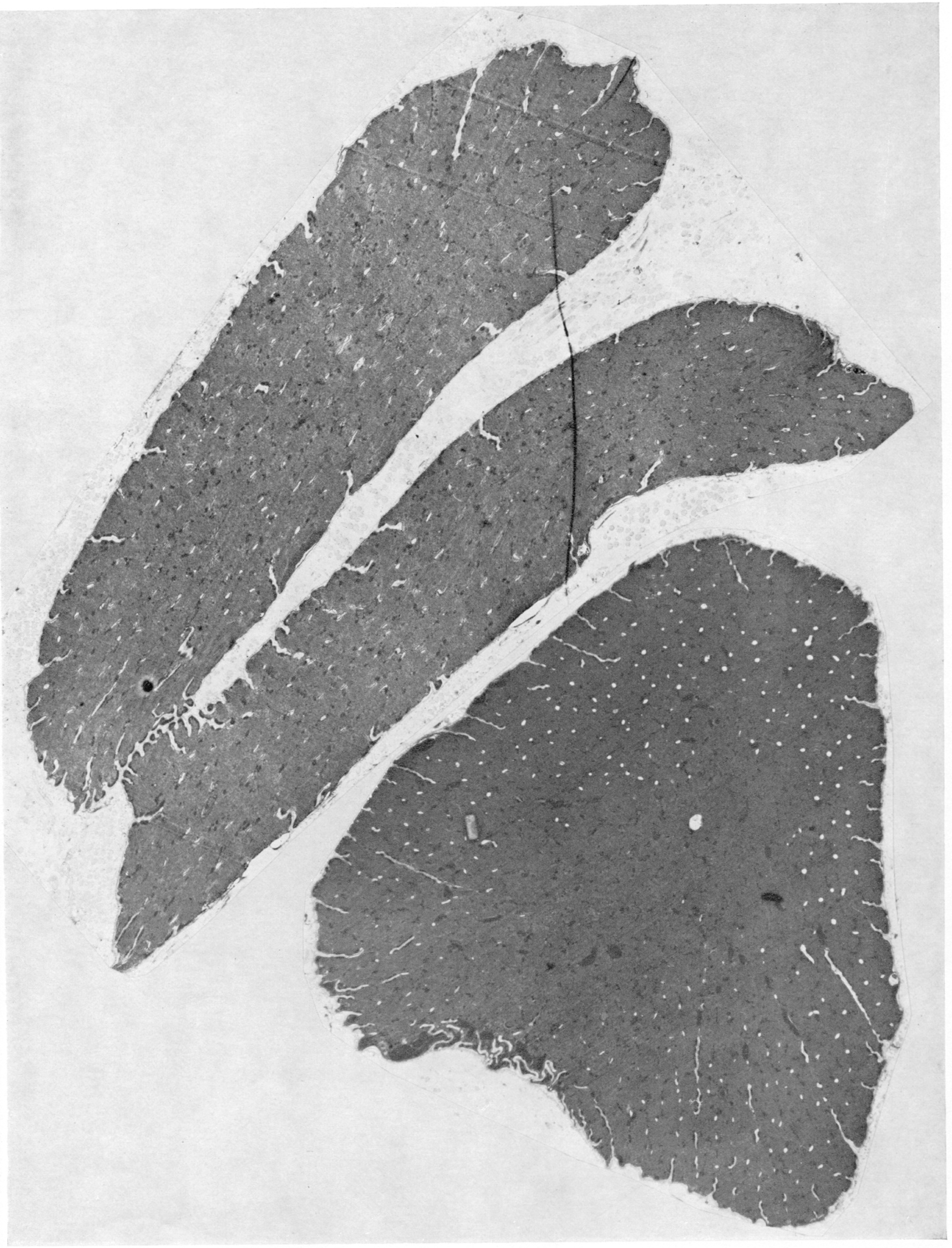

FIGURE 17. For description see p. 500.

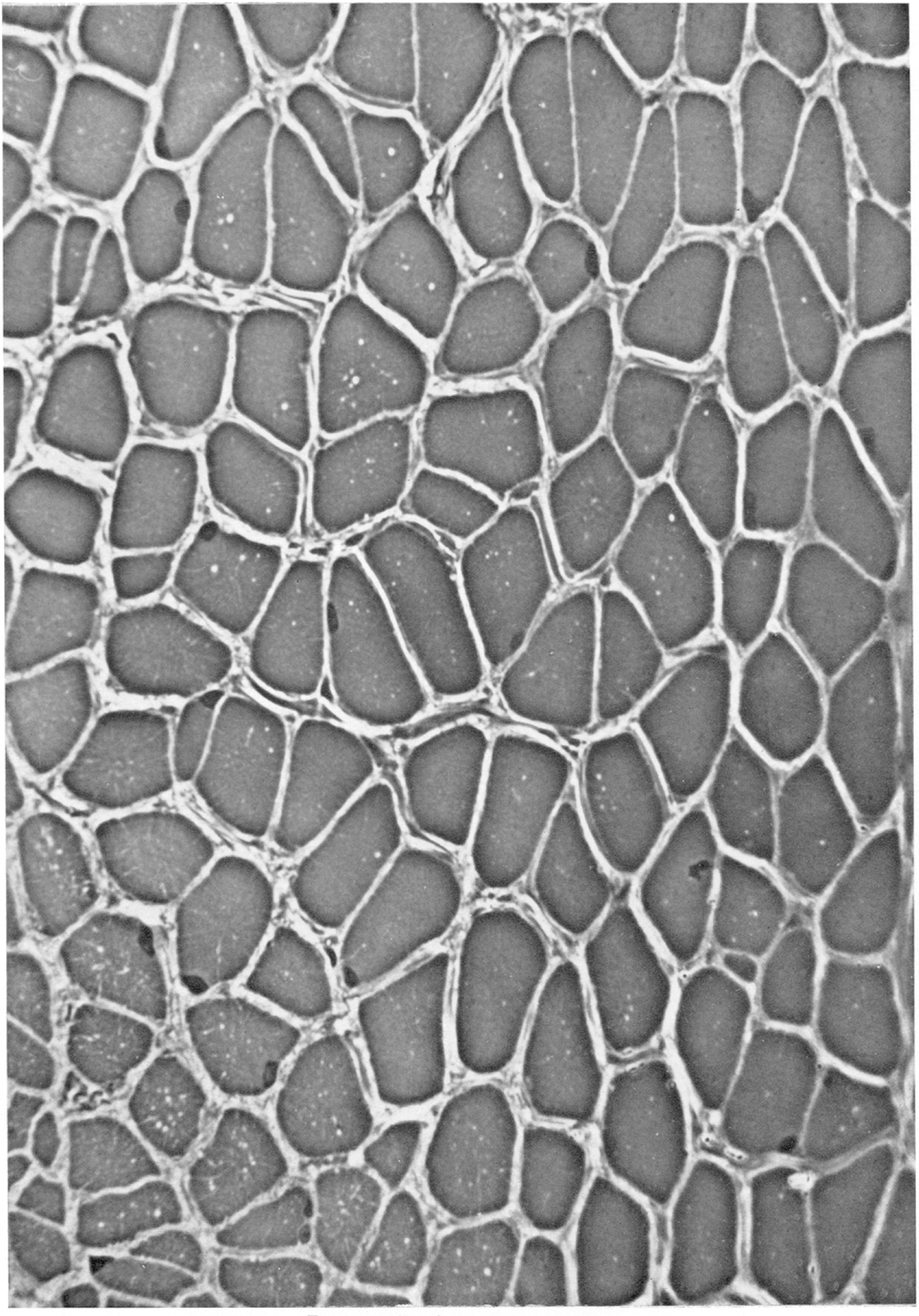

FIGURE 18. For description see p. 500.

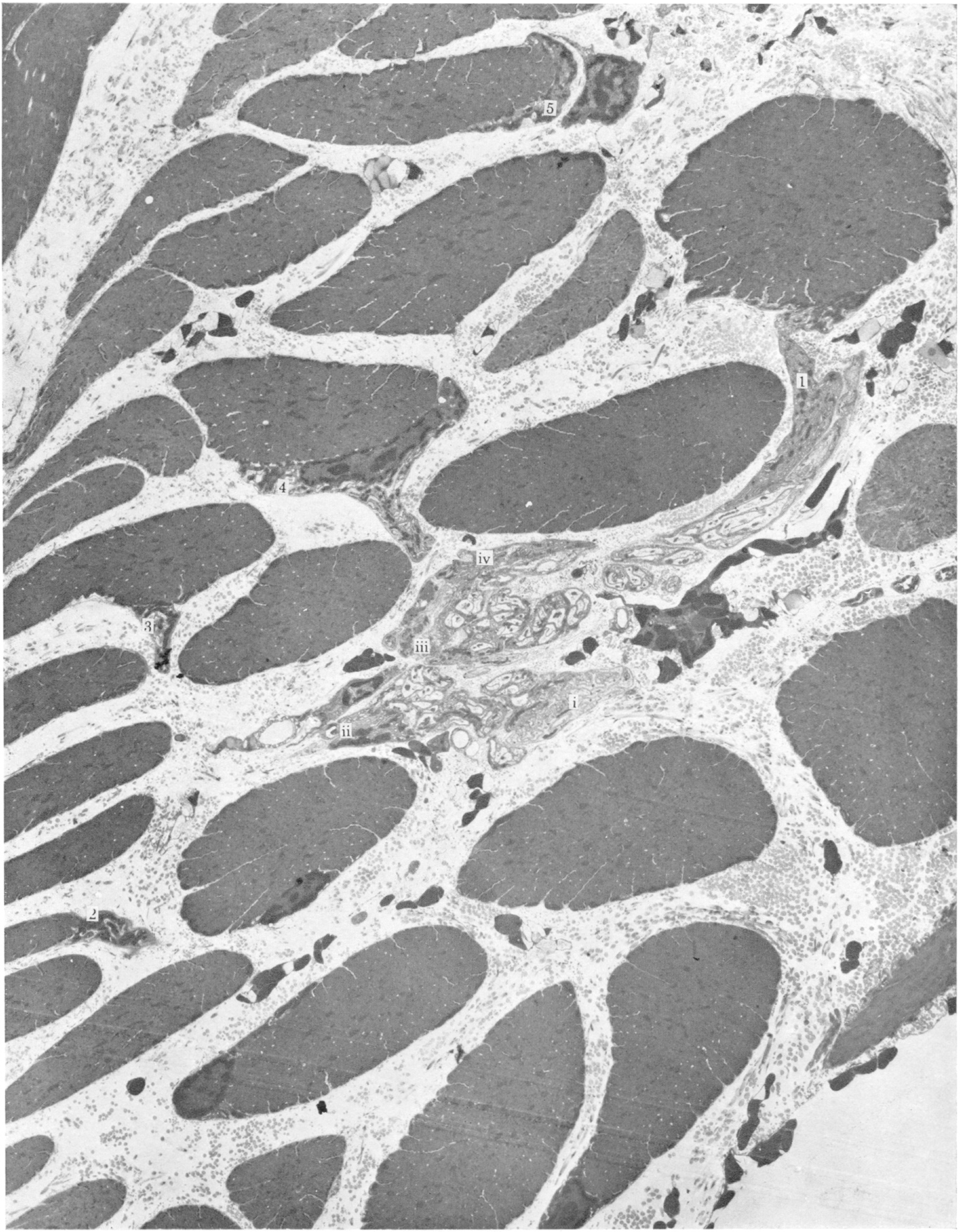

FIGURE 19. For description see p. 500.

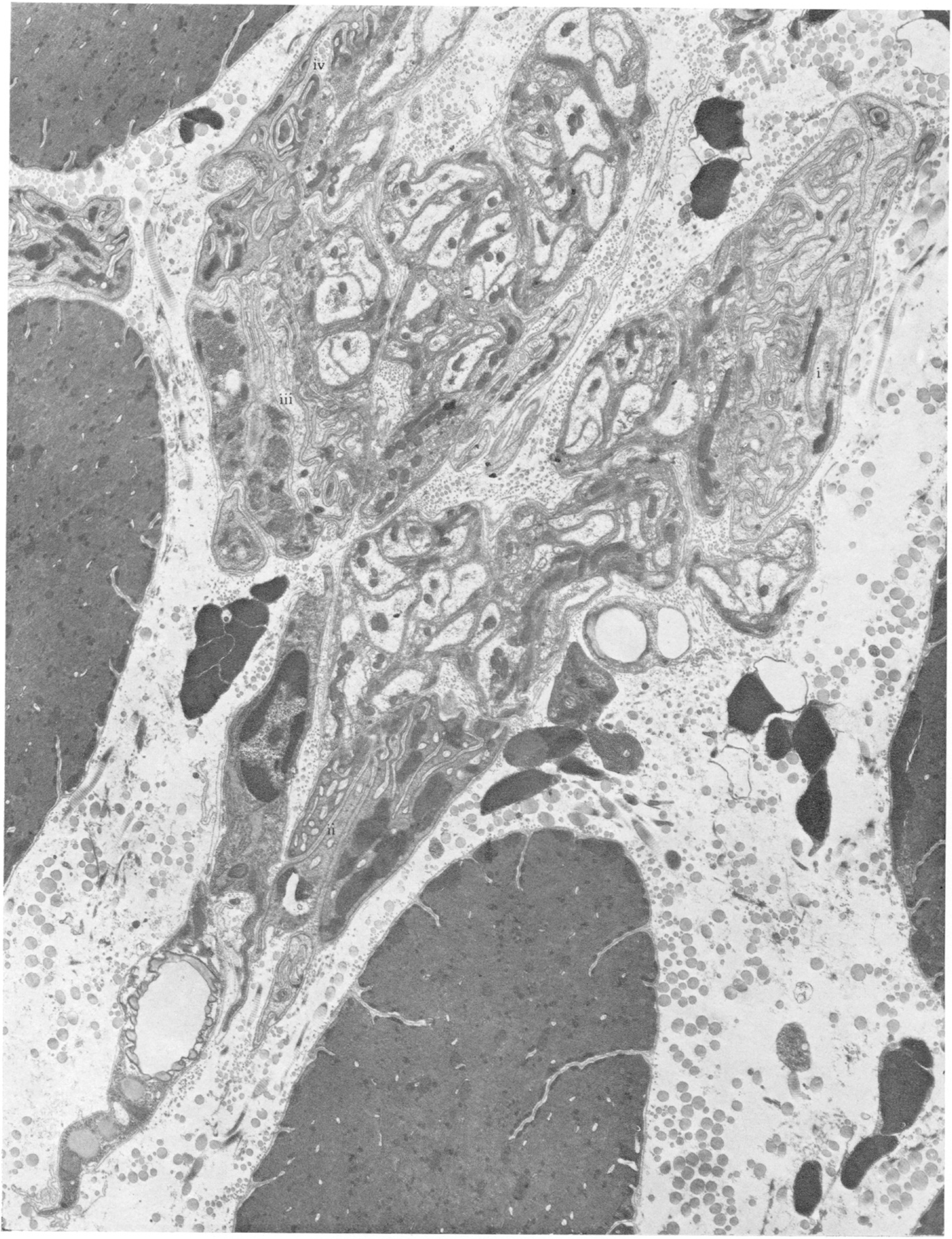

FIGURE 20. For description see p. 500.

FIGURE 21. For description see p. 501.

FIGURE 22. For description see p. 501.

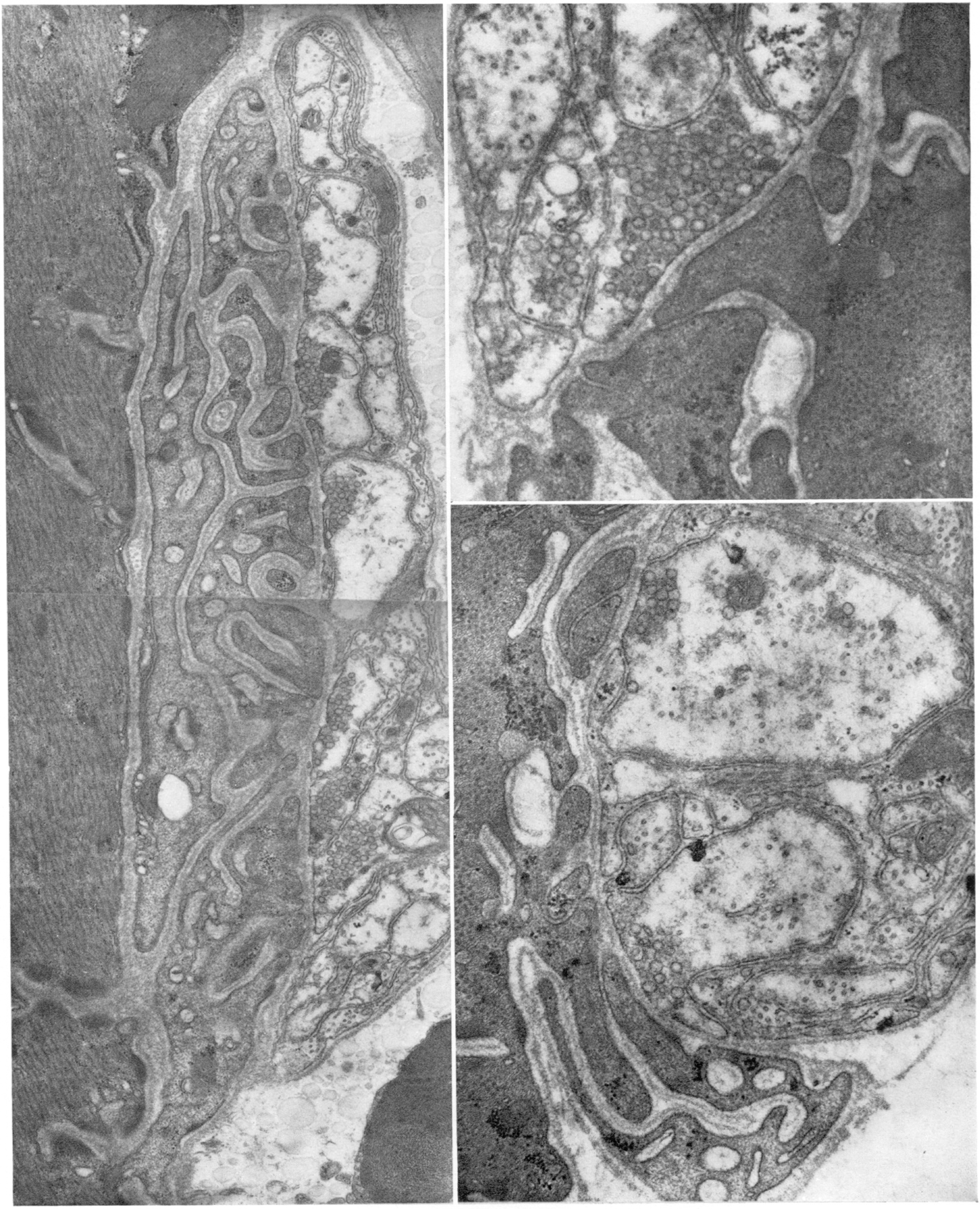

Figure 23. For description see p. 501.