A very singular muscle: the secondary muscle of chaetognaths

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SUMMARY

Chaetognaths show in their trunk and tail a type of muscle fibre that is at present unique in the animal kingdom. It was identified and first described by Grassi in 1883, who termed it the secondary muscle. It is less than 1 % of the tissue wall volume. This muscle forms four longitudinal bands, two mediodorsal and ventral and two lateral, suggesting an antagonistic functional role. It is related to myoepithelial cells, which may adjust the tonus of the hydroskeleton.

The secondary muscle shows a unique mode of cross-striation: two sarcomere types (s1 and s2) alternate in series along the longitudinal axis of the contractile apparatus. Successive Z bands are common to these two sarcomeres types. s1 sarcomeres have irregular A bands, about 1.7 μm in length; their loose architecture, poor lateral cohesion and F1:F2 = 1:9 or 1:10, probably reflects a slow mode of functioning. s2 sarcomeres are pleomorphic. They are composed of filaments assembled laterally in various ways and embedded in a loose matrix; they have no A bands, their length can vary from < 1 μm to > 10 μm without apparent change in myofibrillar density.

The functioning of s1 sarcomeres is in accordance with the sliding filament model, but that of the s2 functioning is not; the s2 sarcomeres look like stress-fibres. These two sarcomere types seem to have a relatively independent function.

It is suggested that the chaetognath secondary muscle shows a contractile architecture partly related to more general cytoskeletal structures and partly related to a true myofibrillar contractile apparatus. It is a highly dynamic contractile architecture which seems to be able to modulate strongly the muscular contraction. It profoundly differs from all other chaetognath muscles.

This muscle is peculiar to chaetognaths; it does not assist in relating them to phyla.

1. INTRODUCTION

The chaetognath body wall surrounds a large cavity which contains very few organs: only the intestine and the reproductive apparatus (Hertwig 1880; Grassi 1883; Ghirardelli, 1968).

The trunk of *Sagitta* is cylindrical (figure 1). It is constructed around an hydroskeleton whose main components are: (i) an aqueous phase in the general cavity, and (ii) a dense fibrous connective tissue (the 'basement membrane') which separates the multilayered epidermis and the nervous system from the underlying tissues (Duvert & Salat 1990). Surrounding the general cavity there are: (i) four important longitudinal muscle quadrants (more than 80 % of the trunk wall volume (Duvert 1989)), (ii) two mesenteries (mediodorsal and medioventral) and two epithelial lateral fields. The trunk musculature distinguishes the two main chaetognath groups, the Aphragmophora and the Phragmophora (Tokioka 1965*a*, *b*; Casanova 1986). In Aphragmophora the muscular tissue contains two kinds of cross-striated muscles: the primary and the secondary muscles, identified and localized for the first time by Grassi (1883). A third kind, the ventral transversal muscle, is only found in the Phragmophora (John 1933).

The structure of the primary muscle was first described by Hertwig (1880) and Grassi (1883), (reviewed in Duvert (1989)) and some aspects of the locomotory muscle physiology were given by Bone & Pulsford (1984), Duvert & Savineau (1986), Savineau & Duvert (1986), Bone *et al.* (1987). Very few studies have been done on the secondary muscle (*musculatura generale secondaria* of Grassi), some reports are in Duvert (1969, 1975, 1989) and Duvert & Salat (1979). As Grassi (1883) pointed out, this muscle is very peculiar, he said *singolarissima*; its mode of striation is unique at present in the animal kingdom. It is constituted of four bands of fibre bundles, two mediodorsal and medioventral at the level of the mesenteries, two ventrolateral near the lateral fields (figure 1).

This ultrastructural study of the secondary muscle characterizes some of its architectural variations and considers the aspects of its remarkable structure.

2. MATERIALS AND METHODS

Sagitta setosa Müller and *S. friderici* Müller were collected in the plankton from the Bassin d'Arcachon. In some cases, three sets of experiments were done. Animals were fixed: (i) at resting length (i.e. unstretched or uncontracted); (ii) during a contraction

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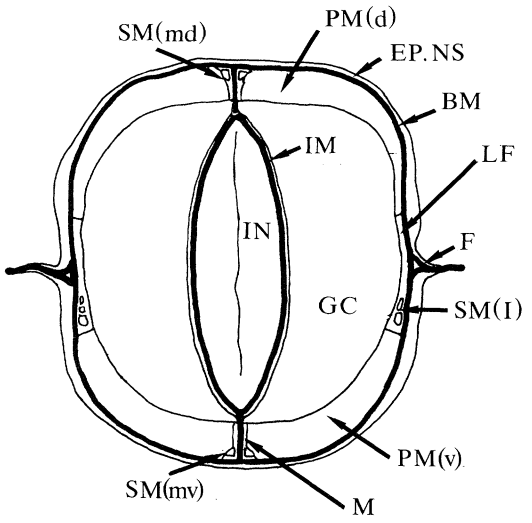


Figure 1. Diagram of a transverse section in the trunk of *Sagitta* showing the localization of the primary and of the secondary muscles. The asymmetry of the dorsal and of the thicker ventral bands is accentuated by the localization of the ventral secondary muscle bands.

induced by 10^{-5} M acetylcholine; (iii) stretched during a slight reversible stretch. The contractile state was recorded during fixation; the tension did not noticeably change because of the large variations mechanically induced.

The animals were fixed for 1 h in 2.5% glutaraldehyde, sucrose 0.6 M Na Cacodylate–HCl buffer 0.1 M, pH 7.6, $CaCl_2$ 30 mg%. They were dried on blotting paper and post-fixed in OsO_4 1%, Na-Cacodylate–HCl buffer 0, 1 M, pH 7.5, for several hours at 0–4 °C. Some animals were fixed in buffered OsO_4 alone.

For light microscopy, the animals were fixed in Carnoy (chloroform: 3V, ethanol 100:6V); 5 μm sections were stained with Levanol fast cyanide 5 N (Puchtler *et al.*, 1969). Semi-thin sections were stained with Unna's stain. In some experiments, after double fixation, animals were stained *en bloc* with haematoxylin (Gabe 1968).

Cells were isolated as in Hernandez-Nicaise *et al.* (1984), using a mixture of Ca^{2+}-free artificial sea water containing 0.1% trypsin and 0.1% hyaluronidase. The cell suspension was fixed and stained with Groat haematoxylin (Gabe 1968): drops of this cell suspension were observed directly in the light microscope.

Mechanical recording, solutions and drugs were performed as in Duvert & Savineau (1986). Physiological and cytochemical studies of Ca^{2+} were as in Savineau & Duvert (1986). Ca^{2+} ATPase activity was localized as in Akisaka & Gay (1985). In morphometric studies, the various dimensions are expressed as the mean of n measures with standard deviation.

Other chaetognaths were provided by Dr J.-P. Casanova (Université d'Aix-Marseille I, Biologie animale – Plancton); the animals were collected and then fixed in formalin sea water for several months, for anatomical studies. They were washed in distilled water, post-fixed in OsO_4 in distilled water and studied on semi-thin sections stained with Unna's stain.

3. RESULTS

In Aphragmophora as well as in the archaïc Phragmorphora studied, the four secondary muscle bands are present throughout the body. No secondary muscle was seen in the head. The two ventro-lateral bands are more or less developed according to the genus studied; they seem to be larger in genera or species where lateral fields are well developed.

(*a*) *Identification and localization*

In *Sagitta* the four secondary muscle bands constitute less than 1% of the tissue wall volume. All the quantitative values given are purely indicative, since animals grow throughout life; they were measured in large animals at sexual maturity.

Figure 2 shows part of a transverse section of a dorsal and a ventral primary muscle quadrant, separated by a lateral field; the ventral quadrant is thicker than the dorsal one. A secondary muscle band is seen between the primary muscle and the lateral field, on the ventral side. There is also another band at the extremity of the other ventral quadrant. Each band is about 10–13 μm to 5–10 μm thick (against the primary muscle) and 2–5 μm (against the lateral field).

Two other secondary muscle bands exist on each side of the origins of the ventral and dorsal mesentery

Figures 2–7.

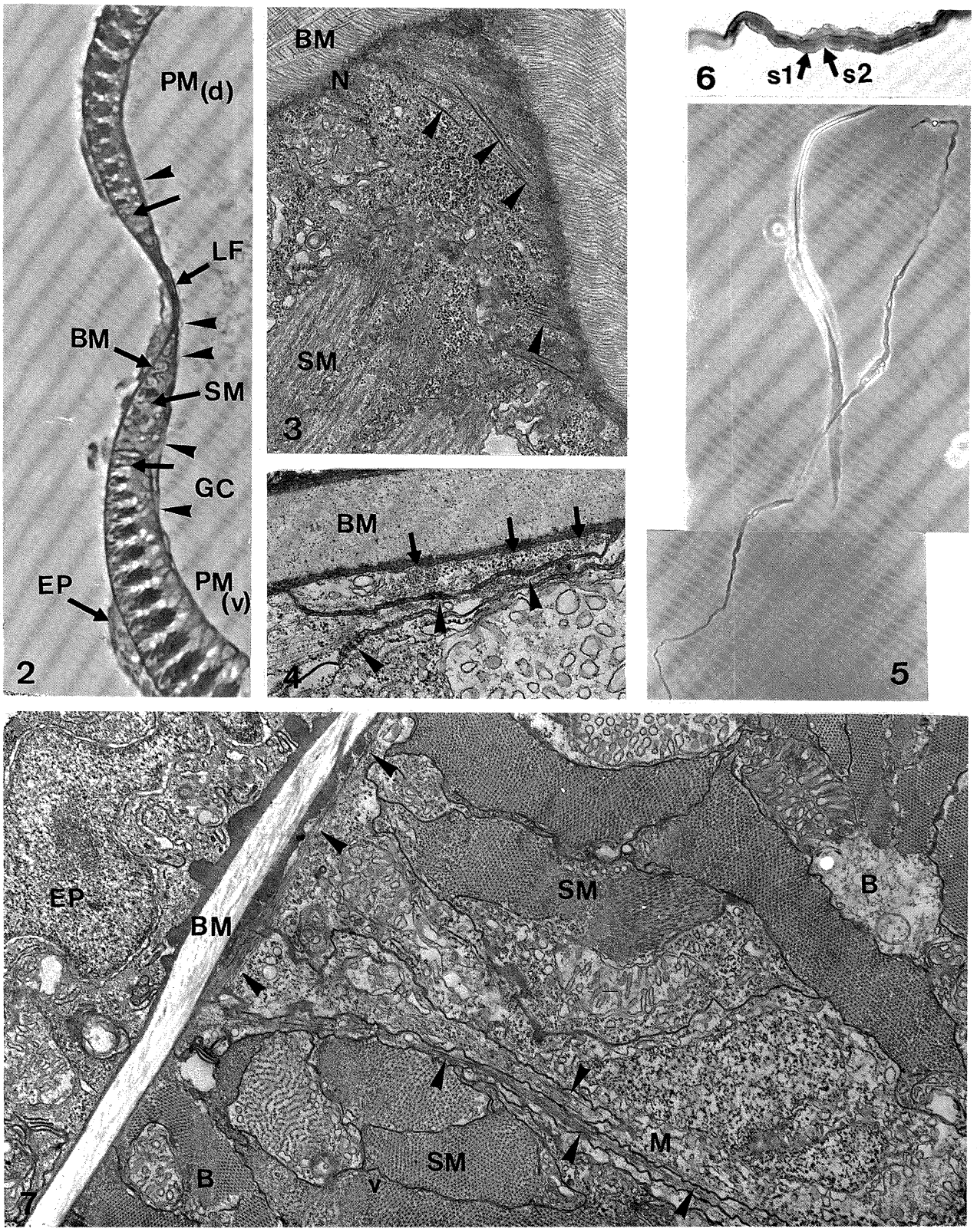
Figure 2. Semi-thin transverse section at the lateral field level. Note the thicker ventral primary muscle with the typical A fibre groups which originate from the myogenesis zones (arrows). The secondary muscle is clearly delimited between the lateral field and the ventral primary muscle. It is covered by the thin endothelium lining the general cavity (arrowheads). Haemotoxylin en bloc; magn. × 680.

Figure 3. Tangential section of myofibrillar apparatus in myoepithelial cells at the level of a secondary muscle ventro-lateral band. Arrowheads point to thick filaments; magn. × 17 600.

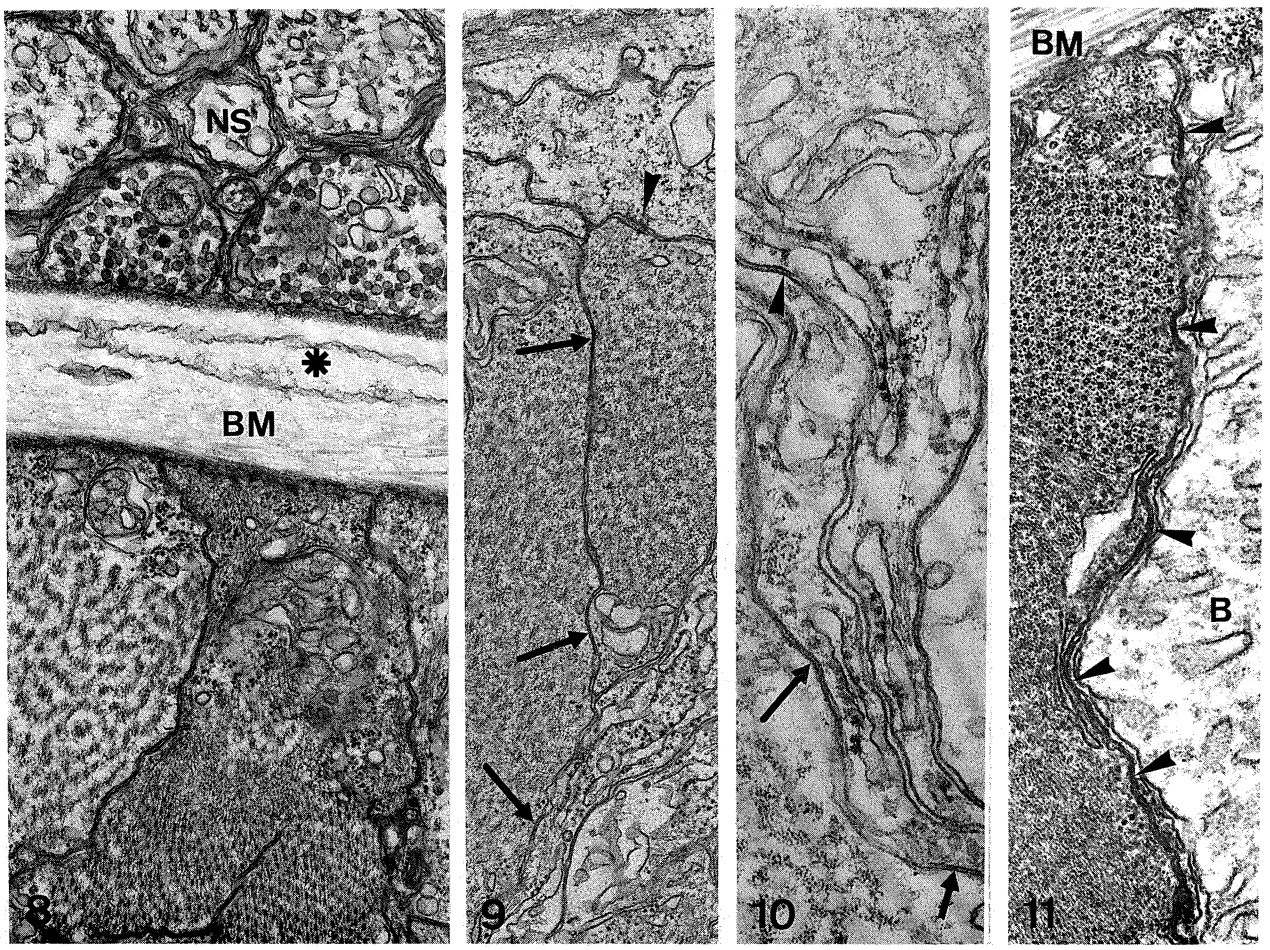
Figure 4. Transverse section at the level of the myofibrillar apparatus of myoepithelial cells showing clusters of thin and thick myofilaments (arrows). Arrowheads show columnar junctions with their associated cytoskeleton; magn. × 18 000.

Figures 5 and 6. Isolated large primary and secondary muscle fibres. The latter is very thin and long; magn. × 250; inset: detail of the striation in the secondary muscle cell showing s1 and s2 sarcomeres; magn. × 850.

Figure 7. Transverse section at the mesentary level. The secondary muscle forms two bands on each side of the mesentery; the contractile part of the myoepithelial cells is indicated (arrowheads), it lines the mesentery and extends under the basement membrane. These bands are also in contact with B-fibre groups; magn. × 11 300.



Figures 2–7. For description see opposite.



Figures 8–11.

Figure 8. Nerve ending-like structures at the level of a ventral secondary muscle band. Note the 'basement membrane' with a connective cell (*) between the nerve and the muscle; magn. × 22 000.

Figure 9. Cell junctions between secondary muscle fibres: gap junctions (arrows) and columnar junction (arrowhead). Under the 'basement membrane' can be seen the contractile part of the myoepithelial cells (top), which shows a coated vesicle; magn. × 24 800.

Figure 10. Gap junctions between secondary muscle and myoepithelial cells (arrows) and between myoepithelial cells (arrowhead); magn. × 43 400.

Figure 11. At the mesentery level, numerous gap junctions between a secondary muscle fibre and a B fibre (arrowheads); tannic acid treatment, magn. × 39 000.

(figures 1, 7, 13 & 34). They have a roughly triangular shape, in transverse section: about 8–13 μm wide against the epidermis and 4–10 μm along the mesentery; the mediodorsal and medioventral bands lie against primary muscle fibres. Each band contains *ca.* 2–5 fibres on each side of the mesentery. The secondary muscle ventral bands are not in contact with the thin endothelium lining the general cavity (figure 2), they are made of various muscle fibre bundles; at this level, the muscle is covered by various cell expansions (figures 39–41). Obviously, the mediodorsal bands are never in contact with the general cavity.

The number of muscle cells in each band is a function of the length of the animal, i.e. its age, but this variation is much more important for the lateral bands than for the medial ones. Myogenesis zones comparable to those described in the primary muscle (Duvert 1989) were not found; they presumably exist at least in the ventral bands, since the fibre bundles are more numerous in large animals.

(*b*) *Cell shape and architecture of the muscle*

When the locomotor muscle is dissociated, the secondary muscle fibres can be distinguished from the primary fibres; they are very long, probably mononucleated, and thin ovoid or ribbon-shaped in section. Their small diameter is about 1 μm, the larger about 3–5 μm. Figure 5 shows a large primary muscle fibre and a secondary fibre. At higher magnification (figure 6) the peculiar mode of striation of the myofibrillar apparatus can be seen, which characterizes the secondary muscle fibre, in contrast to the primary muscle fibres, myotendinous junctions were not seen against the 'basement membrane'.

Again, in contrast to the primary muscle, the

secondary muscle is always associated with myoepithelial cells. At the lateral field level (figures 9, 30, 33 & 35), the myoepithelial cells separate, completely or partially, bundles of 2–4 or more muscle fibres (figures 39 and 41). The apex of these myoepithelial cells (frequently a secretory pole) is directed towards the general cavity. Each muscle cell bundle contains from 2–10 cells, more often 3–4. The myofibrillar part of myoepithelial cells lie against the 'basement membrane', i.e. between this connective tissue and the lateral field epithelial cells or the secondary muscle fibres. In the mesentery, the situation is simple. The mesentery is a composite structure made of a bundle of cells that may or may not be crossed by a 'basement membrane' sheet, directed towards the intestine.

Myoepithelial cells send cytoplasmic processes between the 'basement membrane' and the secondary muscle (figure 7), these processes contain an abundant cytoskeleton. The contractile apparatus of the myoepithelial cells contains two sets of filaments that are not regularly arranged; their longitudinal axis is dorsoventral, i.e. perpendicular to the main axis of the secondary muscle (figures 3, 4 & 7). In this cytoskeleton there are many thin filaments with some thick 20 ± 3 nm ($n = 159$), and long (at least 1 μm) myofilaments (figure 3); they are not regularly assembled (figure 4). So the contractile part of the myoepithelial cells is topographically related to the connective tissue and mesenteries and not to the secondary muscle. A basal lamina covers the myofibrillar part of the myoepithelial cells, it is continuous with the primary muscle basal lamina. At the lateral fields, this basal lamina partly covers part of the secondary muscle fibre membranes (figure 8); when present, this sarcolemma is very reduced at the mesentery level.

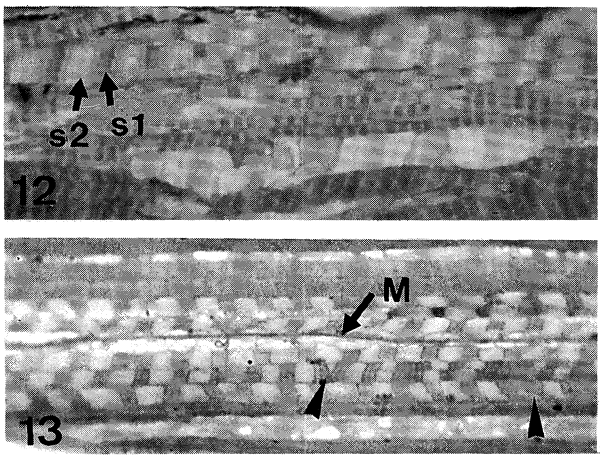
As for the primary muscle, nerves do not cross the 'basement membrane' at the secondary muscle level; nerve-ending like structures are to be seen scattered under the epidermis or at the lateral nerve trunk levels (figure 8). No membrane specializations are noted in the muscle fibres or in the myoepithelial cells in front of the nerve-ending like structures.

As in the primary muscle, this muscle has an epithelial architecture. Its cells are connected by junctions: columnar junctions and more particularly, gap junctions (figure 9). Gap junctions also connect secondary muscle cells to myoepithelial cells (figure 10) and to the B fibre cells group near the mesentery (figure 11).

(*c*) *Muscle fibre ultrastructure*

The contractile apparatus fill practically all the sarcoplasm; plasma membrane invaginations divide it partially or completely (figures 18 and 35), except in small diameter cells where a unique myobril is found with very few or no invaginations (Figure 7).

Because of the special mode of striation of the myofibrillar apparatus, two sarcomere types can be distinguished: s1 and s2 which alternate in series (figures 12 and 13). Sarcomeres s1 and s2 are delimited by Z-bands; only the s1 sarcomeres show A bands, s2 sarcomeres show light bands of variable length. Unlike



Figures 12 and 13.

Figure 12. Semi-thin longitudinal section in the secondary muscle at resting length-slightly stretched, the primary muscle is at resting-length. The two sarcomere types, s1 and s2, are clearly individualized, they are rarely in lateral register; magn. × 900.

Figure 13. Semi-thin longitudinal section in the secondary muscle where the sarcomeres s1 are contracted; the primary muscle is at resting length. Note the C_Z and C_M bands in s1; the s1 edges are more or less oblique with respect to the longitudinal axis of the myofibril; some s2 sarcomeres are not visible or very reduced (arrowheads); magn. × 900.

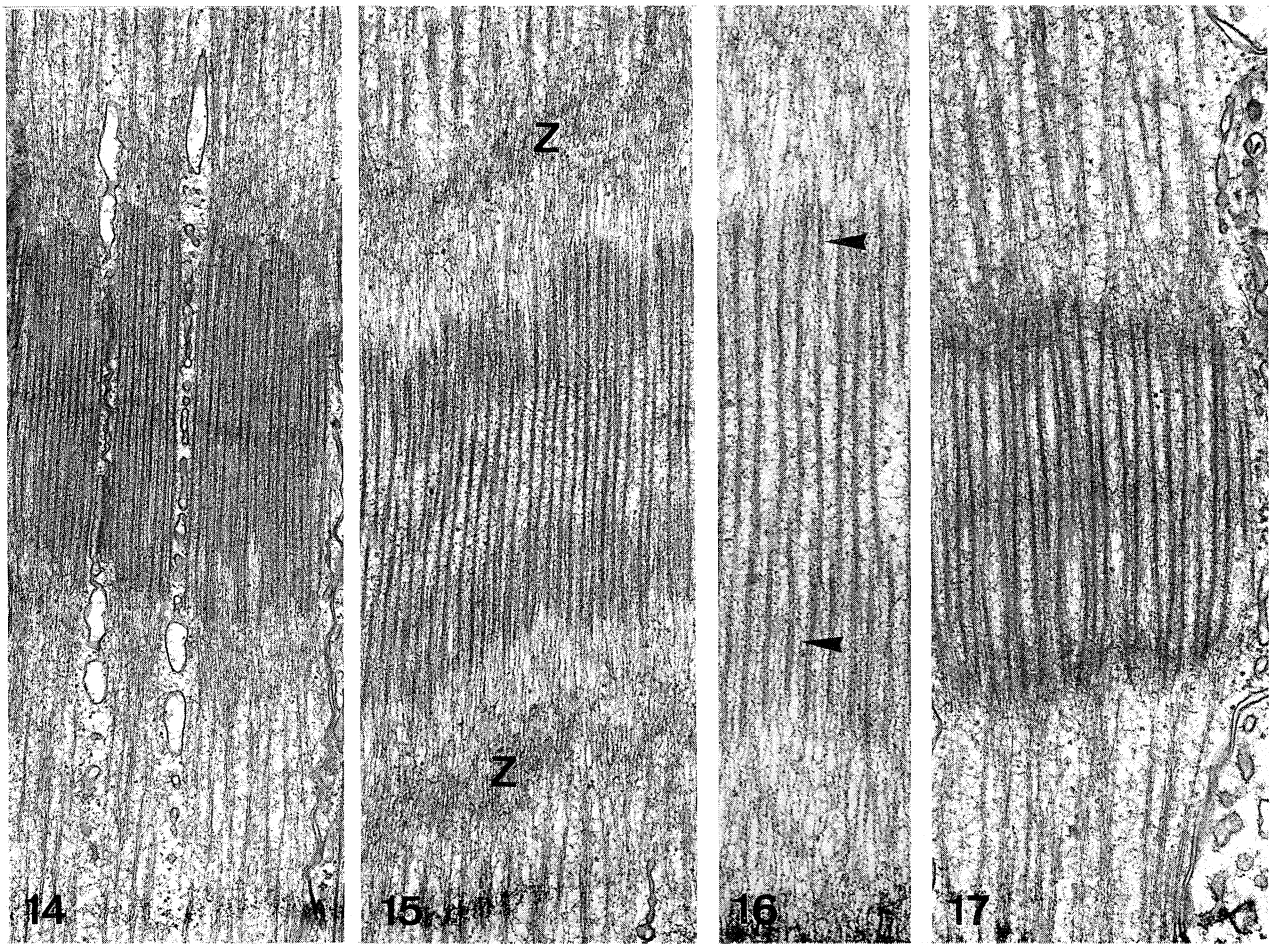
primary muscle cells, the secondary muscle fibres have no clearly individualized myofibrils. In these cells, the contractile apparatus forms a continuum of irregularly shaped, longitudinally arranged myofibril-like columns. In adjoining columns or cells s1 are rarely in register and if so, only in very small areas. Along the same myofibril, s1 are not always regularly spaced, they are never in contact but separated by s2 sarcomeres. On one occasion, a longitudinal section showed, in the same myofibril, part of s1 and s2 sarcomere separated from a s1 sarcomere, by the same continuous Z line (figure 32).

(i) *s1 Sarcomeres*

In some experiments, measurements were done on muscles where the physiological state of the locomotory muscle was previously determined from mechanical recordings (figure 42).

Longitudinal section

Resting length. In light microscope studies, sarcomeres are not easy to delimit since the Z band is not always seen. They are about 2.2 μm long. A bands are 1.7 ± 0.26 mm ($n = 223$) long, their edges are not always straight (figures 14, 22, 23, 26, 32 & 33); primary filaments seem to be continuous in the Z band via very thin C-filaments (figure 26). Z bands are: 0.24 ± 0.09 μm (n 322) wide, they are not clearly delimited with straight edges (figures 14 and 15). The light band between the edge of the A band and the Z-line, is about 0.25 μm. An H band could be seen in the centre of the A band, but an M-band is never observed. In many sarcomeres the thick filaments are not the same length (figures 14 and 22), and sometimes the A



Figures 14–17. s1 sarcomeres: longitudinal sections.

Figure 14. Resting length; in some A bands (right) all thick filaments are seen not to have the same length; magn. × 21 300.

Figure 15. Stretched sarcomere, the Z band is more obvious than at resting length; × magn. 24 500.

Figure 16. Detail of a stretched sarcomere; no M band is seen, lateral bridges between thick and thin myofilaments are observed (arrowheads); magn. × 39 600.

Figure 17. Contracted sarcomere, the A band shows a loose lateral cohesion between thick filaments; magn. × 31 000.

band length changes (Figures 17 and 33). Changes between consecutive A bands can also be seen (figure 24) they may be attributed to a more or less oblique section plane.

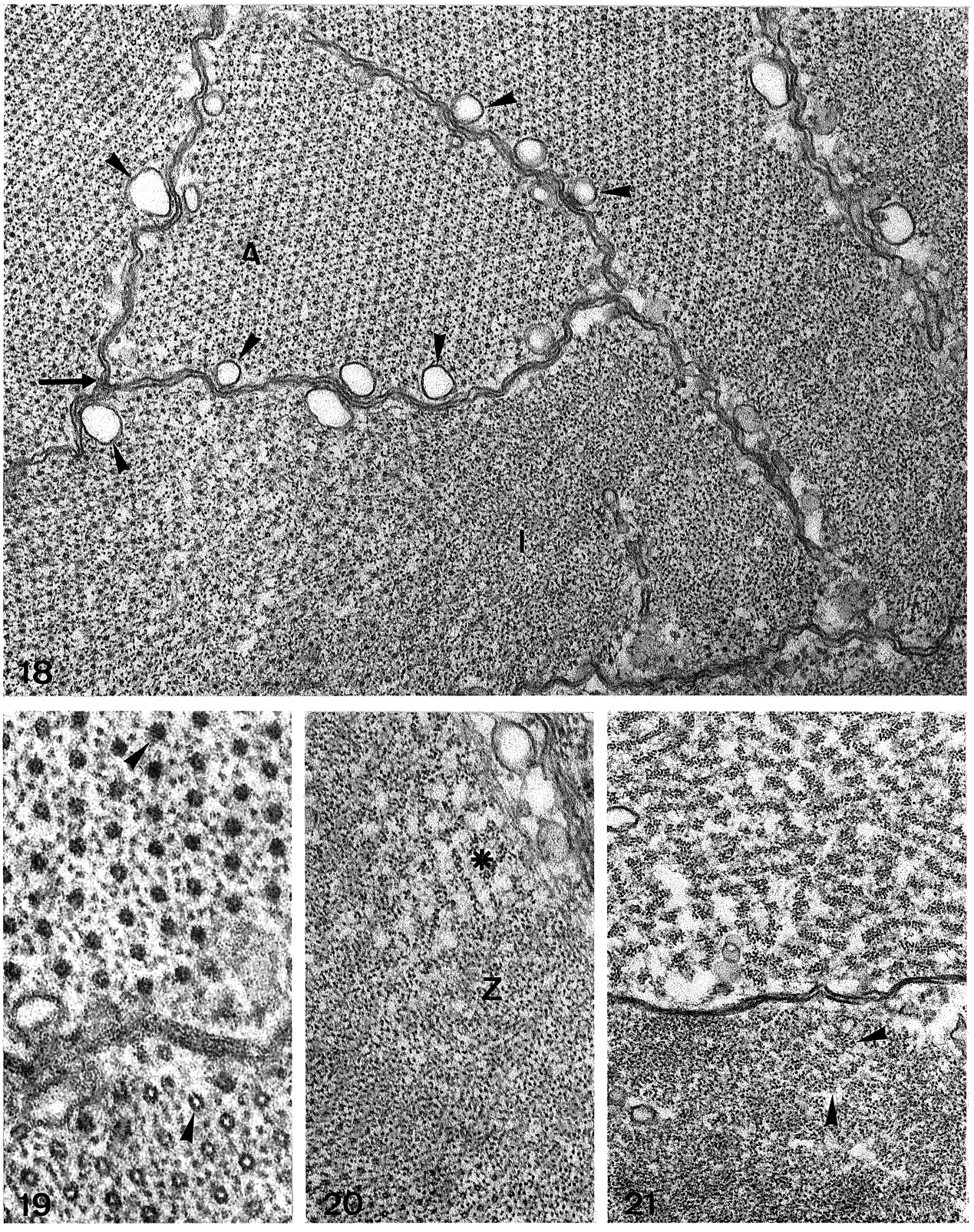
Stretched sarcomeres. Small stretches were applied to animals that were fixed during tension recording. The sarcomere length could attain at least 3 μm (figure 15). In one experiment, the values obtained were 2.85 ± 0.58 μm ($n = 113$). We must note that this value shows little variation; although separated by s2 sarcomeres all the s1 sarcomeres of a same muscle generally respond in the same way. The H-band is correlatively larger than at resting length. In these preparations, the centre of the A bands is more clearly seen, it never shows a thickness that corresponds to M bands (figure 16). Thick filament transverse bridges are clearly seen (figure 16). A band edges are never straight and Z bands are somewhat diffuse. This loose architecture strikingly contrasts with the one found in the primary muscle.

Contracted sarcomeres. The sarcomeres show a typical banding pattern: two dark bands are seen at the edges of the A band, with a central dark band (figure 13). The distance between the two A band dark edges is less than 2 μm; in acetylcholine-contracted muscle, it is: 1.93 ± 0.34 μm ($n = 116$). Again, although separated by s2 sarcomeres, the s1 sarcomeres length of a contracted muscle show similar values.

The Z-band filaments enter the edges of A bands (figure 17). The fully contracted state is correlated with a looser architecture of the A band where the primary filaments lose their straight parallel diposition and lateral cohesion. In many contracted myofibrils all the s1 sarcomeres lost their tranverse orientation and became more or less oblique (figure 13).

Transverse sections

Resting length. A band: the primary filaments have an hexagonal arrangement (figure 18) with a distance, from centre to centre of 52.6 ± 9.1 nm ($n = 185$). They are round and hollow, 20 ± 3.3 nm ($n = 83$) in diameter. The cortex seems to be made of numerous subunits (figure 19). One thick filament is surrounded by 9 to 10 thin filaments, about 8 nm in diameter.



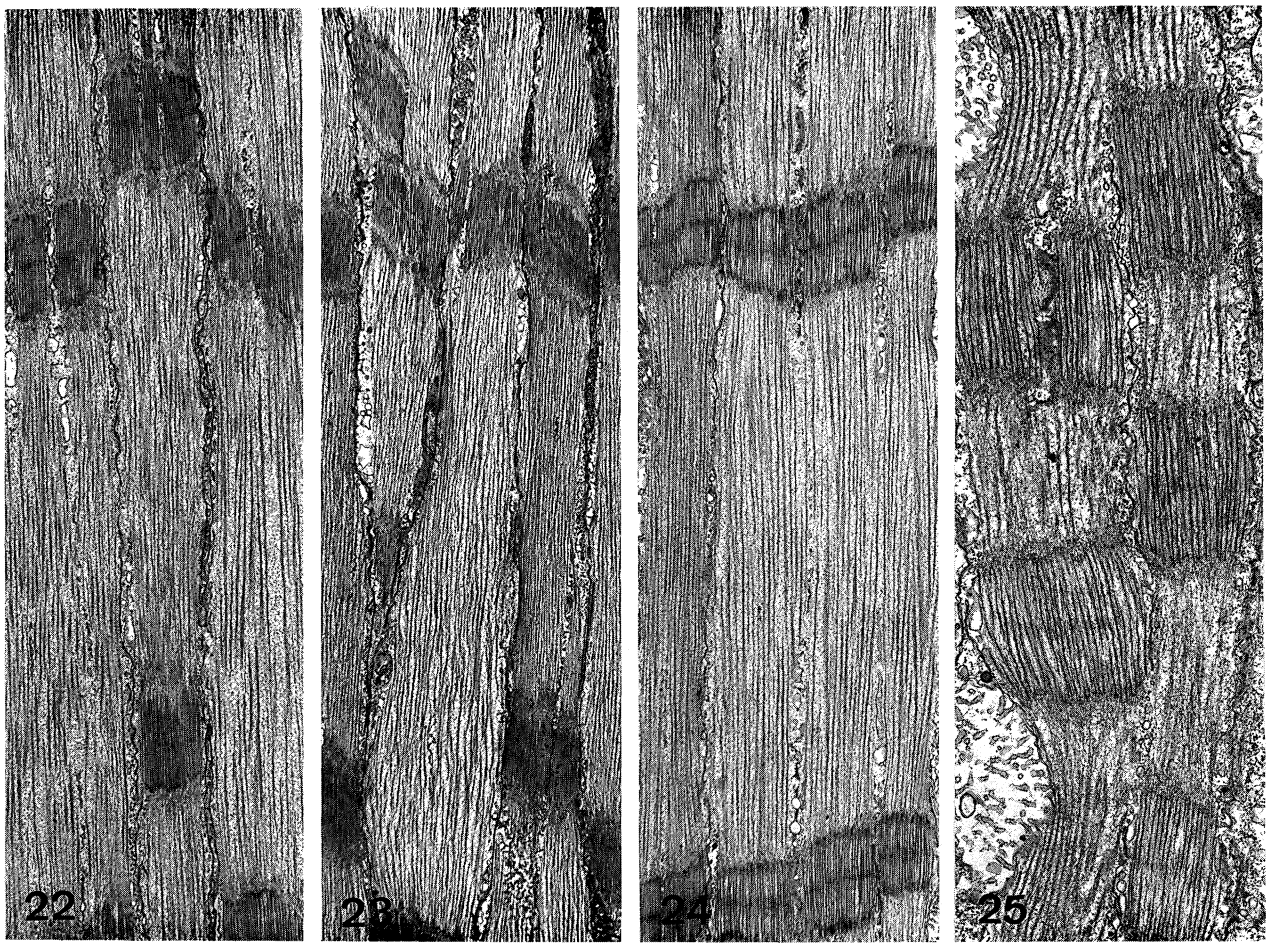
Figures 18–21. s1 sarcomeres: transverse sections.

Figure 18. Slightly oblique section showing the A and I bands. Invaginations of the plasma membrane (arrow) penetrate deeply in the contractile apparatus and give rise to myofibril-like units; at their level and at the plasma membrane level, S.R. tubules show many couplings (arrowheads); magn. × 49 000.

Figure 19. M band (top) and A band (below): note subunits in the thick filaments (arrowheads): magn. × 170 000.

Figure 20. Extremity of an A band; the Z band is not precisely defined, it extends between the A band and the beginning of the s2 sarcomere (asterisk); at least two classes of filaments (dense and light in section) are to be seen; magn. × 61 200.

Figure 21. s1 Z-band (below) and s2 sarcomere (top). Note that in the Z band there is a tendency of the filaments to form clusters (arrowheads). magn. × 52 500.



Figures 22–25. s2 sarcomeres longitudinal sections.

Figure 22. s2 sarcomeres in three fibres where s1 are at resting length or slightly stretched; magn. × 77000.

Figure 23. s2 sarcomeres in five fibres where s1 are stretched; magn. × 5800.

Figure 24. s2 sarcomeres in two fibres where s1 are contracted. In the same fibre, s1 sarcomeres lie in register. Note the irregular length of the s1 sarcomere A bands; magn. × 7700.

Figure 25. s2 sarcomeres with very reduced length in a fibre where s1 are contracted; the s2 band density does not change with respect to the others stretched and/or resting state s2 sarcomeres; magn. × 13900.

H band: the primary filaments are not hollow, show numerous subunits (figure 19), and they are thicker, 22 ± 3.6 nm ($n = 77$). Some interfilament connections can be seen.

Z band: it contains a dense population of filaments which may correspond to extensions of the secondary and primary filaments. Measurements suggest two kinds of filaments, some 7 nm diameter, others 11 nm diameter (figures 20 and 21).

Contracted sarcomeres. The central A band dark band

Figures 26–32. s2 sarcomeres: longitudinal and transverse sections.

Figure 26. Longitudinal section showing the continuity in the architecture of s1 and s2 sarcomeres. At the Z band level (asterisks) secondary thin filaments and C filaments (arrowheads) are reassembled and meet the s2 bundle of filaments. In s2, an interbundle matrix shows numerous lateral connections (arrows); magn. × 29900.

Figure 27. In some short s2 sarcomeres, groups of filaments are very regularly disposed. Lateral bridges are noted, in the interfilament matrix (arrowheads); s1 sarcomeres are at resting length or slightly contracted. Note that s1 and s2 sarcomere longitudinal axes are not parallel to the same myofibrillar axis; magn. × 24800.

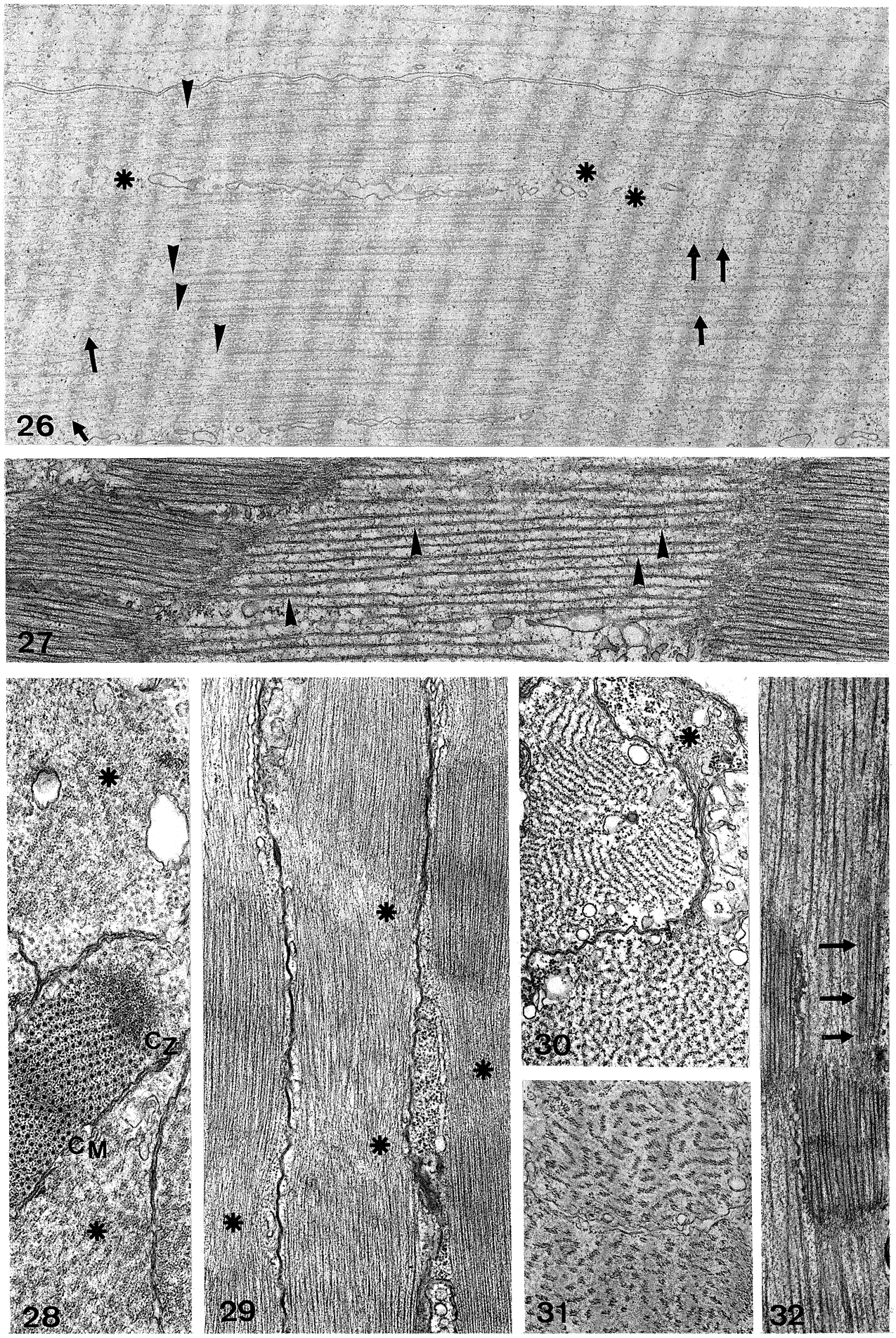
Figure 28. Transverse section in a secondary muscle with highly contracted s1 sarcomeres (C_M and C_Z bands are obvious), s2 show bundles and sheets in a dense matrix (asterisk); magn. × 40700.

Figure 29. Very narrow s2 sarcomeres (asterisks) after freezing and freeze-substitution procedures. As in figure 25, these very short s2 sarcomeres do not show any additional density compared to the long s2 sarcomeres; magn. × 21100.

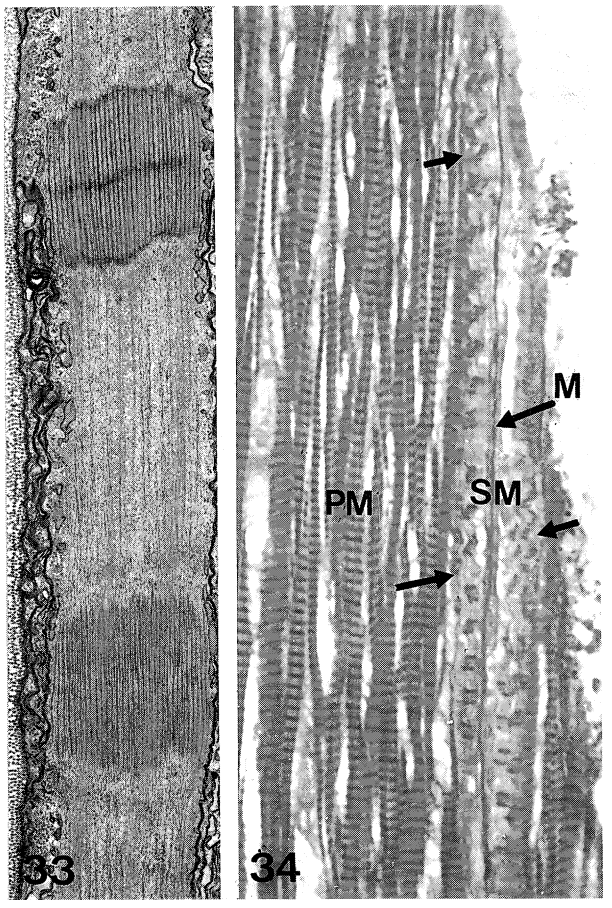
Figure 30. Two architectures of s2 sarcomeres in two fibres partly separated by a myoepithelial cell (asterisk); magn. × 21900.

Figure 31. After freezing and freeze substitution the s2 interfilament matrix is very dense; magn. × 30200.

Figure 32. Part of a s1 sarcomere in a s2 one (arrows); magn. × 13700.



Figures 26–32. For description see opposite.



Figures 33 and 34.

Figure 33. Longitudinal section of the same myofibril showing two contiguous s1 sarcomeres. One is slightly contracted (top, with a C_M band), the other is stretched (below, with an obvious H band); magn. × 11 400.

Figure 34. Longitudinal semi-thin section showing two secondary muscle bands on each side of the mesentery. The primary muscle is contracted as the s1 secondary muscle sarcomeres. The secondary muscle bundles are pleated (arrows); magn. × 1100.

corresponds to a very high density of thin filaments (figure 28). At the edges of the A band, the dark band represents the Z line penetrated by the extremities of the thick filaments.

(ii) *s2 Sarcomere*

An s2 sarcomere is the myofibrillar part between two consecutive s1 sarcomeres. These sarcomeres are polymorphic and difficult to characterize, they do not show A-bands.

Morphometric data

Over large distances, on the same myofibril, the length of the s2 sarcomeres varies. A first estimation made on various animals, shows that when s1 sarcomeres are at rest, the s2 sarcomere lengths lie between 2–9 μm. These values are < 5 μm in myofibrils with contracted s1 and between 2–11 μm in myofibrils with stretched s1 sarcomeres.

Some animals were examined on light microscope sections stained with levanol fast cyanide 5 N, allowing a clear visualization of A and Z bands. When s1 sarcomeres are contracted the s2 values, over a distance of 3 and 9 μm, disappeared ($n = 130$ in each case: resting state and contraction).

In experiments where mechanical recordings were done (figure 42): (i) for s1 at rest (sarcomere length: 2.14 ± 0.27 μm), s2 lay between 1.5 and 8.5 μm ($n = 27$; 24 values between 1.5 and 3.5 μm); (ii) for s1 contracted (sarcomere length: 1.93 ± 0.34 μm), s2 lay between 0.5 and 12.5 μm ($n = 42$; 28 values between 0.5 and 5.5 μm), and (iii) for s1 stretched (sarcomere length: 2.85 ± 0.58 μm), s2 were between 1.5 and 11.5 μm ($n = 39$, with 28 values between 1.5 and 6.5 μm).

These results suggest an apparent relative independence of the s1 length versus s2 length, but the effective contractile state of the secondary muscle cannot be controlled, only the whole skeleto-muscular functional state of the trunk wall.

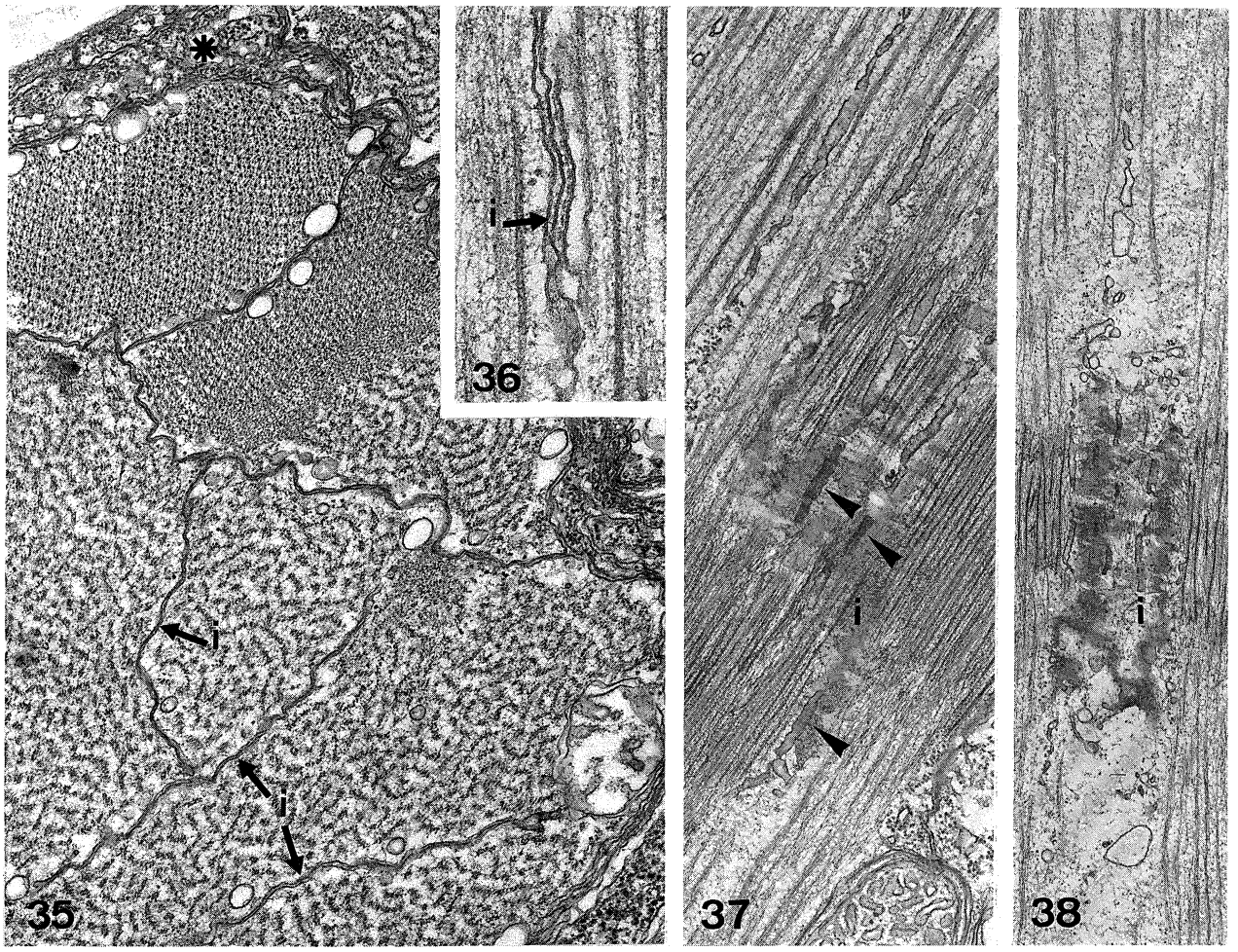
Another technical difficulty is estimating these data. The fibres are narrow and their myofibrils are made of densely ramified thin columns; consequently: (i) paraffin light microscope longitudinal sections may contain a superposition of at least two to three muscle fibres; (ii) the contractile apparatus is not regularly arranged in myofibrils, it has a complex architecture. We cannot assume that a longitudinal thin section contains a unique or various myofibril expansions; it is not always possible to decide if a longitudinal section that contains two s2 sarcomeres pertains to one or to various adjoining columns, more particularly when the plane of section is oblique to the myofibrillar axis. Consequently, the values reported are given with some reserve.

Many transverse connections between various bundles are to be seen (figures 26 and 27), they partly constitute a fibrillo-granular matrix and they may extend to several lateral bundles. These transverse bridges seem to be regularly disposed but the interval between successive bridges varies widely around a mean value of 50 nm. It seems that these bridges are of various kinds, they probably do not constitute an homogeneous functional architecture.

Ultrastructural data

Figures 22–25 show s2 sarcomeres at various lengths. Four points should be noted: (i) s2 sarcomeres are mainly made of parallel filament bundles; (ii) when s2 are very short (s1 being always contracted) the density of the s2 sarcomere does not increase; (iii) in some instances s2 virtually disappears (figures 13 and 29); (iv) when *Sagitta* are fixed in osmium tetroxide alone, s2 sarcomeres are destroyed as are the thin filaments of s1 sarcomeres and in the primary muscle cell myofibrils.

Longitudinal sections. High magnifications show that the parallel strands of filaments are continuous with the Z band filaments (figure 26). These parallel strands seem to be embedded in a diffuse granular matrix where filamentous sections are seen; some of them seem to connect the filament bundles (figures 26 and 27). When bundles disappear in osmium-treated tissues the matrix seems partly preserved.



Figures 35–38.

Figure 35. Transverse section showing S.R. longitudinal tubules and plasma membrane invaginations: these two compartments form structural units which are regularly associated with s1 sarcomeres. Invaginations penetrate deeply in the contractile apparatus and forms myofibril-like units. Part of a myoepithelial cell is seen (asterisk); magn. × 25 300.

Figure 36. Perpendicular longitudinal section in an internal coupling. Magn. × 72 000.

Figure 37. Tangential section at the level of s1 sarcomeres, internal couplings show the parallel arrays of junctional feet at the S.R. level (arrowheads); note the longitudinal tubules of S.R. at s2 sarcomere level; magn. × 20 300.

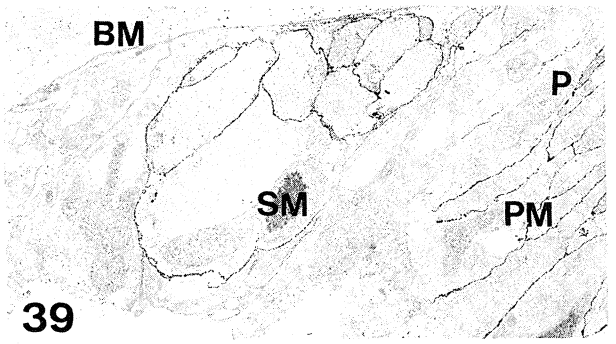
Figure 38. Longitudinal section in a secondary muscle with contracted s1 sarcomeres. Invaginations and S.R. remain associated with s1; magn. × 14 300.

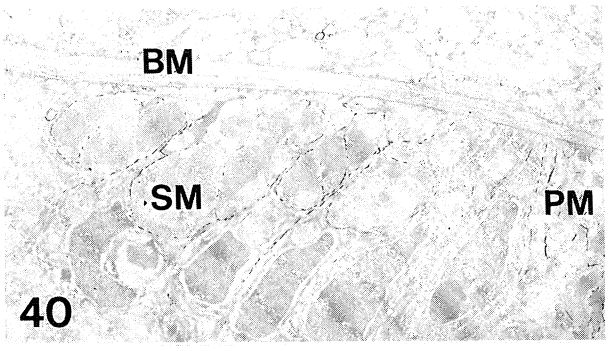
Transverse sections. The pleomorphism of the s2 sarcomere is clearly shown, together with the great difficulty in defining the precise nature of this myofibrillar unit.

(i) The filament strands can form discrete bundles, or ramified sheets of different dimensions showing a high degree of architecture (figure 30). In these strands, filaments are more or less tightly aggregated (figures 21 and 31). Bundle and sheet thickness were measured ($n = 100$), the histograms show various peaks at about 2.5 and 4.5 nm and a very large population at about 7–8 nm. Transition states are to be seen between bundles and sheets (figures 31 and 35). In the same fibre group, some cells show s2 sarcomeres as sheets, others as bundles of filaments. At high magnification, each filament is seen to be composite and contains very few subunits. The filaments are either aggregated or separated from one another; owing to the difficulty in preservation of this s2 sarcomere it is not possible to describe adequately the aggregation states of the filaments and the transition states between sheets and bundles. The impression is that s2 sarcomeres have a dynamic architecture made of more or less tightly arranged filaments (first level of organization) which can form transitional architectures between bundles and sheets (second level of organization) (figure 30).

(ii) In the bundles and sheets, filaments of various thickness are to be seen, with a main population about 7 nm in section (and others perhaps of about 4–5 and 10 nm).

All these bundles and sheets are embedded in a poorly defined matrix. This matrix is very difficult to preserve and observations suggest that: (i) it may change in density according to the contractile state of s2 (figures 28 and 30); (ii) after quick freezing and freeze-substitution, this matrix is dense (figure 31), correlatively, the s1 and s2 myofibrillar electron microscope density is almost comparable, this is more





Figures 39–41. Transverse sections in ventral secondary muscle bands.

Figure 39. Ca^{2+} ATPase activity at the level of external membranes (plasma membrane and invaginations); the same activity is shown in the primary muscle (right) but not in the myoepithelial cells; magn. × 4500.

Figure 40. Lanthanum localization on the secondary muscle (centre) and primary muscle (right) cell membranes. Again, myoepithelial cells show low or no reactivity; magn. × 3600.

Figure 41. Pyroantimonate treatment after incubation in artificial sea water containing twice (20 mM) Ca as in normal sea water. Deposits are dense at the plasma membrane/invagination levels, and, probably in mitochondria (arrowhead); magn. × 4900.

obvious when s1 and s2 are all contracted (figure 29); (iii) it shows variations among Chaetognath species (data not shown).

(iii) *Intracellular compartments*

The secondary muscle contains many organelles comparable to those of the primary muscle: large dictyosomes related to the nuclear envelope, mitochondria with tubular cristae, ribosomes, absence of glycogen and lipid inclusions. Owing to their expected implication in the contractile state of the fibres, the plasma membrane invaginations and the S.R. only are described here.

Invaginations. The plasma membrane invaginates (figures 18 and 35) and forms large sheets that individualize myofibril columns in the large diameter cells. Invaginations are more frequently shown at the s1 level where they are closely related to longitudinal S.R. tubules (figures 37 and 38). They mainly originate at the s1 sarcomere A band level but they may extend over consecutive s1 and s2 sarcomeres.

S.R. The S.R. is essentially made of longitudinal tubules which run against the invaginations and under the plasma membrane (figures 18 and 35). Transverse elements are rare and structural units ('sarcomerization') are not obvious, except at the s1 sarcomere level? (figures 37 and 38).

At the s2 level, the S.R. is less abundant and seems to be less regular than in the s1 sarcomere. In s1, peripheral (plasma membrane level) and internal (invagination level) couplings are very abundant (figure 18). They have the same morphological characteristics as the ones we have already noted in the primary muscle. The distance between apposed membranes is about 17 nm (from face to face), the S.R. membrane shows junctional feet (with a clear central part) about 22 nm thick, the interval between two junctional feet is about 8 nm, they are not in contact with the junctional plasma membrane (figure 36). The junctional feet are in two parallel rows along the S.R. tubules (figure 37).

Myofibrillar and membrane organelles of the secondary muscle cells do not show any difference which could be related to a fibre typology. Similarly in their relative shape, abundance and architecture, such cytochemical data as myofibrillar ATPase activity, or Ca^{2+} localization, do not help to differentiate secondary muscle fibre types. Cytochemically, the secondary muscle shows the same reactivity as the whole primary muscle and as the A fibre groups.

(iv) *Functional aspects*

Contractile apparatus

Some observations suggest functional aspects intrinsic to the secondary muscle or relative to the function of the primary muscle.

(i) In some preparations, the s1 state of contraction can vary along the same myofibril. Figure 33 shows two consecutive s1 sarcomeres, one slightly stretched, the other slightly contracted.

(ii) In many cases, the primary muscle is contracted while the secondary muscle is pleated and the sarcomeres s1 and s2 do not show any sign of contraction. In other cases, on the same pleated muscle, the sarcomere s1 show obvious contracted features (figure 34).

Ca^{2+} and the contraction-relaxation cycle

As previously shown (Savineau & Duvert 1986) the body locomotory muscle fails to contract in the absence of external Ca^{2+}. Contraction is reversibly blocked by lanthanum; it is amplified in the presence of an excess of Ca^{2+} in a normal sea water.

Cytochemical methods for Ca^{2+} fail to dinstinguish the primary muscle A fibres and the secondary muscle fibres (Savineau & Duvert 1986). In the presence of artificial sea water containing 5 mM lanthanum, the

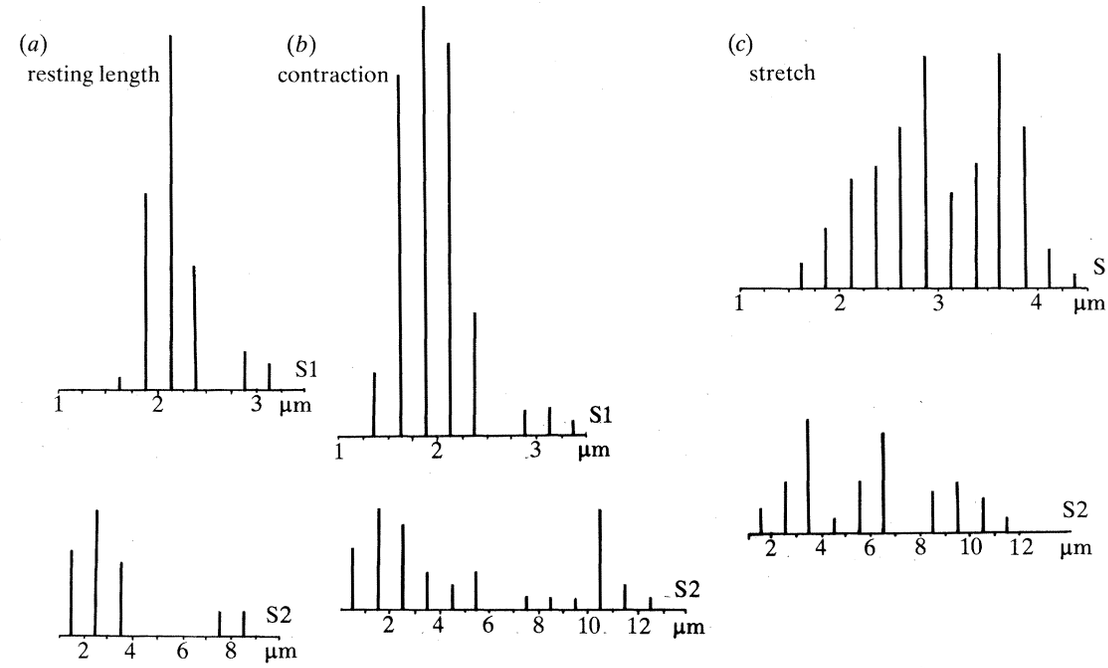


Figure 42. s1 and s2 sarcomere lengths during: (*a*) resting length; (*b*) contraction; (*c*) stretch.

plasma membrane and invaginations show deposits (figure 40), muscular contraction is blocked. When the Ca^{2+} concentration in sea water is twice as much as is normal ($CaCl_2$ 20 mM) pyroantimonate deposits are seen in the sarcoplasm, they are concentrated in small areas which may correspond to mitochondria (figure 41); as in normal sea water, deposits are also seen on plasma membranes and invaginations. Ca^{2+} ATPase activity can be seen along the extracellular compartments, plasma membrane and invaginations (figure 39). Controls are negative. All these reactions in the primary and secondary muscles suggest peculiar modes of functioning. They are distinctive, surrounding myoepithelial cells are almost negative.

Cytoskeleton

As in the primary muscle, this intracellular architecture replaces the connective tissue which is absent between muscle fibres. The precise architecture of the cytoskeleton is unknown, but as in the primary muscle, it is likely to be essentially in contact with columnar junctions (figure 9). The development of this organelle is indirectly evident: (i) sarcomeres lie in lateral register (figure 24); (ii) during contraction s1 sarcomeres remain topographically related to the invaginations and S.R. tubules (figure 38).

4. DISCUSSION

The muscles of various invertebrate phyla are largely unknown, and many of them cannot be described and understood adequately on the basis of the classical division into cross-striated, smooth and oblique striated muscles (Lanzavecchia 1988). Thus it seems more useful to discuss the functional significance of the very singular secondary muscle, only in the context of the Chaetognath body.

(*a*) *Secondary muscle*

The mode of striation of the secondary muscle is peculiar. It was first described by Grassi (1883) in a comprehensive way. Grassi showed the secondary muscle in its actual median localization, and in the lateral fields, on their ventral side. It seems that this author was able to distinguish individual secondary muscle myofibrils and he established that the singular mode of striation was an intrinsic characteristic of the secondary muscles of various if not all Chaetognaths. As with many penetrating pioneering works of the 19th century, the work of Grassi was nearly forgotten. So far as I am aware, no other author has published a study of this muscle, and (owing to its strangeness?) classical works on Chaetognaths (Doncaster 1902; Burfield 1927; John 1933) do not mention it at all. In a similar way, zoological texts or monographs do not mention this muscle, with the exception of Perrier (1897) who reported the observations of Grassi. Curiously, although light microscope studies, using polarizing microscopy were done by Schmidt (1939, 1951, 1952), showing this muscle, he did not recognize its peculiar nature.

In this study, the secondary muscle was found in various Chaetognath genera belonging both to the Phragmophora and Aphragmophora: *Sagitta*, *Pterosagitta*, *Spadella*, *Eukrohnia*, *Heterokrohnia*. The uniqueness of this muscle is its striation mode with this regular succession in series of s1 and s2 sarcomeres, but its salient pecularity is the nature, the architecture and the functional characteristics of the sarcomeres s2. In this work, the word sarcomere is used in its classical sense: myofibrils can be subdivided in longitudinal units called sarcomeres, 'each sarcomere is separated from the text by a tranverse stripe, the Z-membrane' (Picken 1960).

(i) *Structural characteristics*

On a very general basis, the secondary muscle seems to be related to cytoskeletal architectures and more precisely to stress fibres.

The s1 sarcomeres have some characteristics in common with the primary muscle sarcomeres (Duvert & Savineau, 1986): the distance between two neighbouring thick filaments is about the same; C-filaments (Auber & Couteaux, 1963) attach the thick filaments to the Z band; thick and thin filaments have about the same diameter.

However, in contrast, the s1 sarcomeres show many structural features, which are typical of slow fibres, more particularly when we compare these myofibrillar units with those of the primary muscle which are very fast (Duvert & Savineau 1986; Duvert 1989). For these comparisons, the first value refers to the secondary muscle, the second to the primary muscle: sarcomere length = 2.7/1.5 μm; thick filament length is 1.2/1.7 μm; thin filament length is 0.7/1 μm; s1 lacks M band and lateral bridges in I-bands. Each thick filament is surrounded by 9–10 thin filaments in s1, in the primary muscle 1 thick filament is surrounded by 6 thin filaments and, as in very fast-acting arthropod muscles, F2:F1 = 3/1. Large differences are also to be seen between primary and secondary contractile apparatus; they are related to their architecture, to their lateral cohesion, to the individualization of myofibrils and to their shape.

(i) s1 sarcomere A band edges are easily deformed; they rarely remain straight, out of the resting state. Correlatively, Z bands may have a highly sinuous course during mechanical constraints.

(ii) For short sarcomere lengths, the lateral cohesion is lost between these thick filaments devoid of M bands.

(iii) In a s1 sarcomere, all the filaments may have not the same length; A band length may decrease in a somewhat regular way. These data suggest a relative independence for constructing and assembling thick filaments during sarcomerogenesis.

The s2 sarcomere looks like the cytoskeleton of some cells, especially the 'stress fibres':

(i) It has a pleomorphic structure showing a highly polarized architecture made of filament bundles.

(ii) These sarcomeres seem to be able to contract; in some cases they virtually disappear. Curiously, in the supposed contracted states, the density of the sarcomeres does not change. These data suggest that the filamentous components of the s2 sarcomeres may constitute highly dynamic architectures which reflect equilibrium states between polymerized–depolymerized, assembled–disassembled, elements of the sarcoplasm.

(iii) In spite of a loose matrix giving a lateral cohesion, the filament bundles may be relatively independent. These bundles are related to the Z band which seems to have a poor lateral cohesion. This framework helps us to understand why during stretches and strong contractions, the sarcomeres s1 edges are more or less irregular and oblique relative to the myofibrillar axis.

So far, there is no information about the nature of the s2 sarcomere components but as the filament diameters suggest, actin may constitute a major structural protein; they are destroyed after OsO_4 treatment alone, as are all other secondary filaments in the primary muscle and in the s1 sarcomeres.

The whole contractile apparatus of the secondary muscle seems to be related to a slow contractile architecture: loose arrangement of the myofibrillar apparatus, apparent irregular s1 sarcomeres which are related to the pleomorphic s2 sarcomeres. In the absence of any direct evidence as to the function of the secondary muscle, and of any similar muscle in any other animal, any speculation about its role is perhaps premature. Nevertheless, it seems reasonable to suggest from its morphology and position that it may be related to the maintenance of the appropriate pressure of the fluid in the body cavity. The hydroskeleton formed by the body cavity and the collagenous basal membrane external to the primary musculature may thus be most efficiently 'tuned' for the operation of the primary musculature during locomotion. Such a possible function could well be served by the coordination of the s1 and s2 sarcomeres which are in series rather than in parallel.

Further work remains to be done at the structure level particularly: (i) optimal preservation of the s2 sarcomere ultrastructure and more particularly of the 'matrix' between filament bundles (compare figure 30 with 31); (ii) to correlate the changing aspect of the s2 (filament bundles versus sheets) to contractile state of these sarcomeres; to establish the relative independence of the functioning of s1 and s2 sarcomeres; to elucidate the framework giving cohesion to this contractile apparatus, and relating sarcoplasmic components to the cell membrane.

(ii) *Functional characteristics*

Together with this 'slow' morphology of the contractile apparatus, sarcomeres s1 and s2 have a poorly developed S.R., as is the case in A and B primary muscle fibres. This observation suggests that it mainly depends on extacellular Ca^{2+} for contraction, as we have previously established for the whole locomotory muscle (Savineau & Duvert 1986); correlatively, La^{3+} fixes on the extracellular membranes (plasma membrane and invaginations) where a Ca^{2+}-ATPase activity could be visualized. All these data were already reported for primary muscle. It cannot be decided if the loose arrangement of S.R. and invaginations in the s2 sarcomeres, compared to the arrangement in the s1 sarcomeres has a physiological significance in the context of the Ca^{2+} activation–deactivation cycles.

During contraction and stretch, s1 sarcomere A band length remains constant in spite of the loose lateral connection and misalignment of the thick and thin filaments. The half I bands (between the A band edges and the contiguous Z bands) change in length according to the s1 contractile state. These morphological and morphometric data are in good accord with the sliding filament hypothesis (Huxley 1969). The functional basis of the s2 sarcomere changes in length remains obscure; particularly the causes and the

consequences of the filament aggregation states and of the conversion between bundle and sheet states are unknown; it has not been possible to correlate structural changes with functional data. Our observations suggest that filament bundles or sheets may have a somewhat autonomous functioning in spite of the matrix lateral bridges. Bundles and sheets may represent two kinds of organization; the s2 sarcomere architecture seem to oscillate between these two organizations and observations, not reported, suggest that (in some animals at least) the interfilament bundle/sheet matrix may play a central role in the architecture of the s2 sarcomeres.

The data suggest a relative independence of functioning:

(i) inside the secondary muscle, between s1 and s2 sarcomeres in the same fibre and/or myofibril;

(ii) between secondary and primary muscles, in spite of the fact that the secondary muscle fibres and the primary muscle B-fibres located near the mesentery, are linked by gap-junctions. Gap-junctions also link secondary muscle cells and myoepithelial cells. Obviously, some kind of selectivity in gap-junction nature and/or physiology may be assumed. Like the columnar junctions together with their cytoskeleton (Duvert *et al.* 1980), gap junctions seem to be necessary to the functional integrity of this epithelio-muscular tissue (devoid of connective tissue, and excretory, respiratory and circulatory apparatus) (Duvert 1989).

The secondary muscle, because of its regular position in all Chaetognaths studied (Grassi 1883), i.e. at the edges of the dorsalventral and lateral axis, may participate in the *orientation* of the body; locomotion is driven by the large and powerful primary muscles. This function may be 'tonic' as its peculiar structure suggests. The pleated aspect of the secondary muscle when the primary is much contracted, are not at odds with this hypothesis. Histological data also suggest that the mediodorsal and ventral bands associated with the mesenteries, do not act in the same way as the discrete fibre bundles of the lateral bands associated with the lateral fields.

The tonic function of the secondary muscle seems a very peculiar one. It seems to involve a dual mechanism which may be related to two coordinated functions: (i) s1 sarcomere contraction may be more rapid than the s2 sarcomere contraction: possibly it adjusts rapidly the muscular tension giving fine gradual changes of low amplitude; (ii) s2 sarcomere contraction may provide sustained and large amplitude changes. One may suggest that one mechanism (the first ?) is related to body orientation changes, the other (the second ?) may affect the whole body and more precisely the adjustment of the cavity pressure (for an optimization of the primary muscle functioning) during locomotor movements.

(*b*) *Myoepithelial cells*

These cells have not previously been described in Chaetognaths. Burfield (1927) reported: 'a thin lateral band muscle along the mid-lateral line', this 'lateral muscle' (sometimes cited in zoological text books) which hardly correspond to the myoepithelial cells, has no existence.

These cells are part of the lateral fields made of an epithelium with various cell types; many are secretory and act as an endocrine system (Duvert 1989). Myoepithelial cells, are also 'glial-like' elements which surround, at least partly, bundles of secondary muscle cells. Their contractile apparatus is very different from the primary and secondary muscle fibres and from the transverse muscles of Phragmophora. Sarcomeres have not been seen and are probably absent.

It is suggested that these myoepithelial cells may have principal and accessory functions:

(i) As in many animals provided with an hydrostatic skeleton, the contractile state of these 'muscular fields' (Barrington 1979) may adjust the pressure in the aqueous general cavity to give optimal functioning conditions for the powerful locomotory musculature. Various observations support this hypothesis: the internal pressure in the general cavity may vary according to the age (i.e., the diameter) of the animal, the degree of development of the genital apparatus, and also in case of parasitism, during feeding, etc. (Duvert 1989). Myoepithelial cells are good candidates to adjust the general cavity volume, their contractile apparatus lie in a dorsoventral position.

(ii) Because of their activity, the myoepithelial fields may have many secondary functions. At the lateral field only, they may contribute to the secretion of the various substances synthesized by the lateral field cells, as excretion of the secretory products are directed against the pressure developed by the hydroskeleton.

(iii) Myoepithelial cells are also structurally related to secondary muscle cells or bundles but the functional architectural relations between these two contractile structures remain obscure (it may have a phylogenetic significance).

(*c*) *Phylogenetic considerations*

The adult locomotory muscle of chaetognaths (*Sagitta* and *Spadella* at least) is a neoformation that appears after the obliteration of the embryonic coelom and during the construction of the general cavity of the adult (Hertwig 1880; Kowalevski 1871; Bütschli 1873; Doncaster 1902; John 1933). The embryonic origin of the muscles and of many other adult tissues remain to be established, particularly so for the tissues lining the general cavity. Nothing is known about the origin of the secondary muscle. Chaetognaths are totally isolated in the animal kingdom. Their powerful locomotory musculature and the very singular secondary muscle do not help us to determine their affinities.

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ABBREVIATIONS USED IN THE FIGURES

A A band
B B fibres group
BM basement membrane
C_M contraction band at M level
C_Z contraction band at Z level
EP epidermis
F lateral fin
GC general cavity
I I band
i invagination
IN intestine
IM intestine muscle
LF lateral field
M mesentery
NS nervous system
PM primary muscle (d, dorsal quadrant; v, ventral quadrant)
s1 s1 sarcomere
s2 s2 sarcomere
SM secondary muscle (l, lateral; md, mediodorsal; mv, medioventral)
Z Z band

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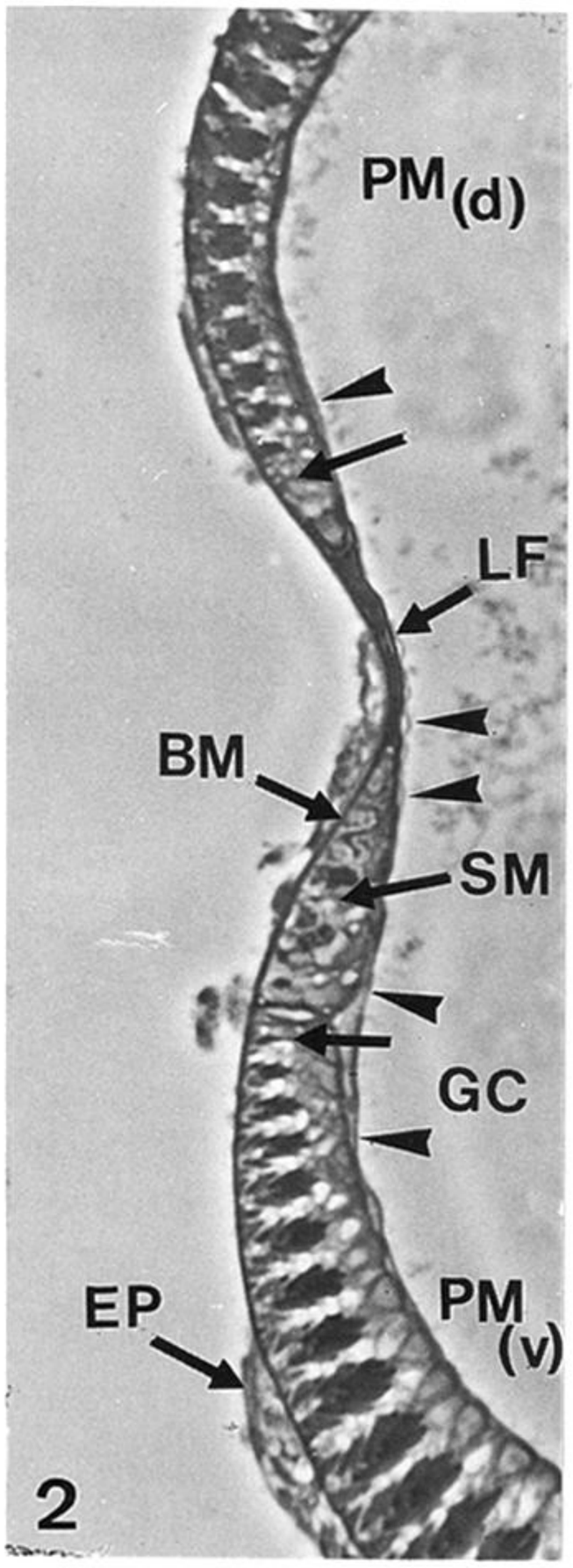
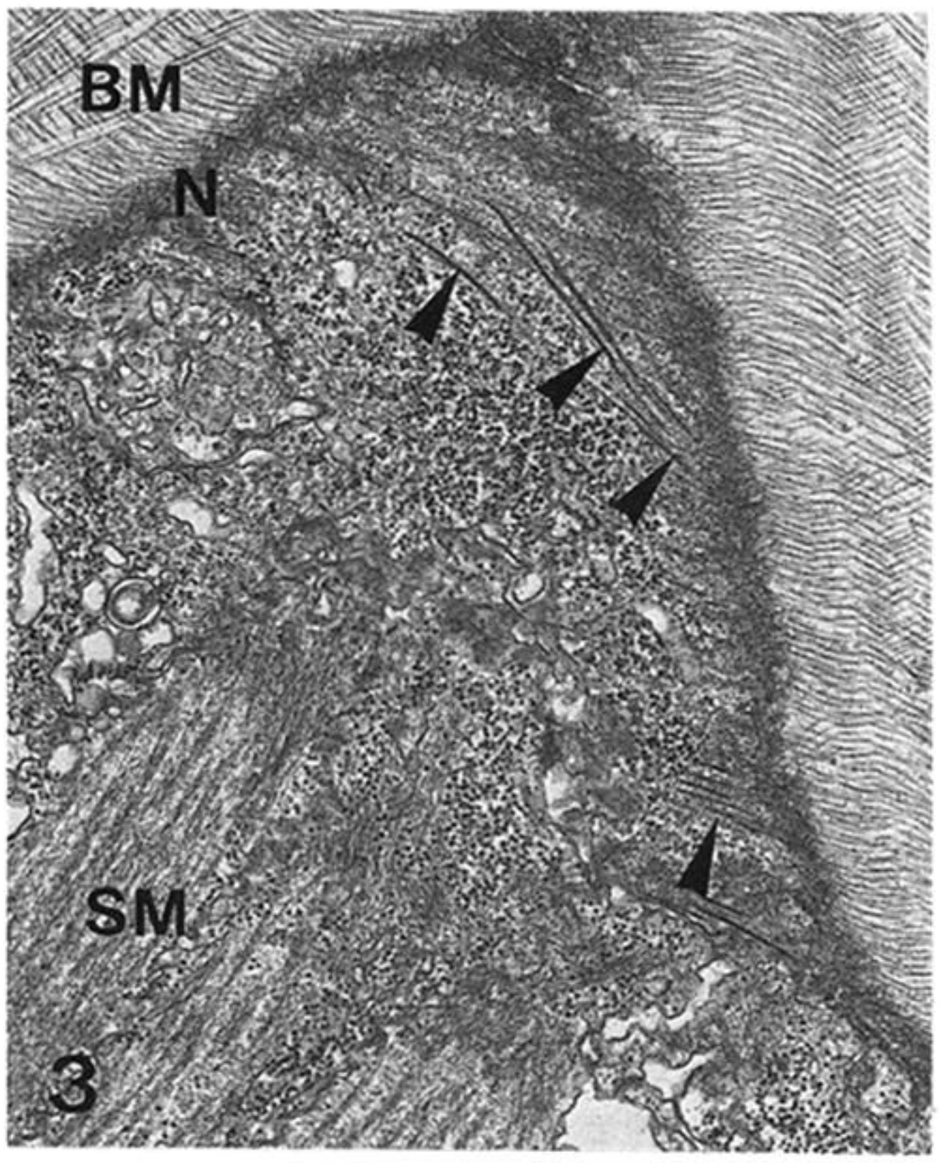
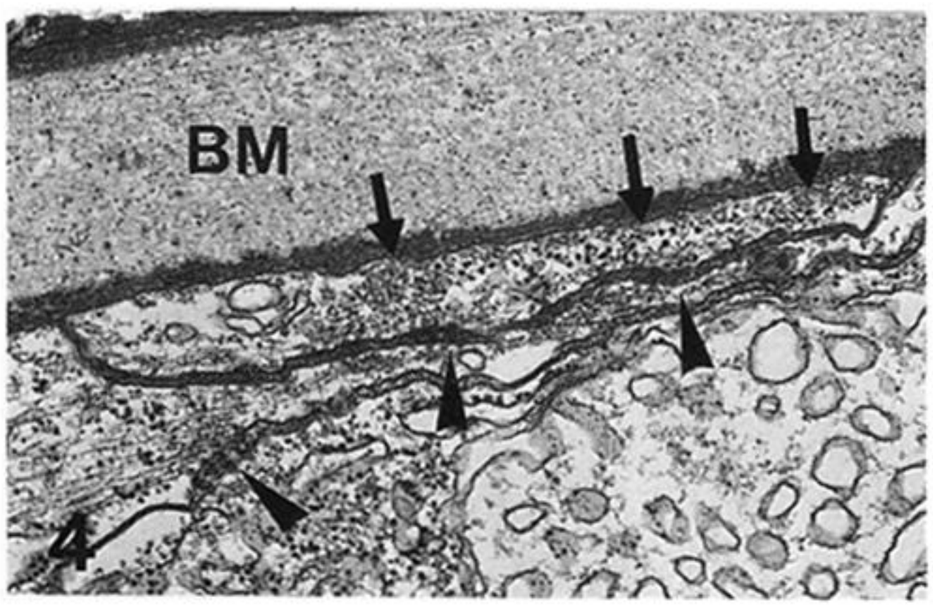
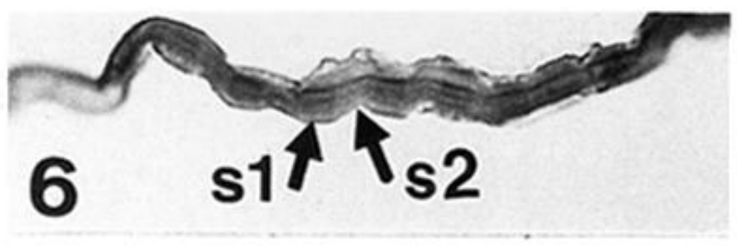
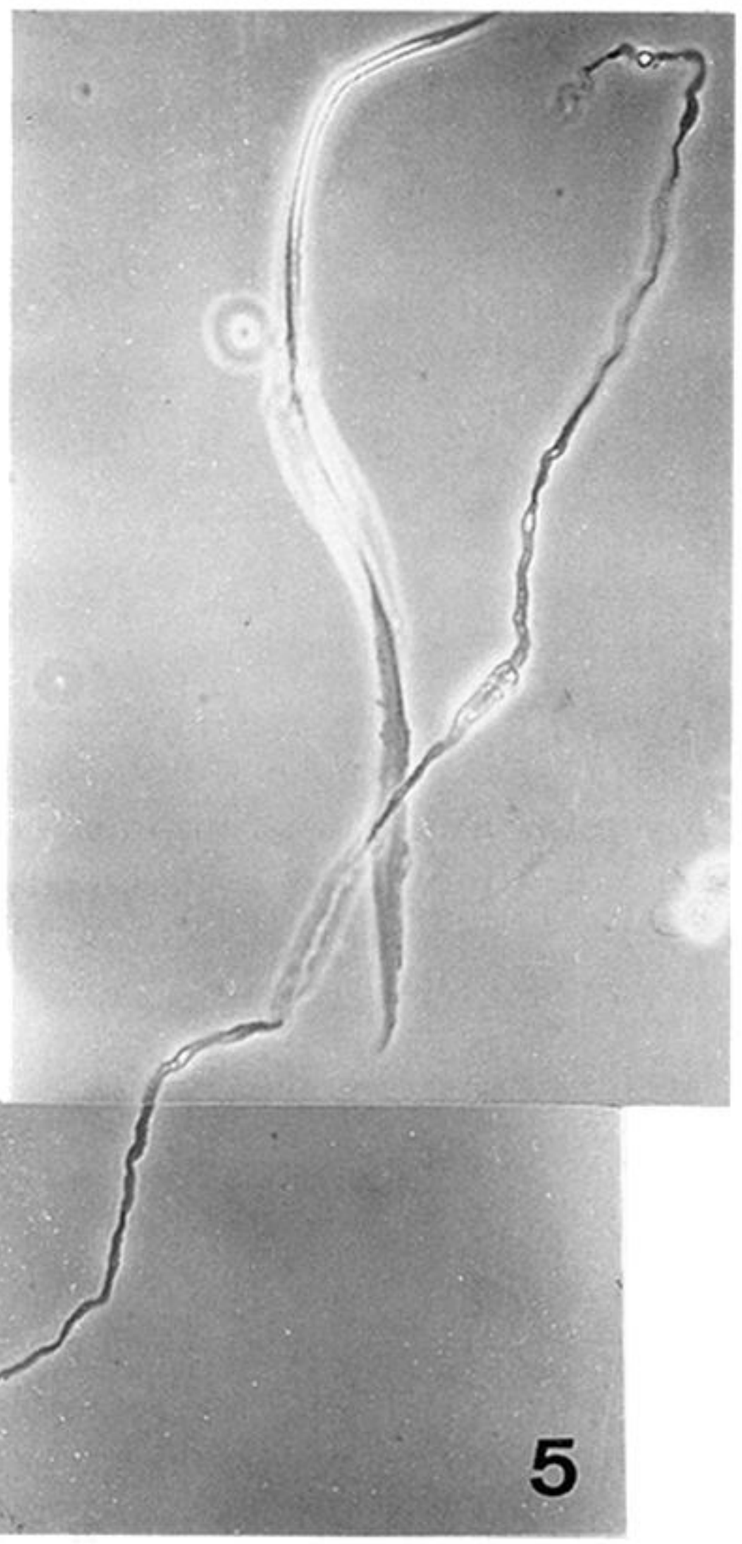
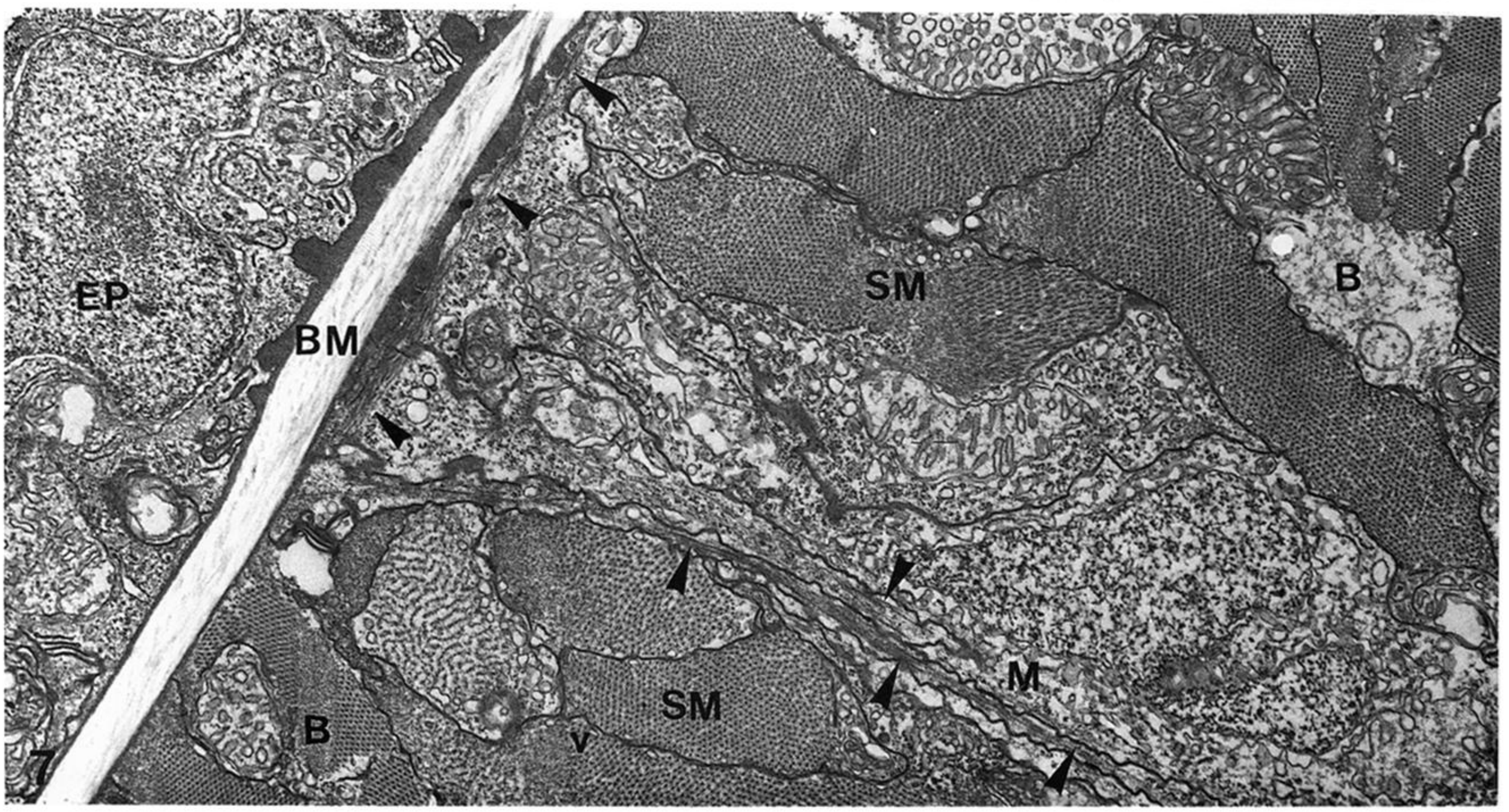




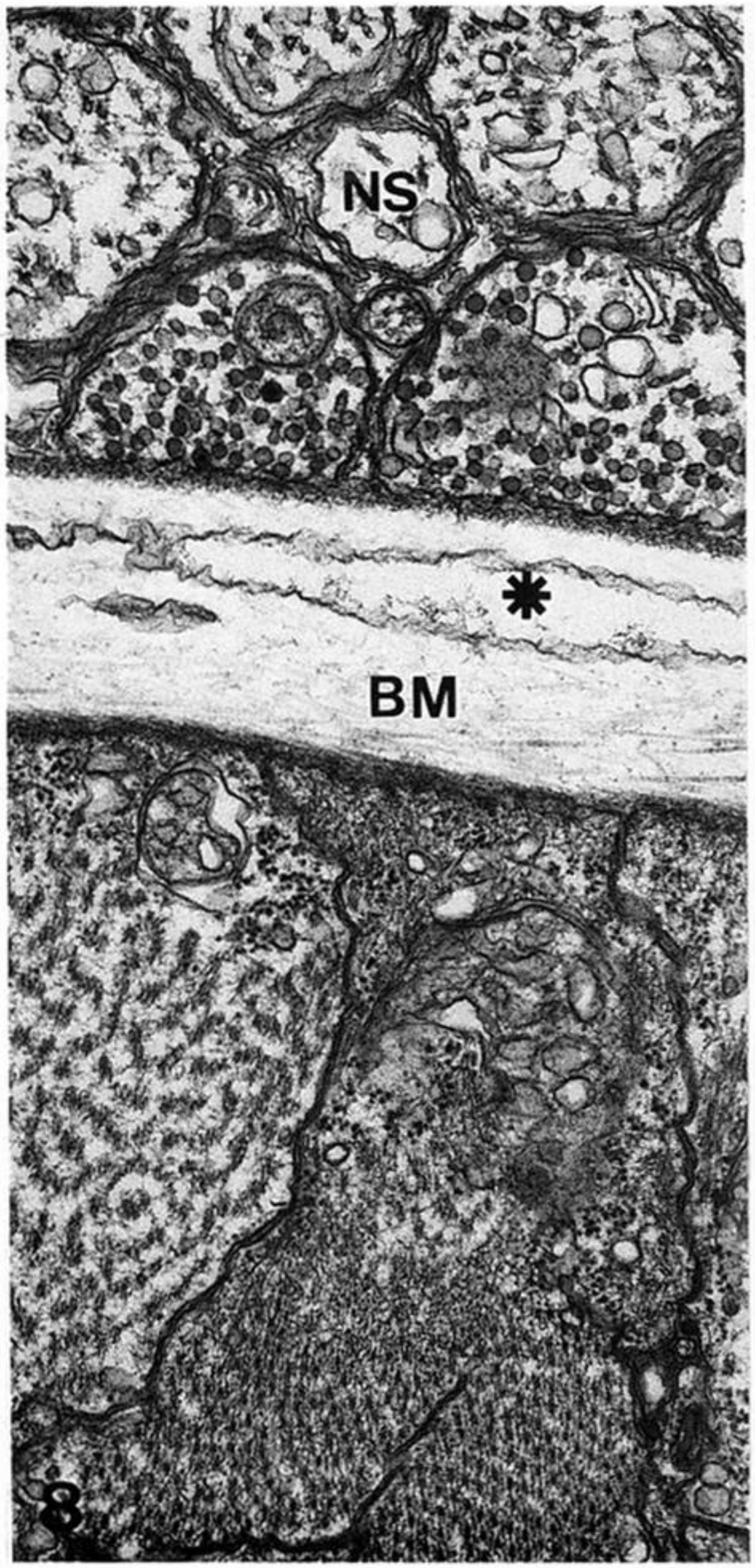


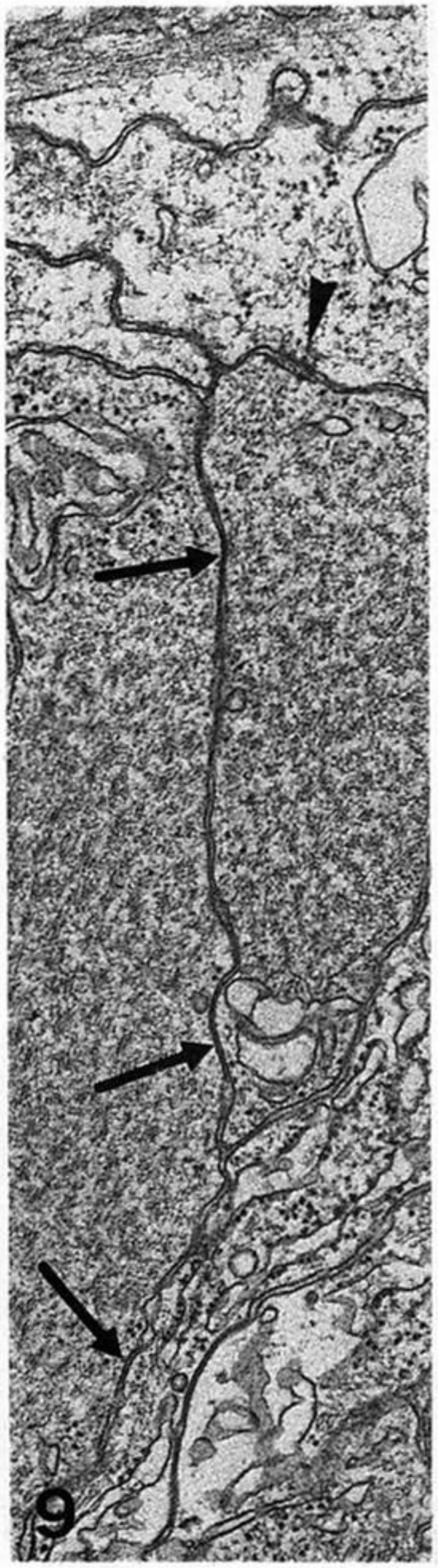


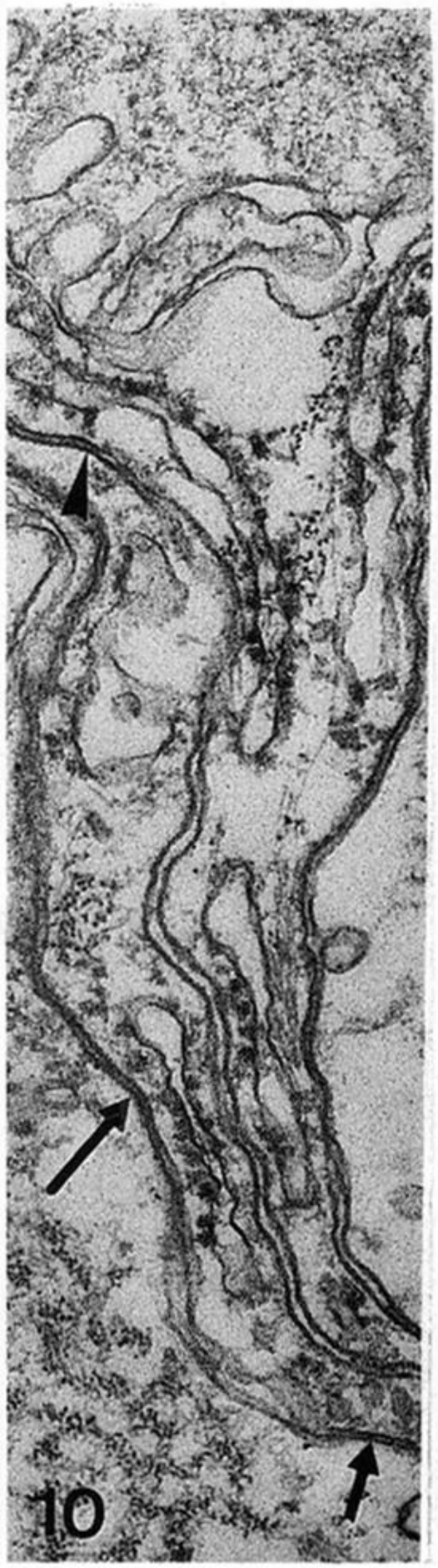


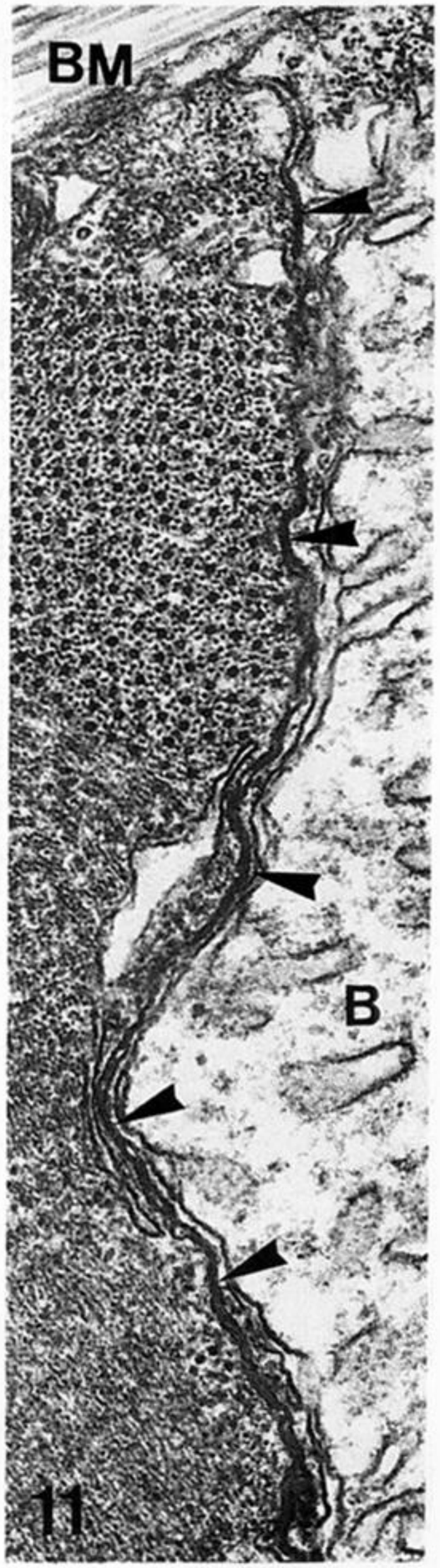


Figures 2–7. For description see opposite.









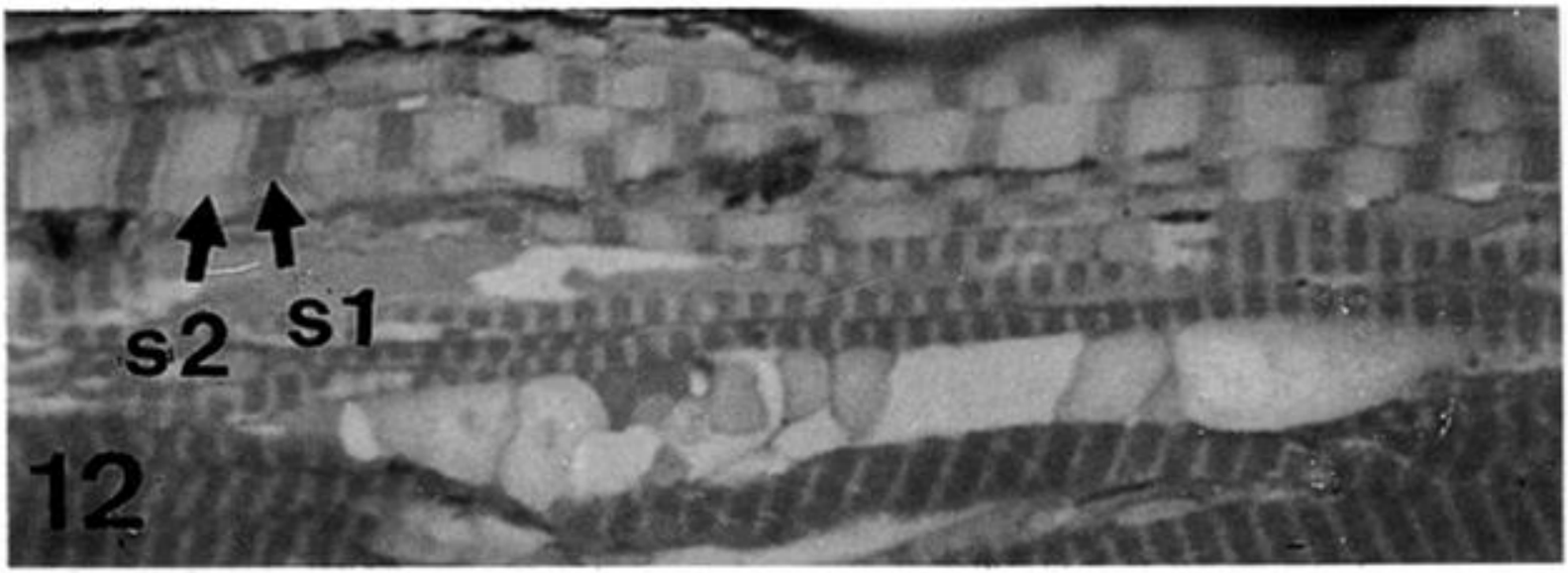
Figures 8–11.

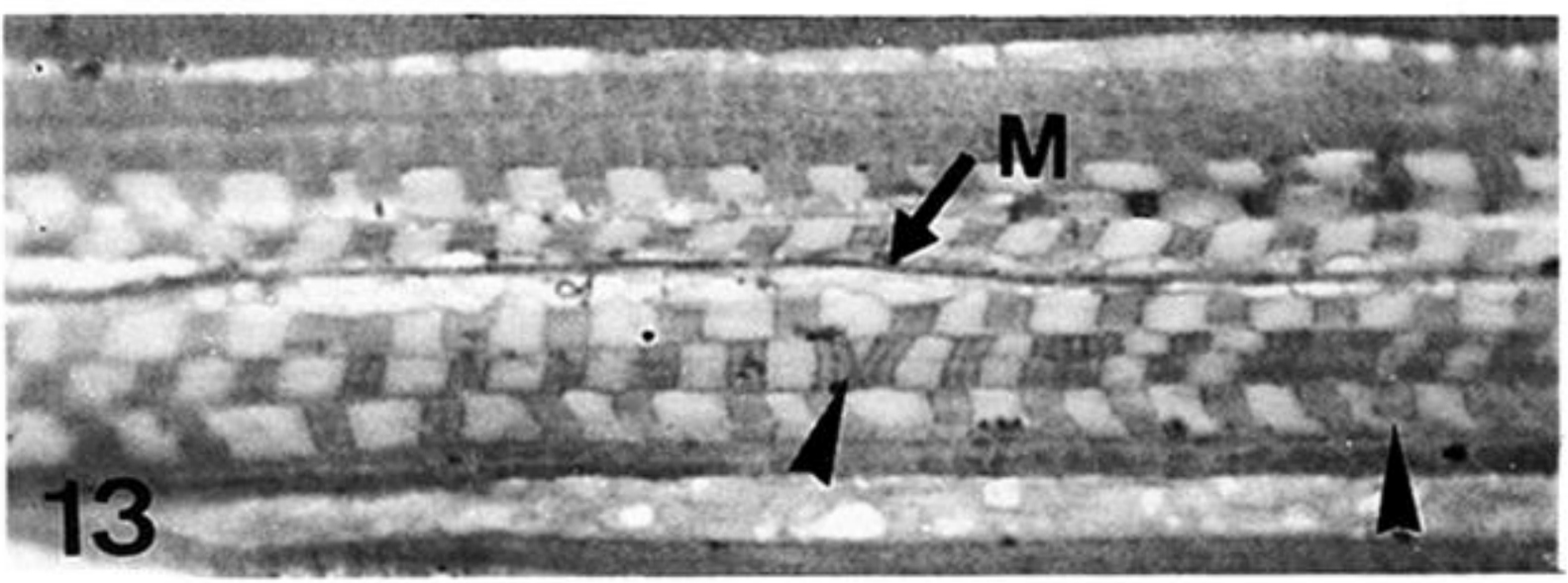
Figure 8. Nerve ending-like structures at the level of a ventral secondary muscle band. Note the 'basement membrane' with a connective cell (*) between the nerve and the muscle; magn. × 22 000.

Figure 9. Cell junctions between secondary muscle fibres: gap junctions (arrows) and columnar junction (arrowhead). Under the 'basement membrane' can be seen the contractile part of the myoepithelial cells (top), which shows a coated vesicle; magn. × 24 800.

Figure 10. Gap junctions between secondary muscle and myoepithelial cells (arrows) and between myoepithelial cells (arrowhead); magn. × 43 400.

Figure 11. At the mesentery level, numerous gap junctions between a secondary muscle fibre and a B fibre (arrowheads); tannic acid treatment, magn. × 39 000.

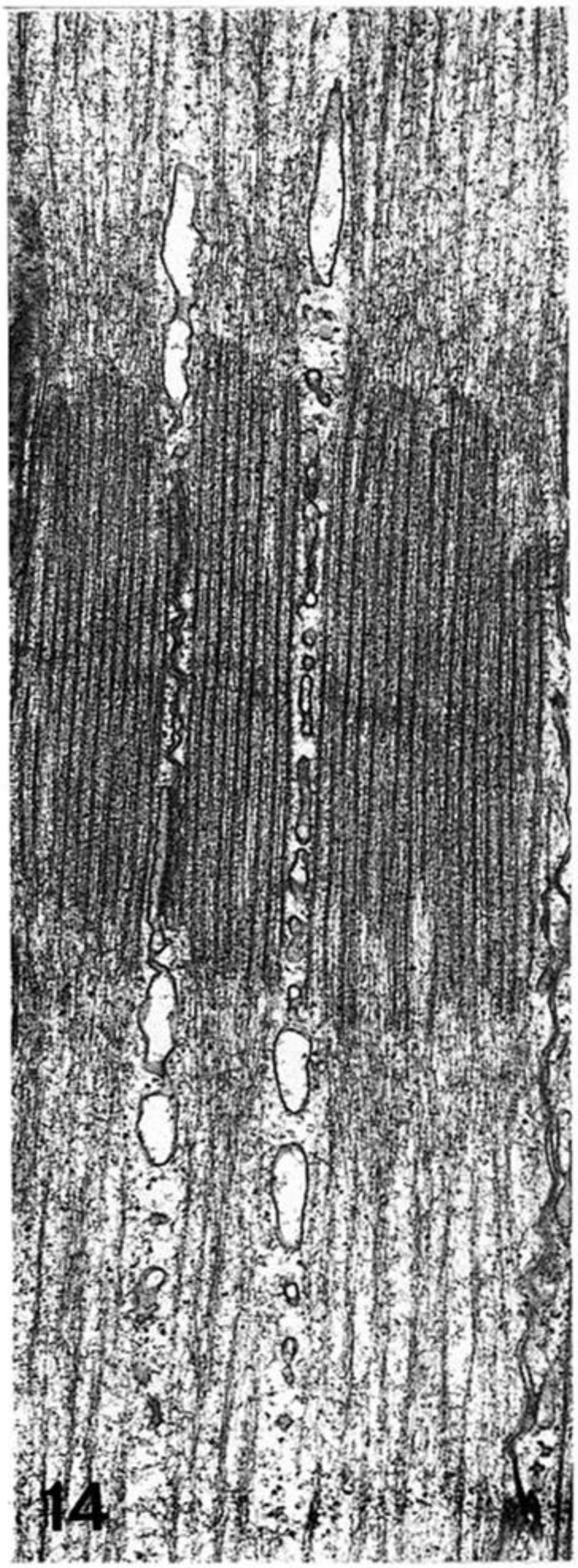


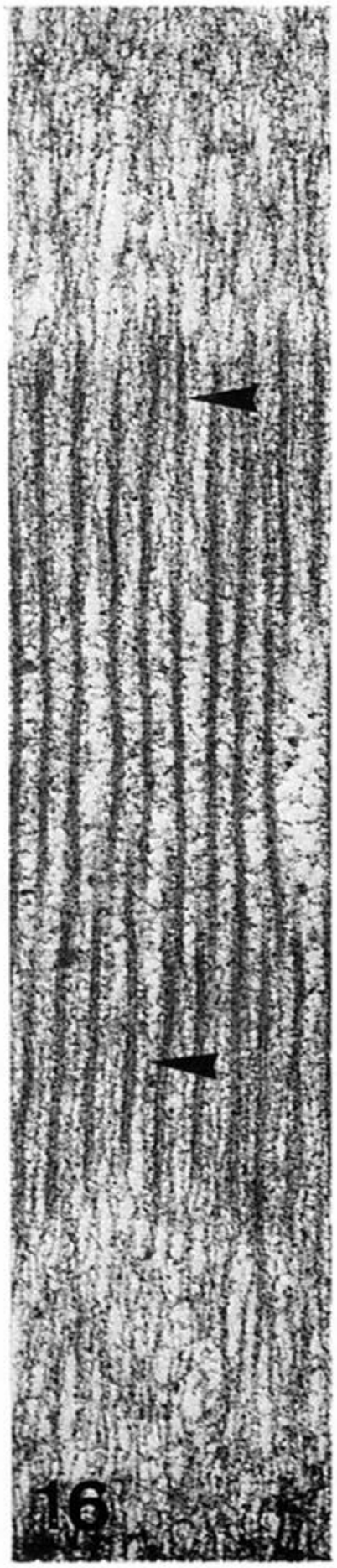


Figures 12 and 13.

Figure 12. Semi-thin longitudinal section in the secondary muscle at resting length-slightly stretched, the primary muscle is at resting-length. The two sarcomere types, s1 and s2, are clearly individualized, they are rarely in lateral register; magn. × 900.

Figure 13. Semi-thin longitudinal section in the secondary muscle where the sarcomeres s1 are contracted; the primary muscle is at resting length. Note the C_Z and C_M bands in s1; the s1 edges are more or less oblique with respect to the longitudinal axis of the myofibril; some s2 sarcomeres are not visible or very reduced (arrowheads); magn. × 900.





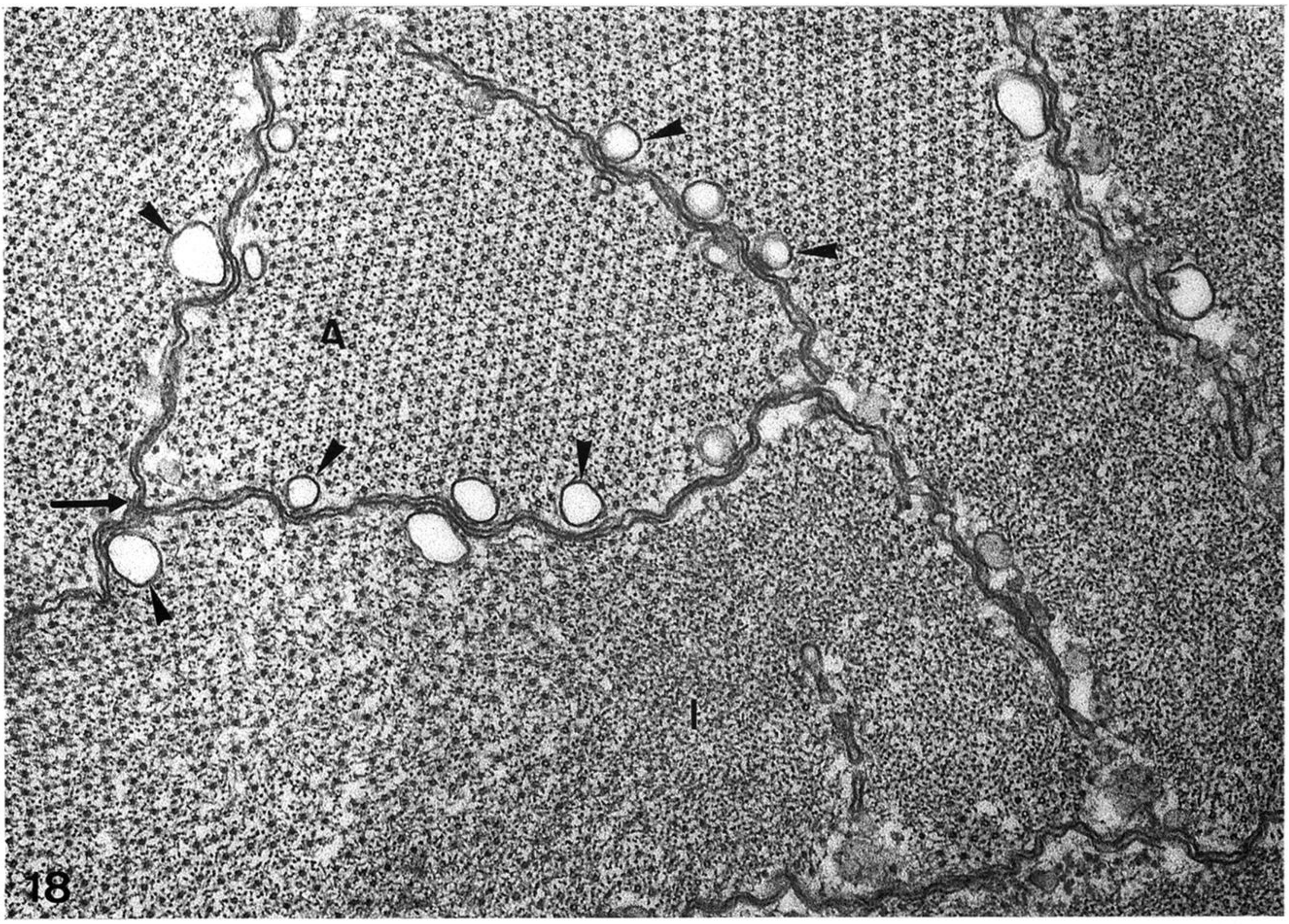
Figures 14–17. s1 sarcomeres: longitudinal sections.

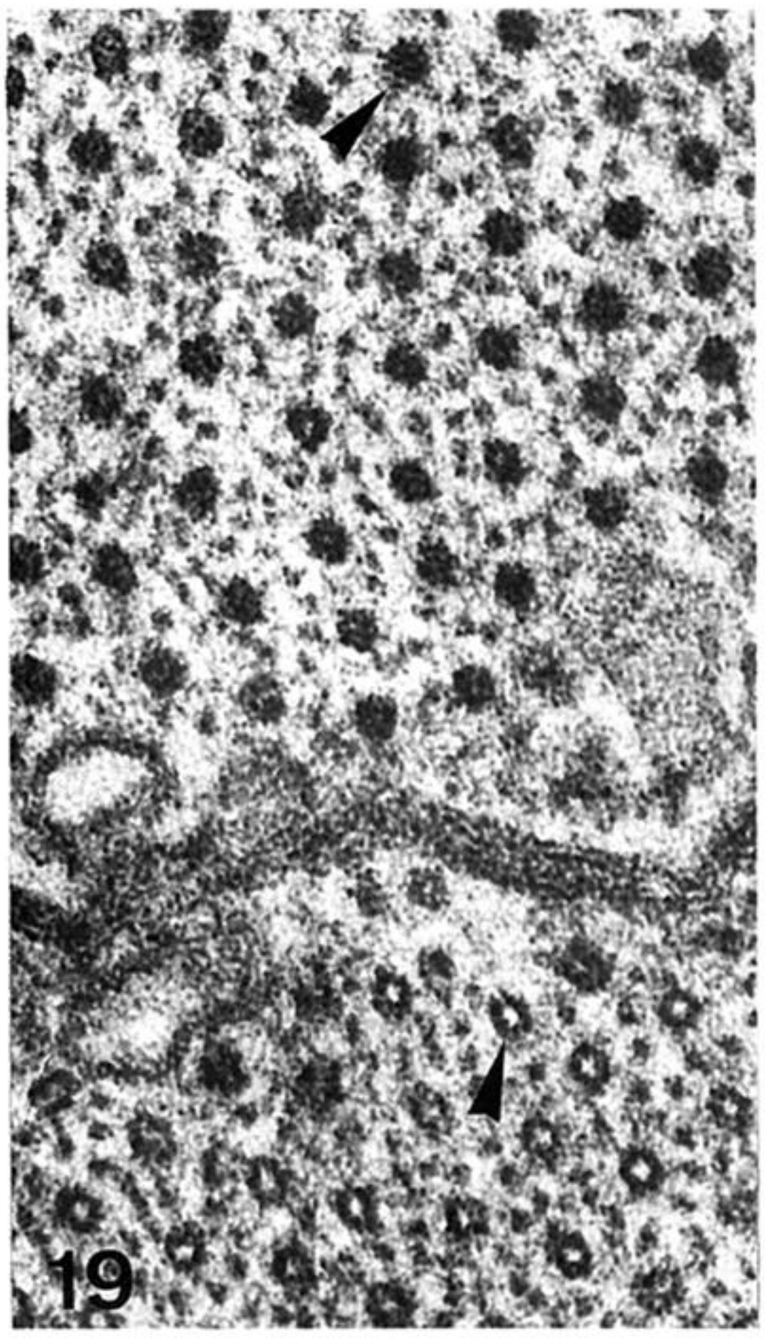
Figure 14. Resting length; in some A bands (right) all thick filaments are seen not to have the same length; magn. × 21 300.

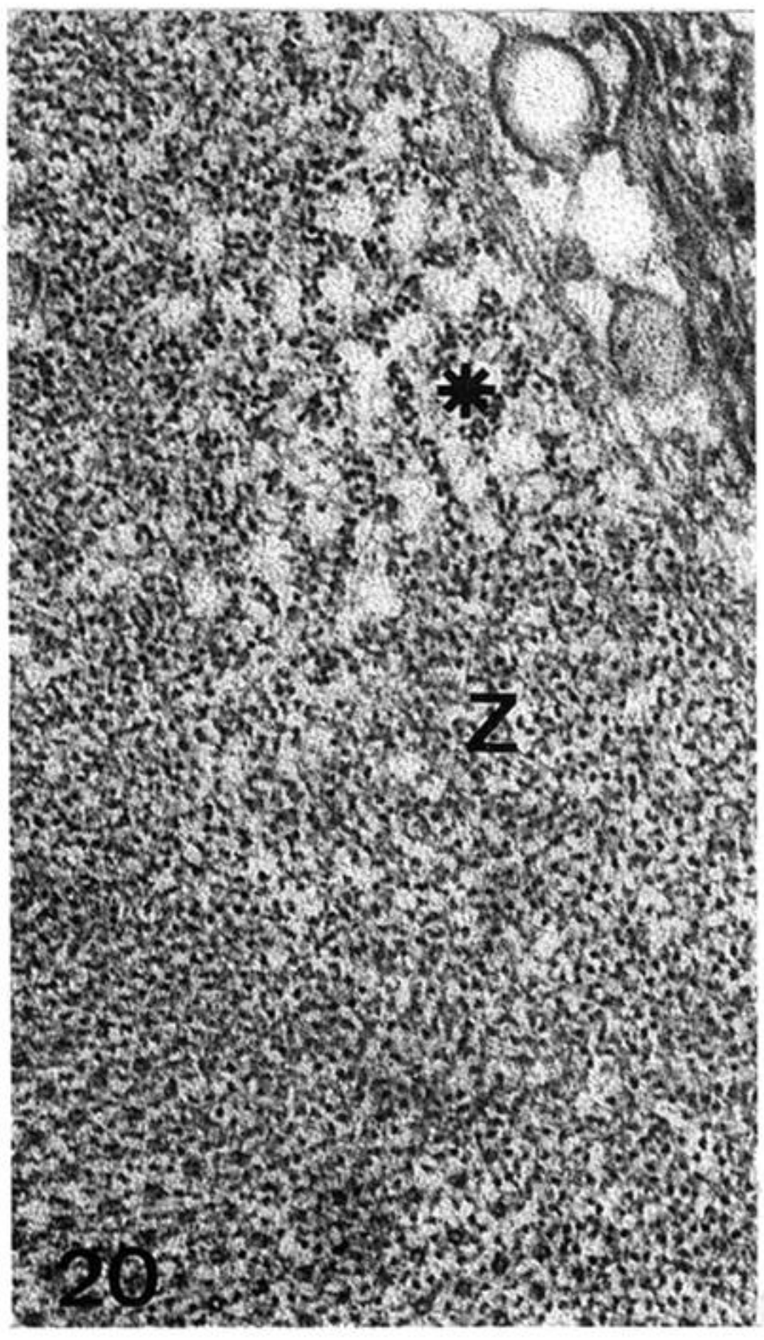
Figure 15. Stretched sarcomere, the Z band is more obvious than at resting length; × magn. 24 500.

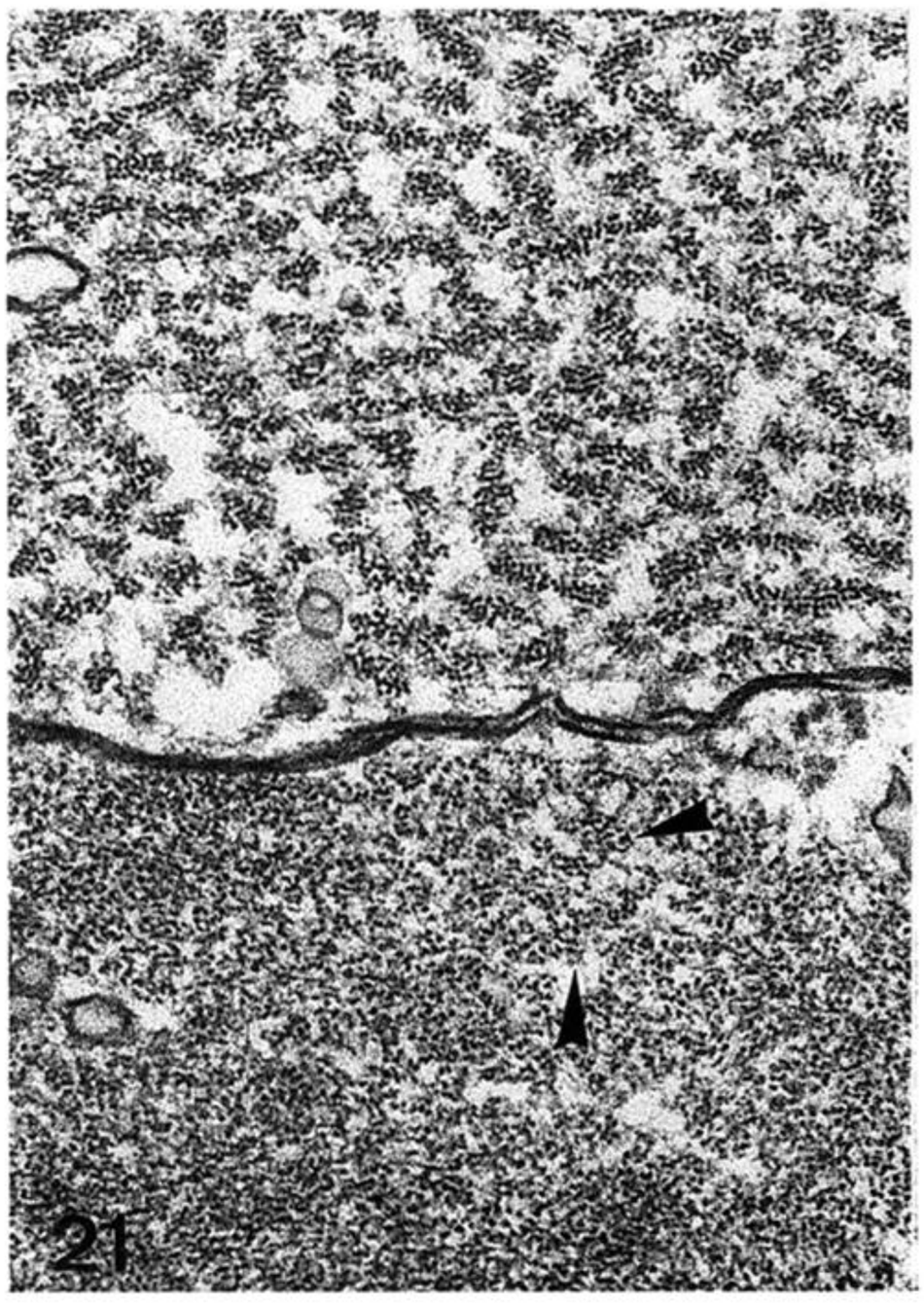
Figure 16. Detail of a stretched sarcomere; no M band is seen, lateral bridges between thick and thin myofilaments are observed (arrowheads); magn. × 39 600.

Figure 17. Contracted sarcomere, the A band shows a loose lateral cohesion between thick filaments; magn. × 31 000.









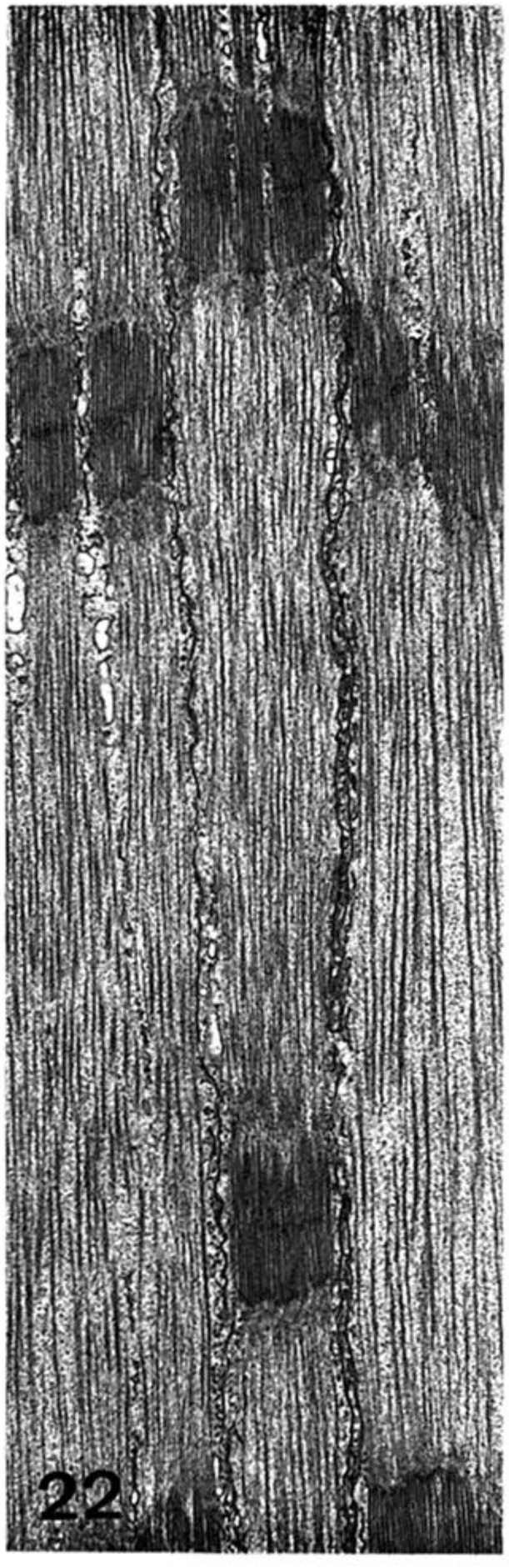
Figures 18–21. s1 sarcomeres: transverse sections.

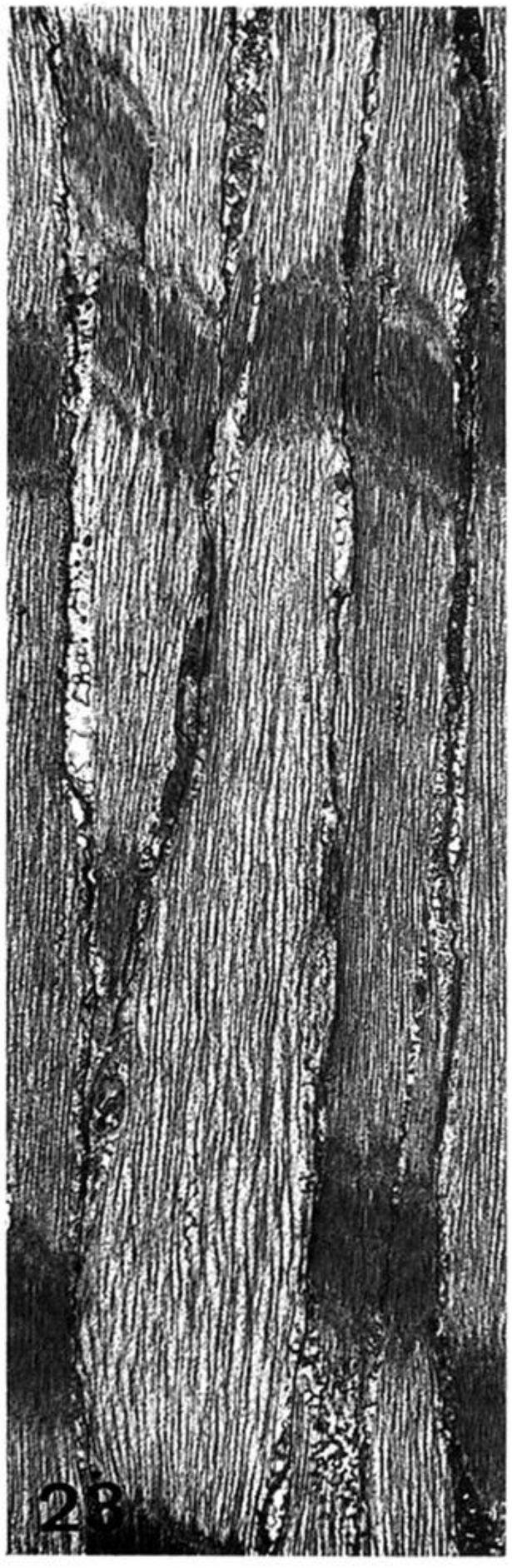
Figure 18. Slightly oblique section showing the A and I bands. Invaginations of the plasma membrane (arrow) penetrate deeply in the contractile apparatus and give rise to myofibril-like units; at their level and at the plasma membrane level, S.R. tubules show many couplings (arrowheads); magn. × 49 000.

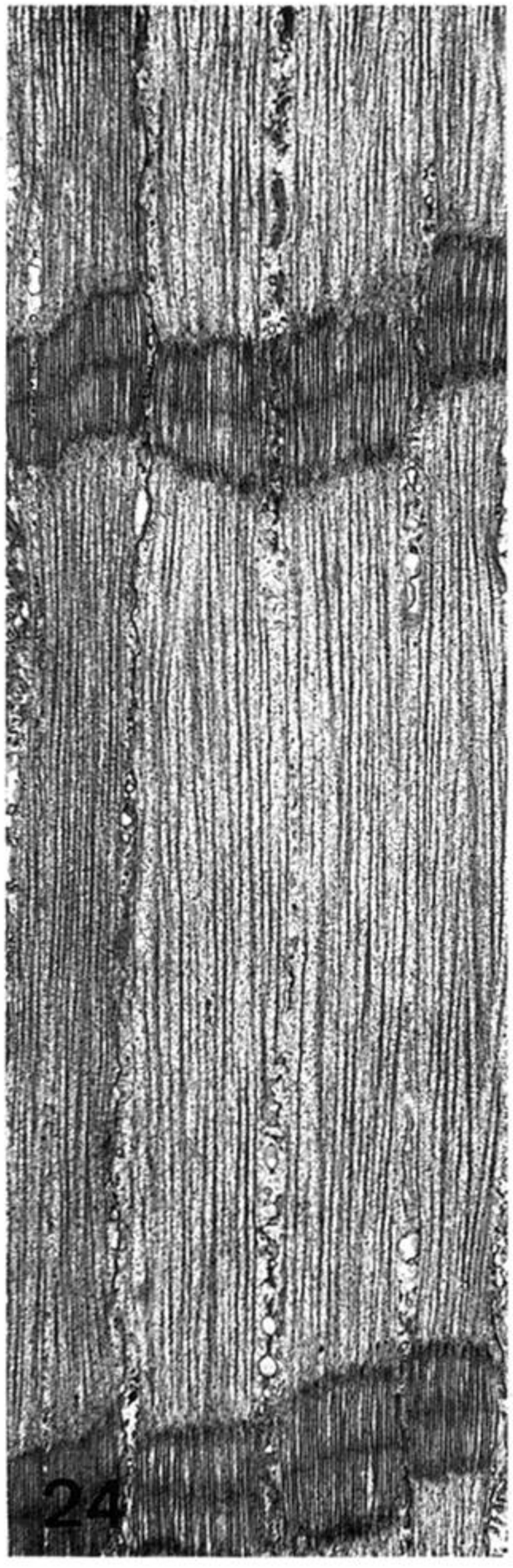
Figure 19. M band (top) and A band (below): note subunits in the thick filaments (arrowheads): magn. × 170 000.

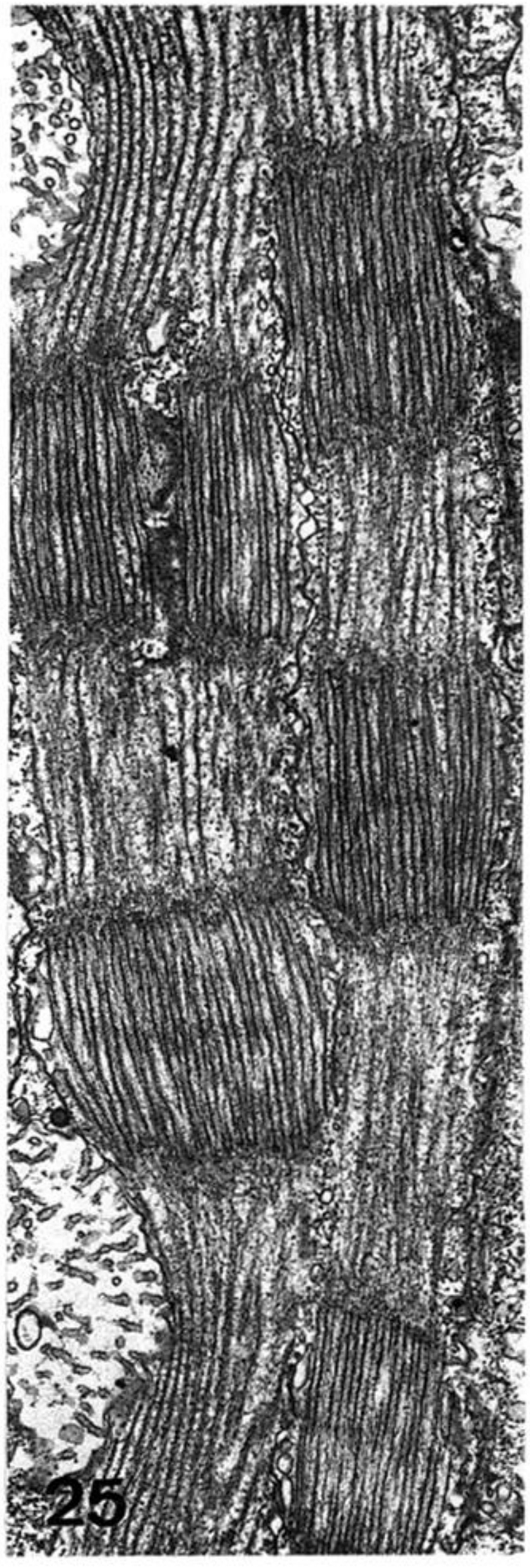
Figure 20. Extremity of an A band; the Z band is not precisely defined, it extends between the A band and the beginning of the s2 sarcomere (asterisk); at least two classes of filaments (dense and light in section) are to be seen; magn. × 61 200.

Figure 21. s1 Z-band (below) and s2 sarcomere (top). Note that in the Z band there is a tendency of the filaments to form clusters (arrowheads). magn. × 52 500.









Figures 22–25. s2 sarcomeres longitudinal sections.

Figure 22. s2 sarcomeres in three fibres where s1 are at resting length or slightly stretched; magn. × 77000.

Figure 23. s2 sarcomeres in five fibres where s1 are stretched; magn. × 5800.

Figure 24. s2 sarcomeres in two fibres where s1 are contracted. In the same fibre, s1 sarcomeres lie in register. Note the irregular length of the s1 sarcomere A bands; magn. × 7700.

Figure 25. s2 sarcomeres with very reduced length in a fibre where s1 are contracted; the s2 band density does not change with respect to the others stretched and/or resting state s2 sarcomeres; magn. × 13900.

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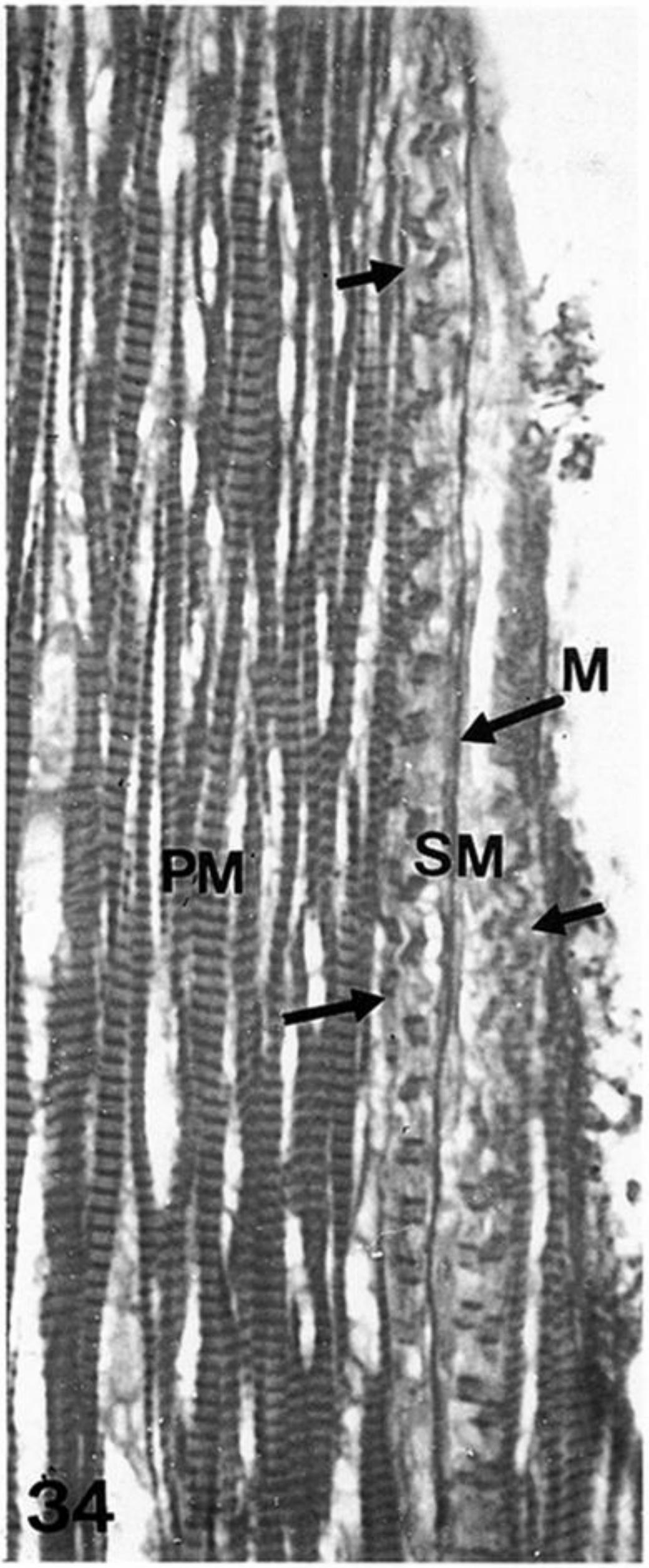
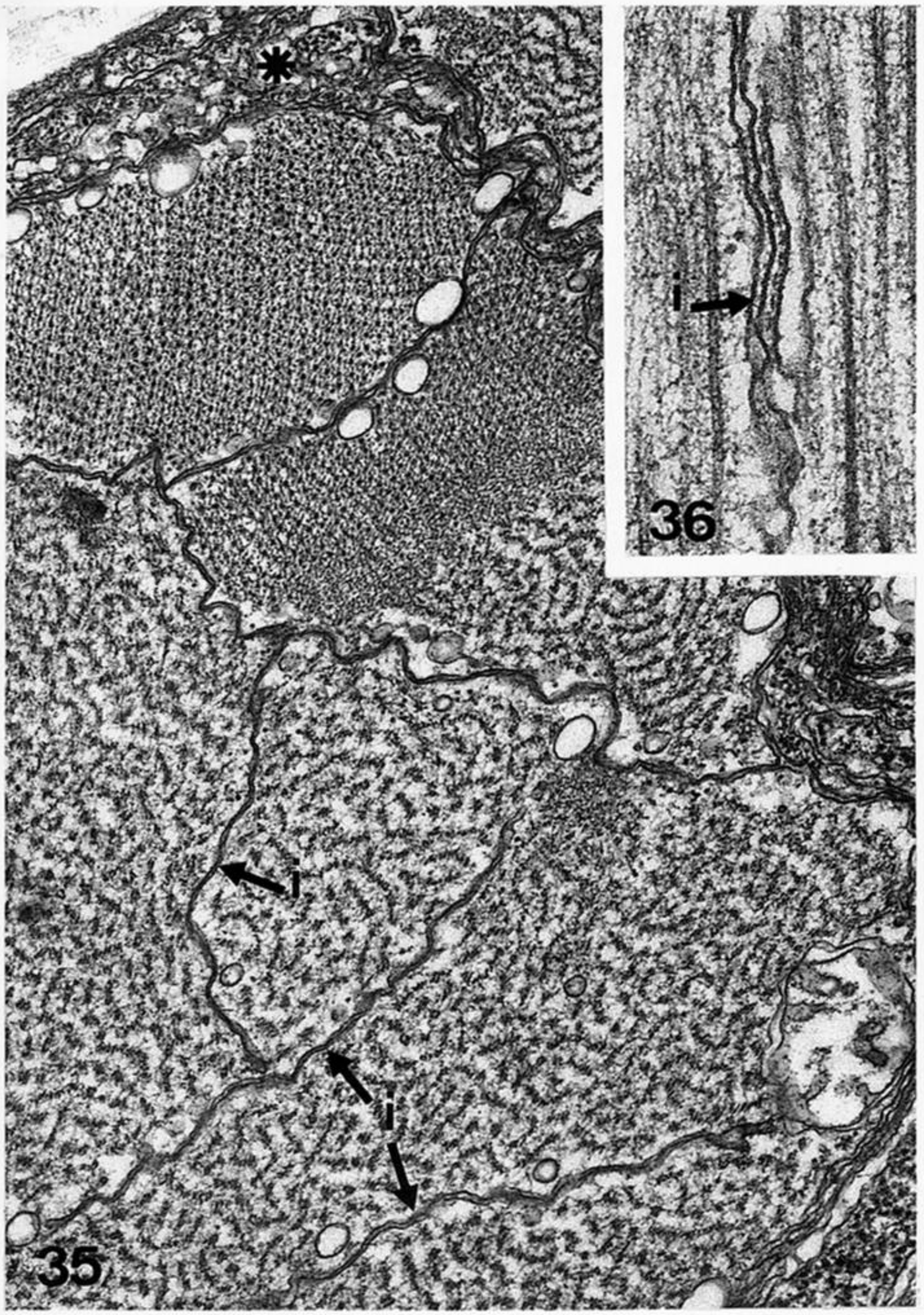
Figures 26–32. For description see opposite.

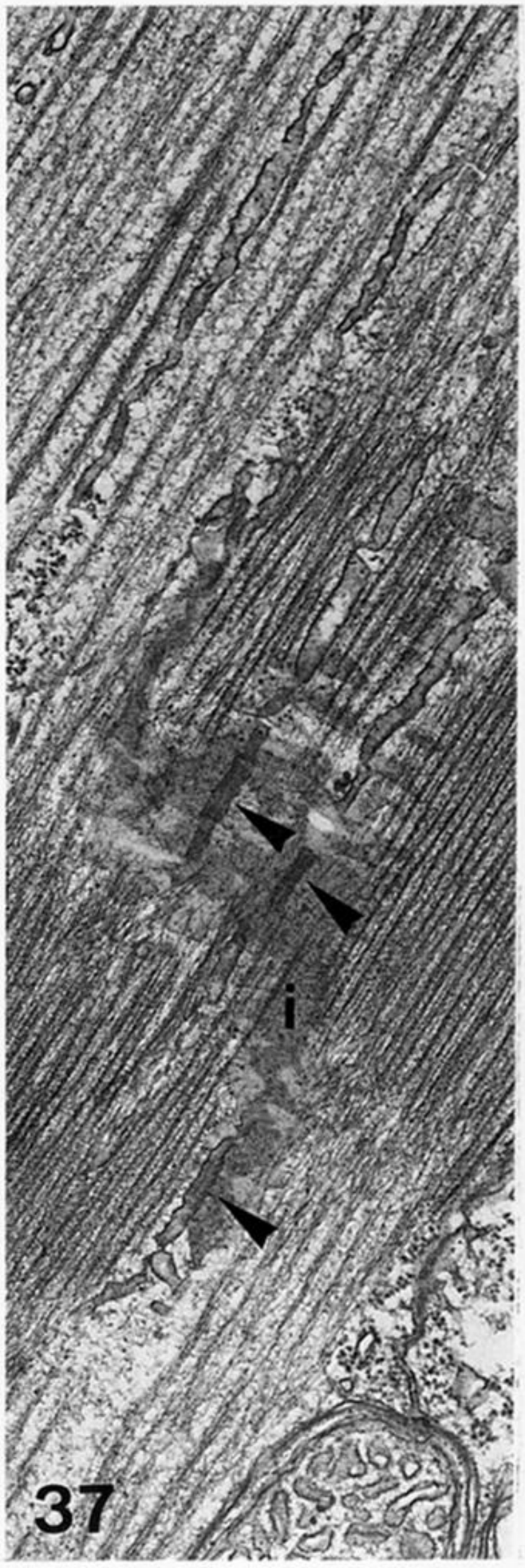


Figures 33 and 34.

Figure 33. Longitudinal section of the same myofibril showing two contiguous s1 sarcomeres. One is slightly contracted (top, with a C_M band), the other is stretched (below, with an obvious H band); magn. × 11400.

Figure 34. Longitudinal semi-thin section showing two secondary muscle bands on each side of the mesentery. The primary muscle is contracted as the s1 secondary muscle sarcomeres. The secondary muscle bundles are pleated (arrows); magn. × 1100.





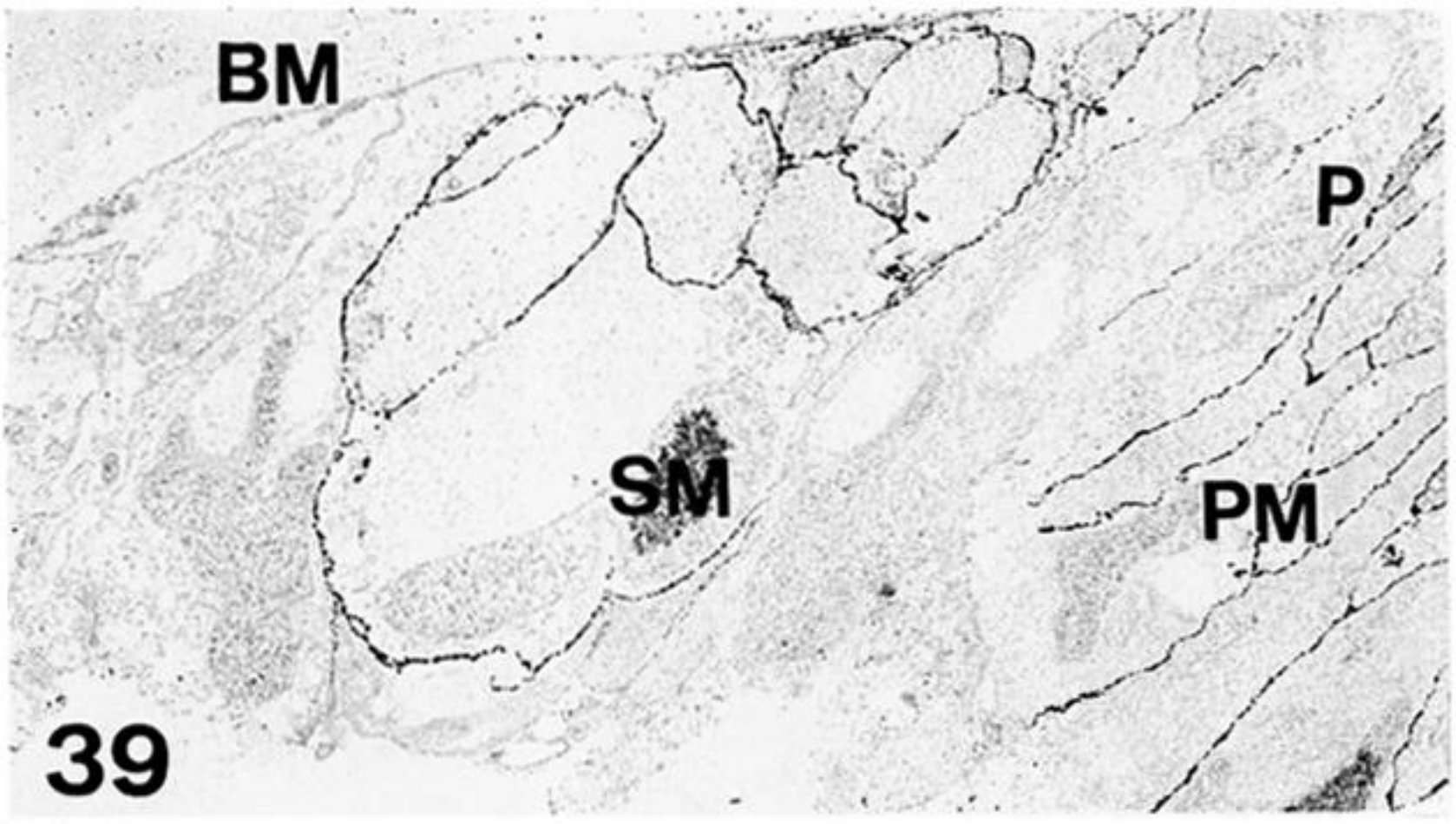
Figures 35–38.

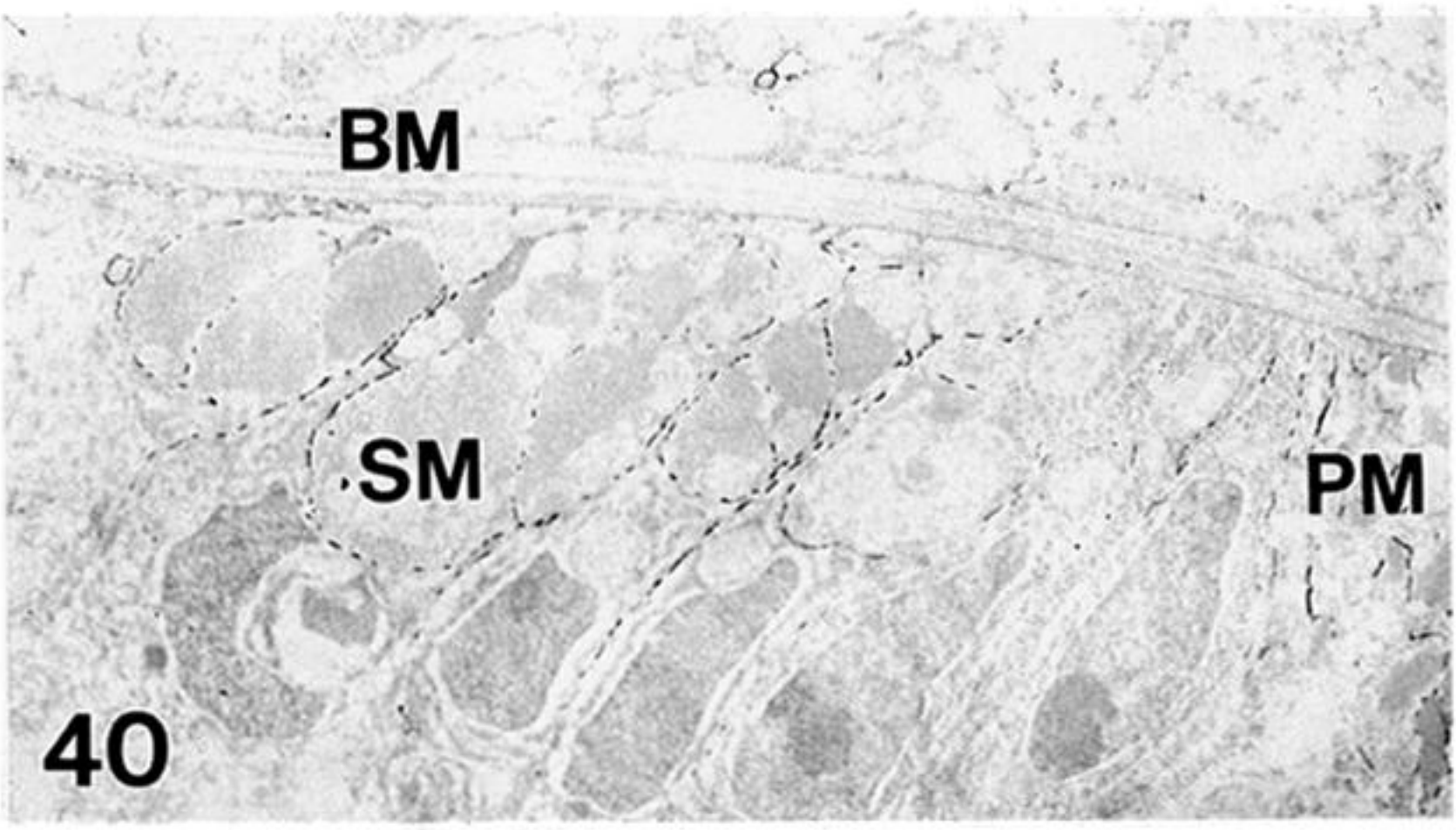
Figure 35. Transverse section showing S.R. longitudinal tubules and plasma membrane invaginations: these two compartments form structural units which are regularly associated with s1 sarcomeres. Invaginations penetrate deeply in the contractile apparatus and forms myofibril-like units. Part of a myoepithelial cell is seen (asterisk); magn. × 25 300.

Figure 36. Perpendicular longitudinal section in an internal coupling. Magn. × 72 000.

Figure 37. Tangential section at the level of s1 sarcomeres, internal couplings show the parallel arrays of junctional feet at the S.R. level (arrowheads); note the longitudinal tubules of S.R. at s2 sarcomere level; magn. × 20 300.

Figure 38. Longitudinal section in a secondary muscle with contracted s1 sarcomeres. Invaginations and S.R. remain associated with s1; magn. × 14 300.





Figures 39–41. Transverse sections in ventral secondary muscle bands.

Figure 39. Ca^{2+} ATPase activity at the level of external membranes (plasma membrane and invaginations); the same activity is shown in the primary muscle (right) but not in the myoepithelial cells; magn. × 4500.

Figure 40. Lanthanum localization on the secondary muscle (centre) and primary muscle (right) cell membranes. Again, myoepithelial cells show low or no reactivity; magn. × 3600.

Figure 41. Pyroantimonate treatment after incubation in artificial sea water containing twice (20 mM) Ca as in normal sea water. Deposits are dense at the plasma membrane/invagination levels, and, probably in mitochondria (arrowhead); magn. × 4900.