Phil. Trans. R. Soc. Lond. B. **265**, 223–229 (1973) [223]
Printed in Great Britain

Filament organization in vertebrate smooth muscle

By A. P. Somlyo, C. E. Devine,† Avril V. Somlyo and R. V. Rice
The Departments of Pathology, Presbyterian-University of Pennsylvania Medical Center, 51 North Thirtyninth Street, Philadelphia, Pennsylvania, 19104, and the School of Medicine, University of Pennsylvania; the Carnegie–Mellon University, Pittsburgh, Pennsylvania, U.S.A.

[Plates 27 to 31]

Thin (actin), thick (myosin) and intermediate filaments are described in vertebrate smooth muscle. The thick filaments are present in relaxed, contracted, stretched and unstretched vertebrate smooth muscle and bear lateral projections suggestive of cross-bridges. The relatively regular thick filament lattice of the rabbit portal-anterior mesenteric vein can be aggregated by hypertonic solutions and excessive stretch. The intermediate filaments are morphologically distinct and clearly not breakdown products of thick filaments.

The ultrastructural organization of contractile proteins in vertebrate smooth muscle has been, until recently, uncertain and highly controversial. The major unsettled question has been whether, in living smooth muscle, myosin is dispersed or organized into thick filaments (or some other structures), or whether thick filaments are absent from relaxed muscle and are assembled only at the time of contraction. The experimental basis of the conflicting theories was a negative observation: the inability of several laboratories to consistently demonstrate thick filaments in numbers that would account for the biochemically demonstrable myosin content of vertebrate smooth muscles (for review see Needham & Shoenberg 1967; Somlyo & Somlyo 1968; Burnstock 1970). Thus, although the biochemical evidence and the characteristics of the length–active tension curve of vertebrate smooth muscle were highly suggestive of a sliding filament mechanism of contraction (Needham & Shoenberg 1967; Somlyo & Somlyo 1968), until recently, definitive ultrastructural evidence including the consistent demonstration of cross-bridge bearing thick filaments in vertebrate smooth muscle has been lacking.

In the following, some of the ultrastructural evidence favouring the view that myosin is organized into thick filaments in vertebrate smooth muscle will be presented. We shall attempt to show that thick filaments are stable structures in vertebrate smooth muscles, and failures to visualize them are due to inadequate preparatory techniques for electron microscopy, and that they may be aggregated into ribbon-like structures under unphysiological conditions. We shall also present some evidence regarding a third, intermediate width filament in vertebrate smooth muscles: such round, intermediate filaments are neither break-down products of organized myosin nor aggregation products of the thin, actin filaments.

Methods

Smooth muscle preparations, unless otherwise stated, were dissected from the animals, stretched to approximately 1.5 times their excised length, and incubated for half an hour in a modified Krebs solution before fixation. Tissues were fixed (*a*) in 2 to 3 % glutaraldehyde in

† Present address: Wellcome Medical Research Institute, University of Otago, Dunedin, New Zealand.

0.075 mol/l cacodylate with 4.5 % sucrose for 2 h, followed by 2 % osmium in 0.05 mol/l cacodylate buffer for 2 h; (*b*) in a 3 % glutaraldehyde–2 % formaldehyde–1 % acrolein fixative (Kalt & Tandler 1971) modified for smooth muscle ('trialdehyde fixation', Somlyo & Somlyo 1973) followed by 2 % osmium; or (*c*) fixed directly in 2 % osmium. Following fixation the strips were block-stained with saturated aqueous uranyl acetate for 1 to 2 h, dehydrated in graded alcohols and embedded in Spurr's resin (Spurr 1969). (Details of the methods have been previously described in Devine & Somlyo 1971; Rice, McManus, Devine & Somlyo 1971; Somlyo, Somlyo, Devine & Rice 1971*a*; Somlyo, Devine, Somlyo & North 1971*b*; Somlyo, Devine & Somlyo 1971; Devine, Somlyo & Somlyo 1972). The filament diameters cited are ranges of two standard deviations about the mean of 65 to 70 measurements each, obtained from trialdehyde-fixed material.

Thin filaments

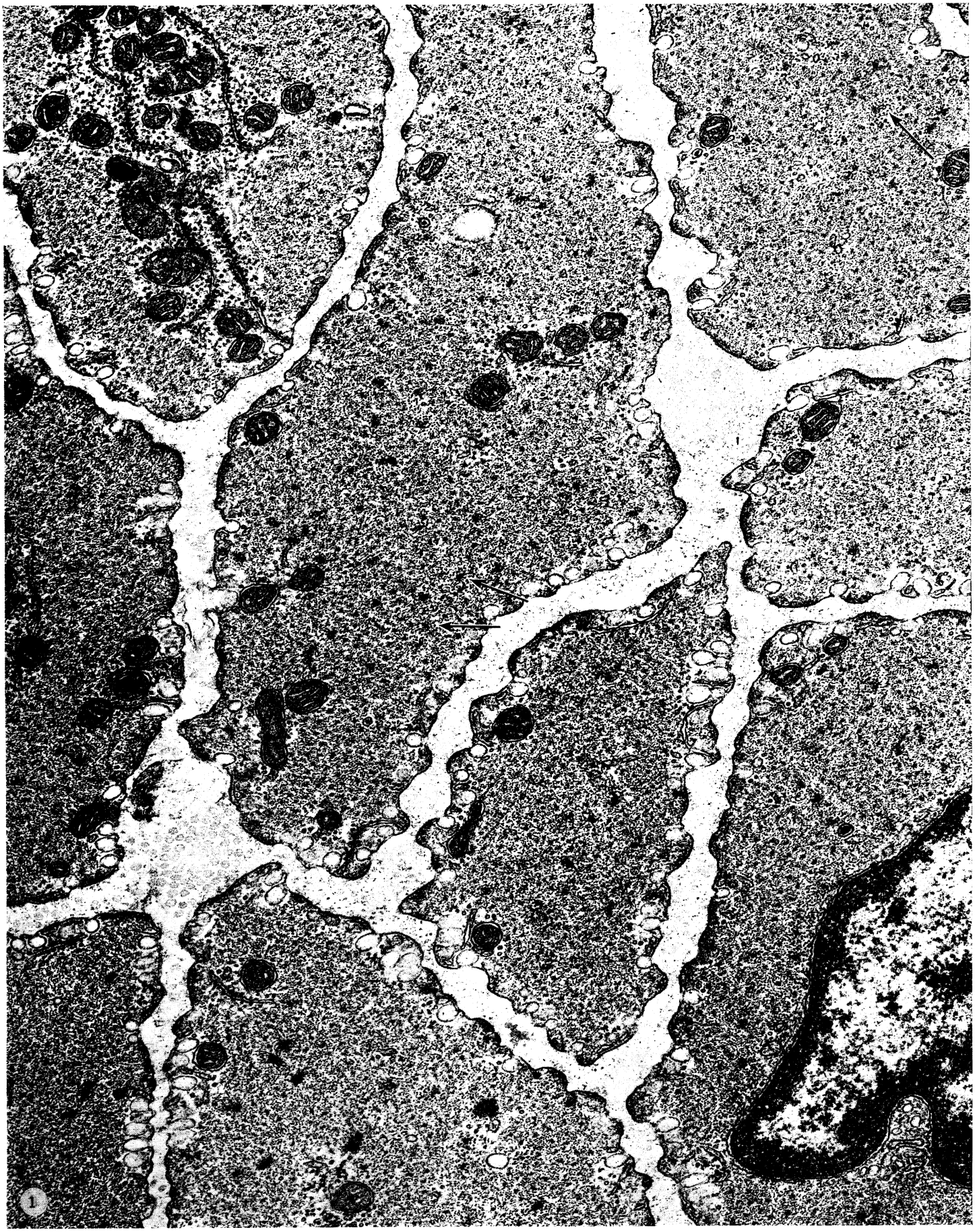
Thin (4.8 to 8.0 nm in diameter) filaments were found in greatest numbers in normal smooth muscle fibres. The thin:thick filament ratio, in the best ordered preparations, was approximately 15:1. In several smooth muscles examined in detail (e.g. rabbit main pulmonary artery, portal-anterior mesenteric vein) rosettes consisting of approximately fifteen thin filaments surrounding a thick filament were found (figures 1 and 2). Two to three rows of regularly alined thin filaments, running between adjacent rows of thick filaments, were also seen. In some exceptionally well-oriented sections, the appearance of two rows of thin filaments seemed to be due to the alinement of the thin filaments belonging to the orbits of two adjacent thick filaments (figure 2). Less commonly, large groupings of thin filaments are packed in an approximately hexagonal fashion (Rice *et al.* 1970; Heumann 1970; Somlyo *et al.* 1971*b*). Such large, regular groupings of thin filaments, without accompanying thick filaments, may be analogous to the I-bands of striated muscles. Preservation of the thin filaments is generally better after prefixation with an aldehyde than after primary fixation with osmium (figure 3). The identification of thin filaments with the organized form of actin (Hanson & Lowy 1963) is generally accepted.

The centre to centre spacing of the thin to thick filaments in our best preparations, including the most regular rosettes and rows adjacent to thin filaments, was 25.7 nm ($\pm$ 5.9 nm s.d., 40 measurements), corresponding to a (computed) surface to surface distance of approximately 14.7 nm.

The thin filaments enter dense bodies (Devine & Somlyo 1971): this relationship and the characteristic position of some of the dense bodies at the plasma membrane (e.g. figure 5 in Devine *et al.* 1972) is consistent with the view that the dense bodies are functionally analogous to the Z-bands of striated muscles.

Intermediate filaments

Filaments with a relatively regular, round profile and approximately 10 nm in diameter (7.7 to 11.7 nm in the present series) have been observed in a variety of smooth muscles (Rice *et al.* 1970; Lowy & Small 1970; Cooke & Chase 1971; Somlyo *et al.* 1971*a*; Uehara, Campbell & Burnstock 1971), and are readily distinguishable from the thin and thick myofilaments (figures 1 and 2). In transverse section the centre of the round filaments is sometimes more



All illustrations, unless noted, are unpublished electron micrographs of rabbit portal-anterior mesenteric vein, taken by A. P. Somlyo.

FIGURE 1. Transverse section of a bundle of smooth muscle fibres illustrating the regular spacing of the thick filaments and the relatively large number of thin myofilaments. Several groups of intermediate filaments (some shown by arrows) associated with dense bodies are present. This preparation was incubated for half an hour, under physiological stretch, in a Na-free, Ca-free, 230 mmol/l sucrose substituted solution before glutaraldehyde fixation. (Magn. × 34000.)

(*Facing p.* 224)

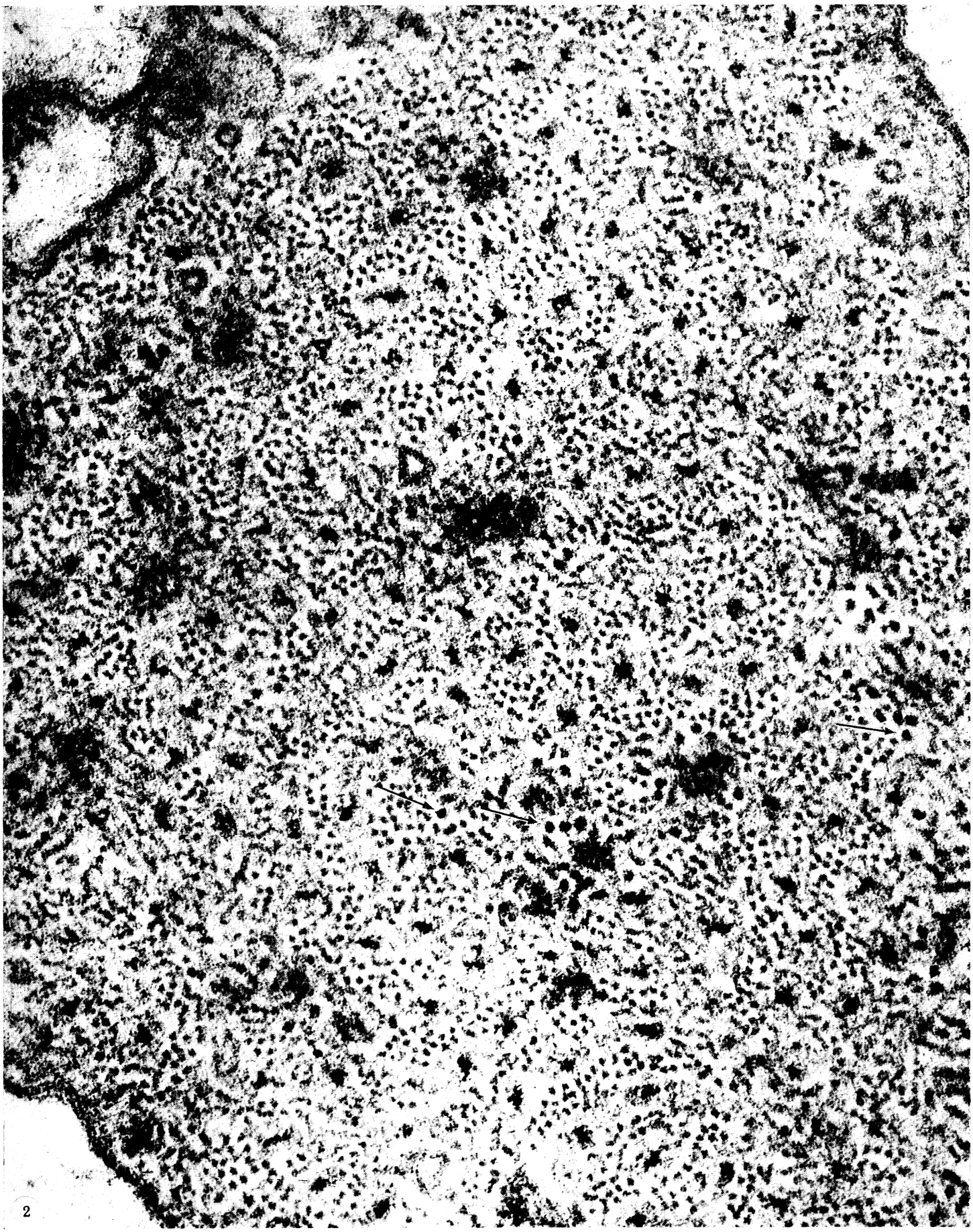


FIGURE 2. Transverse section of a regular arrangement of thick and thin myofilaments with a number of rosettes present. Some of the adjacent thick filaments are connected by an amorphous grey substance. Intermediate filaments (arrows) at the periphery of the dense bodies are also present. (Glutaraldehyde fixation; magn. × 220000; from Somlyo *et al.* 1971*a*.)

electron lucent than the darkly staining peripheral portion of the filament (Rice *et al.* 1970; Somlyo *et al.* 1971*a*). In longitudinal sections bundles of intermediate filaments can be followed over several micrometers (Somlyo *et al.* 1971). The intermediate filaments are more consistently preserved during fixation with osmium alone (figure 3) than are the thin filaments (Somlyo *et al.* 1971*a*). Intermediate filaments survive extraction (Cooke & Chase 1971) or swelling of the smooth muscle fibres induced by equimolar substitution of KCl for NaCl (Jones, Somlyo & Somlyo, in preparation): procedures that disperse the thin and thick filaments or interfere with their preservation for electron microscopy. It is probable that these intermediate filaments are composed of neither actin nor myosin: preliminary studies suggest that they contain a major protein of molecular mass about 81000 (Rice 1972). In transverse section of intact fibres the intermediate filaments are often assembled around the periphery of dense bodies (Somlyo *et al.*; 1971; Lowy & Small 1970) and the two structures remain associated after extraction from the fibres (Cooke & Chase 1971; Rice 1972). The intermediate filaments associated with dense bodies may function in a manner analogous to the cytoskeleton of the obliquely striated invertebrate muscles (Somlyo *et al.* 1971*a*).

Intermediate filaments that are not clearly organized around the periphery of dense bodies occur, with varying frequency, in the centre of smooth muscle fibres. In extreme cases, the intermediate filaments may occupy a large proportion of the cross-section of the cell, leaving small islands of thin and thick myofilaments (figure 4). Comparison of the intermediate with the thin and thick myofilaments in such fibres clearly indicates the distinguishing morphological features of the three types of filaments, eliminating the possibility that apparent differences in intermediate, thin and thick filaments may have been due to fortuitous variations in fixation. Intermediate filaments have been observed in large numbers in cultured and developing smooth muscle (Uehara *et al.* 1971), and the extreme examples of adult smooth muscle fibres containing large masses of intermediate filaments (figure 4) may represent a response to injury. It is clear, however, that the intermediate filaments are not disaggregation products of the thick (myosin) filaments.

Thick filaments

Thick filaments (in the present series 13.5 to 17.5 nm in diameter) have been observed initially in small numbers (Nonomura 1968) or not altogether consistently (in every preparation) in vertebrate smooth muscle (Kelly & Rice 1969; for review see Somlyo & Somlyo 1968; Burnstock 1970). Optimal and, within the limitations of electron microscopy, consistent visualization of thick filaments was found to be facilitated by incubating smooth muscle (stretched to approximately L_0) in a warm, oxygenated physiological salt solution (Devine & Somlyo 1971; Garamvölgyi, Vizi & Knoll 1971). However, neither contraction nor stretch is essential for the preservation of thick filaments (Devine & Somlyo 1971; Rice *et al.* 1971; Somlyo *et al.* 1971; Cooke & Fay 1972), and they are a consistent feature of stretched, unstretched (figure 8), contracted and relaxed vertebrate smooth muscle. In transverse section the thick filaments have a somewhat irregular profile and at high magnification the presence of subunits can be resolved (Devine & Somlyo 1971).

Measurements of longitudinal sections of thick filaments (figure 5) are subject to considerable uncertainty due to the difficulty of properly orienting the smooth muscle fibres. In examining thicker sections, end to end overlap of two thick filaments within the focal plane may lead to overestimation of filament length. Measurements of both the longest thick filaments fortuitously

included in ultrathin (grey) sections and of maximal filament length in lower magnification views of thicker sections, suggest that the length of thick filaments in rabbit portal-anterior mesenteric vein is approximately 1.5 μm.

There are approximately 160 thick filaments per square micrometre cytoplasmic area (excluding nuclei and mitochondria) in the rabbit portal-anterior mesenteric vein, and the distribution of thick filaments in the guinea-pig portal vein is also similar (Somlyo *et al.* 1971). The thick filaments are not arranged in a completely random fashion (figure 1), but are separated from each other at a usual spacing of approximately 60 × 80 nm forming a quasi-rectangular lattice (Rice *et al.* 1971). In transverse section an amorphous material is sometimes seen connecting some of the adjacent thick filaments (figure 2). The possibility has been considered (Somlyo *et al.* 1971*a*), that this material is analogous to the M-substance of striated muscle (Pepe 1971), but this suggestion remains to be verified by more specific identification of this material in smooth muscle. The myosin content of the different smooth muscles may be variable, because of the varying amount of connective tissue and non-filamentous elements (e.g. mitochondria and sarcoplasmic reticulum) in different preparations. In the large elastic arteries, myofilaments occupy a smaller area of the fibre cross-section and the proportion of sarcoplasmic reticulum and mitochondria is relatively large (Devine *et al.* 1972).

Cross-bridges are not as readily identified at low magnifications in smooth muscles as in striated muscles. The less regular alinement and greater spacing between adjacent thick filaments in smooth muscle would not be expected to produce the fine axial period due to the cross-bridges in striated muscles (Huxley 1957; Reedy 1968). Furthermore, after conventional glycerination, vertebrate smooth muscle is not preserved adequately for electron microscopy (Shoenberg 1969; A. P. Somlyo, unpublished observation). In non-glycerinated smooth, as in striated (Huxley 1957), muscles the background granularity due to precipitated soluble proteins makes the clear identification of cross-bridges difficult. Nevertheless, electron micrographs of ultrathin sections of our best preserved material (figures 6 and 7) show lateral projections suggestive of a periodicity on the thick filaments. The presence of cross-bridges in vertebrate smooth muscle is also supported by the 14.4 nm meridional X-ray diffraction recently observed in taenia coli (Lowy, Poulsen & Vibert 1970). In transverse sections of selected rosettes there is also a suggestion of cross-bridges, but a definitive identification of these structures is difficult. The imperfect preservation of this non-glycerinated material and the lack of precise information about section thickness do not permit us to decide the significance of the occasional 'flared' appearance of cross-bridges in transverse sections.

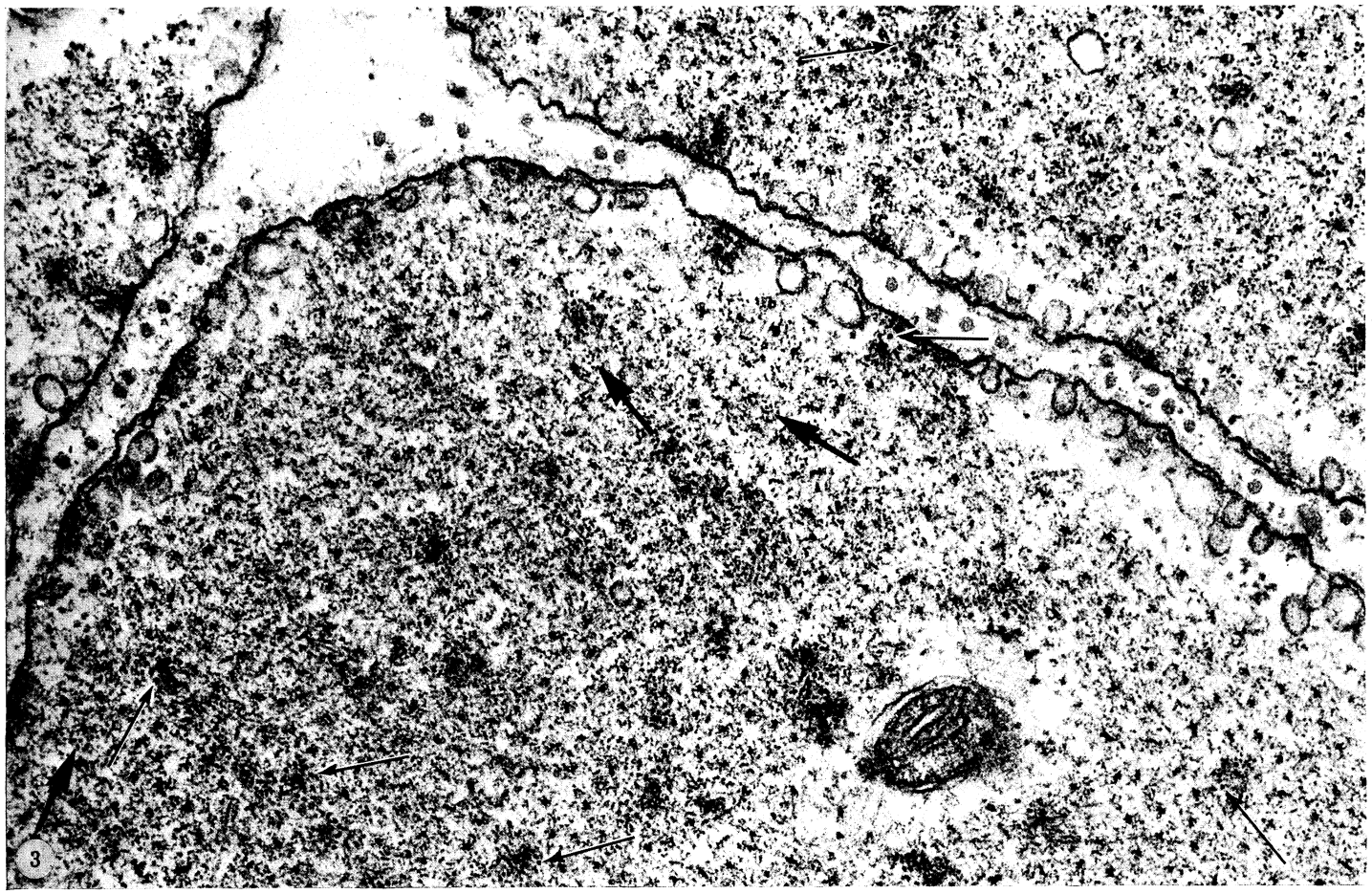
Previous difficulties in demonstrating thick filaments in vertebrate smooth muscle were presumably due to suboptimal preparatory techniques. Swelling (gain in NaCl and water) of smooth muscle during dissection is probably a particularly common deleterious factor con-

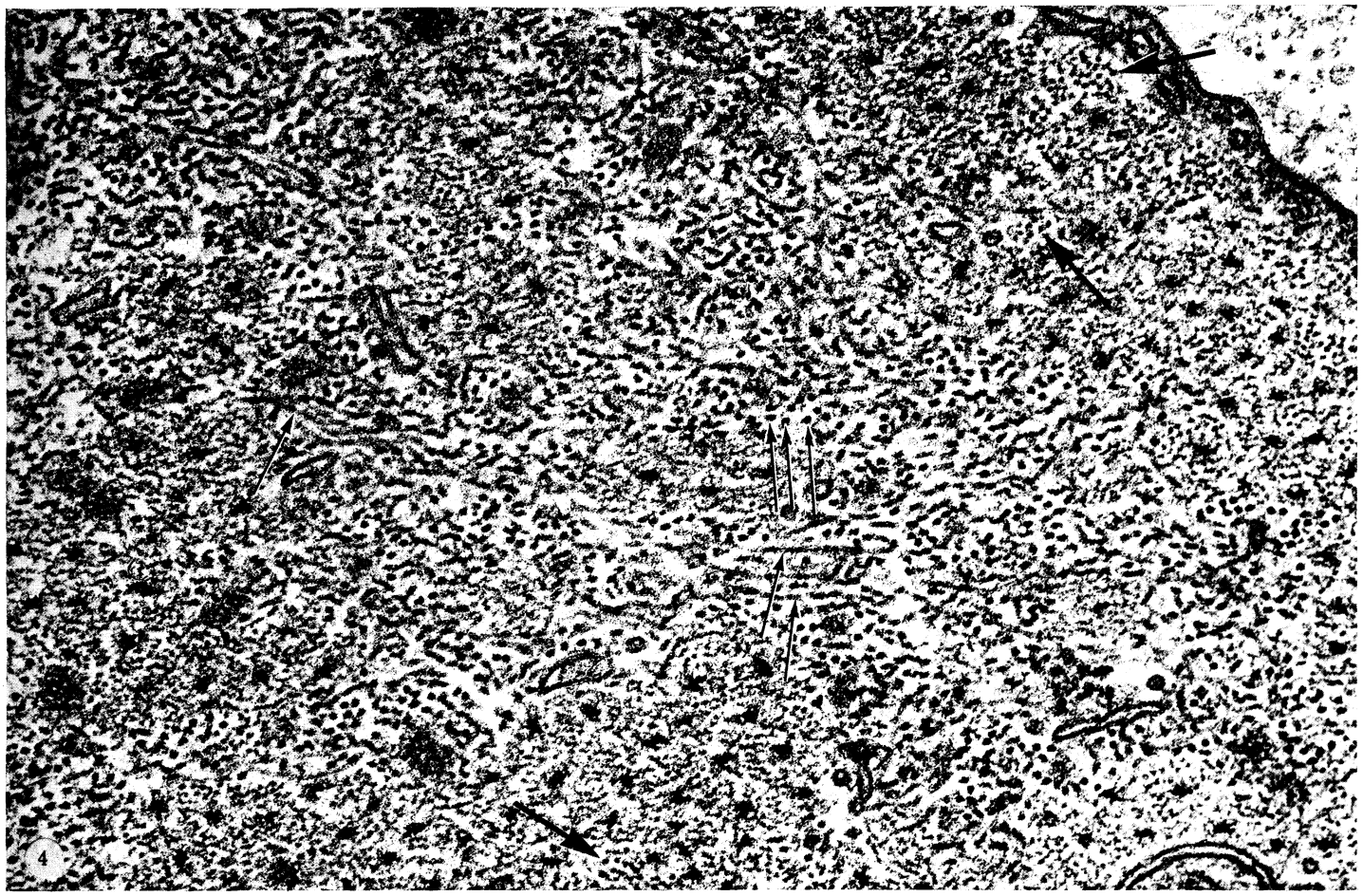
---

Description of plate 29

Figure 3. Transverse section of smooth muscle after primary fixation with osmium. The spacing of the thick filaments is well preserved and the intermediate filaments (small arrows) associated with dense bodies are present, but the thin filament profiles (large arrows) are indistinct. (2% osmium fixation; magn. × 65000.)

Figure 4. Near transverse section of a smooth muscle fibre containing an abnormally large number of intermediate filaments (small arrows) that occupy much of the central portion of the electron micrograph and are seen in both transverse section and obliquely passing through the plane of section. Comparison with the regions occupied by the thick and thin myofilaments (large arrows) shows the clear difference between these three types of filaments. (Glutaraldehyde fixation; magn. × 93000.)





FIGURES 3 AND 4. For legends see facing page.

(Facing p. 226)

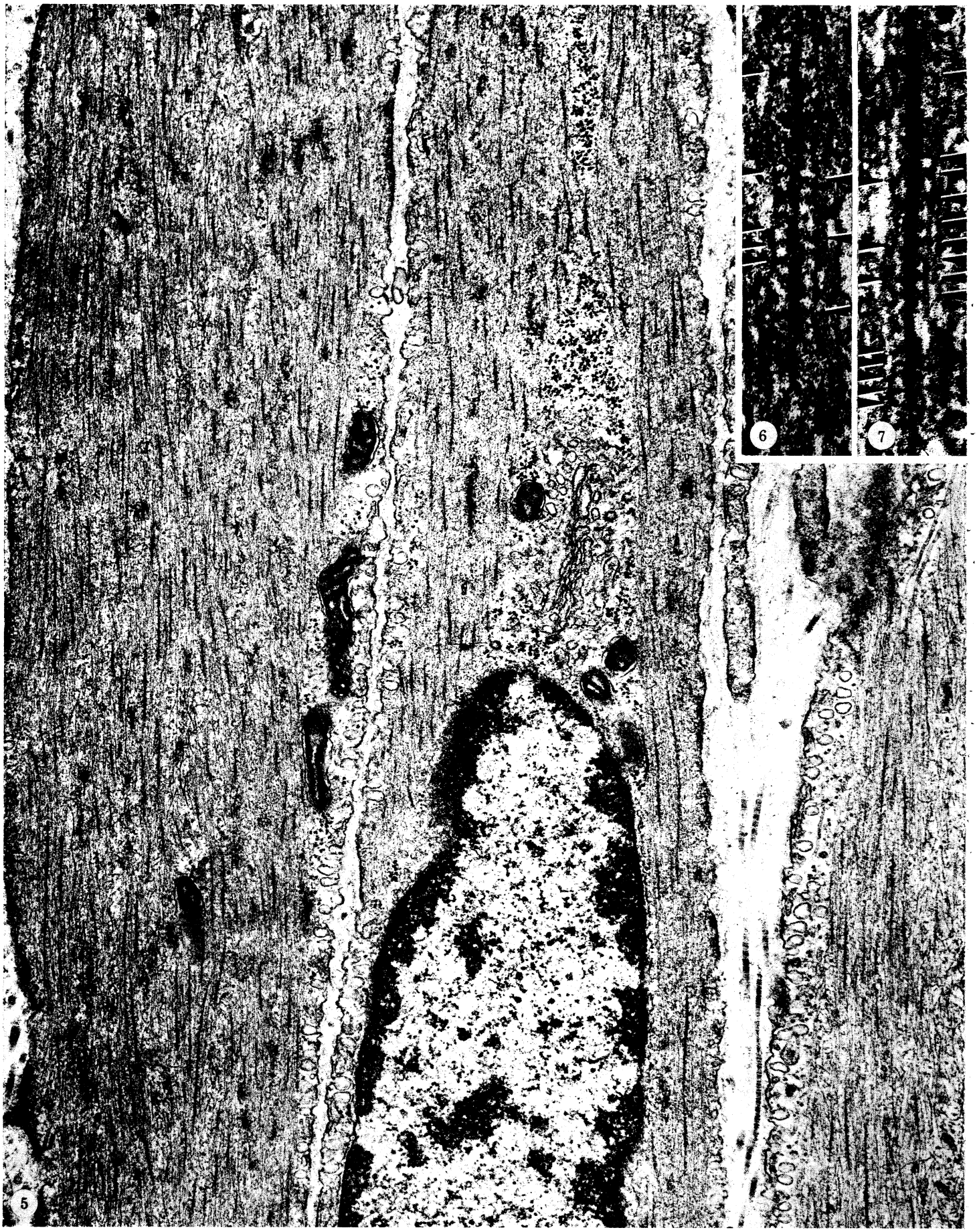


FIGURE 5. Longitudinal-oblique section of parts of three muscle fibres. Incubated for 30 min in the Krebs solution under physiological stretch before trialdehyde fixation. The variable length of the thick myofilaments is due to the somewhat oblique orientation of the section. (Magn. × 38000.)

FIGURES 6, 7. Longitudinal sections of thick filaments bearing lateral projections (arrow heads) suggestive of cross-bridges connecting with adjacent thin filaments. (Contrast enhanced, trialdehyde fixation; magnifications: figure 6, × 187000; figure 7, × 162000.)

tributing to the inadequate preservation of thick filaments (Somlyo *et al.* 1971). In smooth muscle fibres that have been swollen (gained KCl and water) through incubation in solutions in which NaCl has been replaced with KCl, the thick filaments (and thin filaments as well) are destroyed, probably during fixation with osmium (Jones *et al.*, in preparation). The thick and thin filaments are preserved if sufficient sucrose is included in the KCl solution to prevent swelling or if an impermeant anion (SO_4) instead of Cl is present in the high K solution (Jones *et al.*, in preparation).

Aggregates of thick filaments

Hypertonic solutions increase the electron opacity (for a given section thickness and stain) of smooth muscle ('dark cells') (Somlyo *et al.* 1971 *a*, *b*). In smooth muscles incubated in hypertonic solutions and fixed in hypertonically buffered aldehyde, ribbon-like structures may be seen in transverse section (figure 9). Ribbons considered to be aggregation products of the thick filaments and the adjacent amorphous material, are particularly numerous in hypertonically shrunken and overstretched (beyond the contractile limit) smooth muscles (Somlyo *et al.* 1971 *a*). It is uncertain whether the ribbons are present in living muscle or whether they are produced through the additional shrinkage imposed by processing for electron microscopy of an osmotically reduced lattice of thick filaments. Physiological studies have shown that in hypertonic solutions smooth muscle behaves as a near-perfect osmometer (Arvill, Johansson & Jonsson 1969; Brading & Setekliev 1968). We suspect that at least the majority of the ribbon-like structures identified by others (Lowy & Small 1970) as the organized form of myosin are aggregates of the thick filaments and adjacent amorphous substance in smooth muscles maintained under rather unphysiological conditions before fixation.

Conclusions

The organization of thin and thick myofilaments is consistent with biochemical and physiological evidence that a sliding filament mechanism of contraction, comparable to that originally proposed for striated muscles (Huxley & Hanson 1954; Huxley & Niedergerke 1954), also operates in vertebrate smooth muscle. Thick filaments, considered to represent the organized form of myosin in vertebrate smooth muscle, may appear dispersed (absent) or aggregated (ribbons) in electron micrographs of, respectively, osmotically swollen or shrunken preparations. The relationship of the thin filaments to the dense bodies is consistent with the view that the latter are functionally analogous to the Z-line of striated muscles. The intermediate size (10 nm) round filaments seen in association with dense bodies in normal smooth muscle fibres and seen in large conglomerations in certain unusual or developing smooth muscle fibres are distinct from the thin (actin) and thick (myosin) filaments.

A more detailed analysis of filament organization will have to account for the extensive shortening of vertebrate smooth muscle. Earlier studies of smooth muscle strips have shown that vertebrate smooth muscle can shorten by an amount considerably in excess of 50 % of its physiological length, but the possibility that such shortening may have been due to internal folding in a multicellular preparation could not be excluded. Recently, a single-cell toad stomach muscle preparation has been described (Bagby *et al.* 1971). Observations with phase interference microscopy show such single fibres shorten from a mean length of 254 μm ($n = 27$) to a mean length of 90 μm ($n = 42$) when stimulated with acetylcholine (Avril V. Somlyo,

unpublished observations). Furthermore, this is probably not the maximal shortening of these muscles, because depolarization with potassium produces somewhat greater peak tension in strips of toad muscle then does stimulation with acetylcholine, and because it is unlikely that the isolated fibres are at the maximally extended length before stimulation. The ability of single smooth muscle fibres to shorten considerably below 50 % of their initial length will have to be accounted for in a future, more definitive, description of filament organization.

Supported by National Institutes of Health Grant HE 08226, and the George L. and Emily McMichael Harrison Fund for Gynecological Research. A.P.S. is recipient of the United States Public Health Service Career Program Award K3-17833. The electron microscope used in these studies was purchased by National Institutes of Health General Research Support Grant FR 05610. Dr C. E. Devine was a George L. and Emily McMichael Harrison Research Fellow. We thank Mr J. Lang for the measurements of the filament dimensions and spacings and Miss Carol Lindsey for excellent technical assistance.

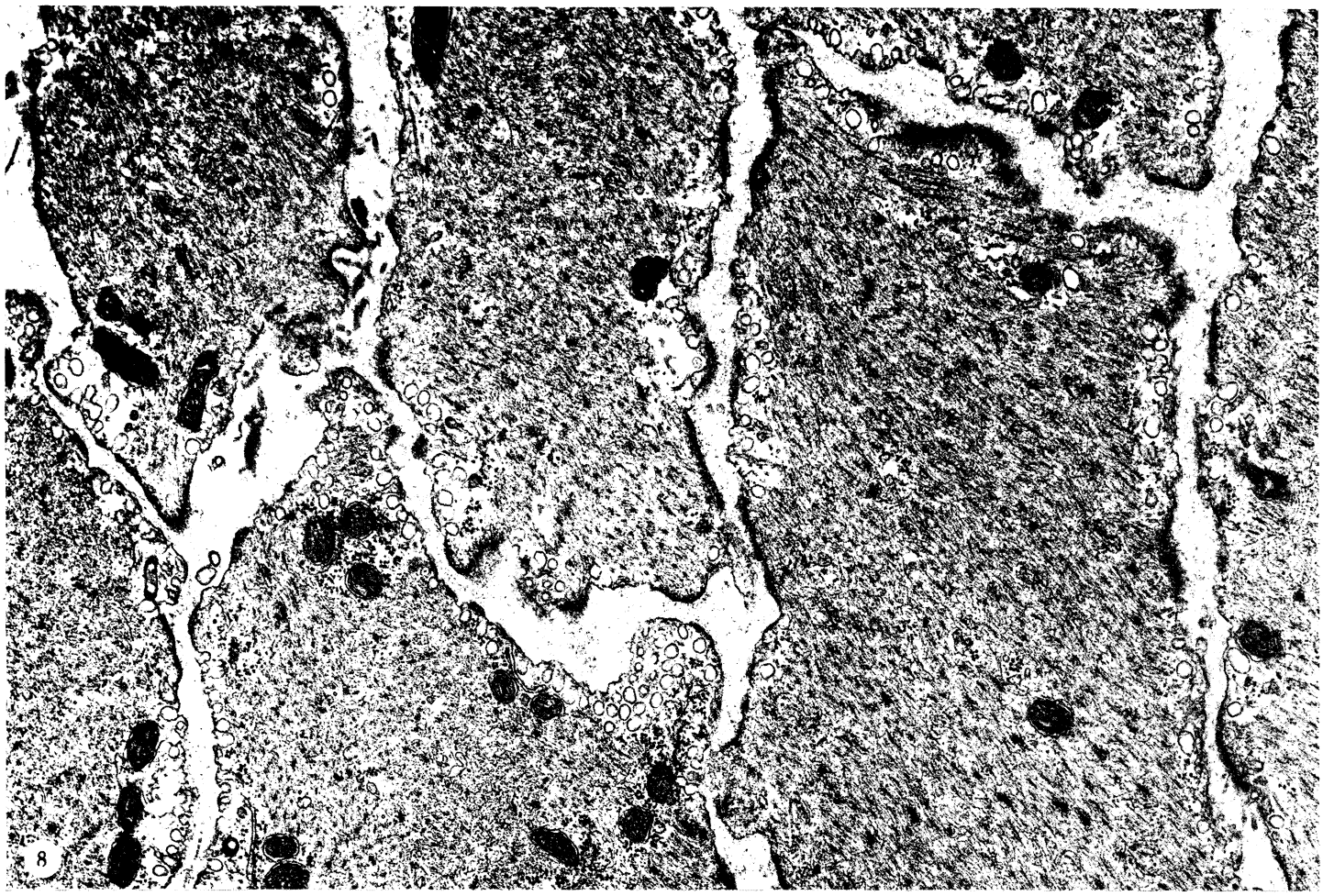
References (Somlyo *et al.*)

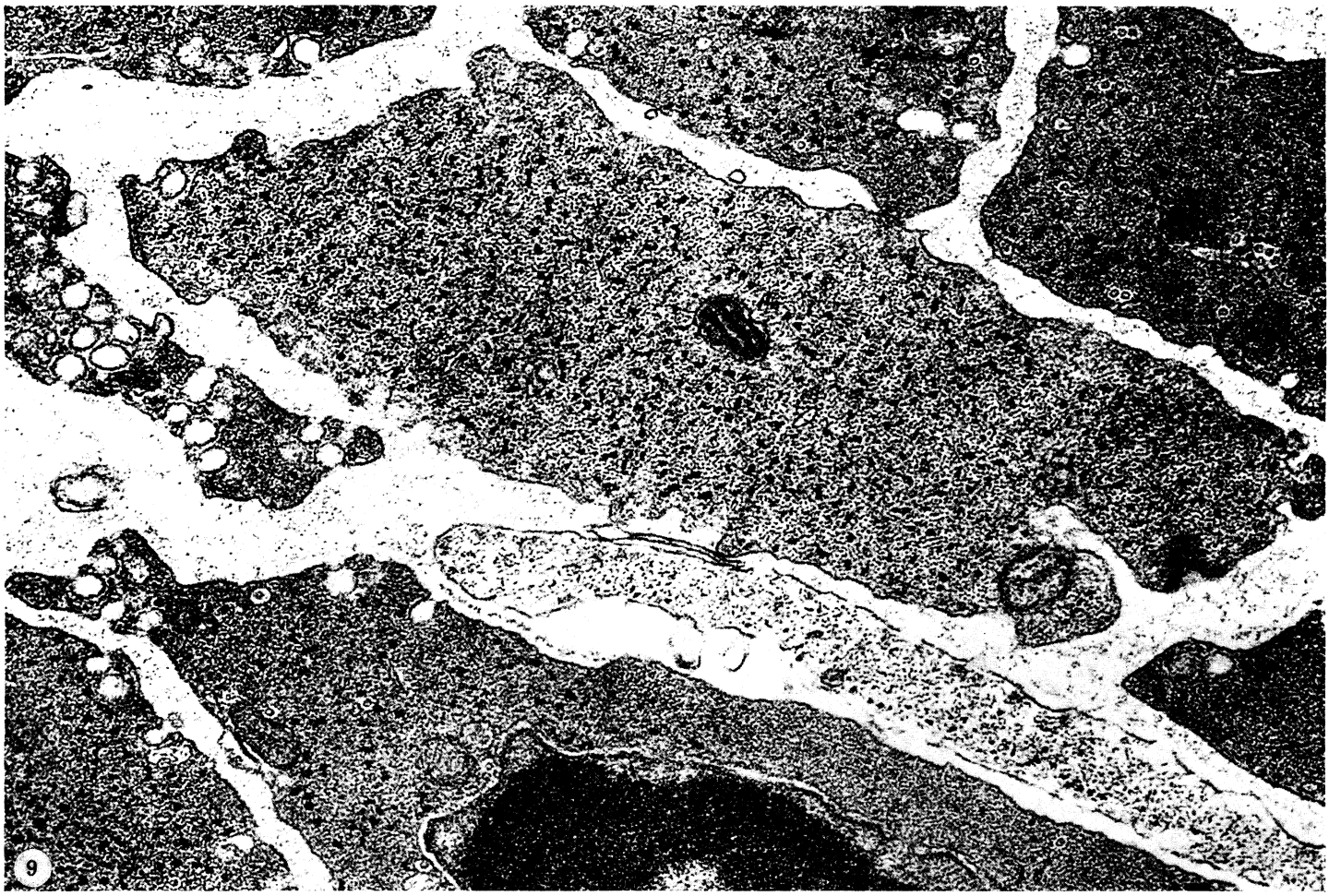
Arvill, A., Johansson, B. & Jonsson, O. 1969 *Acta physiol. scand.* **75**, 484–495.
Bagby, R. M., Young, A. M., Dotson, R. S., Fisher, B. A. & McKinnon, K. 1971 *Nature, Lond.* **234**, 351–352.
Brading, A. F. & Setekleiv, J. 1968 *J. Physiol., Lond.* **195**, 107–118.
Burnstock, G. 1970 Structure of smooth muscle and its innervation. In *Smooth muscle* (ed. E. Bülbring, A. Brading, A. Jones and T. Tomita), pp. 1–69. London: Edward Arnold.
Cooke, P. H. & Chase, R. H. 1971 *Expl Cell Res.* **66**, 417–425.
Cooke, P. H. & Fay, F. S. 1972 *Expl Cell Res.* **71**, 265–272.
Devine, C. E. & Somlyo, A. P. 1971 *J. Cell Biol.* **49**, 636–649.
Devine, C. E., Somlyo, A. V. & Somlyo, A. P. 1972 *J. Cell Biol.* **52**, 690–718.
Garamvölgyi, N., Vizi, E. S. & Knoll, J. 1971 *J. Ultrastruct. Res.* **34**, 135–143.
Hanson, J. & Lowy, J. 1963 *J. molec. Biol.* **6**, 46–60.
Heumann, H.-G. 1970 *Experientia* **26**, 1131–1132.
Huxley, A. F. & Niedergerke, R. 1954 *Nature, Lond.* **173**, 971–973.
Huxley, H. E. 1957 *J. biophys. biochem. Cytol.* **3**, 631–648.
Huxley, H. E. & Hanson, J. 1954 *Nature, Lond.* **173**, 973–976.
Jones, A. W., Somlyo, A. P. & Somlyo, A. V. 1973 In preparation.
Kalt, M. R. & Tandler, B. 1971 *J. Ultrastruct. Res.* **36**, 633–645.
Kelly, R. E. & Rice, R. V. 1969 *J. Cell Biol.* **42**, 683–694.
Lowy, J., Poulsen, F. R. & Vibert, P. J. 1970 *Nature, Lond.* **225**, 1053–1054.
Lowy, J. & Small, J. V. 1970 *Nature, Lond.* **227**, 46–51.
Needham, D. M. & Shoenberg, C. F. 1967 In *Cellular biology of the uterus* (ed. R. M. Wynn), pp. 291–352. New York: Appleton Century Crofts.
Nonomura, Y. 1968 *J. Cell Biol.* **39**, 741–745.
Pepe, F. A. 1971 *Biological Macromolecules Series. Subunits in Biological Systems* (ed. S. N. Timasheff and G. D. Fasman), vol. 5, part A, pp. 323–353. New York: Marcell Dekker.
Reedy, M. K. 1968 *J. molec. Biol.* **31**, 155–176.
Rice, R. V. 1972 In *Proc. Cold Spring Harbor Laboratory's 37th Ann. Symp. on the Mechanism of muscle contraction* (in the Press).

Description of plate 31

Figure 8. Section of several variably oriented smooth muscle fibres illustrating thick filament profiles from near transverse to very oblique, in a manner often seen in unstretched smooth muscle. Incubated for 30 min in the Krebs solution without stretch before trialdehyde fixation. (Magn. × 25000.)

Figure 9. Transverse section of smooth muscle fibres stretched beyond contractile limit during incubation in a hypertonic (with 12 % sucrose added) Krebs solution. The electron opacity of the fibres is increased, the nuclear chromatin is condensed and ribbon-like structures, presumably aggregates of adjacent thick filaments due to shrinkage, are present. (Trialdehyde fixation with 12 % sucrose in fixative buffer; magn. × 65000.)





FIGURES 8 AND 9. For legends see facing page.

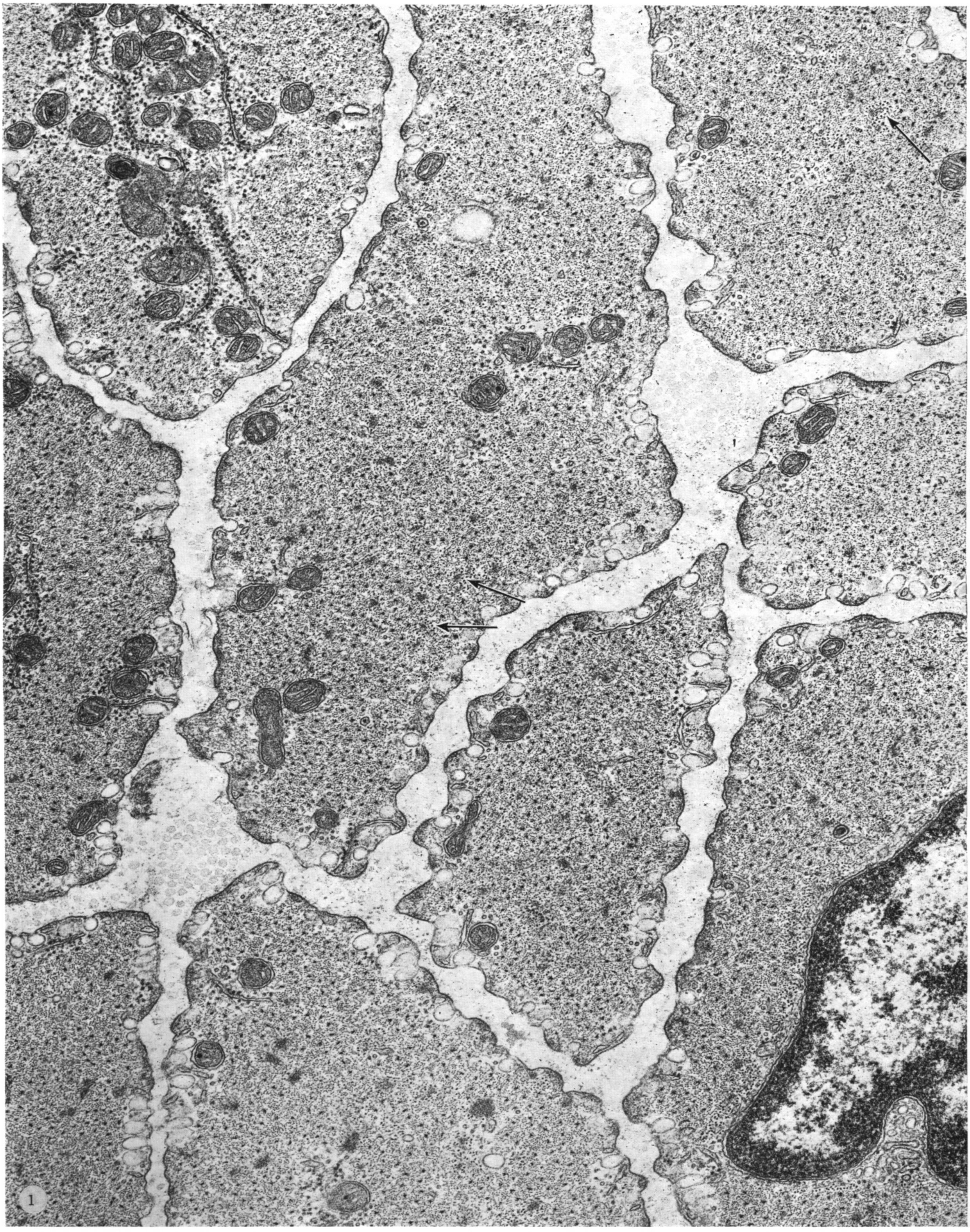
(*Facing p.* 228)

Rice, R. V., McManus, G. M., Devine, C. E. & Somlyo, A. P. 1971 *Nature, Lond.* **231**, 242–243.
Rice, R. V., Moses, J. A., McManus, G. M., Brady, A. C. & Blasik, L. M. 1970 *J. Cell Biol.* **47**, 183–196.
Shoenberg, C. F. 1969 *Tissue and Cell* **1**, 83–96.
Somlyo, A. P., Devine, C. E. & Somlyo, A. V. 1971 *Nature, Lond.* **233**, 218–219.
Somlyo, A. P., Devine, C. E., Somlyo, A. V. & North, S. R. 1971*b* *J. Cell Biol.* **51**, 722–741.
Somlyo, A. P. & Somlyo, A. V. 1968 *Pharmacol. Rev.* **20**, 197–272.
Somlyo, A. P., Somlyo, A. V., Devine, C. E. & Rice, R. V. 1971*a* *Nature, Lond.* **231**, 243–246.
Somlyo, A. P. & Somlyo, A. V. 1973 In *Methods in pharmacology. Smooth muscle* (ed. E. E. Daniel and D. M. Paton), vol. III. New York: Appleton Century Crofts.
Spurr, A. R. 1969 *J. Ultrastruct. Res.* **26**, 31–43.
Uehara, Y., Campbell, G. R. & Burnstock, G. 1971 *J. Cell Biol.* **50**, 484–497.

Discussion

R. T. TREGEAR, (*Department of Zoology, Oxford*): Dr Squire and I have estimated the mass ratio of actin to myosin in guinea pig taenia coli muscle, using SDS polyacrylamide gels prepared either from fresh or glycerol-extracted muscle. There is two to three times as much actin as myosin in these preparations. On the face of it this does not appear to be consistent with the published observation of Rice *et al.* (*J. Cell Biol.* **47**, 183, 1970) that there are about 60 times as many actin filaments as myosin filaments observable in an average cross-section of the muscle; in this case one would expect a much higher relative actin content, unless the myosin filaments had a very much greater number of myosin molecules per unit length than is found in striated muscle.

A. P. SOMLYO: The thin to thick filament ratio in the rabbit portal-anterior mesenteric vein is approximately 15:1. The average diameters are 6.4 nm and 15.5 nm respectively. Calculation of the actin to myosin area ratios from these values yields a figure that is as consistent with your results with chemical analyses as are the calculations based on the electron micrographs showing ribbons. This is, of course, what one would expect if the ribbons were due to the aggregation of thick filaments, and I am not certain whether biochemical analysis can decisively settle the question regarding the normal organized form of myosin vertebrate smooth muscle.



All illustrations, unless noted, are unpublished electron micrographs of rabbit portal-anterior mesenteric vein, taken by A. P. Somlyo.

FIGURE 1. Transverse section of a bundle of smooth muscle fibres illustrating the regular spacing of the thick filaments and the relatively large number of thin myofilaments. Several groups of intermediate filaments (some shown by arrows) associated with dense bodies are present. This preparation was incubated for half an hour, under physiological stretch, in a Na-free, Ca-free, 230 mmol/l sucrose substituted solution before glutaraldehyde fixation. (Magn. × 34000.)

(*Facing p.* 224)

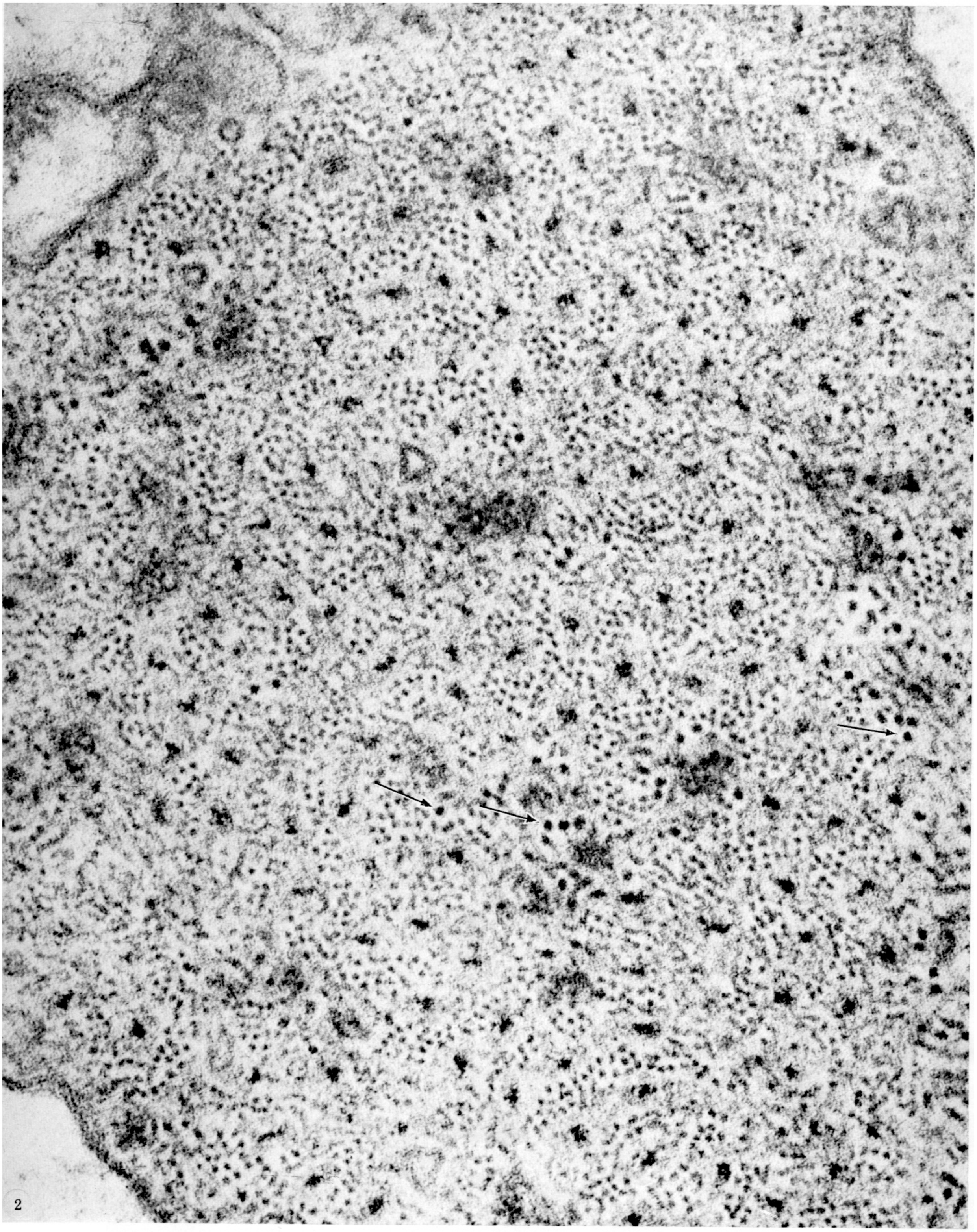
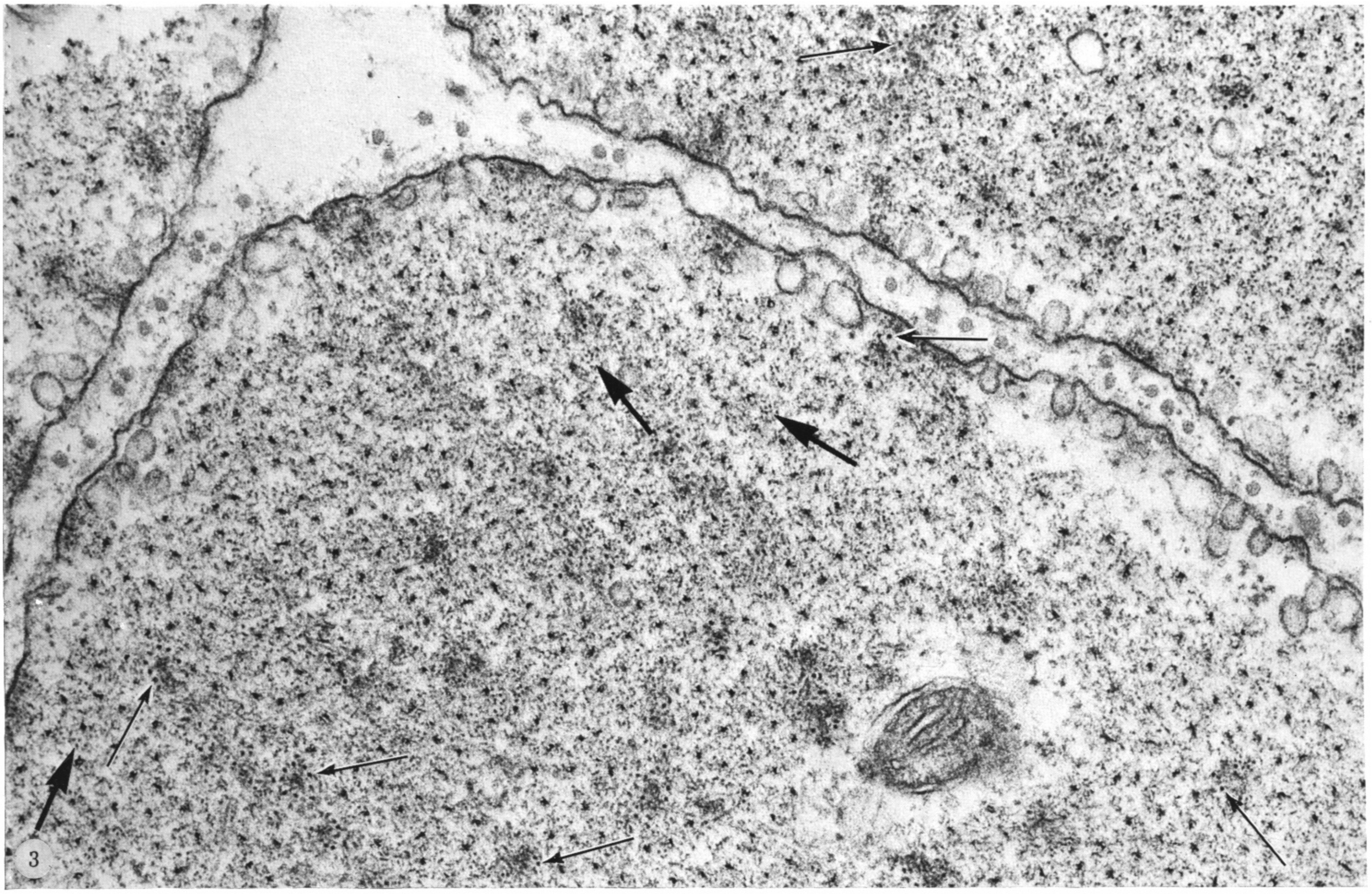
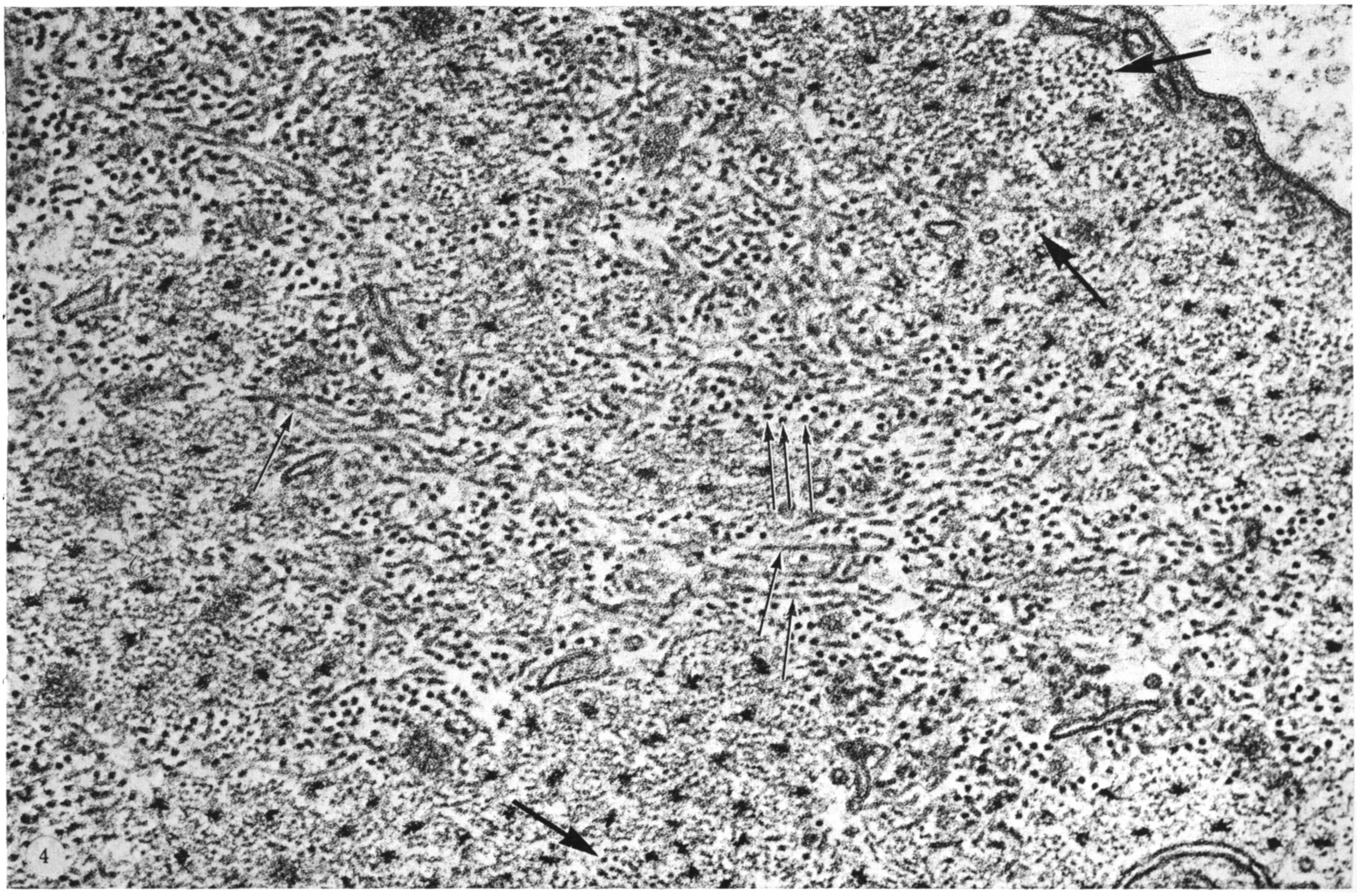


FIGURE 2. Transverse section of a regular arrangement of thick and thin myofilaments with a number of rosettes present. Some of the adjacent thick filaments are connected by an amorphous grey substance. Intermediate filaments (arrows) at the periphery of the dense bodies are also present. (Glutaraldehyde fixation; magn. $\times 220000$; from Somlyo *et al.* 1971*a*.)





FIGURES 3 AND 4. For legends see facing page.

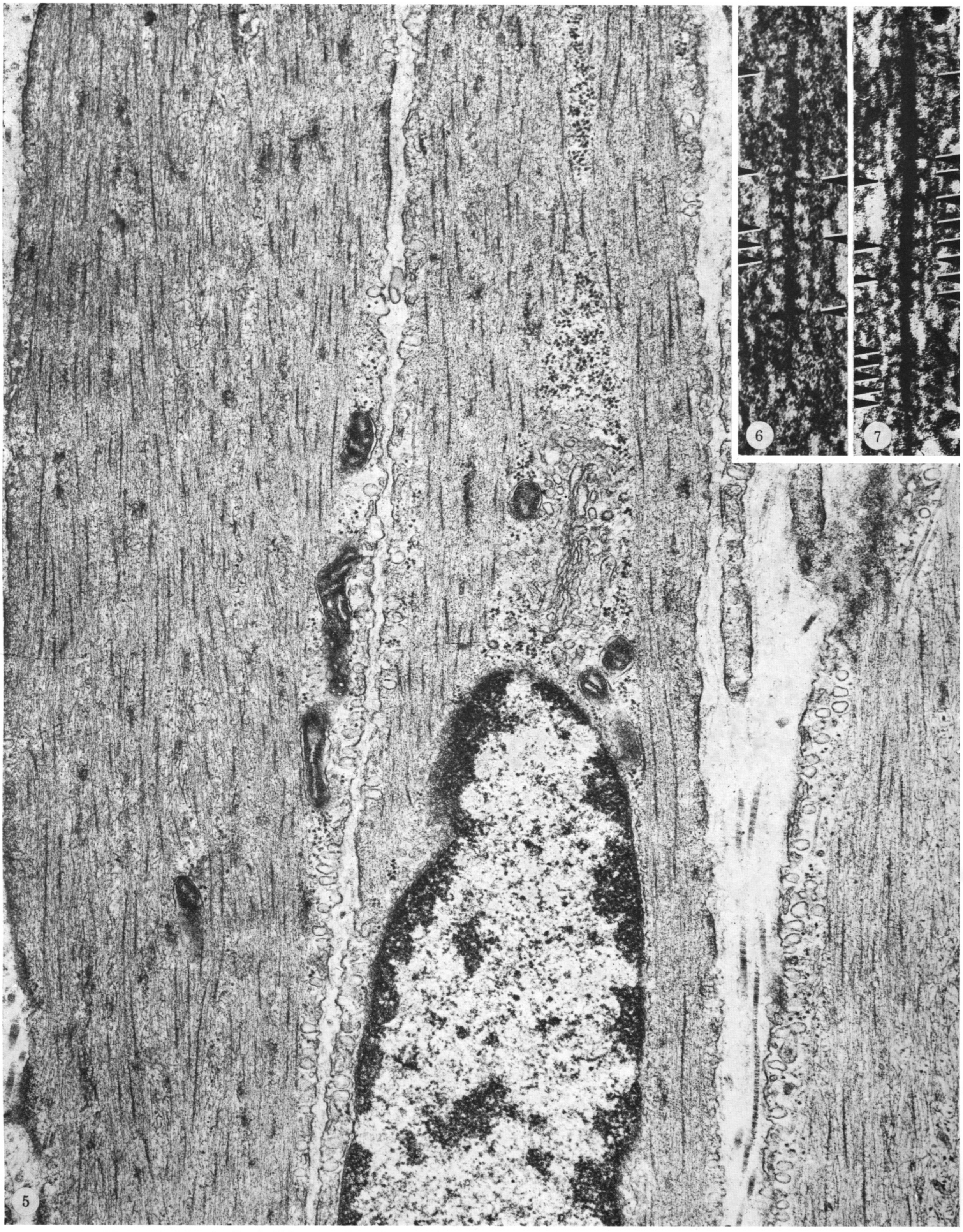
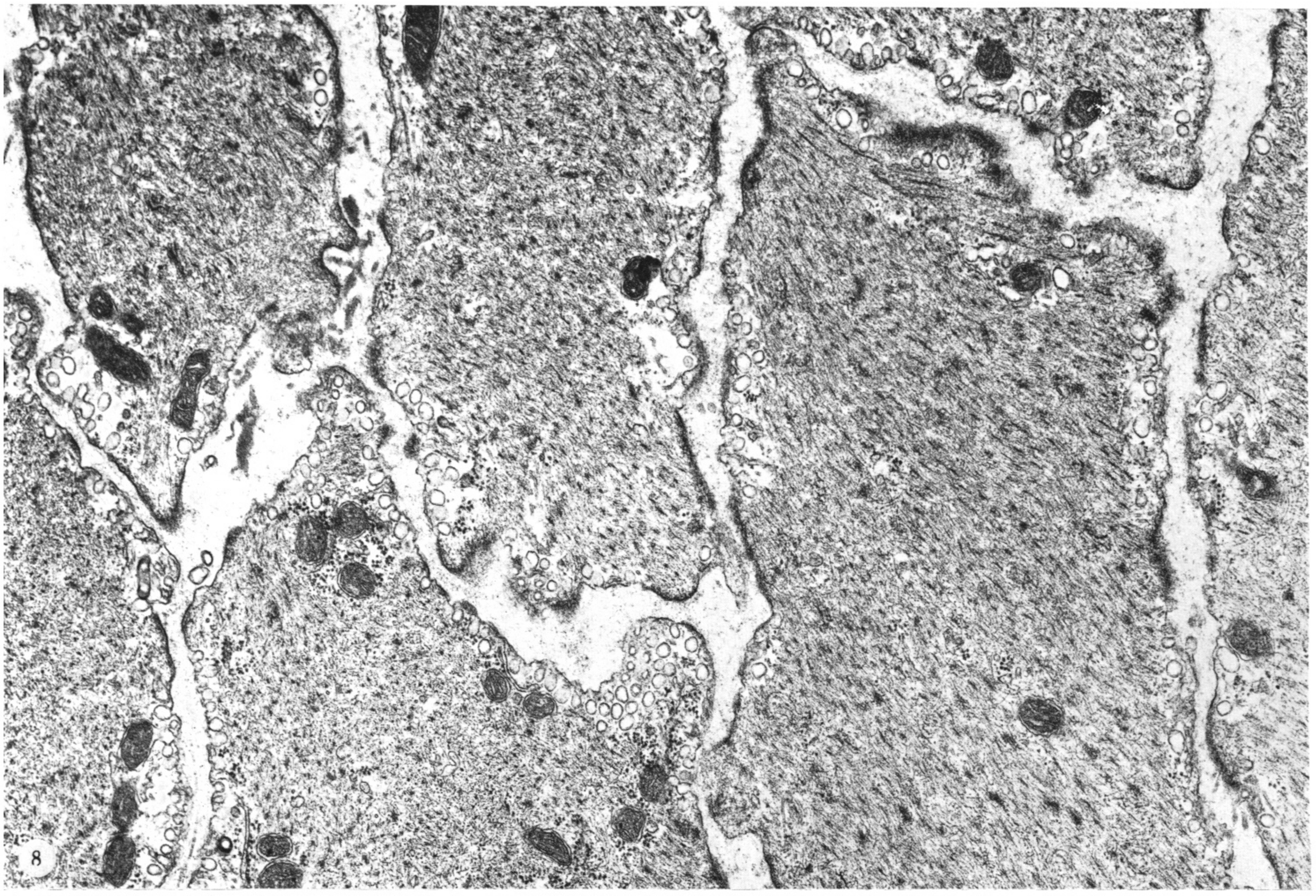
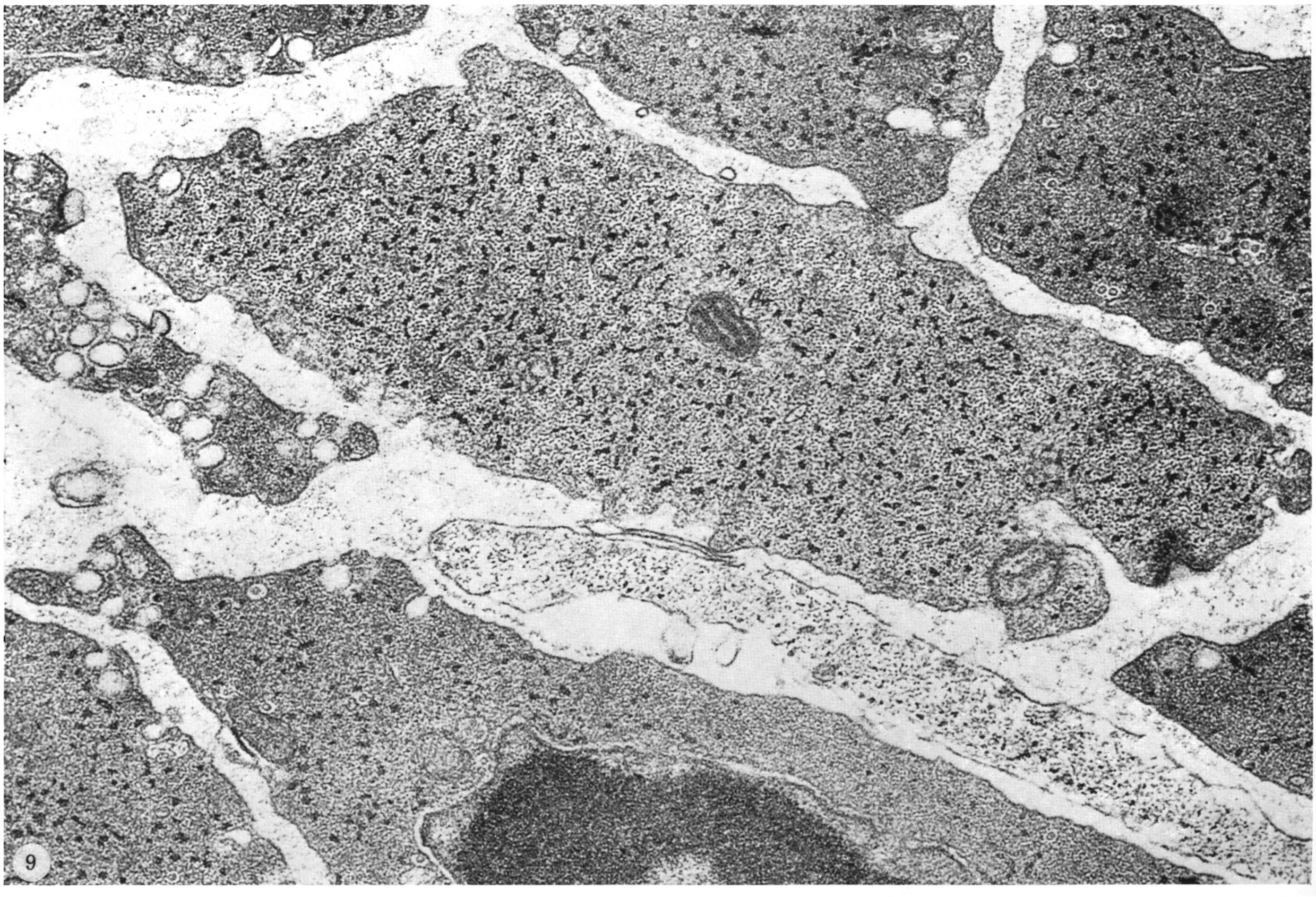


FIGURE 5. Longitudinal-oblique section of parts of three muscle fibres. Incubated for 30 min in the Krebs solution under physiological stretch before trialdehyde fixation. The variable length of the thick myofilaments is due to the somewhat oblique orientation of the section. (Magn. × 38000.)

FIGURES 6, 7. Longitudinal sections of thick filaments bearing lateral projections (arrow heads) suggestive of cross-bridges connecting with adjacent thin filaments. (Contrast enhanced, trialdehyde fixation; magnifications: figure 6, × 187000; figure 7, × 162000.)





FIGURES 8 AND 9. For legends see facing page.