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I. CELLULAR STRUCTURES AND ELECTRO-PHYSIOLOGICAL BEHAVIOUR

Fine structure of smooth muscle

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[Plates 1 to 8]

The size of smooth muscle cells is different in different organs but varies little within the same tissue. Great changes in cross-sectional area are observed in distended and contracted smooth muscle cells and during contraction and relaxation in neighbouring layers. Two basic types of intercellular junction are described: (*a*) attachment plaque, where myofilaments end and where the intercellular gap is wide; (*b*) nexus, where the adjoining cells come close together; the gap or the degree of fusion of membranes is affected by preparation procedures. The occurrence of nexuses is generally correlated with electrical coupling, although sometimes, e.g. in intestinal longitudinal muscle, there is electrical coupling but nexuses are virtually absent. *Caveolae* are spread over the surface of smooth muscle cells. They appear early in embryonic development. It is suggested that they represent a compartment situated between extracellular space proper and intracellular space; its ionic composition may be more readily controlled by the muscle cell itself. Caveolae disappear from muscle cells after treatment with hyaluronidase *in vitro*. Sacs, cisternae and tubules of sarcoplasmic reticulum are observed in the superficial region of cells in visceral muscles, closely related to caveolae and plasma membrane. Mitochondria are frequently associated with the reticular system. The suggestion that sarcoplasmic reticulum may play a similar role in smooth muscle as in skeletal muscle, in storage and release of calcium, is discussed. Accumulation of divalent cations by sarcoplasmic reticulum and the effect of incubation in Ca-rich solutions support this interpretation, although the crucial evidence for it is still lacking.

Fine structure of smooth muscle

In this paper an attempt is made to outline some contributions of morphology, mainly electron microscopy, to our knowledge of smooth muscle physiology. Attention is focused on the smooth muscle at the cellular level, and particularly on the superficial region of the smooth muscle cell. However, it is necessary to stress the importance of morphological studies at supra-cellular level, especially when muscle layers and bundles interact in the complex motor activity of the whole organ.

Size of smooth muscle cells

In a given smooth muscle the sizes of the smooth muscle cells are relatively homogeneous, in contrast to the situation in many skeletal muscles, but there are great and consistent differences among various smooth muscles (see Burnstock 1970). In the muscularis mucosae, for example, and in blood vessels, muscle cells are much smaller than in the taenia coli and in the vas deferens.

A peculiar disposition is observed throughout the whole length of the small intestine of the guinea-pig. In the muscularis externa muscle cells are 2 to 4 μm in diameter, but in the innermost part of the circular coat there is a single-celled layer of muscle whose cells are more electron-dense and have a significantly smaller diameter (figure 1, plate 1). An earlier report on this innermost circular layer seen by light microscopy was published by Li (1940). The specific function of this muscle layer is unknown, but it is worth noting that many nerve bundles

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(with numerous vesiculated nerve processes) are found between this layer and the main component of the circular coat (figure 2, plate 1). The distribution of nerve fibres in the intestinal muscularis externa appears rather uneven. Almost 60 % of all the nerve fibres found in the circular coat (and 63 % of vesiculated nerve boutons) are situated between its two muscular components. Another thick network of nerve bundles is found between the circular and longitudinal muscle layers (figure 2, plate 1), as well as the ganglia and primary meshes of the myenteric plexus. In the latter group of nerve fibres, vesicle-containing boutons are very numerous and are preferentially situated near the muscle cells of the longitudinal layer (figure 2, plate 1). The great majority of vesiculated nerve processes of the muscularis externa are then situated (*a*) between the two components of the circular layer, or (*b*) between the circular and longitudinal layers, either in small bundles or at the surface of myenteric plexus ganglia (Gabella 1972*a*). The gap between the surfaces of the bouton and the muscle cell is most commonly of the order of hundreds of nanometres. Vesiculated nerve processes in the proximity of Schwann cells or of interstitial cells are not uncommon.

In the abdominal tract of the rat's oesophagus large smooth muscle cells are mixed up with striated muscle fibres (figure 3, plate 2). Thus this organ provides a model for a mixed type of musculature.

There is no consistent relation between muscle cell size and body size of the animal. Mitoses are sometimes observed in visceral and vascular smooth muscle cells even in adult animals (figure 4, plate 2). When there is increased functional demand on the tissue (for example in the small intestine in case of hyperfunction produced by partial stenosis of the lumen), the smooth muscle cells increase both in number by mitosis and in size (hypertrophy). Mitoses have been observed in regenerating taenia coli after crush injury (McGeachie 1971) and after transplant in the anterior eye chamber (Campbell, Uehara, Malmfors & Burnstock 1971).

Dramatic changes in cell diameter occur during contraction and relaxation. When two lengths of small intestine a few centimetres apart are fixed in contracted and distended conditions, in the cross-sectioned circular layer up to 200000 smooth muscle cells per square millimetre are counted in the distended muscle, and approximately 50000/mm^2 in the contracted muscle.

These differences between contracted and relaxed muscle are enhanced by the fact that in most of the viscera smooth muscle occurs in layers which are at an angle with one another, and contraction and relaxation in one layer greatly affects the shape of the cells in the other layer. The interaction between the various contractile units is also evident when the muscle directly controls the volume and tension of a hollow space. This approach is far from new and, for example, Wood & Perkins (1970) recently examined the mechanical interaction between

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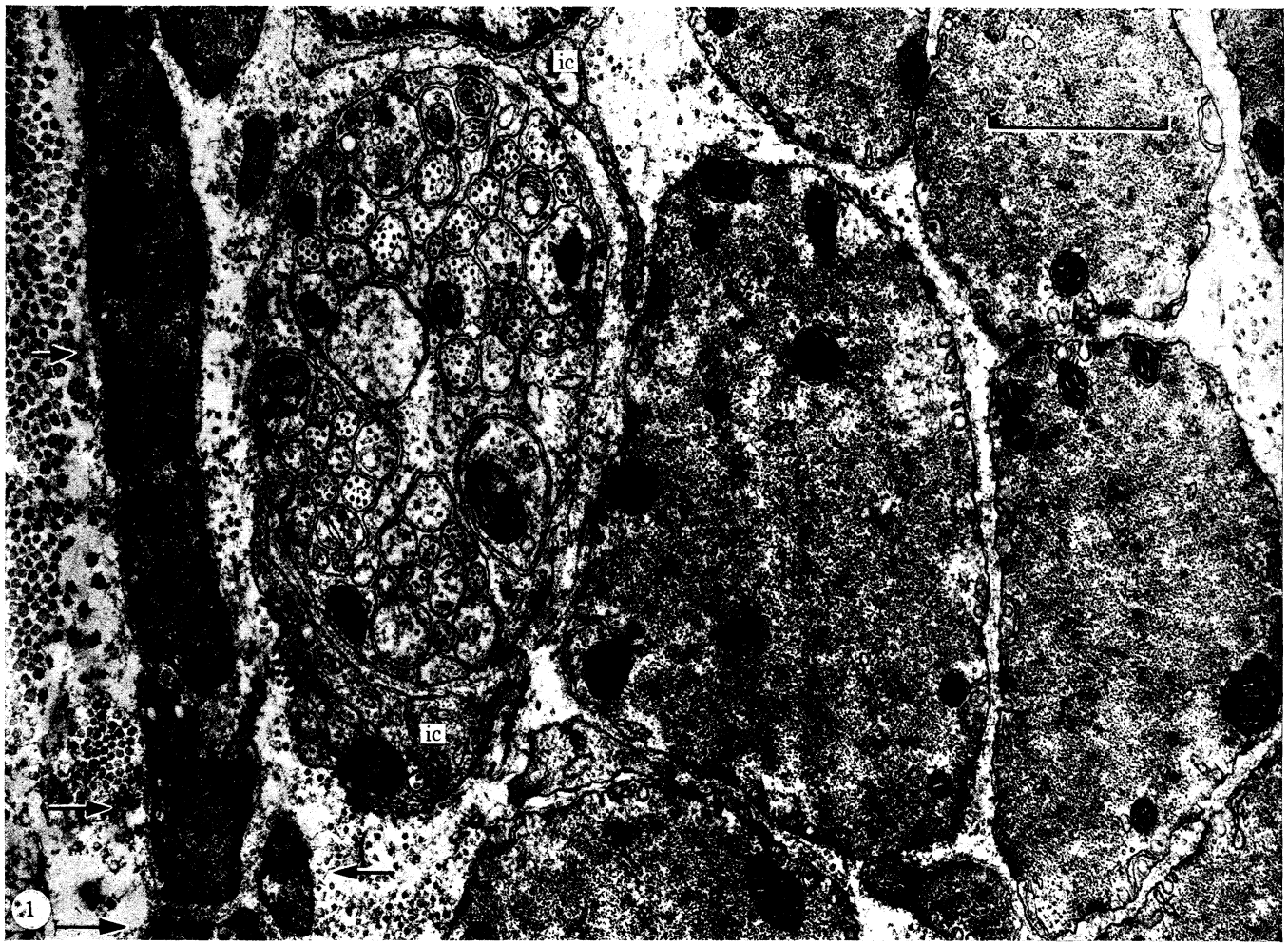
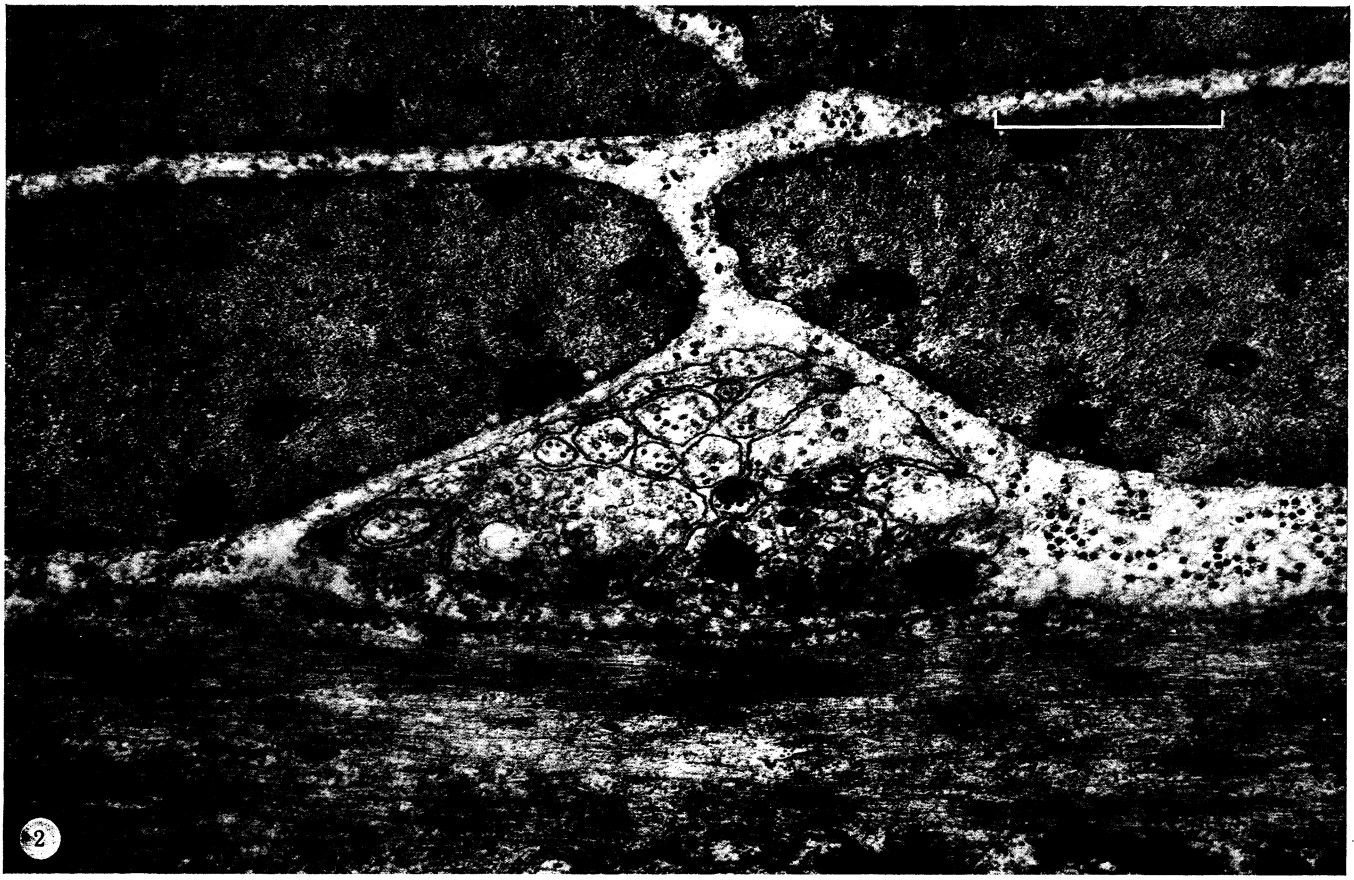
Description of plate 1

(Unless otherwise stated methods are as reported in previous papers [Gabella 1971, 1972*a*].)

Figure 1. Guinea-pig ileum. Circular muscle coat. The main component of the circular layer is formed by several layers of smooth muscle cells 2 to 4 μm in diameter. The innermost layer of the circular coat (to the left) is formed by smooth muscle cells which are flattened, more electron-dense and significantly smaller (arrows). Between the two muscle components is a thick nerve network. *ic*, interstitial cell. (Magn. × 25000; calibration 1 μm.)

Figure 2. Guinea-pig ileum. Parts of both the longitudinal (bottom) and circular (top) muscle coats are present in this section. Intervening between the two is a thin nerve bundle with two agranular vesicle-containing boutons. They are both near the longitudinal muscle cells. (Magn. × 29500; calibration 1 μm.)





FIGURES 1 AND 2. For legend see facing page.

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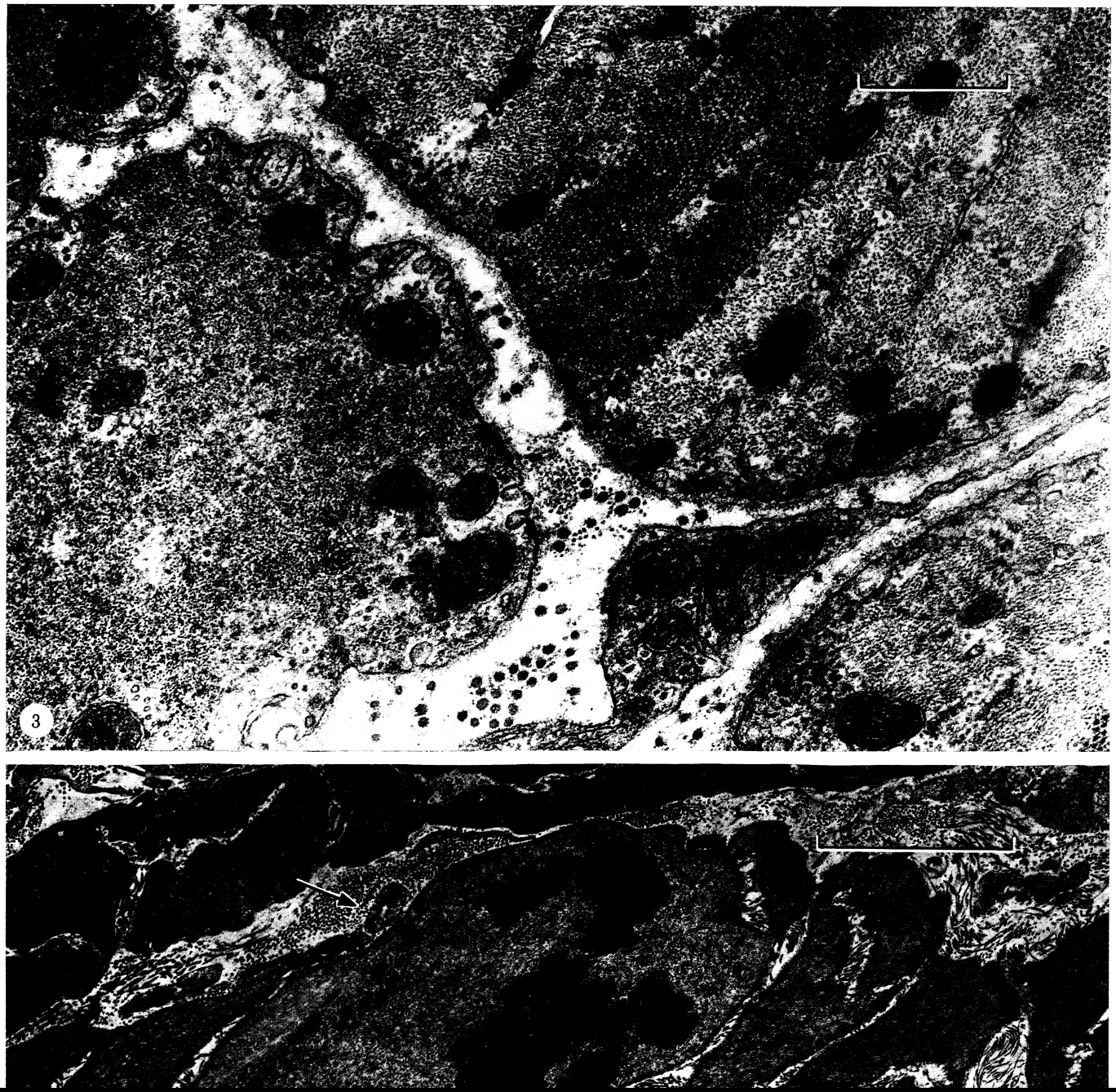
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longitudinal and circular muscle in the cat's intestine, showing that relaxation in the longitudinal axis is a mechanical consequence of circular muscle contraction rather than of nervous inhibition of the longitudinal muscle.

On the other hand, when physiological experiments are performed *in vitro*, the geometrical pattern of smooth muscle cells of the organ is often disrupted. An extreme example of geometrical disruption is a blood vessel when spirally cut and mounted as a single longitudinal strip. Even in the case of taenia coli special care and dissecting ability are necessary to isolate a bundle of pure longitudinal muscle (Bülbring 1954; Burnstock, Campbell & Rand 1966). Otherwise it is easy to obtain a preparation which includes the myenteric plexus and some circular muscle.

Intercellular junctions

The problem of intercellular relationships in smooth muscle has been under investigation for more than half a century. Electron microscopy has shown the cellular individuality of contractile units in smooth muscle and more recently the existence of characteristic intercellular junctions. The nature of these junctions is far from understood and the role played by preparation artefacts is still not completely clear. It seems, however, safe to say that there are two basic types of intercellular junctions. Intermediate aspects are found, but only on rare occasions.

One type of junction, the attachment plaque, is characterized by a thick layer of electron dense material on the cytoplasmic aspect of the two membranes. In this dense material bundles of myofilaments penetrate and merge. At the attachment plaque the gap between the two cells can be up to 20 to 30 nm or so, in which case an intervening layer of extracellular dense material is observed (figure 5, plate 3), or be reduced to 10 nm or less. Although it seems likely, it has not yet been proved that these intercellular connexions are permanent structures. Besides these symmetrical attachment plaques, frequently only one half of this structure is observed: a dense patch at the cytoplasmic aspect of the plasma membrane, where myofilaments end, and related to an area of thicker and denser basement lamina. When the muscle cell contracts, these non-symmetrical attachment plaques are pulled inwards (or the intervening portions of the cell are pushed outwards), clearly showing their continuity with the myofilaments (Lane 1965) (figures 6, 7, plate 3).

The second type of junction is characterized by a close apposition of the membranes of the two cells (figure 8, plate 4); no dense material occurs at the cytoplasmic side of the membranes. This junction was called a nexus by Dewey & Barr (1962). It was originally claimed that at the nexus the adjoining membranes are partially fused (as in tight junctions between epithelial cells); in further experiments it was shown that colloidal lanthanum can penetrate the nexus (as in the gap junctions between epithelial cells) (Revel, Olson & Karnovsky 1967). Other authors have confirmed the occurrence of gap junctions (with a gap of approximately 2 nm)

Description of plate 2

Figure 3. Rat oesophagus. Abdominal tract. Cross-sectioned, parts of two striated muscle fibres and of two smooth muscle cells. Note the different size and pattern of various myofilaments. (Magn. × 23500; calibration 1 μm.)

Figure 4. Small mesenteric artery of adult (approx. 5-month-old) guinea-pig. One muscle cell is undergoing mitosis. The chromatin masses are grouped in the central region of the cell, whereas myofilaments, sarcoplasmic reticulum, and mitochondria are packed underneath the plasma membrane. A nerve fibre (arrow) and several elastic fibres run close to the dividing cell. *r*, Red blood cell; *e*, endothelium; *i*, inner elastic membrane. (Magn. × 6250; calibration 5 μm.)

between smooth muscle cells (Uehara & Burnstock 1970). There is so far not enough evidence to suggest the occurrence of more than one type of junction with close apposition; the difference observed (particularly the occurrence and the extent of the gap between the membranes) may be attributed to differences in the preparation procedures. Permanganate fixation, especially with acetone dehydration, preferentially produces fusion of the membranes, whereas with aldehyde fixation a gap between the membranes is generally seen. In the central nervous system it has been shown that permanganate fixation transforms gap junctions into tight junctions (Brightman & Reese 1969). In the gap junctions of the mouse liver after fixation with glutaraldehyde and dehydration with acetone, the 2 nm gap disappears and the junction assumes a pentalaminar aspect (Goodenough & Revel 1970). In the present paper the term *nexus* is used to indicate the close junctions between smooth muscle cells, irrespective of the occurrence of a 2 nm gap or of fusion of the membrane outer leaflets. Taxi (1965) has been able to show that in the mouse duodenum, nexuses occupy approximately 6 % of the surface of smooth muscle cells.

Nexuses are currently believed to be the morphological correlate of electrical coupling between smooth muscle cells. There are, however, smooth muscles where there is good evidence of electrical coupling, but few or no nexuses have been observed. For example, in the canine duodenum Henderson, Duchon & Daniel (1971) found that nexuses are confined to the circular muscle layers, and no nexus was observed in the longitudinal layer. Similar results have been obtained from the guinea-pig ileum (Gabella 1972*b*). Nevertheless, Kuryama, Osa & Toida (1967) have shown electrical coupling in the longitudinal muscle coat of guinea-pig ileum. Similarly, in the vas deferens, there is good evidence of electrical coupling (Tomita 1967), but nexuses are rare (see references in Burnstock 1970). Without further experiments, therefore, it should not be assumed that the nexus is the only morphological correlate of electrical coupling. Similarly, in the nervous system the occurrence of electrical coupling is not always related to the presence of tight junctions, one well-known example being the ciliary ganglion of the pigeon (Hess, Pilar & Weakley 1969).

When the taenia coli, the ileum or the vas deferens are fixed with the least mechanical strain, a number of finger-like processes protruding from one muscle into another are observed (figure 9, plate 4). Occasionally they are large mushroom-like processes, with a thin stalk (figure 10, plate 4). Although these processes have been described by several authors (e.g. Lane & Rhodin (1964), in the mouse vas deferens), their function is still unknown. It may well be that they play a role in intercellular communication. Similarly, complex indentations

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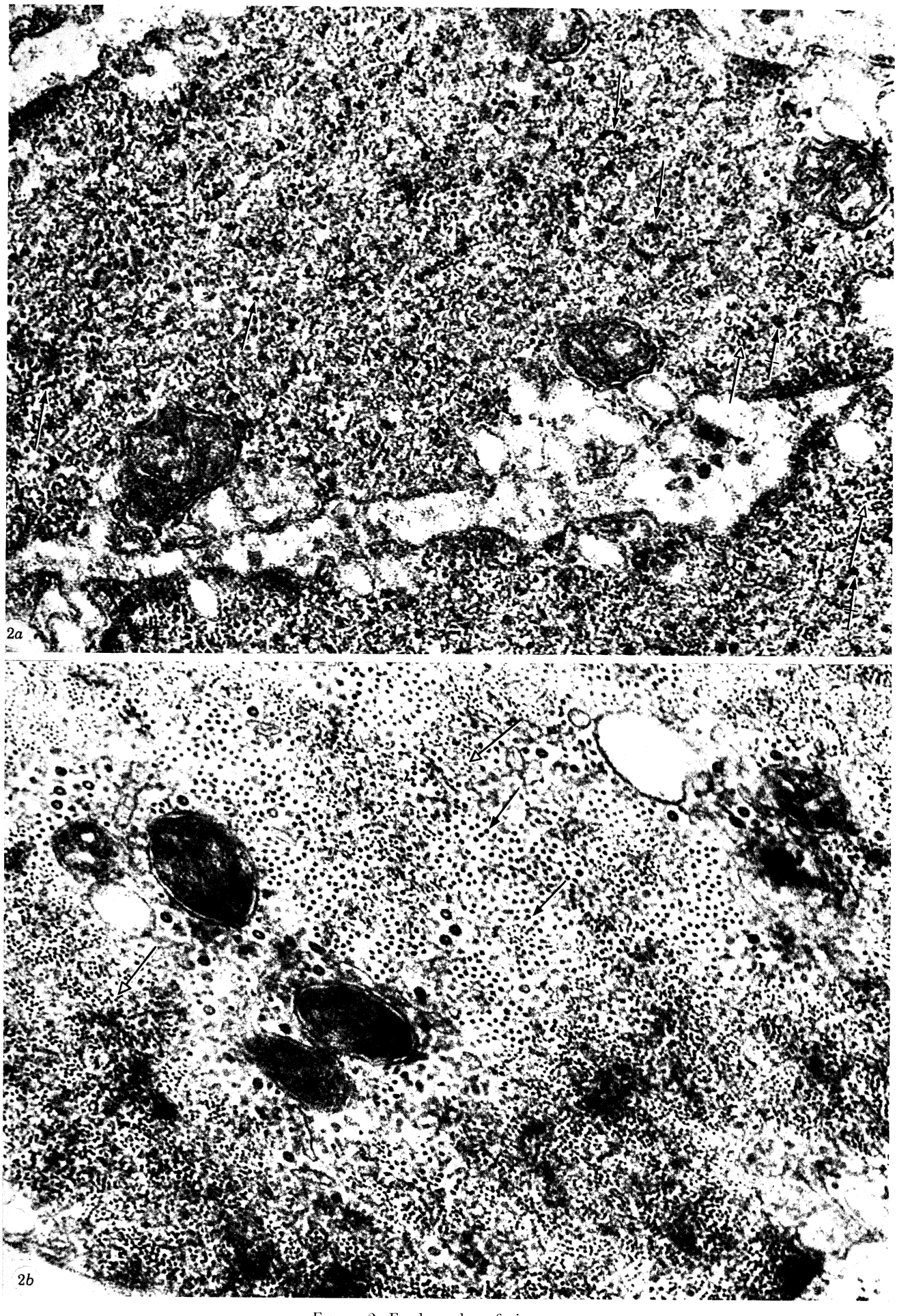
Description of plate 3

Figure 5. Guinea-pig ileum. Circular muscle coat. An attachment plaque between two muscle cells. Note the incrustation of electron-dense material at the cytoplasmic aspect of membranes and the densities in the intervening extracellular gap. (Magn. × 81000; calibration 0.25 μm.)

Figure 6. Guinea-pig colon. Circular muscle coat. A grazing section through the surface of a smooth muscle cell. The section includes dense patches of the cell surface and shows their continuity with myofilaments. In the intervening regions a few sacs of sarcoplasmic reticulum and caveolae are cross-sectioned. Collagen fibres in the extracellular space (to the right). (Magn. × 41500; calibration 1 μm.)

Figure 7. Guinea-pig ileum. Longitudinal muscle coat. In contracted smooth muscle cells, the cell membrane is pulled inwards in correspondence of the attachment plaques (arrows), which are continuous with myofilaments, and pulled outwards in correspondence of the intervening regions. Note a number of caveolae and of sacs of sarcoplasmic reticulum (*r*). (Magn. × 16000; calibration 2 μm.)



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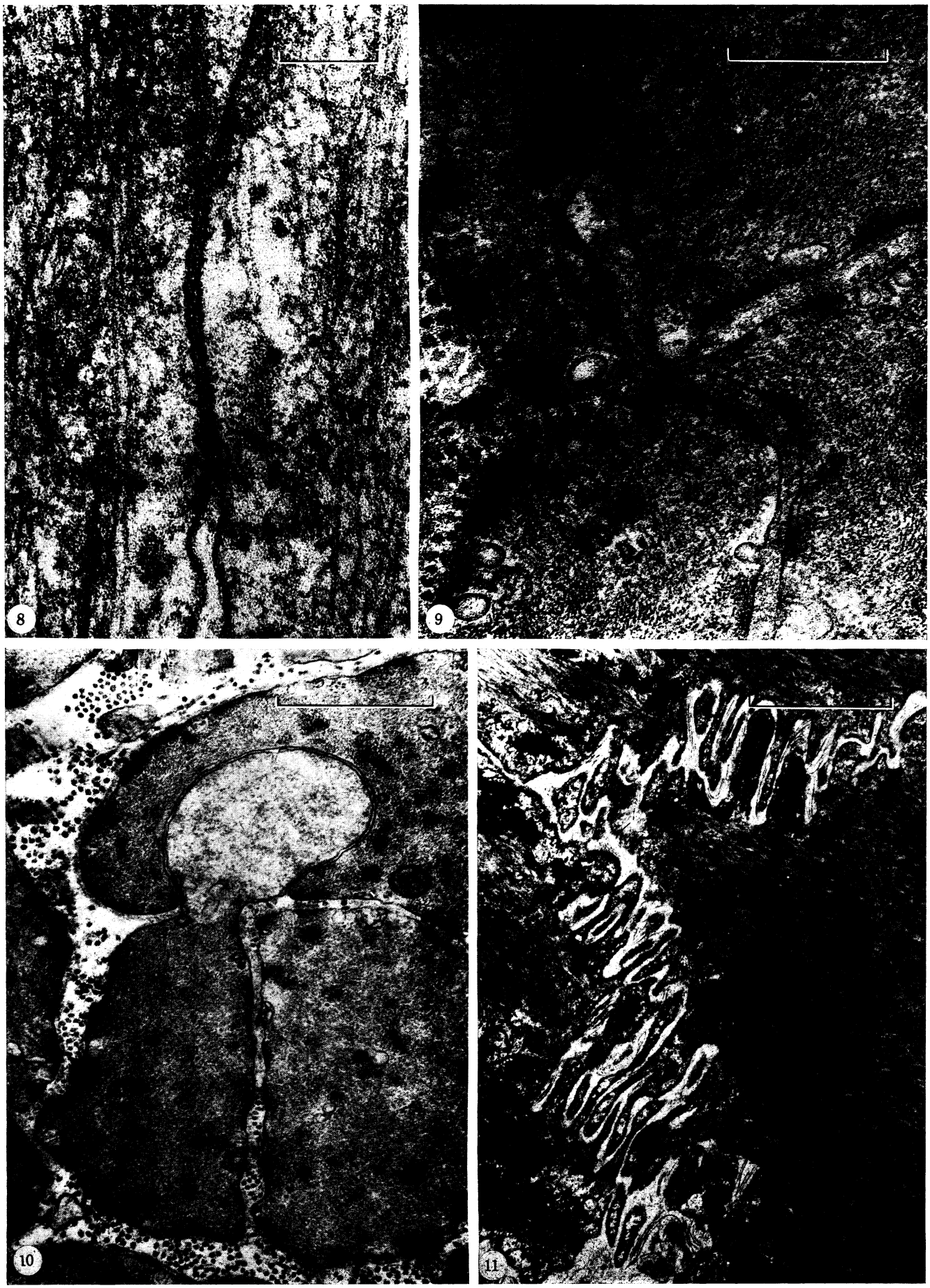

FIGURES 8 TO 11. For legend see facing page.

arising between adjacent muscle cells during contraction (figure 11, plate 4) are possible site of interactions between cells. These complex reciprocal interdigitations show very many caveolae, sarcoplasmic reticulum and mitochondria.

Surface of smooth muscle cells

In cross-section, smooth muscle cells are round or oval, and this is the case for example in the intestinal musculature when it is fixed in relaxed and unstretched conditions. In other muscles the shape is more irregular, and varies from flattened to stellate profiles.

In the vas deferens, thin and long invaginations of the cell membrane can be observed running deeply into the cells. They are sometimes occupied by collagen fibrils which cross the basement lamina surrounding the cell and covering the opening of these invaginations.

In the ano-coccygeus muscle, a muscle recently introduced by Gillespie (1971) for pharmacological investigations, large and irregular invaginations of the plasma membrane are spread over the whole length of the muscle cells (figure 12, plate 5). These cavities increase significantly the volume of the so-called extracellular space. They are not penetrated by collagen fibres and are mainly occupied by a fuzzy material of the same electron density as the basement lamina. Dense patches and areas rich in caveolae are observed in the plasma membrane which surrounds these cavities.

Characteristic flask-shaped invaginations or pits of the plasma membrane, called *caveolae*, are seen at the surface of the smooth muscle cells, arranged in rows parallel to the main axis of the cell. Their number varies from one muscle to the other, but they have been observed in all smooth muscles. Notwithstanding their common occurrence, caveolae are rather enigmatic structures and their significance remains obscure.

Caveolae differ from micropinocytotic vesicles or plasmalemmal vesicles (Palade & Bruns 1968) of endothelial cells, being slightly smaller and more homogeneous in size. Micropinocytotic vesicles often show a diaphragm (interpreted by Palade & Bruns as evidence of fusion of a vesicle with the plasma membrane and of its opening to the outside) or are completely isolated from the surface membrane (and are then true vesicles).

In smooth muscle virtually all the caveolae are connected with the outside of the cell. In fact, when the surface of the cell is loaded with uranyl acetate used as a postfixative tracer, all the caveolae are labelled and thus they must communicate with the extracellular space, although in many cases the actual connexion is not included in the plane of the section (figures 13, 14, plates 5, 6). More complicated arrangements can be observed, e.g. several caveolae opening into a common cavity which in its turn opens at the outside of the cell. Caveolae significantly

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Description of plate 4

Figure 8. Guinea-pig ileum. Circular muscle coat. A nexus between two smooth muscle cells. (Magn. × 182000; calibration 0.1 μm.)

Figure 9. Guinea-pig taenia coli. Cytoplasmic process protruding from one muscle cell into another. The gap between the two membranes is fairly regular and approx. 25 nm wide. (Magn. × 60000; calibration 0.5 μm.)

Figure 10. Guinea-pig taenia coli. Mushroom-shaped cytoplasmic process deeply embedded in a neighbour muscle cell. (Magn. × 29500; calibration 1 μm.)

Figure 11. Guinea-pig ileum. Longitudinal muscle coat. Parts of two adjacent smooth muscle cells form during contraction long interdigitations. (Magn. × 9000; calibration 3 μm.)

increase the surface of the cell, by 25 % in the longitudinal muscle of mouse small intestine (Rhodin 1962) and by more than 70 % in guinea-pig taenia coli (Goodford 1970).

Similar caveolae are observed in muscles other than smooth muscle. An interesting example is the rat heart, where Forssmann & Girardier (1970) found a large number of caveolae, but only in those cells of the atria which lack a transverse system; the other atrial cells and the ventricular cells have a well-developed transverse system and no caveolae. In the ventricular muscle of the lizard, where transverse tubules are absent, many caveolae are observed (Forbes & Sperelakis 1971). This would suggest some similarity between transverse tubules and caveolae, although the caveolae do not penetrate deeply into the cells; but this would hardly be necessary, because of the small size of the smooth muscle cells.

The whole muscle cell is covered by a basement lamina which never penetrates into the caveolae and does not show any structural differences opposite the caveolae.

Caveolae appear rather early in embryonic development (figure 17, plate 7). In the intestinal musculature of the guinea-pig they are already seen in embryos 40 mm long. At that stage smooth muscle cells are already completely surrounded by a basement lamina. From the early embryonic stages caveolae show a close relation with the sarcoplasmic reticulum (figure 18, plate 7).

The neck of the caveola has a lumen of more than 20 nm. It should be noted, however, that the trilaminar membrane that is observed in ordinary electron micrographs, is only the so-called inner zone of the greater membrane (Revel & Ito 1967), which includes – at least in those tissues where this problem has been investigated – an outer coat, a fuzzy layer, some 10 nm thick, mainly consisting of mucopolysaccharides. If this is true for the plasma membrane of smooth muscle cells, the actual neck of the caveola would be significantly smaller than appears in the electron micrographs.

When colloidal lanthanum (Revel & Karnovsky 1967; Goodenough & Revel 1970) is used as an extracellular space tracer, all spaces between smooth muscle cells are filled with electron opaque lanthanum, and the caveolae and all superficial recesses are penetrated by the tracer (figure 15, plate 6). Lanthanum (which is added to the fixative) was never observed penetrating into the cells, although some sites at the inner aspect of the plasma membrane, including the caveolae, appear to accumulate some lanthanum.

In the superficial regions of the specimens, during the preparative procedure, part of the lanthanum is washed out. A thin layer of lanthanum, however, is generally retained on the plasma membrane, where it is probably bound to the glycocalyx or to some extracellular substance (Doggenweiler & Frenk 1965) and lanthanum remains filling the caveolae (figure 16,

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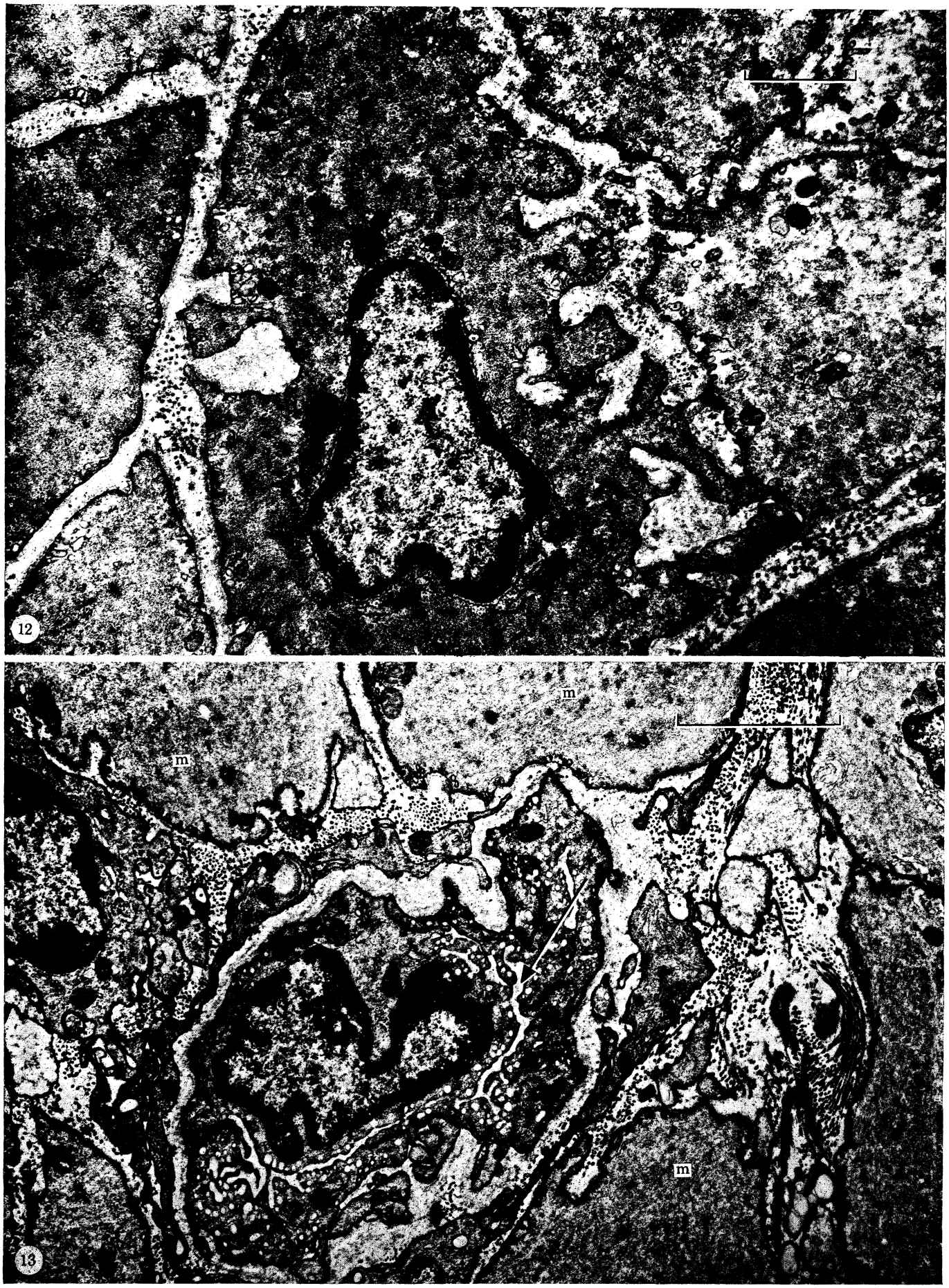
Description of plate 5

Figure 12. Rat ano-coccygeus muscle. Large invaginations of the cell surface are not penetrated by collagen fibres and show a fuzzy material. These invaginations are spread over the whole length of the cell including the region of the nucleus. Caveolae, sarcoplasmic reticulum and mitochondria in the superficial region of the cell are well developed. Nexuses are numerous and the muscle is densely innervated. (Magn. × 21000; calibration 1 μm.)

Figure 13. Guinea-pig taenia coli. Tissue fixed ½ h with glutaraldehyde, then with unbuffered 2 % osmium; treated with uranyl acetate in 70 % ethanol. The uranium is condensed all over the surface of cells. It outlines the surface of smooth muscle cells (*m*), including the caveolae, and of interstitial cells (*ic*). The outer surface of endothelial cells is stained, with some of the micropinocytotic vesicles; however, the tracer is arrested at the junctions between endothelial cells and does not penetrate into the lumen of the vessel (arrow). (Magn. × 15500; calibration 2 μm.)



FIGURES 12 AND 13. For legend see facing page.

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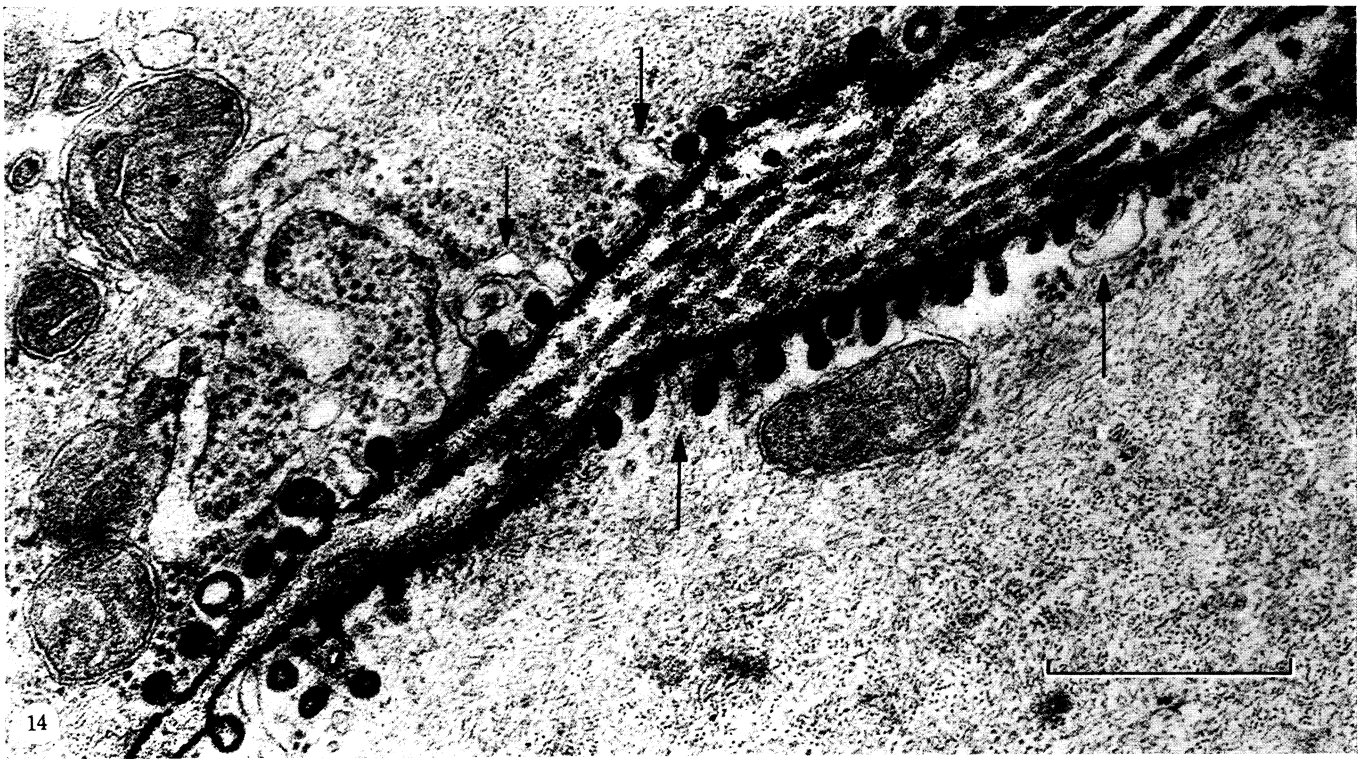

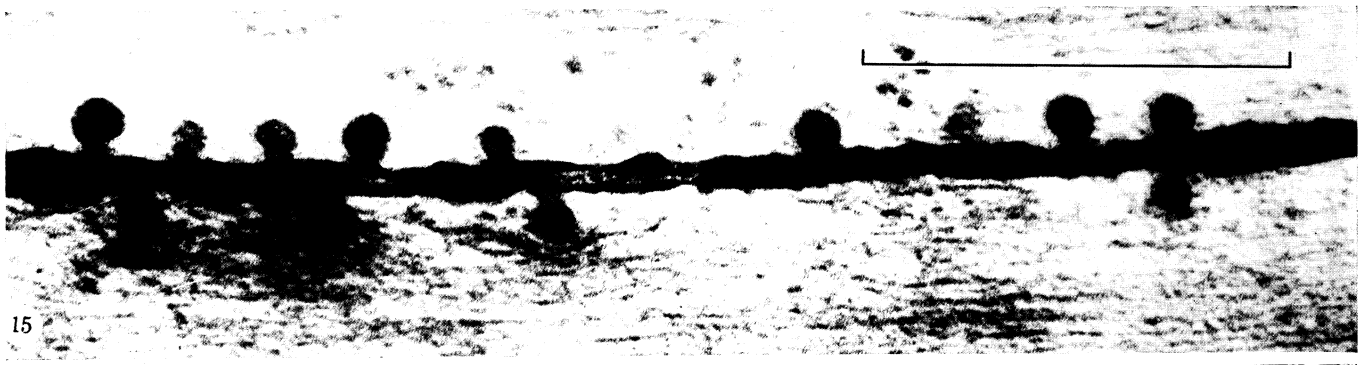

FIGURES 14 TO 16. For legend see facing page.

plate 6). There appears to be no easy depletion of the tracer from the caveolae during the washing out.

Ferritin, another extracellular space tracer, penetrates the caveolae when incubated for 30 min in Krebs solution containing 20 to 30 % ferritin by volume (Somlyo, Devine, Somlyo & North 1971).

All the evidence shows that caveolae are in communication with the extracellular space. However, the fact that tracers added to the fixative or tracers added to the incubation medium for extensive lengths of time, and still present in the tissue during the whole histological procedure, penetrate the caveolae, does not give any indication of how easily an ion or a molecule can reach the caveolae from the bulk of the extracellular space or vice versa.

When the intestinal smooth muscle is treated *in vitro* with hyaluronidase at a concentration of 15 i.u./ml, in the majority of muscle cells caveolae disappear, the basement lamina is less well defined and over large areas becomes separated from the plasma membrane (figure 19, plate 7). The plasma membrane has an undulating aspect and these undulations are reminiscent of caveolae as if they had been stretched and opened. With a similar concentration of hyaluronidase Goodford & Leach (1966) observed a significant increase of the extracellular space ('extracellular inulin space') and a significant decrease of the intracellular space. Moreover, it has been shown that in taenia coli, after incubation in hypertonic solution, caveolae are often found to be more numerous than in control taenia and to be more elongated, flask-like and narrow-necked (Nishihara 1970).

Thus it may be suggested that the space delimited by the caveolae, although in communication with the bulk of the extracellular space, is not a simple extension of it, and it is possible that its ionic composition is more readily controlled by the muscle cell itself. This space would create an additional compartment at the cell surface besides the compartment of the extracellular space proper. Moreover, the occurrence of a glycocalyx and a basement lamina provides the membrane with an outer coat which may play a significant role in ion transport and storage.

The superficial region of smooth muscle cells

Closely related to the plasma membrane and the caveolae, but never in continuity with them, is a well-developed system of endoplasmic reticulum (sarcoplasmic reticulum). The presence of a superficially located sarcoplasmic reticulum has been reported by several investigators since 1960. Prosser, Burnstock & Kahn (1960) for example reported the presence of two types of vesicle at the surface of smooth muscle cells – they are clearly identified with what is now

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Description of plate 6

Figure 14. As figure 13. The tracer outlines the surface of two muscle cells with numerous invaginations (caveolae); in some of them the connexion with the extracellular space is visible, in others the section has missed it and they appear as vesicles. Numerous sacs of smooth and rough sarcoplasmic reticulum are seen (arrows); they are not reached by the tracer. Mitochondria, sarcoplasmic reticulum and caveolae are closely associated. (Magn. × 64000; calibration 0.5 μm.)

Figure 15. Guinea-pig ileum. Circular muscle coat. Colloidal lanthanum (lanthanum hydroxide) added to the fixative (Revel & Karnovsky 1967) fills the caveolae and the space between muscle cells. (Magn. 113000; calibration 0.5 μm.)

Figure 16. Lanthanum is frequently washed out from the superficial portion of the specimens during the preparation procedure. A thin layer of lanthanum bound to the plasma membrane and the tracer filling the caveolae are generally not washed out in these conditions. (Magn. × 68000; calibration 0.5 μm.)

called caveolae and sarcoplasmic reticulum. However, until fairly recently, it was assumed that sarcoplasmic reticulum is poorly represented in smooth muscle cells (Dewey & Barr 1968; Burnstock 1970).

The occurrence of abundant sarcoplasmic reticulum is now well established and documented – in vascular (Somlyo *et al.* 1971) as well as visceral smooth muscle (Gabella 1971). It has been consistently observed in the taenia coli, uterus, vas deferens, small and large intestine and stomach. Moreover, sarcoplasmic reticulum (Golgi complexes, isolated cisternae or sacs of rough and smooth endoplasmic reticulum) is observed in the deep region of the cell, mainly in the cytoplasmic areas at the poles of the nucleus.

In the superficial regions of the cell, the sarcoplasmic reticulum appears as sacs and tubules mainly developed along the cell's longitudinal axis (figure 20, plate 8). Smooth sarcoplasmic reticulum predominates but sacs of rough sarcoplasmic reticulum are not uncommon as well as sacs formed partly by smooth and partly by rough membranes. The occurrence of tubules of sarcoplasmic reticulum running between rows of caveolae or of labyrinthine sarcoplasmic reticulum spread among the caveolae is better seen in tangential sections which graze the cell surface. Other cisternae of sarcoplasmic reticulum form a kind of gutter for a row of caveolae and reach the plasma membrane at both sides of it. Still other sacs of sarcoplasmic reticulum run immediately underneath the plasma membrane. All the superficial sarcoplasmic reticulum then appears to contact the plasma membrane at some point, although the relationship with the caveolae seems too extensive to be fortuitous.

Many of the mitochondria of smooth muscle cells lie close to the caveolae, but frequently a sac of sarcoplasmic reticulum intervenes between the two.

These topographical relations between superficial structures of the smooth muscle cell suggest some specific functional interaction between them. But the possibility, by the analysis of electron micrographs, of getting much closer to the dynamic state of organelles and to the intense traffic of metabolites between organelles in the living state appears remote.

Quantitative determinations of the volume of sarcoplasmic reticulum in smooth muscle cells have been published. For the taenia coli a value of 2 % of the cell cytoplasm volume for all the sarcoplasmic reticulum (superficial and deep) of the cell has been obtained (Devine, Somlyo & Somlyo 1972). It is of interest to know the *surface* of sarcoplasmic reticulum (i.e. the amount of membrane) present in a smooth muscle cell: in the intestinal musculature the area of the sarcoplasmic reticulum present in the superficial regions of the cell is approximately 1.5 times the area of the cell membrane (excluding the caveolae). How functionally homogeneous this superficial sarcoplasmic reticulum may be is not known. In developing aorta, during postnatal growth, and in hormone-stimulated uterus, smooth muscle cells show an intense

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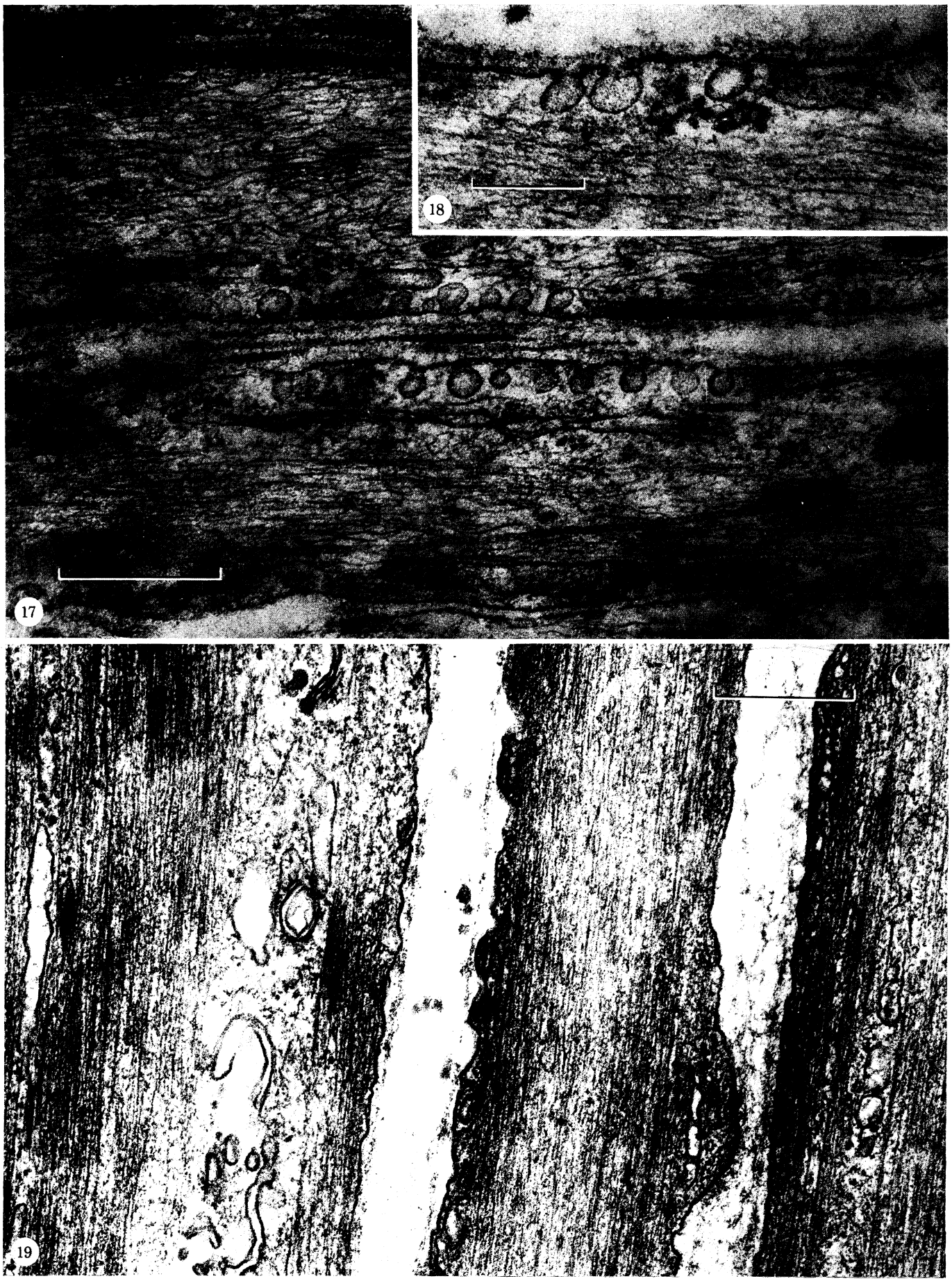
Description of plate 7

Figure 17. Guinea-pig embryo (50 mm CR). Circular muscle coat of ileum. Three smooth muscle cells longitudinally sectioned, showing numerous caveolae and sacs of sarcoplasmic reticulum. (Magn. × 70500; calibration 0.5 μm.)

Figure 18. Higher magnification showing details of caveolae, plasma membrane, sarcoplasmic reticulum and basement lamina. (Magn. × 103000; calibration 0.2 μm.)

Figure 19. Guinea-pig ileum. Circular muscle coat. Tissue incubated for 30 min in Krebs's solution containing 15 i.u./ml of testicular hyaluronidase. Caveolae have disappeared from most of the muscle cells, the basement lamina is at points separated from the plasma membrane. The plasma membrane has an undulating aspect. (Magn. × 26000; calibration 1 μm.)



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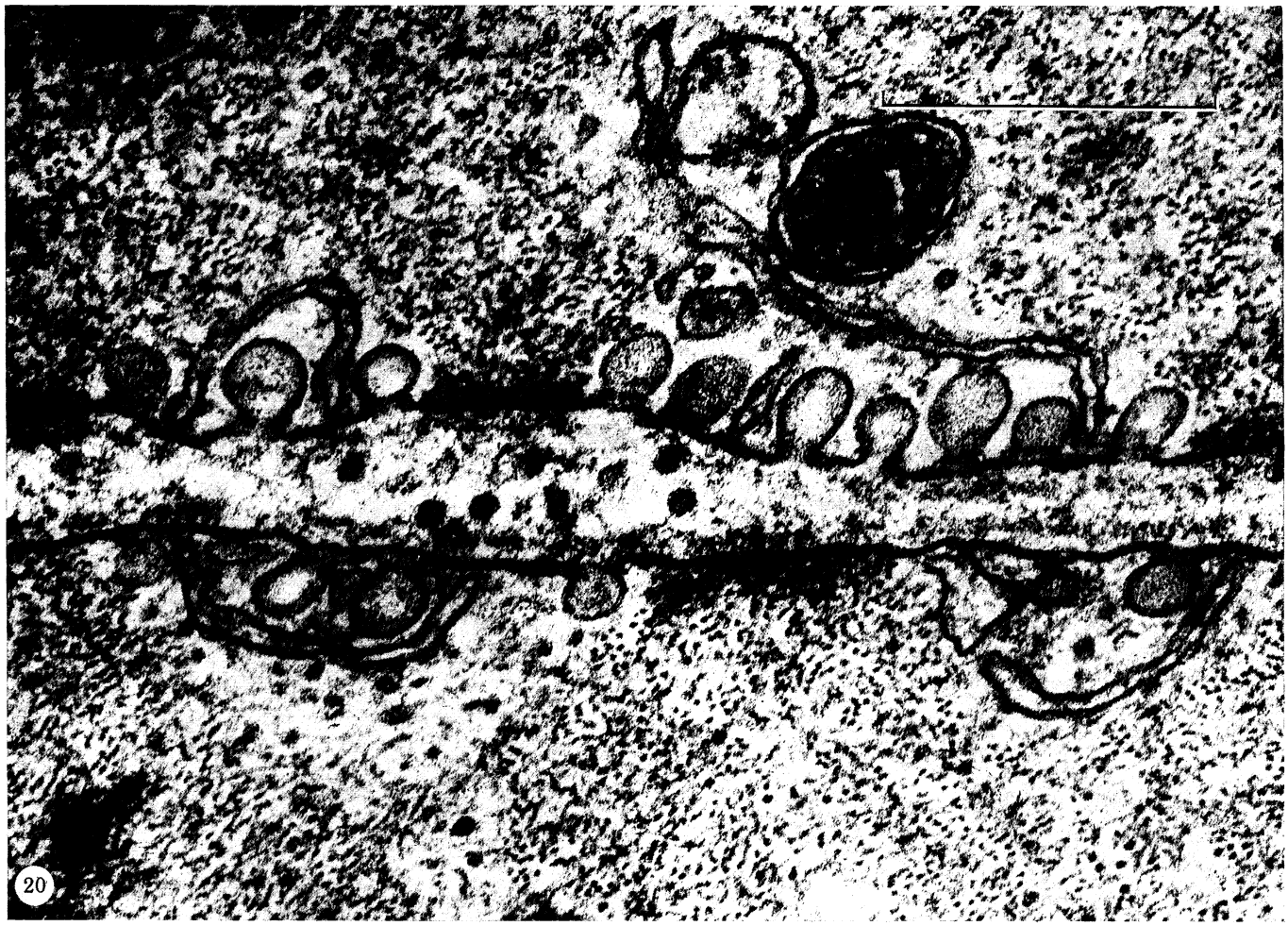

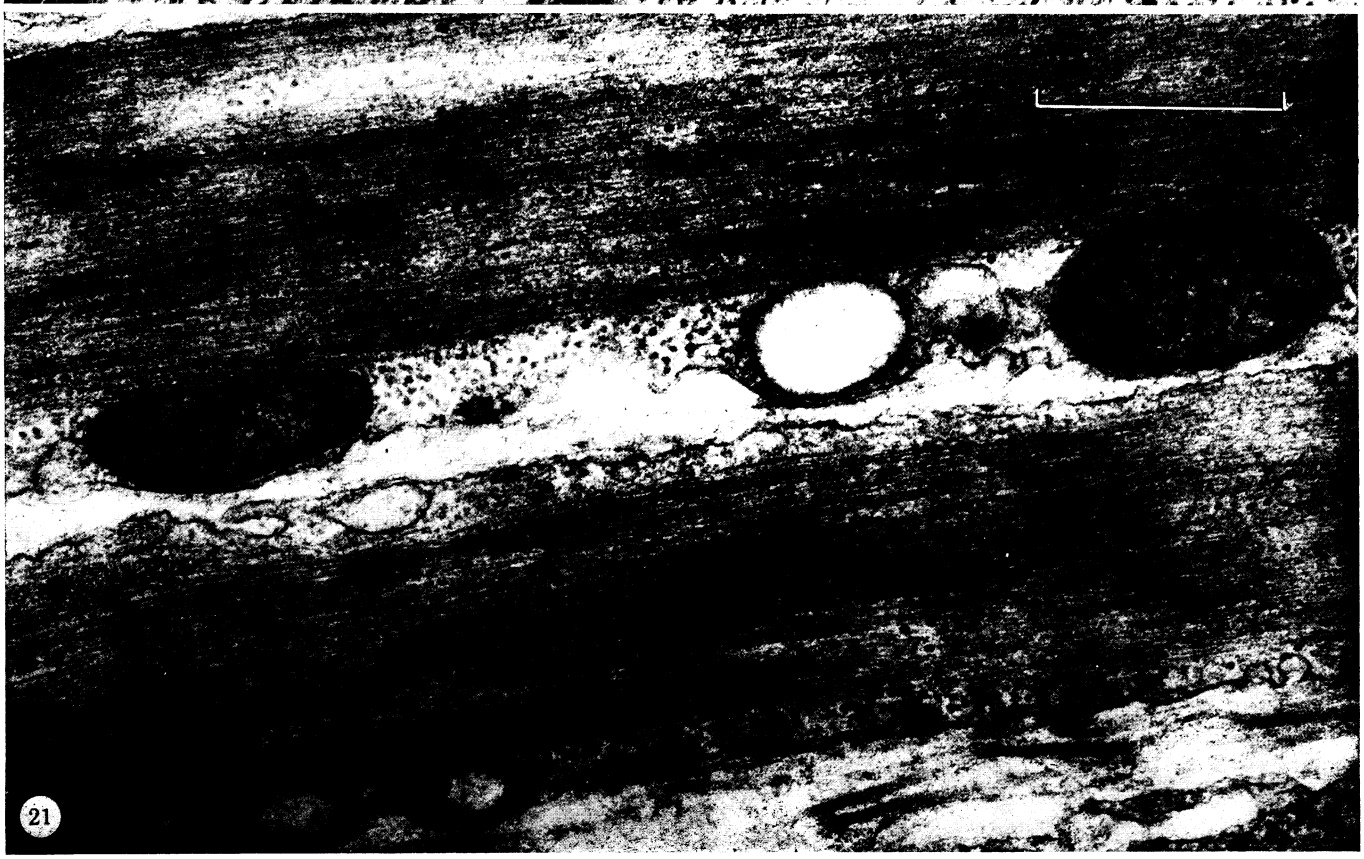

FIGURES 20 AND 21. For legend see facing page.

ability to synthesize extracellular proteins (see Ross & Klebanoff 1971). The occurrence of some rough sarcoplasmic reticulum sacs suggests that this ability may still occur in fully developed vascular and visceral smooth muscle, especially since fibroblasts are absent or very rare in the tunica media of blood vessels and in the muscularis externa of the intestinal wall (apart from interstitial cells).

Because of its strategic position close to the cell surface, and because of the analogy with striated muscle, it has been suggested that sarcoplasmic reticulum may play an essential role in storage and transport of calcium in smooth muscle (Gabella 1971). No direct evidence for this hypothesis has yet been provided. It has recently been shown that sarcoplasmic reticulum accumulates the divalent cation strontium when the tissue is incubated for 1 h in a strontium rich solution (Somlyo & Somlyo 1971). Mitochondria also accumulate strontium, and barium, which is not taken up by sarcoplasmic reticulum (Devine *et al.* 1972). However, the ability of sarcoplasmic reticulum to release the accumulated strontium (and, more to the point, calcium) has not yet been proven.

When intestinal smooth muscle is incubated in media containing a high concentration of calcium (up to 64 mmol Ca^{2+}) the main morphological change is a swelling of the sarcoplasmic reticulum. Underneath the cell membrane a number of balloon-like sacs are observed; their content is lucent but the membrane has an increased electron density (figure 21). Ribosomes are sometimes attached to these structures.

No direct evidence on the localization of ionic calcium in smooth muscle is at present available. The problem, however, is less to show the ability of sarcoplasmic reticulum to take up, store and release calcium than to know what is the state and significance of the calcium that is stored and released and at what moment of the contraction cycle. Mitochondria are in a no less strategic position for calcium storage and release; and in the heart muscle it has been shown that calcium for contraction is released from the mitochondrial membrane (Lehninger 1970, 1971). In the intestinal musculature mitochondria constitute approximately 6.6 % of the cell volume.

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Description of plate 8

Figure 20. Guinea-pig ileum. Circular muscle coat. Two smooth muscle cells transversely sectioned, showing several caveolae, in close relationship with sacs of sarcoplasmic reticulum. (Magn. × 117 000; calibration 0.5 μm.)

Figure 21. Guinea-pig ileum. Circular muscle coat. Smooth muscle incubated in isotonic solution containing 63 mmol/l Ca^{2+}. The balloon-like structures under the plasma membrane probably correspond to swollen sacs of sarcoplasmic reticulum. They show an enhanced electron density. Most of the caveolae are unaffected by the treatment. (Magn. × 64000; calibration 0.5 μm.)

References (Gabella)

Brightman, M. W. & Reese, T. S. 1969 *J. Cell Biol.* **40**, 648–677.

Bülbring, E. 1954 *J. Physiol., Lond.* **125**, 302–315.

Burnstock, G. 1970 Structure of smooth muscle and its innervation. In *Smooth muscle* (eds. E. Bülbring, A. F. Brading, A. W. Jones & T. Tomita), pp. 1–69. London: Arnold.

Burnstock, G., Campbell, G. & Rand, M. J. 1966 *J. Physiol., Lond.* **182**, 504–526.

Campbell, G. R., Uehara, Y., Malmfors, T. & Burnstock, G. 1971 *Z. Zellforsch.* **117**, 155–175.

Devine, C. E., Somlyo, A. V. & Somlyo, A. P. 1972 *J. Cell Biol.* **52**, 690–718.

Dewey, M. M. & Barr, L. 1962 *Science, N.Y.* **137**, 670–672.

Dewey, M. M. & Barr, L. 1968 Structure of vertebrate intestinal smooth muscle. In *Handbook of physiology*, § 6, *Alimentary canal*, vol. 4, *Motility* (ed. C. F. Code), pp. 1629–1645. Washington: American Physiological Society.

Doggenweiler, C. F. & Frenk, S. 1965 *Proc. natn. Acad. Sci. U.S.A.* **53**, 425–430.

Forbes, M. S. & Sperelakis, N. 1971 *J. Ultrastruct. Res.* **34**, 439–451.

Forssmann, W. G. & Girardier, L. 1970 *J. Cell Biol.* **44**, 1–19.

Gabella, G. 1971 *J. Cell Sci.* **8**, 601–609.

Gabella, G. 1972*a* *J. Anat.* **111**, 69–97.

Gabella, G. 1972*b* *Z. Zellforsch.* **125**, 191–199.

Gillespie, J. S. 1971 *Br. J. Pharmacol.* **43**, 430*P*.

Goodenough, D. A. & Revel, J. P. 1970 *J. Cell Biol.* **45**, 272–290.

Goodford, P. J. 1970 Ionic interactions in smooth muscle. In *Smooth muscle* (ed. E. Bülbring, A. F. Brading, A. W. Jones & T. Tomita), pp. 100–121. London: Arnold.

Goodford, P. J. & Leach, E. H. 1966 *J. Physiol. Lond.* **186**, 1–10.

Henderson, R. M., Duchon, G. & Daniel, E. E. 1971 *Am. J. Physiol.* **221**, 564–574.

Hess, A., Pilar, G. & Weakley, J. N. 1969 *J. Physiol., Lond.* **202**, 339–354.

Kuriyama, H., Osa, T. & Toida, N. 1967 *J. Physiol., Lond.* **191**, 239–255.

Lane, B. P. 1965 *J. Cell Biol.* **27**, 199–213.

Lane, B. P. & Rhodin, J. A. G. 1964 *J. Ultrastruct. Res.* **10**, 470–488.

Lehninger, A. L. 1970 *Biochem. J.* **119**, 129–138.

Lehninger, A. L. 1971 Metabolite carriers in mitochondrial membranes: the Ca^{2+} transport system. In *The dynamic structure of cell membranes* (ed. F. D. Wallach & H. Fisher), pp. 119–128. Heidelberg: Springer.

Li, P.-L. 1940 *J. Anat.* **74**, 348–357.

McGeachie, J. K. 1971 *Experientia* **27**, 436.

Nishihara, H. 1970 *J. Anat.* **107**, 101–114.

Palade, G. E. & Bruns, R. R. 1968 *J. Cell Biol.* **37**, 633–649.

Prosser, C. L., Burnstock, G. & Kahn, J. 1960 *Am. J. Physiol.* **199**, 545–552.

Revel, J.-P. & Ito, S. 1967 The surface components of cells. In *The specificity of cell surfaces* (ed. D. B. Davis & L. Warren), pp. 211–234. Englewood Cliffs, New Jersey: Prentice-Hall.

Revel, J. P. & Karnovsky, M. J. 1967 *J. Cell Biol.* **33**, C7.

Revel, J. P., Olson, W. & Karnovsky, M. J. 1967 *J. Cell Biol.* **35**, 112A.

Rhodin, J. A. G. 1962 *Physiol. Rev.* **42**, suppl. 5, 48–81.

Ross, R. & Klebanoff, S. J. 1971 *J. Cell Biol.* **50**, 159–171.

Somlyo, A. P., Devine, C. E., Somlyo, A. V. & North, S. R. 1971 *J. Cell Biol.* **51**, 722–741.

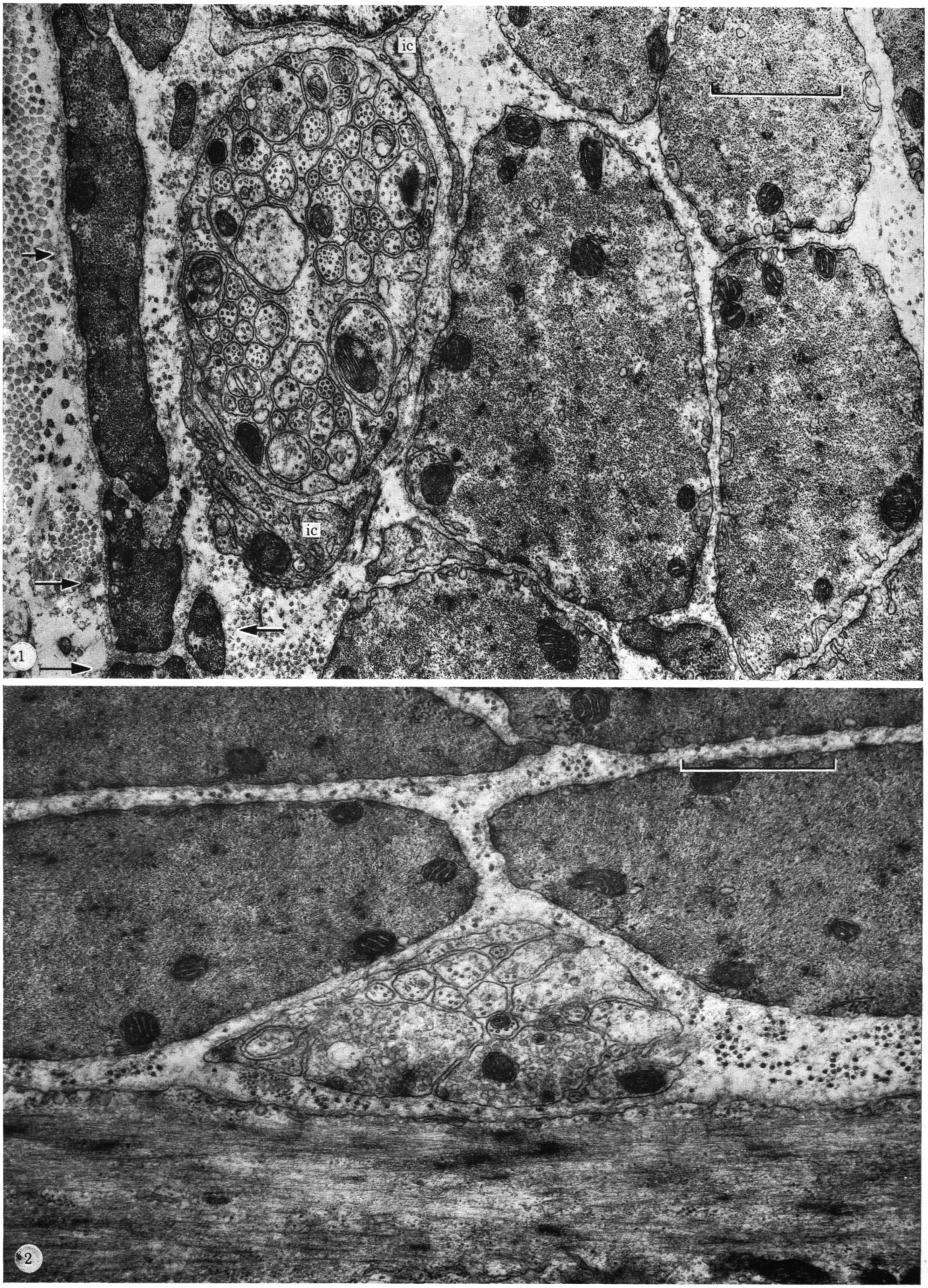
Somlyo, A. V. & Somlyo, A. P. 1971 *Science, N.Y.* **174**, 955–958.

Taxi, J. 1965 *Annls Sci. nat. Zool.* **7**, 413–674.

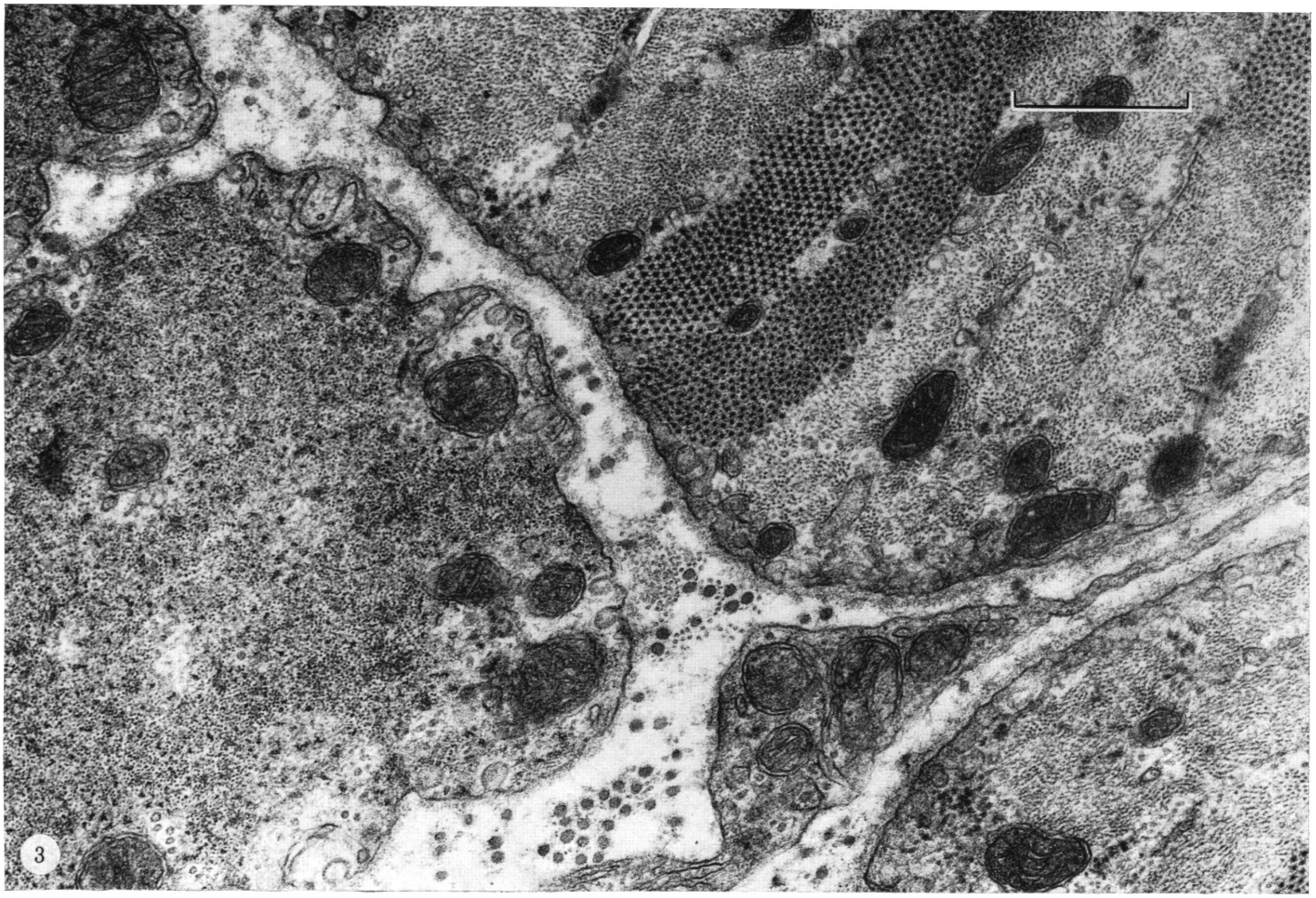
Tomita, T. 1967 *J. Physiol., Lond.* **189**, 163–176.

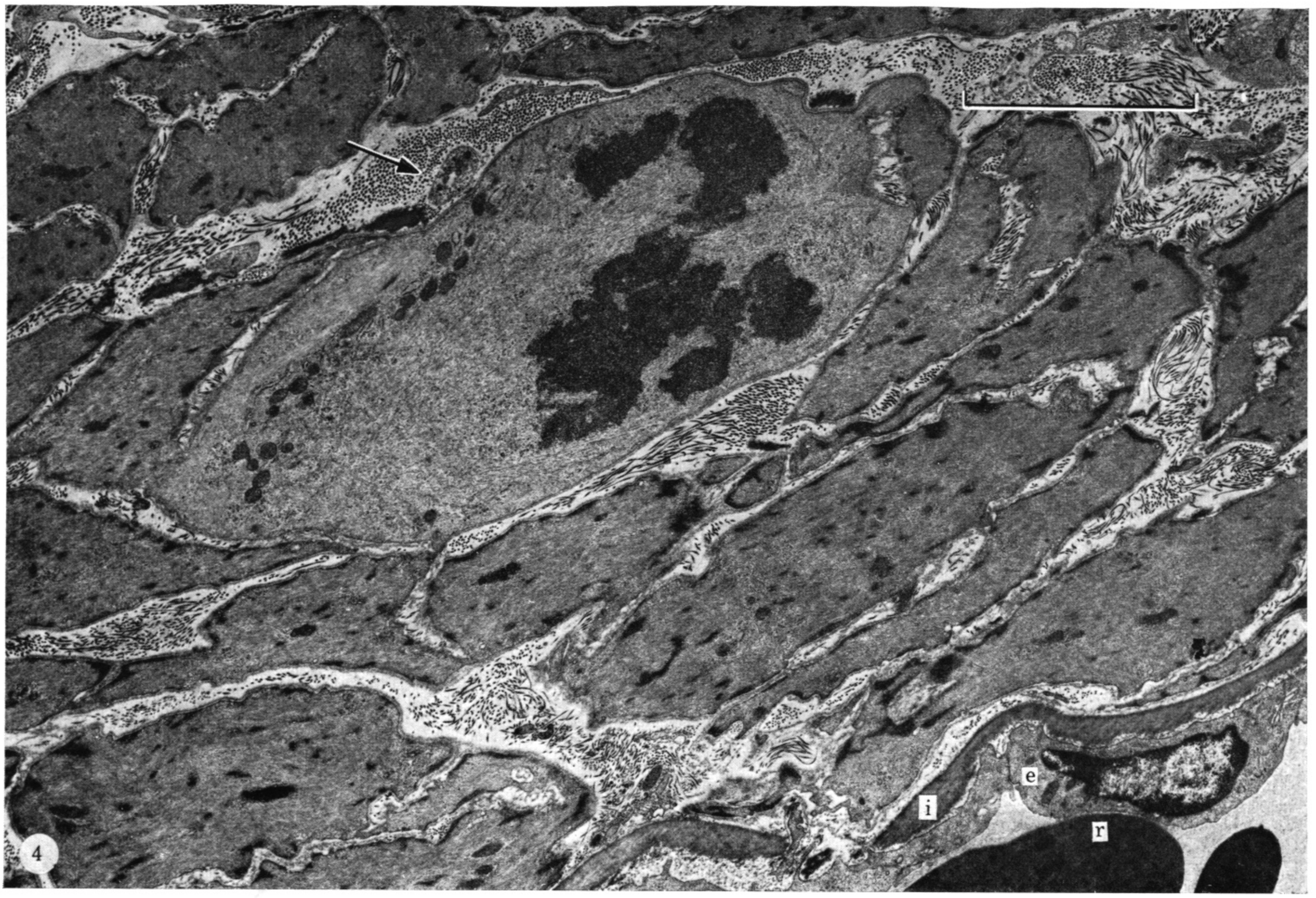
Uehara, Y. & Burnstock, G. 1970 *J. Cell Biol.* **44**, 215–217.

Wood, J. D. & Perkins, W. E. 1970 *Am. J. Physiol.* **218**, 762–768.



FIGURES 1 AND 2. For legend see facing page.





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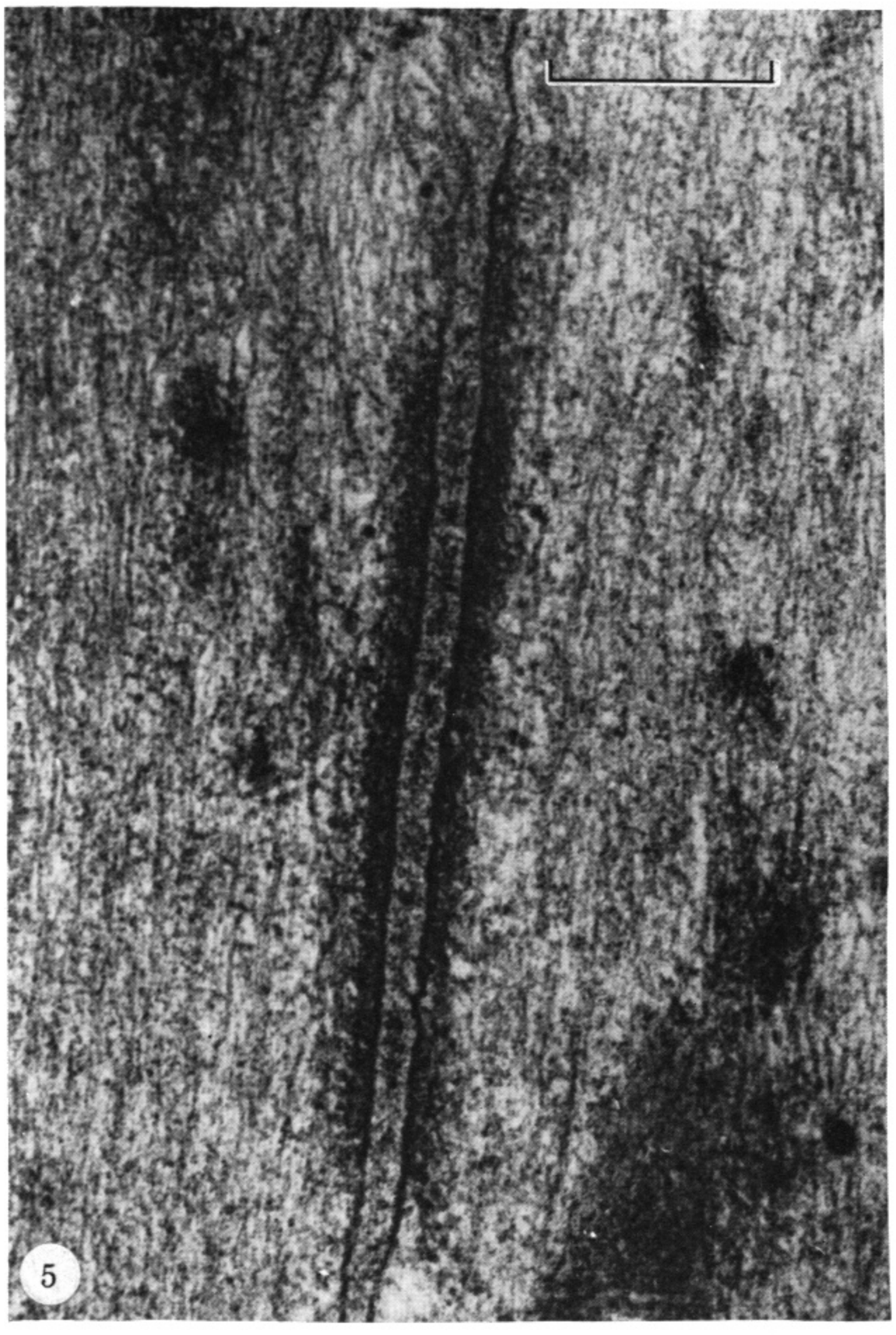

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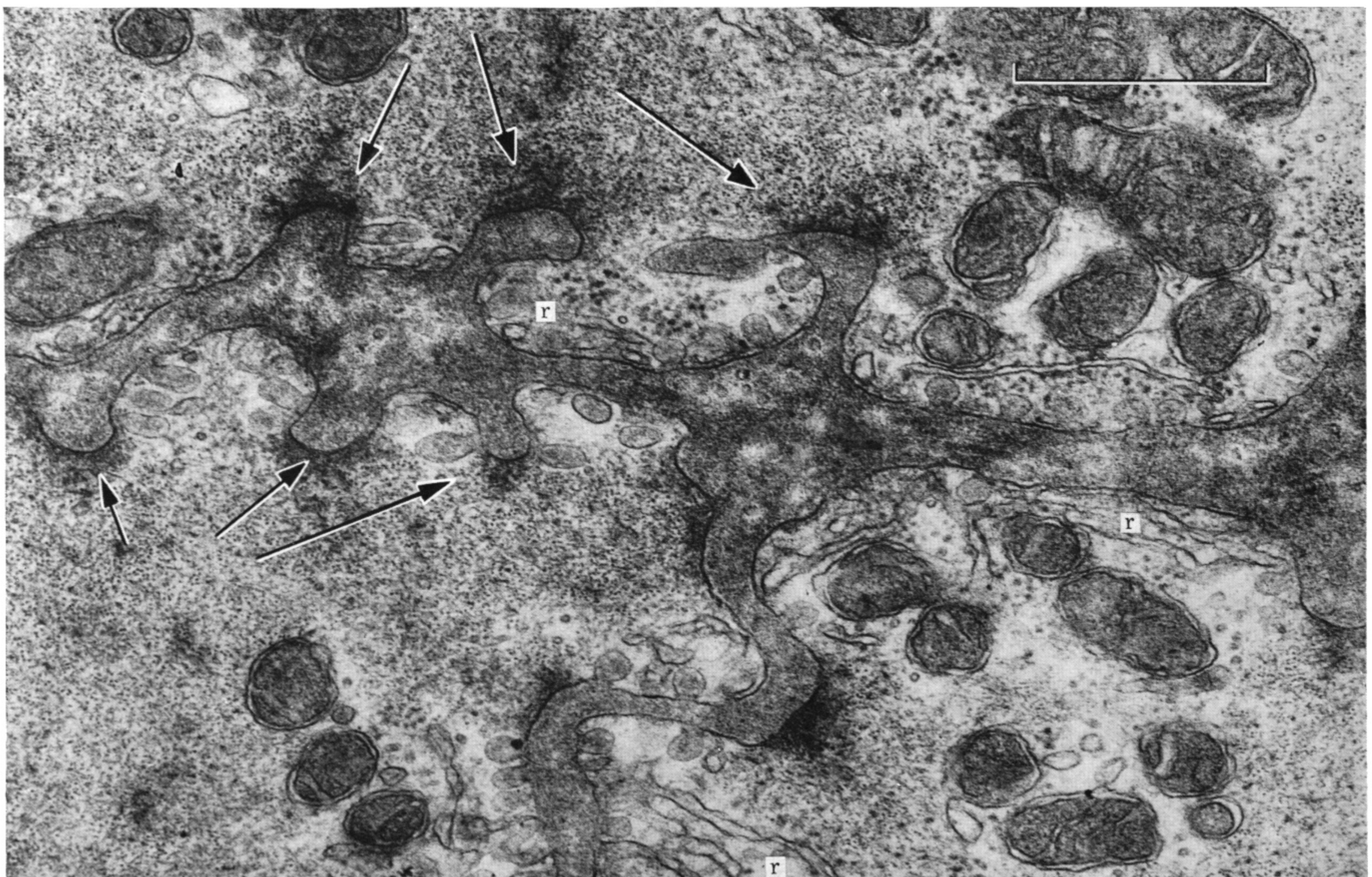

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FIGURES 5 TO 7. For legend see facing page.

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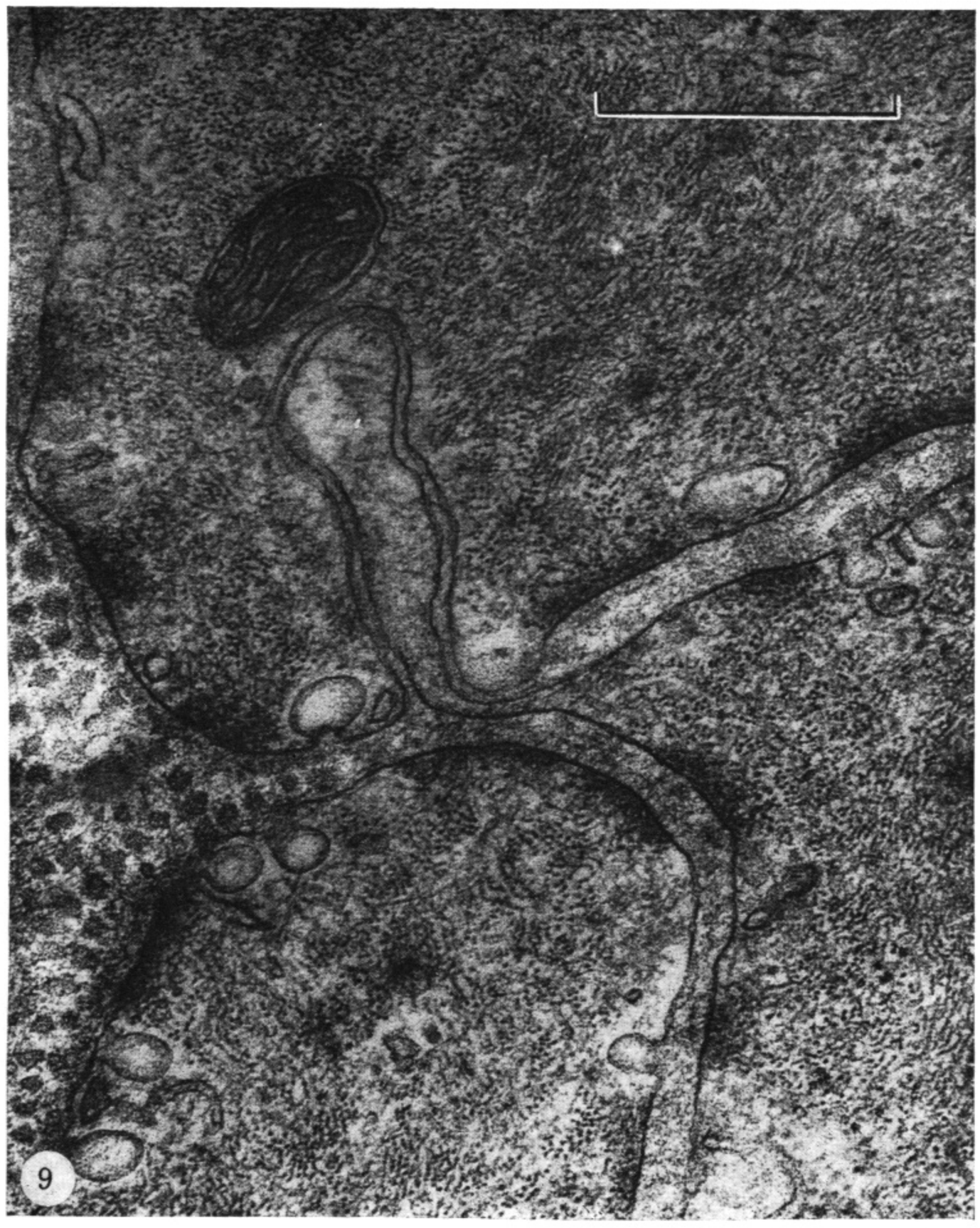

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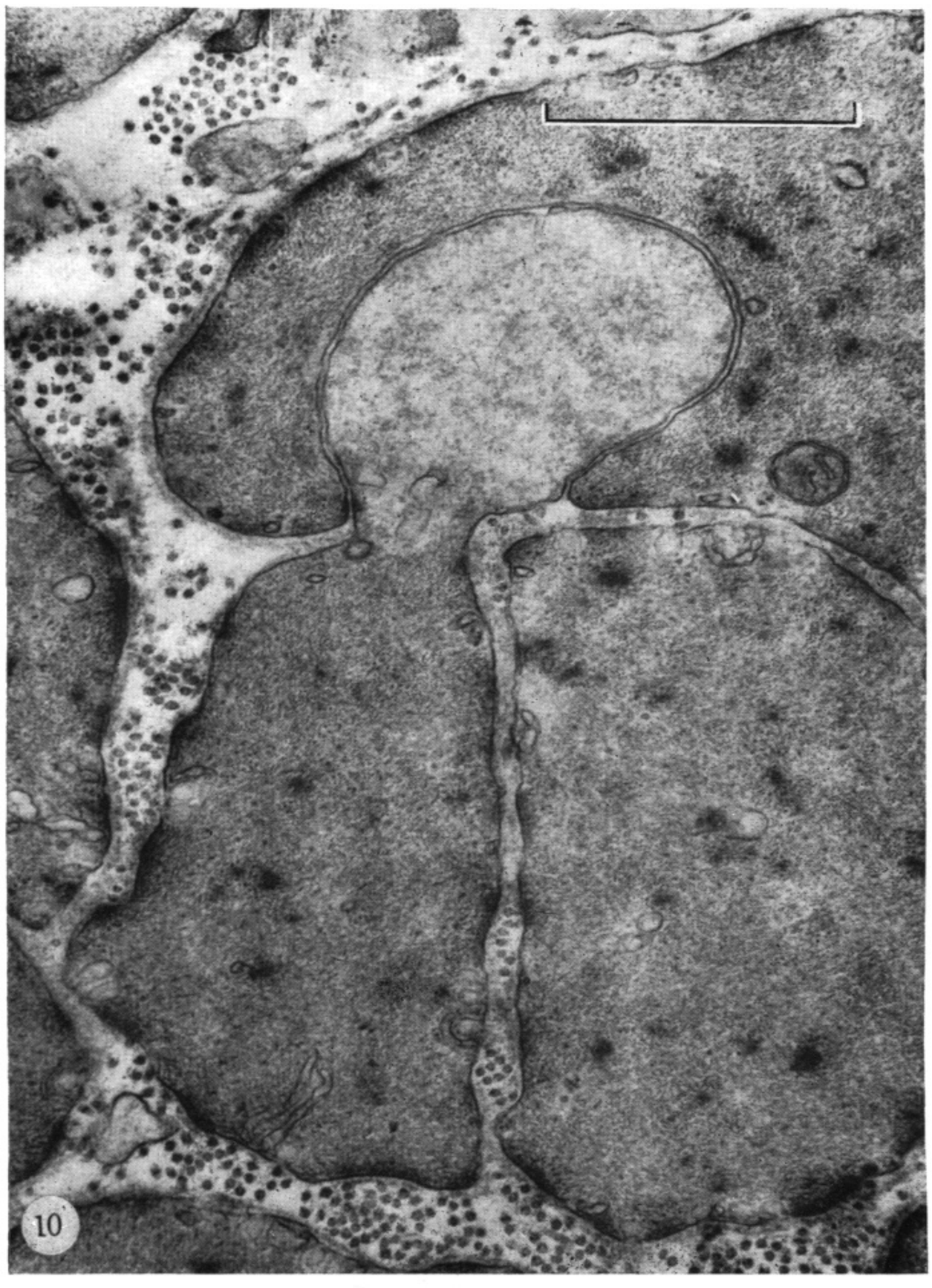

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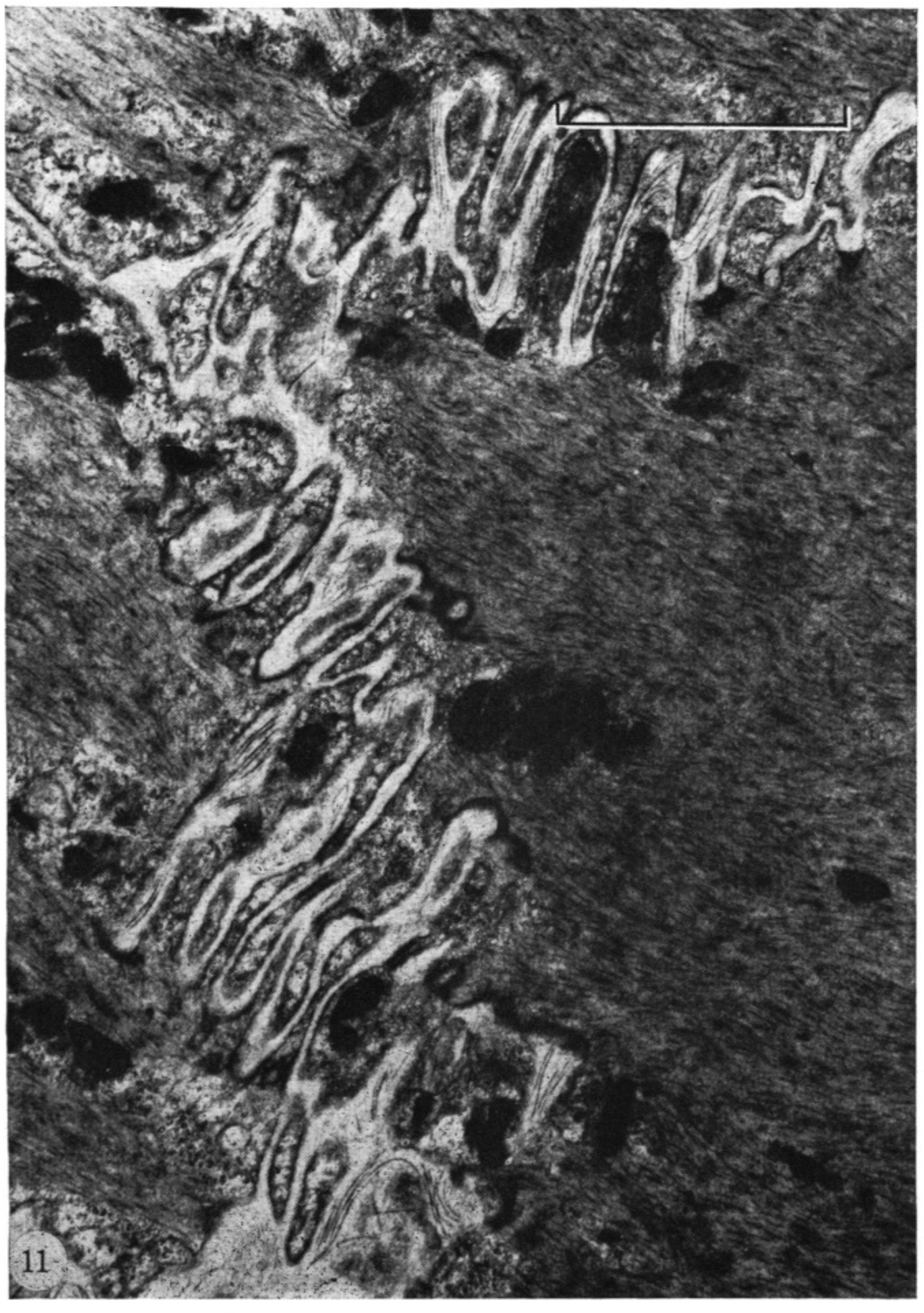

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FIGURES 8 TO 11. For legend see facing page.

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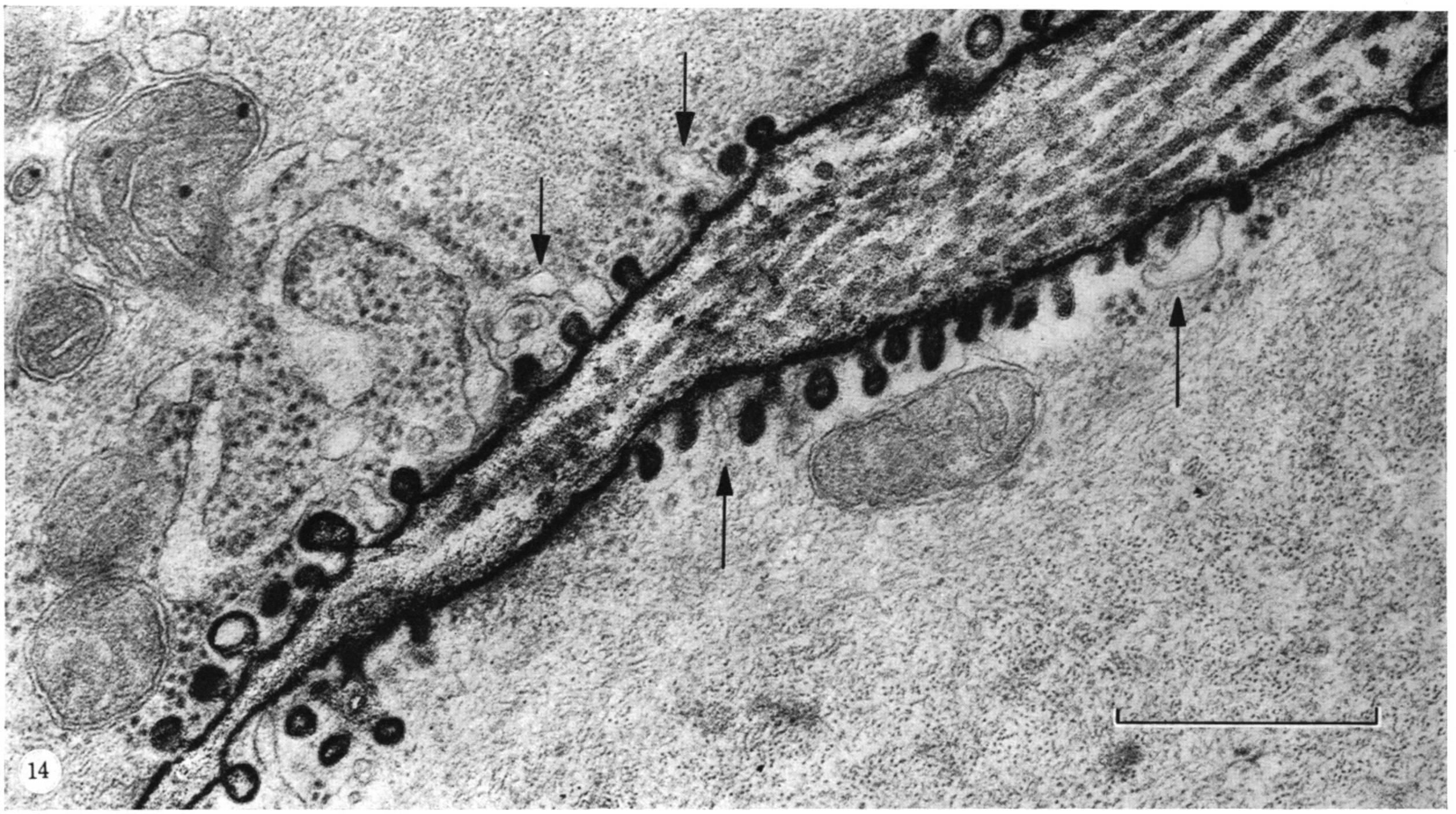
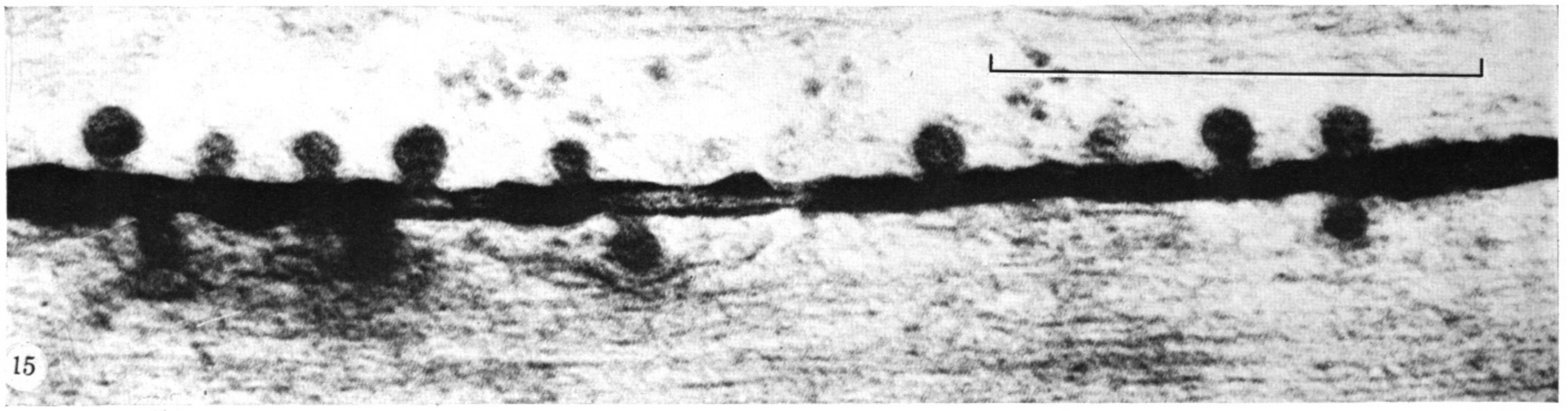
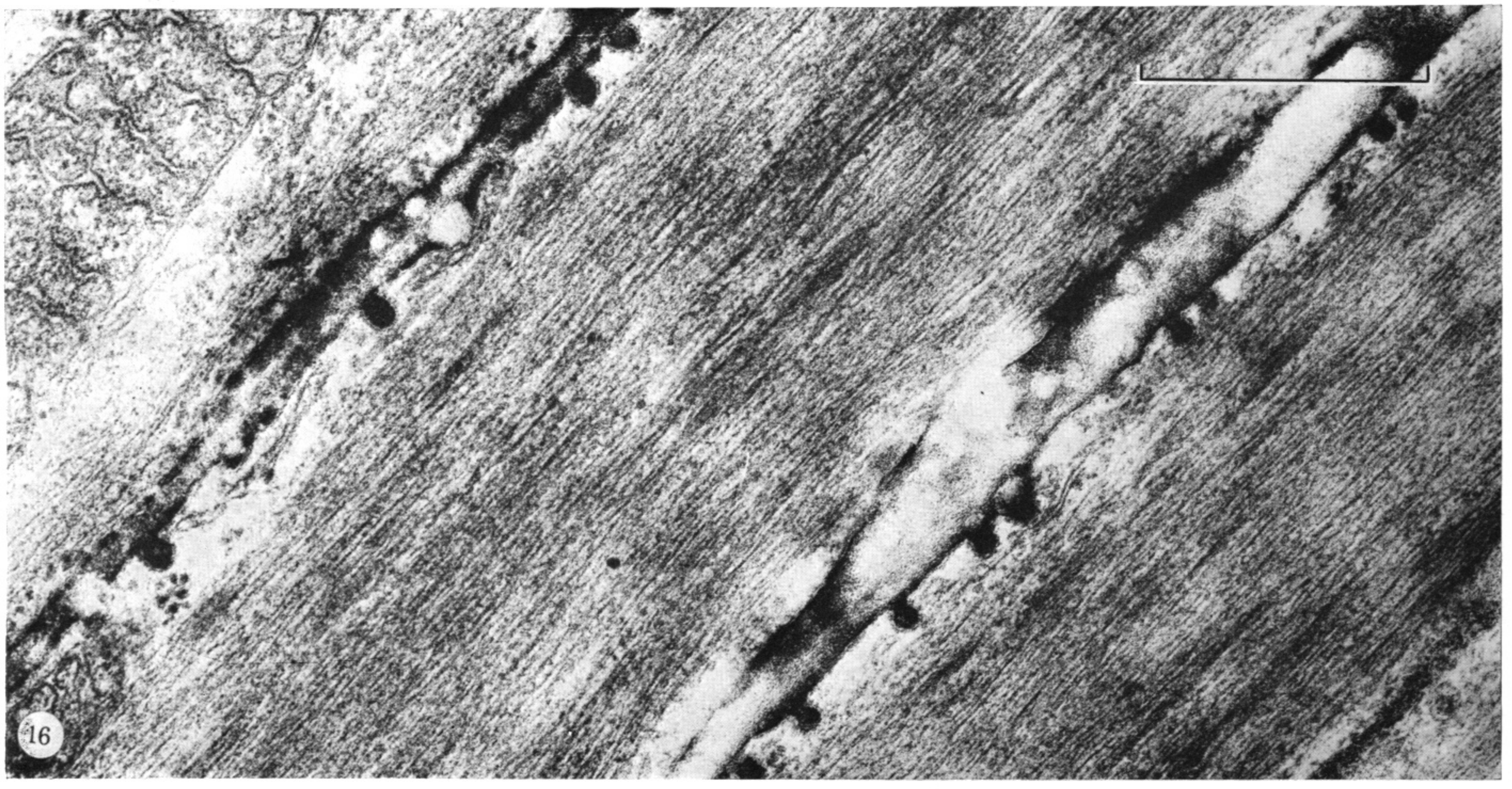
FIGURES 12 AND 13. For legend see facing page.







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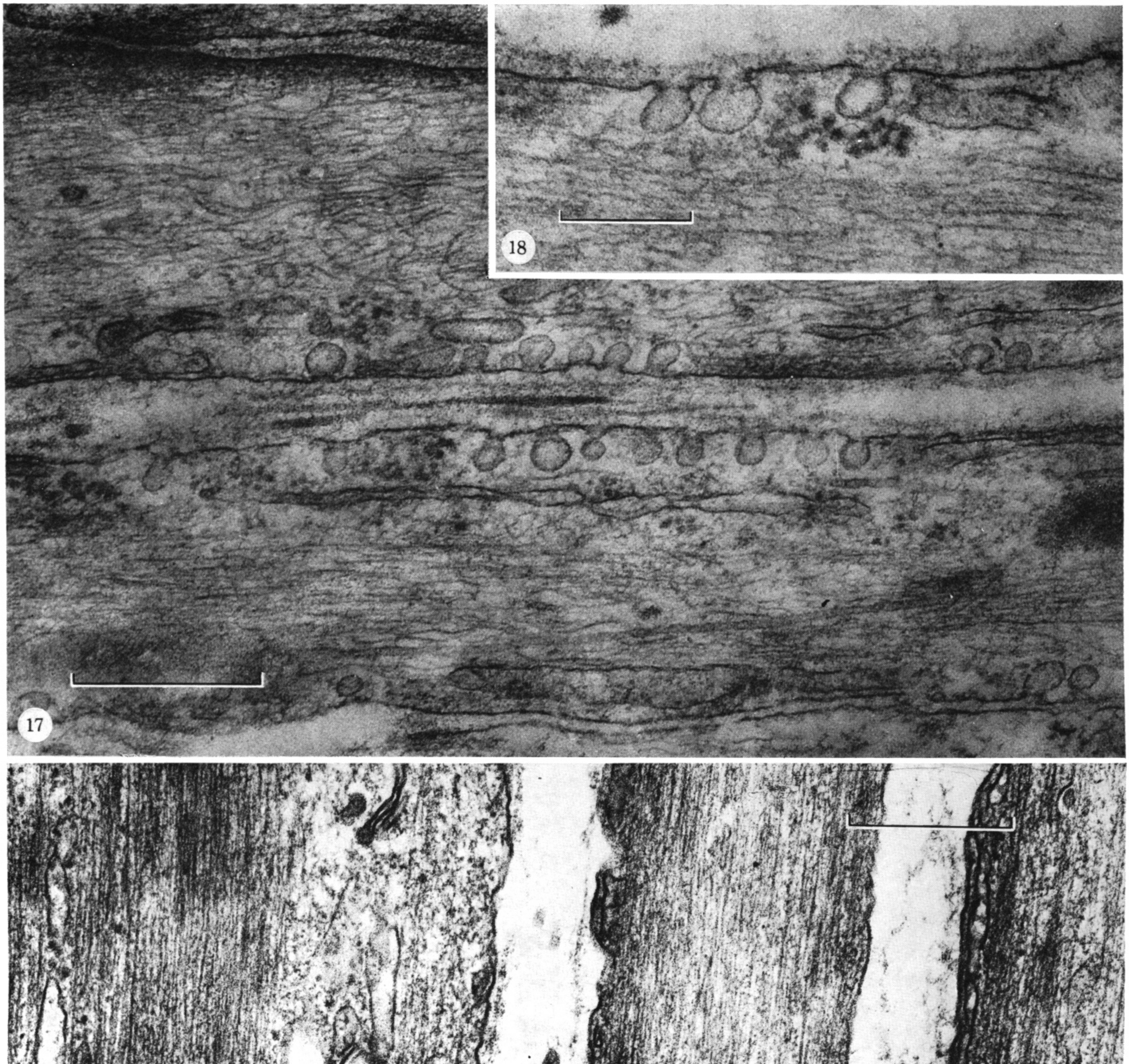

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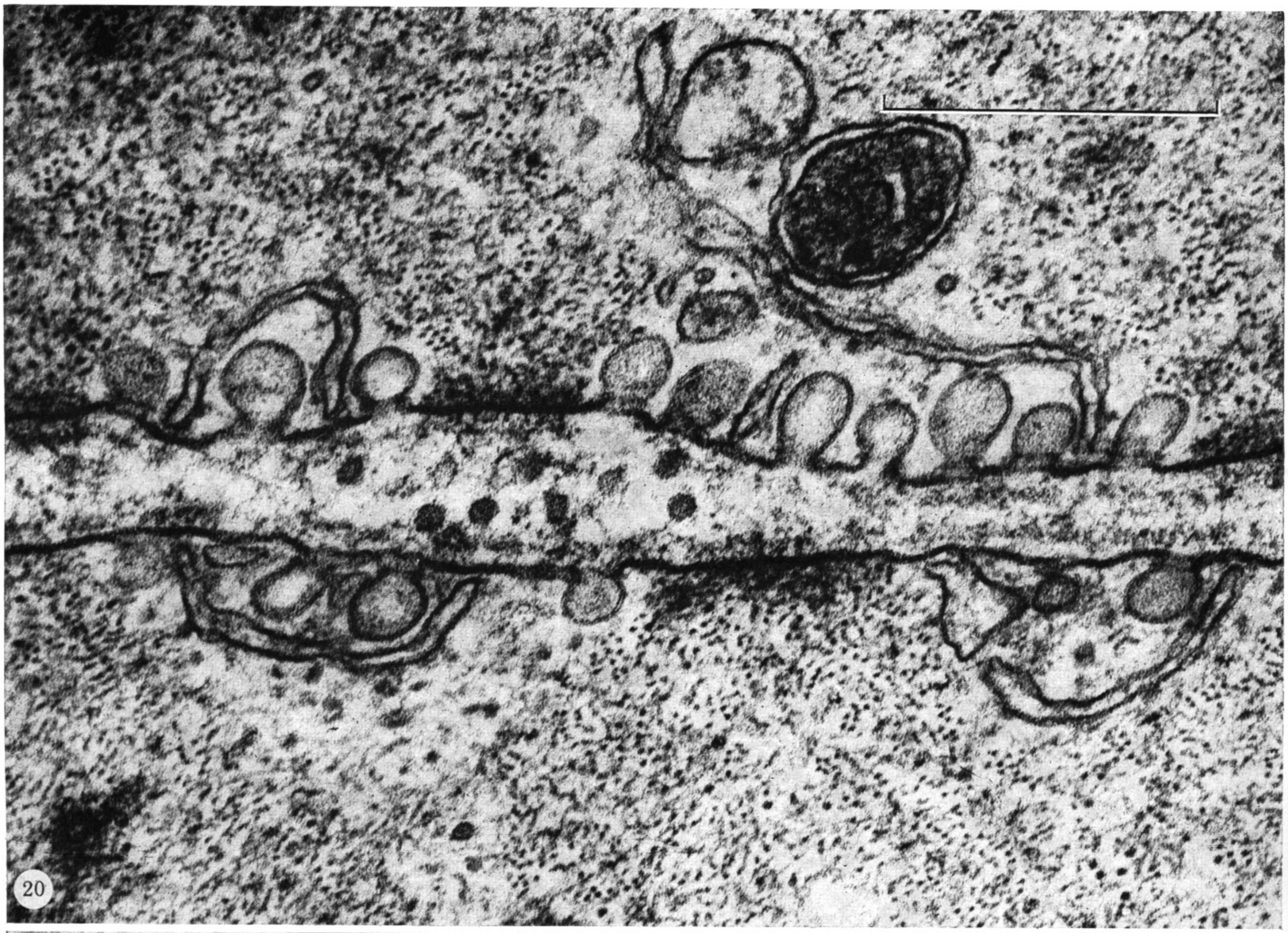

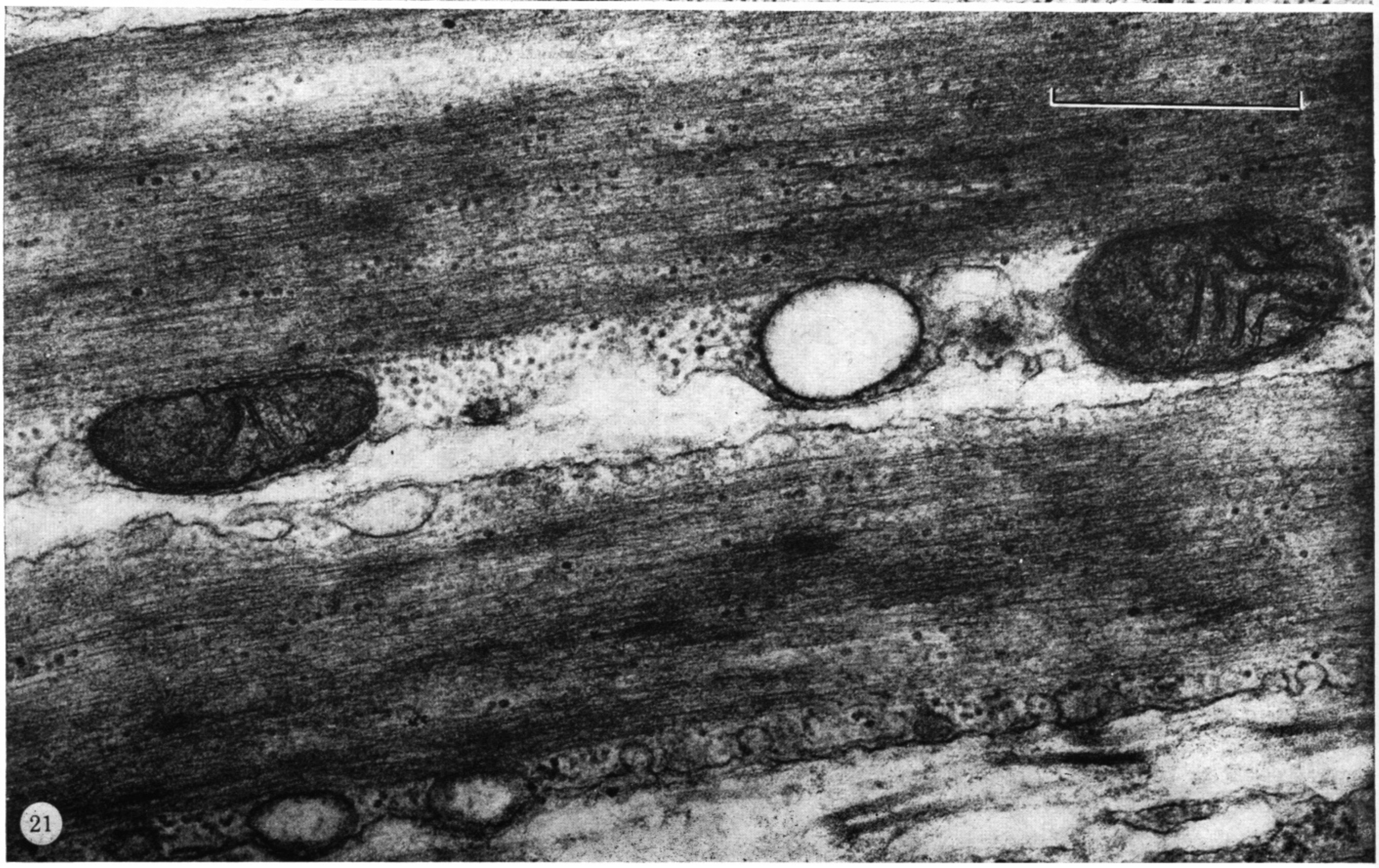

FIGURES 20 AND 21. For legend see facing page.