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STUDIES ON HEXACTINELLID SPONGES. I. HISTOLOGY OF *RHABDOCALYPTUS DAWSONI* (LAMBE, 1873)

By G. O. MACKIE and C. L. SINGLA
Department of Biology, University of Victoria, Victoria, British Columbia, Canada V8W 2Y2

(*Communicated by Sir Eric Smith, F.R.S. – Received* 4 *June* 1982)

[Plates 1–12]

CONTENTS

This paper presents results from the first comprehensive study of hexactinellid tissue organization by electron microscopy.

It is confirmed that the trabecular tissue of *Rhabdocalyptus dawsoni*, which constitutes the bulk of the cellular material in the animal, is a syncytium. The dermal membrane and other similar membranes are specialized regions of the trabecular syncytium, as are thickened regions provisionally equated with the 'cord syncytia' of Reiswig (*Coll. int. Cent. natn. Rech. scient.* no. 291, pp. 173–180 (1979)). Trabecular tissue contributes to the walls of the flagellated chambers and provides the processes that form Reiswig's secondary reticulum.

It is confirmed that choanocytes are absent. The sponge has conventional 'collar bodies' (collar, flagellum and basal cytoplasm) but many collar bodies are syncytially interconnected via narrow 'stolons', and there are no nuclei in these complexes in the fully differentiated state. It is suggested that collar bodies are dehiscent, and are periodically replaced.

A novel feature is the perforate septum, or junctional 'plug'. Plugs are not specialized portions of the cell membranes of adjacent cells. They are complex, disc-shaped structures, probably Golgi secretion products, which are inserted into syncytial bridges and appear to form a filter or partial barrier limiting translocation of materials between differentially specialized portions of the cytoplasm. In this respect they more closely resemble red algal pit connections than junctions found in animals. Gap junctions are absent in *Rhabdocalyptus* (and probably in all sponges) but a type of septate junction is described.

Plugged junctions occur between elements of the trabecular syncytium and collar bodies, and between the latter and cells termed 'choanoblasts', which are probably derived from archaeocytes. A developmental sequence is proposed wherein the collar bodies and their interconnecting stolons are produced as outgrowths from choanoblasts, which may function singly or in syncytial groups during this phase. The cytoplasm is originally continuous throughout these systems, but plugging occurs progressively, leading to segregation of collar body complexes from their mother cells.

Plugged junctions are seen between a variety of cells and the trabecular tissues in which they lie. These cells, whose characteristics are described, are archaeocytes, thesocytes, choanoblasts, granulated cells, spherulous cells and gametes. Sclerocytes, however, appear to lack specialized connections with surrounding tissues. As noted by Okada (1928), spicules are produced intracellularly in hexactinellids.

Spiculation has not been studied in this investigation, and no details have been obtained on embryos or development.

Nerves are absent. The system responsible for impulse conduction is almost certainly the trabecular syncytium. Impulses can probably cross plugged junctions, as pores with internal diameters of about 7 nm are seen in them.

There is no reason to suppose that the tissues lining the openings in the body wall or the internal water passages are contractile. Tests with the dermal membrane show that its pores are not contractile. Regulation of water flow is therefore held to be a property of the sum total of the collar body flagella.

Phagosomes occur both in collar bodies and in the trabecular syncytium. It is assumed that food particles can be taken up throughout the internal surfaces. Mucus nets span the internal lacunae in some places, but information is sketchy. Mucus strands interconnect the collar microvilli and may assist in particle capture. It is suggested that food breakdown products pass directly from collar bodies to choanoblasts and

trabecular issues, crossing junctional plugs, essentially a 'symplastic' transport mechanism as found in plants. Archaeocytes are probably immobile and do not appear to be involved in digestion. External transport of nutrients via the mesolamella is probably of minor importance, and this densely collagenous material is probably not a pathway for cell migration. However, bacteria, presumably symbionts, do occur widely in the mesolamella.

The paper concludes with a review of the phenomenon of syncytialization in plants and animals. Hexactinellids are considered in the same context and the features that set them apart from all other Porifera are listed.

1. General introduction

In most parts of the world hexactinellids live at such great depths that they can be obtained only by dredging. Specimens obtained in this way are usually in poor condition, useless for physiology and even for microscopy. It is not surprising therefore that existing knowledge of the group stresses spiculation and gross anatomy, rather than fine structure, and that there is a dearth of information on the biology of glass sponges: their feeding mechanisms, reproduction, coordination, growth and responses to stimulation. Careful study of a few well preserved specimens around the turn of the century provided most of what is known about the cellular organization of glass sponges. Early claims that they were syncytial have recently been reiterated by Reiswig (1979) from studies with light microscopy, but until the present investigation there was virtually no information on fine structure as revealed by electron microscopy.

Despite uncertainties about the fine structure, enough agreement has been reached among sponge workers for it to be clear that hexactinellids are radically different from other sponges. Indeed the author of a recent book on sponges questions whether the hexactinellids should be classified with the Porifera at all: throughout her text, she finds that 'the Hexactinellida in almost all respects require to be treated separately' (Bergquist 1978). Perhaps it is only the lack of information on so many aspects of the histology of glass sponges that enables specialists to tolerate the present classification of the group on a level with the other three classes of Porifera. An inescapable consequence of the present work is therefore the re-evaluation of the taxonomic status of the Hexactinellida, a matter dealt with in the third paper in this trilogy (Reiswig & Mackie 1983).

The work reported in this paper was begun in order to help us to understand how glass sponges, alone among the Porifera, are able to conduct behaviourially meaningful signals rapidly through their tissues. The system functions to coordinate ciliary arrests. A preliminary announcement of these findings was presented at the same meeting where Reiswig reported on his histological findings (Mackie 1979). The need for an ultrastructural study became doubly obvious. Reiswig had attempted electron microscopy, but had been thwarted by difficulties with fixation. Our early efforts met with similar problems but, after repeated trials with various fixative mixtures, we arrived at a very satisfactory procedure.

The material for both Reiswig's and our own studies comes from coastal waters of British Columbia. It has been known for many years that hexactinellids live in quite shallow water in this area, and they can, in fact, be readily collected in perfect condition by SCUBA divers, but few investigators had taken advantage of this fact until very recently. The present series of studies will, we hope, draw attention to the interesting and unique features of the group, and highlight possibilities for further research on a variety of specific questions.

2. Materials and methods

Specimens were collected by divers from 20–30 m depths in Saanich Inlet, near Victoria, British Columbia, and in the waters of Barclay Sound near Bamfield, British Columbia. Specimens destined for the laboratory were transferred without removal from the water to buckets or plastic bags and transported as gently as possible, in order to minimize mechanical damage and the release of sedimentary material from the ectosomal spicule mass. It was found that specimens would live for weeks in excellent condition in the sea water system at the Bamfield marine station, but deterioration was soon apparent in specimens kept in the tanks at the University of Victoria, where the water is recirculated. Material for electron microscopy was generally cut into small pieces after being brought to the surface and fixed at the collecting site.

Thin pieces of living tissue were examined by phase contrast or Nomarski interference contrast microscopy. Material fixed in 2% OsO_4 or 10% formalin was examined in the same way. The fixative used for electron microscopy† was a freshly prepared mixture of 6.25% glutaraldehyde and 1% osmium tetroxide in 0.2 M s-collidine buffer with 1% sodium chloride (Trump & Bulger 1966), which was applied for 1 h at 4 °C. To remove the sea water carried over into the fixative with the tissue pieces, the glutaraldehyde–osmium mixture was replaced twice. The material was dehydrated through graded alcohols and propylene oxide and was then embedded in Epon 812. To remove silica, small epoxy blocks ready for sectioning and with the surface to be cut exposed were left in a mixture of 2% hydrofluoric acid and 10% acetone in distilled water for 12–24 h at room temperature, rinsed in distilled water and dried overnight in an oven at 60 °C. This will dissolve the spicules within 15–20 μm of the exposed surface. Sections were stained with uranyl acetate and lead citrate. Sections from the same blocks were cut at 0.5 μm thickness and stained with Richardson's stain for light microscopy.

Simultaneous fixation with glutaraldehyde and osmium tetroxide proved to be superior to various fixation methods involving use of the two reagents in sequence, and to Dorey's (osmium–chromate) fixative. This was not found to be the case when fixing local sponges belonging to the class Demospongiae. The best fixation found for *Haliclona*, for example, was cacodylate-buffered glutaraldehyde followed by osmium tetroxide. (Sections of *Haliclona* were prepared to provide a standard of comparison for *Rhabdocalyptus*, and are not referred to specifically in this paper.) It seems that hexactinellids require special fixation.

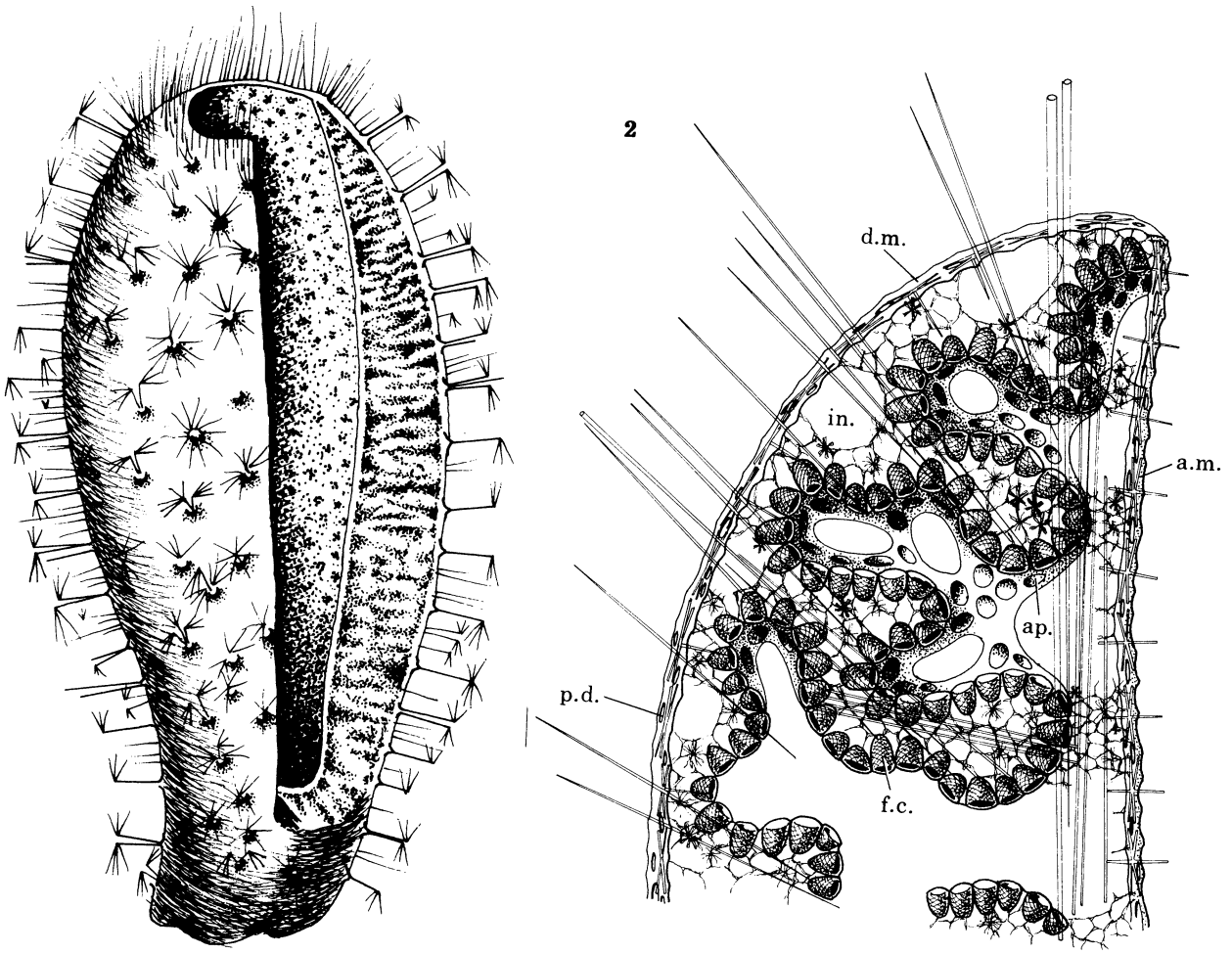
Speed of fixation is extremely important with *Rhabdocalyptus*, as deterioration sets in soon after the sponges are brought to the surface. Fixation after quick freezing in isopentane cooled with liquid nitrogen was attempted in order to minimize these effects. However, tissues fixed in this way showed various artefacts, especially the disruption of ciliary microtubules. In practice, no problems are encountered if the tissue pieces are fixed within a few minutes of being brought to the surface.

3. Existing information

(*a*) *Taxonomy and general anatomy of rosselid sponges*

Most Recent hexactinellids fall within the tribe or subclass Hexasterophora (Schulze 1887), which is divided into two orders, the Dictyonina (with hexactine spicules united into a coherent

† This fixative was used for all the electron micrographs except figure 17.



FIGURES 1 AND 2. General anatomy of rossellid sponges, redrawn from Schulze (1887). 1, Cut-away drawing of *Rossella antarctica*. 2, Internal anatomy of *Acanthascus grossularia*.

Abbreviations: a.m., atrial membrane; ap., apopyle; d.m., dermal membrane, f.c., flagellated chamber; in., incurrent channel; p.d., pore in dermal membrane.

three-dimensional scaffolding), and the Lyssacina (with separate spicules). *Rhabdocalyptus* is a lyssacine genus belonging to the Rossellidae. This family was comprehensively reviewed by Ijima (1904). *R. dawsoni*, the species dealt with in this account, occurs in coastal waters of British Columbia along with several other rossellids (species of *Staurocalyptus* and *Acanthascus*) and members of dictyonine families (*Chonelasma*, *Aphrocallistes*).

R. dawsoni, first described as *Bathydorus dawsoni* by Lambe (1893), was assigned to *Rhabdocalyptus* by Schulze (1897). Ijima (1927) lists 13 species of the genus. Known locally as the 'boot sponge', *R. dawsoni* grows up to 1 m in height and 25 cm in width and has walls from 5 to 22 mm thick (W. C. Austin, personal communication). The species is usually found on sloping or vertical rock faces. Like its close relative *Rosella* (figure 1), it has a dense mass of spicules projecting around the outside, which forms a habitat for many species of invertebrates. Boyd (1981) lists 58 species inhabiting this 'spicule jungle' from specimens of *Rhabdocalyptus*

at one collecting site. In addition, many microscopic invertebrates and microorganisms are present. Water entering the sponge must pass through this layer, which may serve as a 'pre-filter' preventing entry of particles big enough to clog the dermal pores. At the same time, the activities of the epizoites might lead to the conversion of certain materials to dissolved nutrients or bacteria, which the sponge could then use. The interior of the sponge is clean, with few endozoites (Boyd 1981). An account of the taxonomy, distribution and habitats of hexactinellid and other sponges of the coastal waters of British Columbia is in preparation by W. C. Austin†, who has provided much valuable information in the present context.

The general anatomy of rossellid sponges is best illustrated by reference to the classical accounts of Schulze (1887, 1897, 1899) and Ijima (1901, 1904, 1927). The family is a closely knit one and the layout of the trabecular net, flagellated chambers, etc. figured for *Acanthascus* (figure 2) is in good general agreement with what has been observed in *Rhabdocalyptus*.

After passing through the ectosomal spicule mass, water enters the sponge through pores in the dermal membrane (figure 2). The dermal membrane and its spicules (autodermalia) are delicate and may be lost in preserved material (Wilson & Penney 1930), as was evidently the case in the material used by Ijima for the drawing reproduced here as figure 3. This drawing accurately portrays other details of the fine structure of *Rhabdocalyptus* and is included for that reason.

Pore diameters in the dermal membranes of various hexactinellids lie for the most part in the 15–100 μm range, to judge from illustrations by Schulze (1887). According to Tuzet (1973) they are non-contractile. The dermal membrane effectively excludes epizoites from the internal waterways.

Below the dermal membrane lies a subdermal space traversed by trabecular strands. There is no true canal system in hexactinellids. Water percolates through the trabecular net, which is a loose meshwork of tissue strands draped over and around the spicular framework. Incurrent and excurrent waterways are present but they are essentially enlarged spaces within the trabecular mesh rather than being well defined canals. In places, the internal trabecular mesh assumes the character of a continuous membrane, for instance in the membrana reuniens (connecting membrane) of Schulze (1887) which separates incurrent and excurrent waterways by spanning the gaps between the inner ends of the flagellated chambers.

Prosopyles admit water to the flagellated chambers, which open via wide apopyles into spacious excurrent water passages. The effluent current passes to the atrium through perforations in the atrial membrane, a tissue sheet somewhat resembling the dermal membrane but, in *Rhabdocalyptus*, less well defined than it. Water flow through *Rhabdocalyptus* is directional as in sponges of other groups, passing in through the walls and out through the osculum, and not, as Bidder (1923) suggested, lacking any such consistent directionality.

(*b*) *Microscopic anatomy, according to previous accounts*

We owe to Reiswig (1979) a careful review and re-evaluation of the classical histological findings, along with new findings by light microscopy and some preliminary electron microscopy; his electron microscope investigation was however severely limited by difficulties with fixation. The existence of this study, from which a summary diagram is here reproduced as figure 4, makes it unnecessary to provide more than a short review at the present time.

† Khoyatan Marine Laboratory, 4635 Alder Glen Road, Cowichan Bay, British Columbia, Canada V0R 1N0.

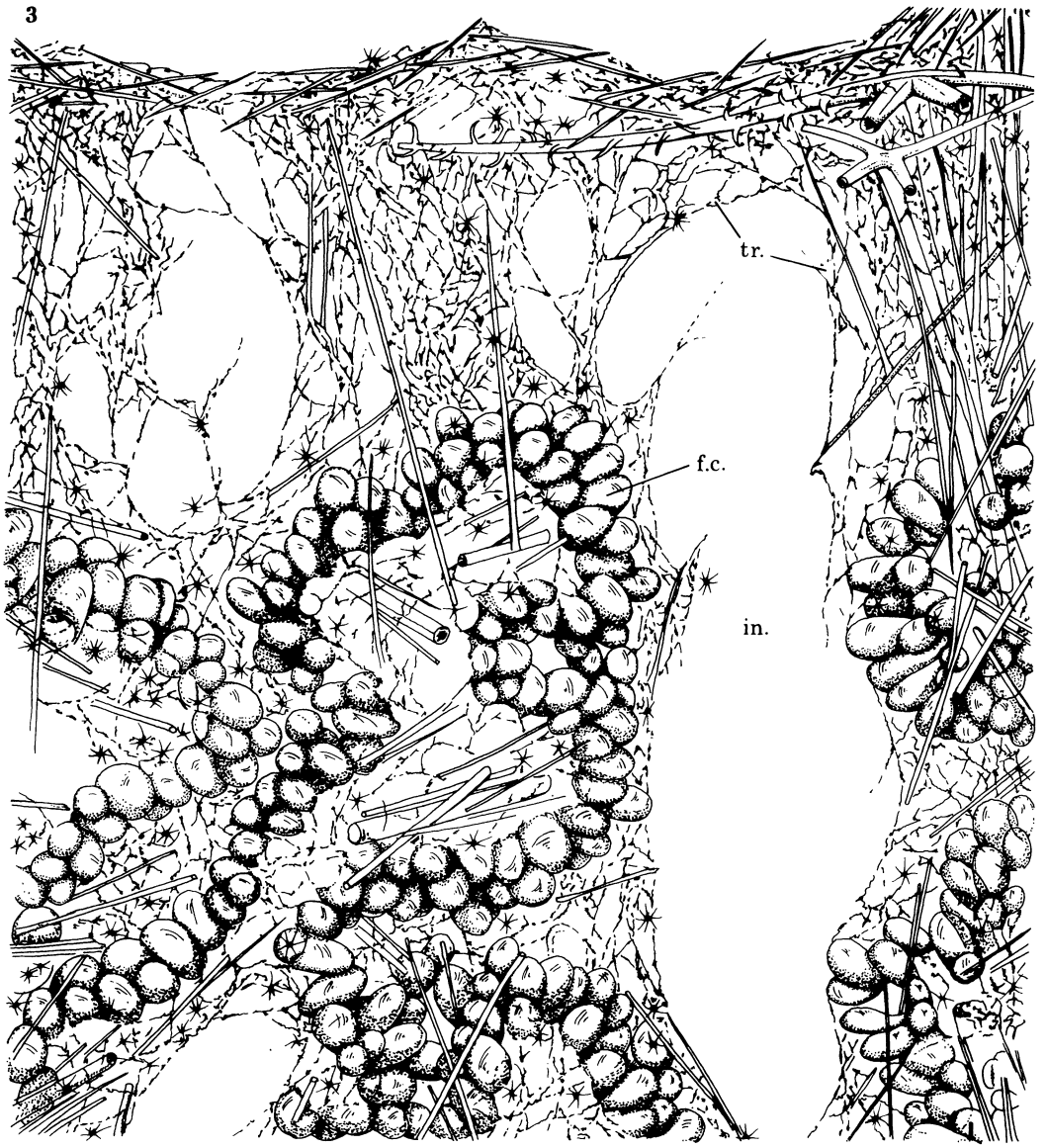
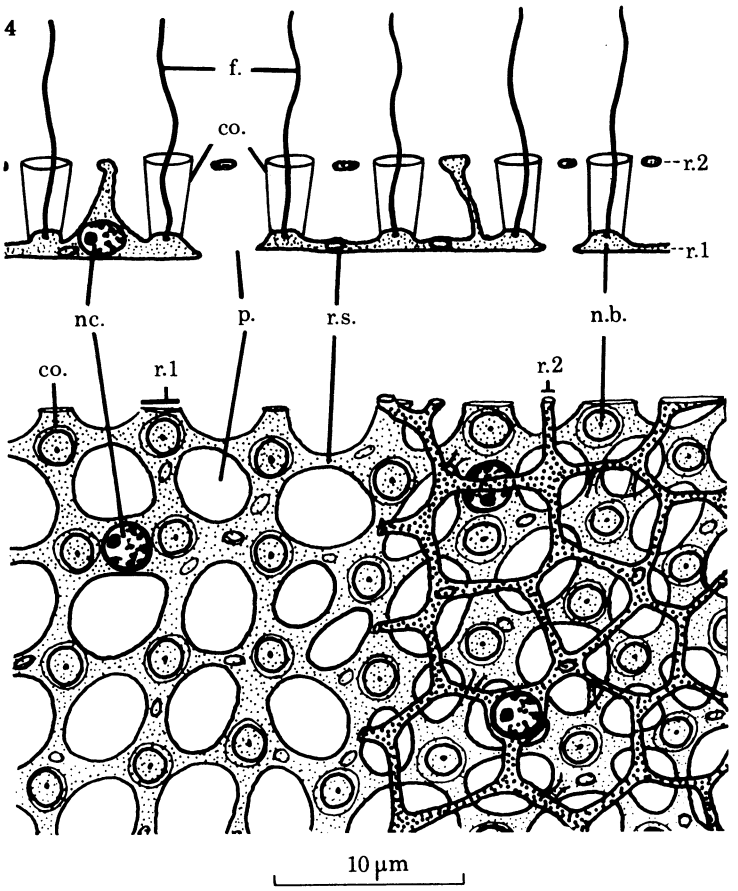


FIGURE 3. For caption see p. 372.

Reiswig discovered that the trabecular tissues are supported by a collagenous mesolamella and he confirmed Ijima's (1901) assertion that the tissues are syncytial. In *Aphrocallistes* and *Chonelasma* he described a new kind of tissue, the cord syncytia, and verified the existence of archaeocytes and thesocytes, the only discrete cells hitherto described apart from germ-line cells and sclerocytes (Okada 1928). Most interestingly, he demonstrated that hexactinellids do not have choanocytes. They have 'collar-flagellar units' (collar bodies†, here) like those of true choanocytes but the collar bodies are attached to a basal cytoplasmic reticulum in which, according to Reiswig, the nuclei are unevenly scattered, rather than occurring in a one-to-one relation with collar units. As will be noted below, the tissue that Reiswig terms 'choanosyncytium' is actually a composite of the true choanosyncytial tissue and extensions of the trabecular tissue, to which the nuclei observed by Reiswig actually belong. When fully

† The term 'collar body' as used here refers to the collar, the flagellum and the (enucleate) cytoplasm from which they both arise.



Abbreviations: co., collar; f., flagellum; f.c., flagellated chamber; in., incurrent lacuna; n.b. nodal body; nc., nucleus; r.1, main reticulum; r.2, secondary reticulum; r.s., connecting strands of main reticulum; tr., trabecular net; p., prosopyle.

FIGURES 3 AND 4. Microscopic anatomy of hexactinellid sponges. 3, *Rhabdocalyptus capillatus*, redrawn from Ijima (1904). 4, Wall of flagellated chamber of *Aphrocallistes astus*, from Reiswig (1979). The wall is shown as consisting of a single tissue, the choanosyncytium, depicted in vertical section (above) and in facial view from the interior of the chamber (below). The secondary reticulum has been removed from part of the lower drawing to show the primary reticulum more clearly. The original lettering has been retained. Points of difference between Reiswig's interpretation and that of the present writers are noted in the text.

differentiated, the choanosyncytium does not have nuclei, but communicates through what we term plugged junctions with tissues that do. Figure 4 correctly shows the reticular nature of the wall of the flagellated chamber. The spaces in the net are the prosopyles. The existence of a secondary reticulum (r.2) has been confirmed in the present study. These processes are trabecular in origin. The distal ends of the collars lie freely and are not attached to each other to form a 'membrane of Sollas' (Schulze 1899), nor are they attached to the processes of the secondary reticulum.

Reiswig notes that the collars are much further apart in hexactinellids than in other sponges. They spring from nodal points in the basal reticulum. He suggests that the secondary reticulum has a hydrostatic function, supporting the collar edges and preventing backflow of water by occluding the spaces between the collars.

Reiswig concludes his account by alluding to the marked histological differences that exist between hexactinellids and other sponges, differences that might 'require formal recognition

at the subphylum level'. The electron microscope findings reported below leave no doubt as to the reality and extent of the differences referred to and they add new features, in particular the plugged intercellular bridges, which set hexactinellids apart not only from other sponges but to some extent from all other animals.

(*c*) *Terminological difficulties*

In dealing with an animal composed largely of syncytia we are faced with problems of cytological terminology. Since the time of Schwann (1839) a 'cell' has conventionally been considered to be a completely membrane-enveloped cytoplasmic domain containing a single nucleus. Can the term be applied to cells like archaeocytes in hexactinellids that are connected to other cells by cytoplasmic bridges containing plugs? A single membrane may envelop several of the connected cells. For practical purposes it seems best to regard archaeocytes as cells in spite of the existence of cytoplasmic bridges connecting them to other cells. They are well differentiated cytological units containing a single nucleus. Plant histologists experience no qualms about use of the word 'cell' in botany, even though plant cells are interconnected by plasmodesmata or other direct bridges and thus form a syncytium in a certain sense (Gunning 1976). Even in animals, epithelial cells are usually interconnected by gap junctions, which are sites where the apposed cell membranes contain high concentrations of hollow membrane particles termed connexons (Unwin & Zampighi 1980) which allow translocation of various substances between the two cells. The difference between hexactinellid cells and the cells of other animals centres around the manner in which the compromise between isolation and communication is achieved. The general question of syncytialization and its significance is discussed further below (p. 393).

In this report we shall apply the term 'cell' to archaeocytes, thesocytes, etc. where there is a single nucleus within a distinctively specialized cytoplasmic domain, regardless of the presence or absence of intercellular bridges. The term 'syncytium'† will be employed to describe the trabecular tissues, where there are open (unplugged) cytoplasmic bridges and many nuclei can be observed within continuous stretches of homogeneous cytoplasm. The 'choanosyncytium' is a special case. In the mature state it consists of enucleate outgrowths of choanoblasts. The word syncytium seems inappropriate here, since syncytia are by definition nucleated. However, the cytoplasm composing a single choanosyncytial domain may derive from several choanoblasts (open bridges occur between them during the formative stages) and so in effect we are dealing with enucleate syncytia.

These terminological problems are mentioned here, not because they represent conceptual barriers, but so that readers will understand the senses in which the various terms are used.

4. New results

The following account represents the results of an ultrastructural survey of desilicated tissues. The spiculation has not been studied. The spaces left by dissolution of spicules are visible in several of the electron micrographs. Some notes are given on sclerocytes (§ 4*d* (vi), p. 385) but we have not attempted to follow the process of spicule formation in any detail and matters rest

† It is of interest that the term 'syncytium' was introduced into biology to describe the outermost tissue layer of calcareous sponges by Haeckel (1872); ironically, this prototype syncytium is now known to consist of separate pinacocytes. The only syncytial sponge tissues are those of hexactinellids.

where Okada (1928) left them. Details of the various spicule types and their distribution are well covered in the taxonomic literature.

It will be convenient to break the subject matter down as follows: (*a*) intercellular connections; (*b*) trabecular tissues; (*c*) choanosyncytium; (*d*) cells; (*e*) extracellular organic components.

(*a*) *Intercellular connections*

(i) *Open cytoplasmic bridges*

Throughout the trabecular tissues (as will be described below) open cytoplasmic bridges of widths varying from 1.0 to 70 μm are seen connecting adjacent expanses of cytoplasm many of which contain their own nuclei. In such situations, the cytoplasm throughout the interconnected regions is similar in terms of density and distribution of organelles.

It would be impossible to prove by electron microscopy that all areas of the cytoplasm in such a system are syncytially connected, for every section will include some areas where perfectly membrane bounded profiles, with or without nuclei, are seen. (By the same token it would be hard to prove, without serial sections, that a cellular animal was not at least partially syncytial.) However, syncytial connections and multinucleate areas of cytoplasm are so often seen that it is justifiable to suggest that the whole trabecular system may actually be one vast syncytium.

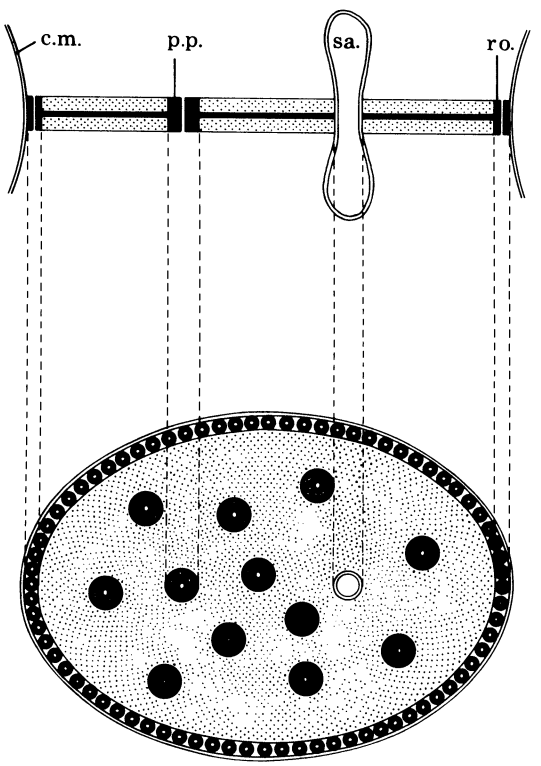


FIGURE 5. Detailed structure of a perforate septum, or 'plug', in cross-section (above) and in facial view (below). Abbreviations: c.m., cell membrane; p.p., pore particle; ro., rodlet; sa., saccule.

It is tentatively assumed that the syncytium is a primary one formed by a failure of cell division following nuclear division, rather than a plasmodium (the result of cell fusion). Pavans de Ceccatty (1982) finds that, during differentiation of aggregates formed from dissociated

tissue, compartmentalization of a primary syncytial 'giant cell' takes place, and we may suppose that something similar to this happens during normal development.

(ii) *Perforate septal partitions* ('plugs')

These structures, consisting of flattened discs containing cylindrical pore particles, are found inserted in intercellular bridges. They are not specializations of the cell membrane, and so cannot be considered in the same category as desmosomes, gap junctions, septate junctions,

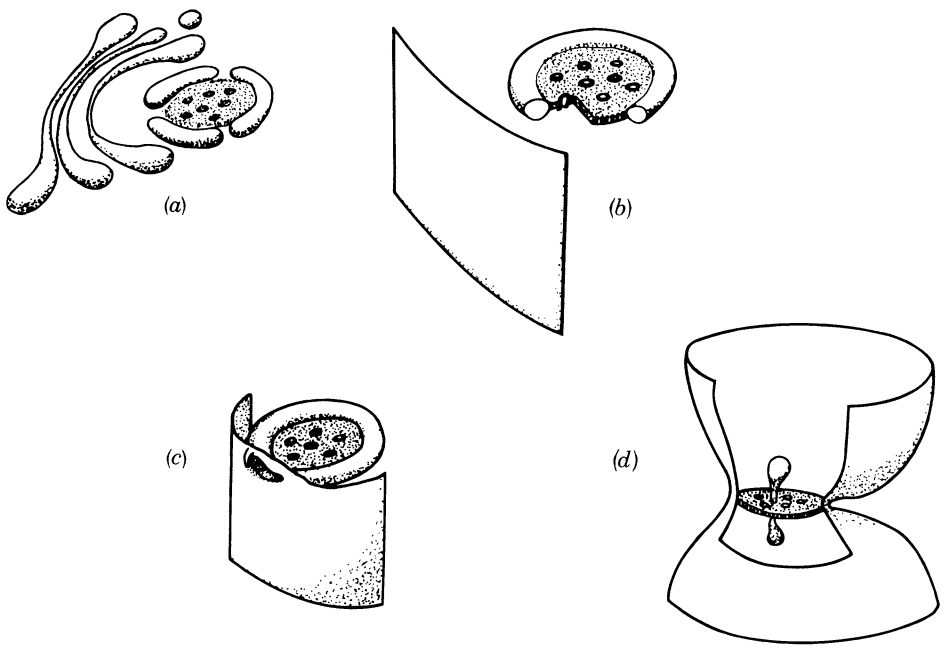


FIGURE 6. Production of a plug, and its insertion into a cytoplasmic bridge, as proposed in the text.

etc. They bear some resemblance to the 'pit plugs' of red algae, and other such junctions found in plants. The components of the mature plug are shown in figure 5, and have been briefly described in a preliminary note (Mackie 1981).

The plug appears to originate in the Golgi region (figures 6*a*, 7, 8†), being seen first as a mass of amorphous, electron dense material lying free in the cytoplasm. Several plugs may be seen developing synchronously in differentiating choanoblasts (figure 7). Early stages of plugs include what appear to be small, clear vesicles lying within the electron dense granular material (figure 8). These rudiments somehow become transformed into a flattened disc in which the pore particles characterizing mature plugs can be recognized (figure 9). It is possible, but not certain, that the pore particles derive directly from the vesicles, but the critical intermediate stages have not been found, and it is not clear how the peripheral rodlets originate. The plug is fully formed while still in the cytoplasm. It lies within a ring-shaped cisterna, shown in cross-section as a circular membrane profile and each end of the plug (figure 10). This annular tube

† The following figures appear on plates: 7–18 (on plate 1), 19–22 (on plate 2), 23–25 (on plate 3), 26–29 (on plate 4), 31–34 (on plate 5), 35–39 (on plate 6), 41–43 (on plate 7), 45 and 46 (on plate 8), 48 and 49 (on plate 9), 51–54 (on plate 10), 56–59 (on plate 11) and 60–62 (on plate 12).

appears to arise by fusion of discrete vesicles which assemble around the edge of the plug, to judge from evidence from redifferentiating tissue aggregates (Pavans de Ceccatty & Mackie 1982). This feature has been incorporated in the interpretative diagram (figure 6).

At this stage the plug moves away from the Golgi zone and into a developing intercellular bridge where it becomes attached to the cell membrane of the bridge (figure 11). The process of attachment probably involves fusion of the annular tube with the cell membrane (figure 6*c*). Plugs are frequently seen attached to the cell membrane at one side, the other side still lying free in the cytoplasm of the bridge, with annular sac still intact. There is therefore a stage (corresponding to figure 6*c*) at which the bridge is still partially open, before membrane fusion has proceeded all the way around the plug, causing it to fill the bridge completely.

Once lodged in its definitive position the plug is closely attached to the inner surface of the cell membrane on all sides. Figures 12 and 13 show the unit membrane applied to the edge of the plug. Peripheral rodlets (figures 13, 14, 16) form an orderly array around the edge of the plug adjacent to the cell membrane. They are 45 nm long and 25 nm in diameter and appear to have a 5 nm channel running through the centre. They are arranged around the edge of the plug with an approximately 30 nm centre to centre spacing. Pore particles (figures 15, 16), up to 13 per plug, are inserted with their ends opening in the cytoplasm on either side. They are 45 nm long cylinders with diameters of 50 nm and with a 7 nm central channel.

The plug has a trilaminar appearance in cross section (figures 5, 15, 17). The outer layers of the sandwich consist of coarsely granular or fibrous material and are each about 11 nm

Description of plate 1

Plug development and structure

Figure 7. Presumed plug rudiment forming on *trans* side of Golgi membrane stack. A mature plug is shown at right (arrow). (Magn. × 17800.)

Figure 8. Another example, at higher magnification. (Magn. × 35200.)

Figure 9. Early (below) and later (above) stages in plug formation in the cytoplasm. (Magn. × 35200.)

Figure 10. Formed plug, still free in the cytoplasm, cut in cross-section. Profiles of the annular sac (arrows) are seen at either end of the plug. (Magn. × 16100.)

Figure 11. Plug attached to cell membrane at one side (arrow), the other side still free. (Magn. × 12000.)

Figure 12. Mature plug cut at an angle close to the horizontal plane, showing the edge of the plug closely applied to the cell membrane (arrows). (Magn. × 105500.)

Figure 13. Cross-section through the edge of a plug where it is attached to the cell membrane (arrow). (Magn. × 112100.)

Figure 14. Section cut tangentially through the edge of a plug, showing rodlets. (Magn. × 65200.)

Figure 15. Cross section through a plug, showing pore particles. (Magn. × 68400.)

Figure 16. Horizontal section through a plug, showing pore particles and rodlets. (Magn. × 62100.)

Figure 17. Dorey-fixed section of a plug, showing trilaminar structure. (Magn. × 136800.)

Figure 18. Plug traversed by a membraneous saccule. (Magn. × 44100.)

Abbreviations: p.p., pore particle; ro., rodlet.

Description of plate 2

Plug formation and evidence of barrier function

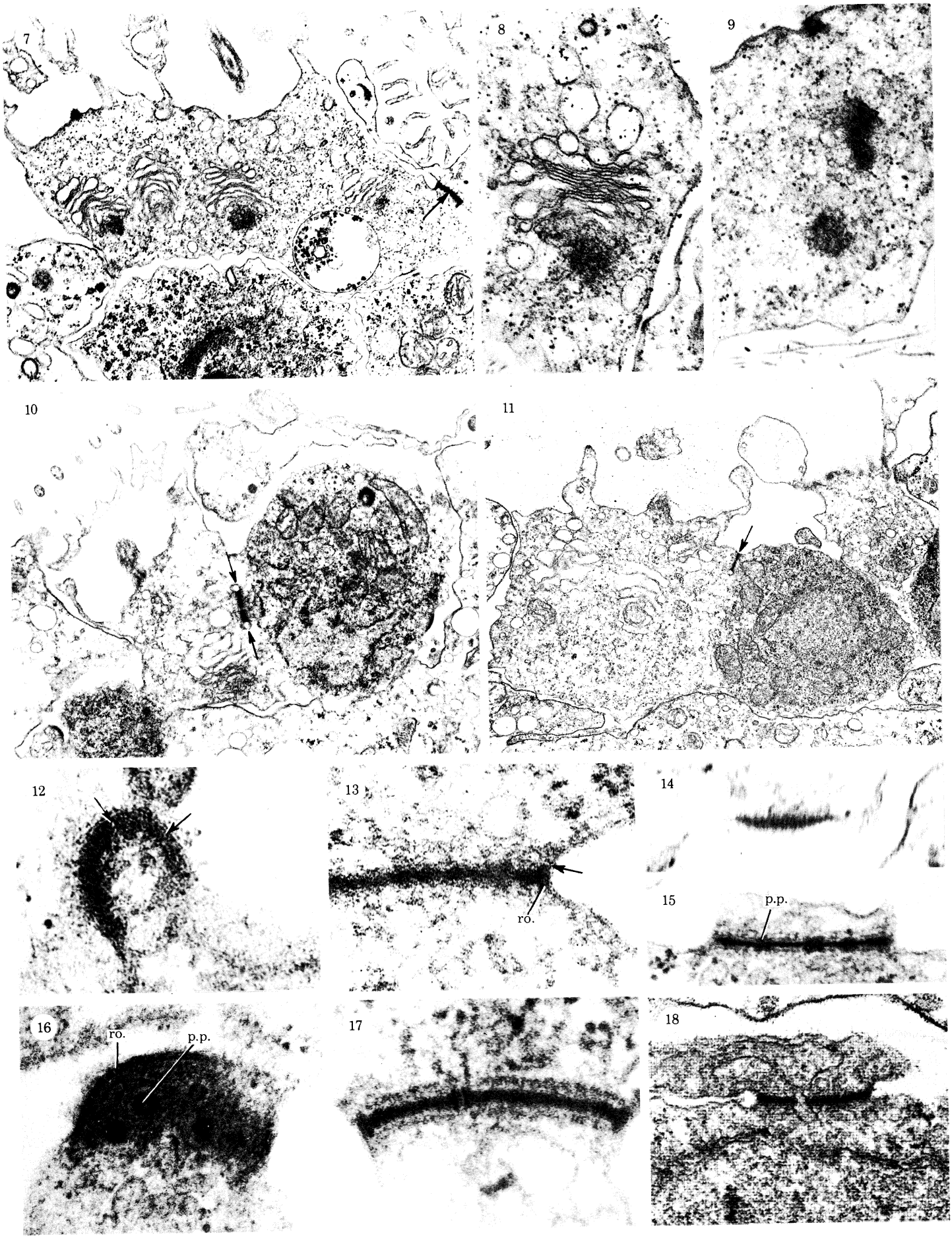
Figure 19. During initial stages of collar body formation, the cytoplasm of the collar body is similar to that of the rest of the choanoblast. (Magn. × 15200.)

Figure 20. In an older collar body, separated from its choanoblast by a plugged bridge, the cytoplasm on the two sides differs markedly. (Magn. × 25700.)

Figure 21. Recently segregated collar body shows darker-staining cytoplasm than choanoblast (below) and trabecular syncytium (right) to which it is attached by plugged bridges. (Magn. × 22400.)

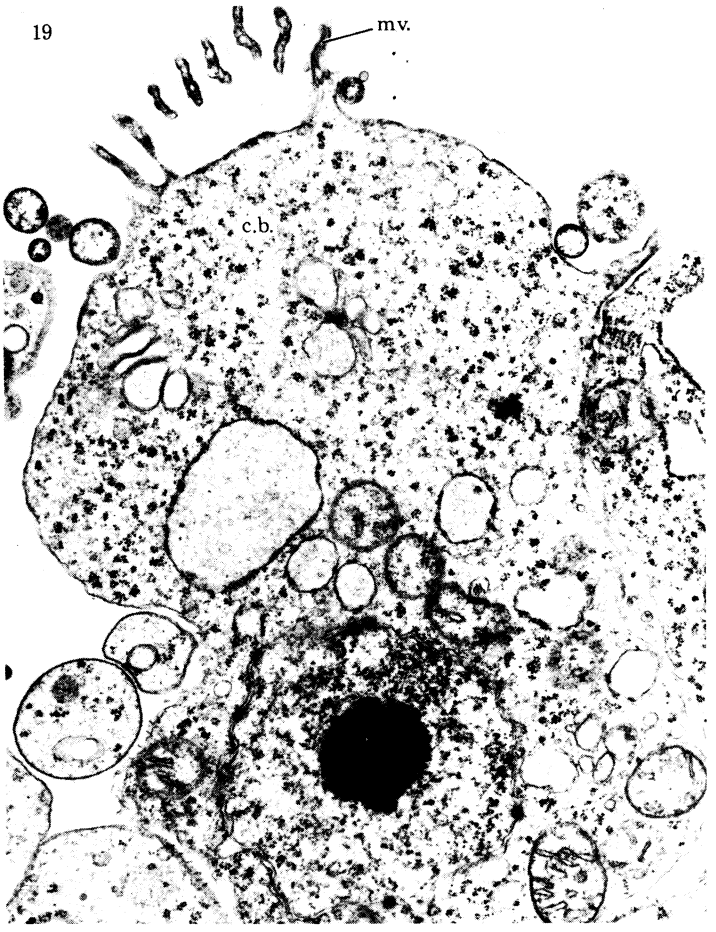
Figure 22. Trabecular syncytium with plugs forming (below) and ensconced (upper middle). (Magn. × 16200.)

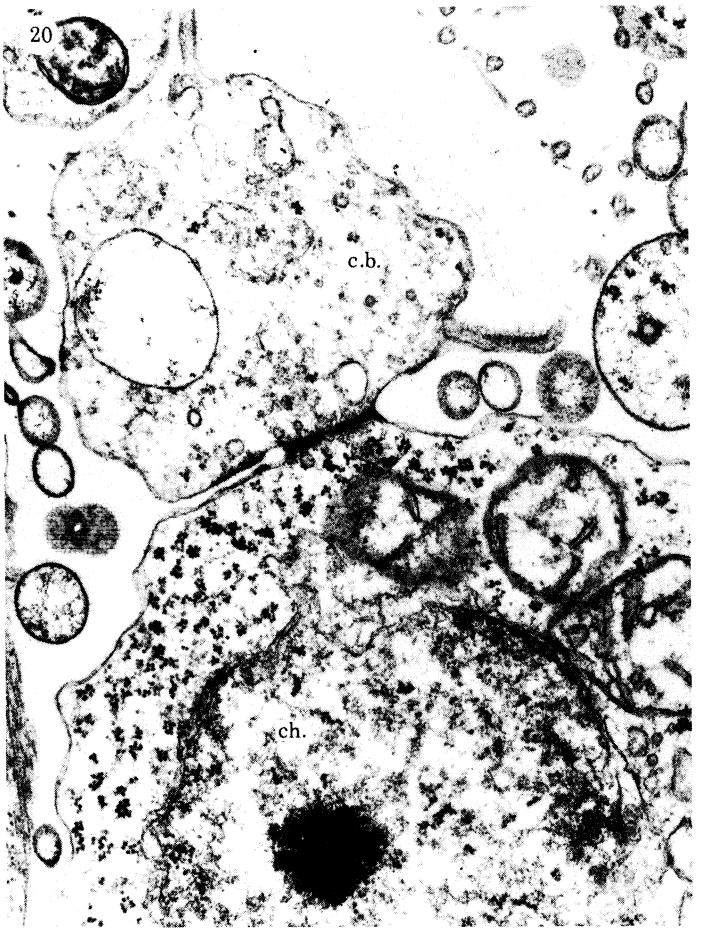
Abbreviations: c.b., collar body; ch., choanoblast; mv., collar microvilli; ph., phagosome.

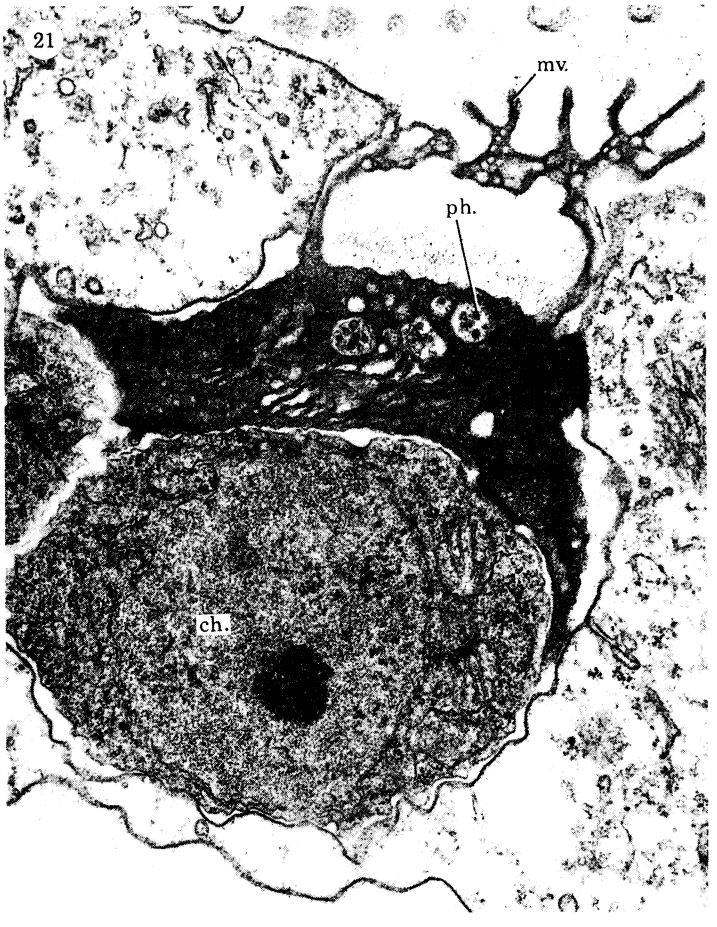


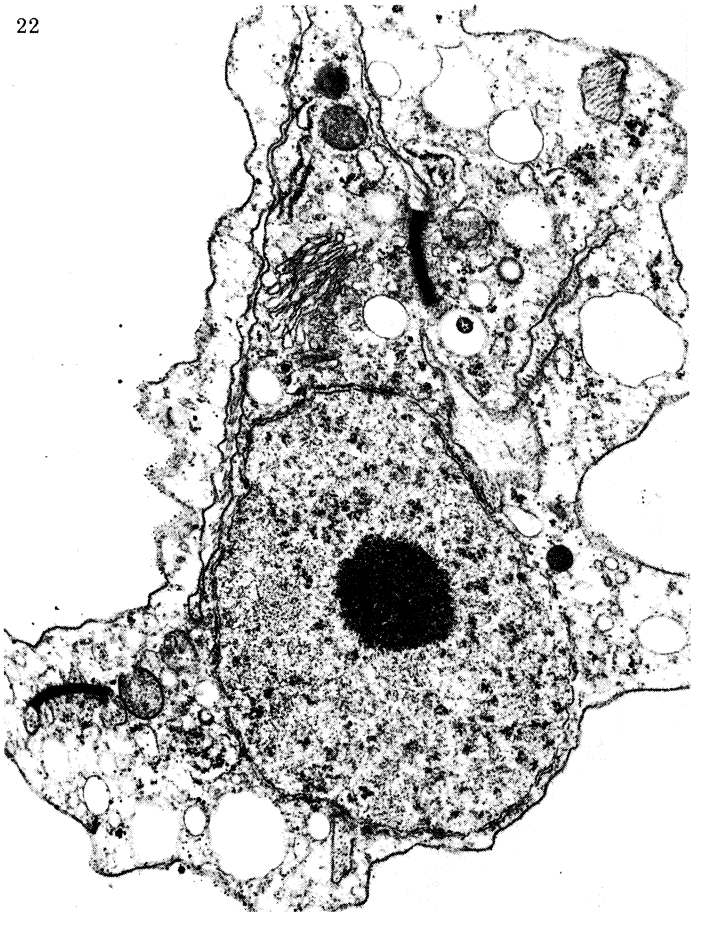
FIGURES 7–18. For description see opposite.

(*Facing p.* 376)









FIGURES 19–22. For description see p. 376.

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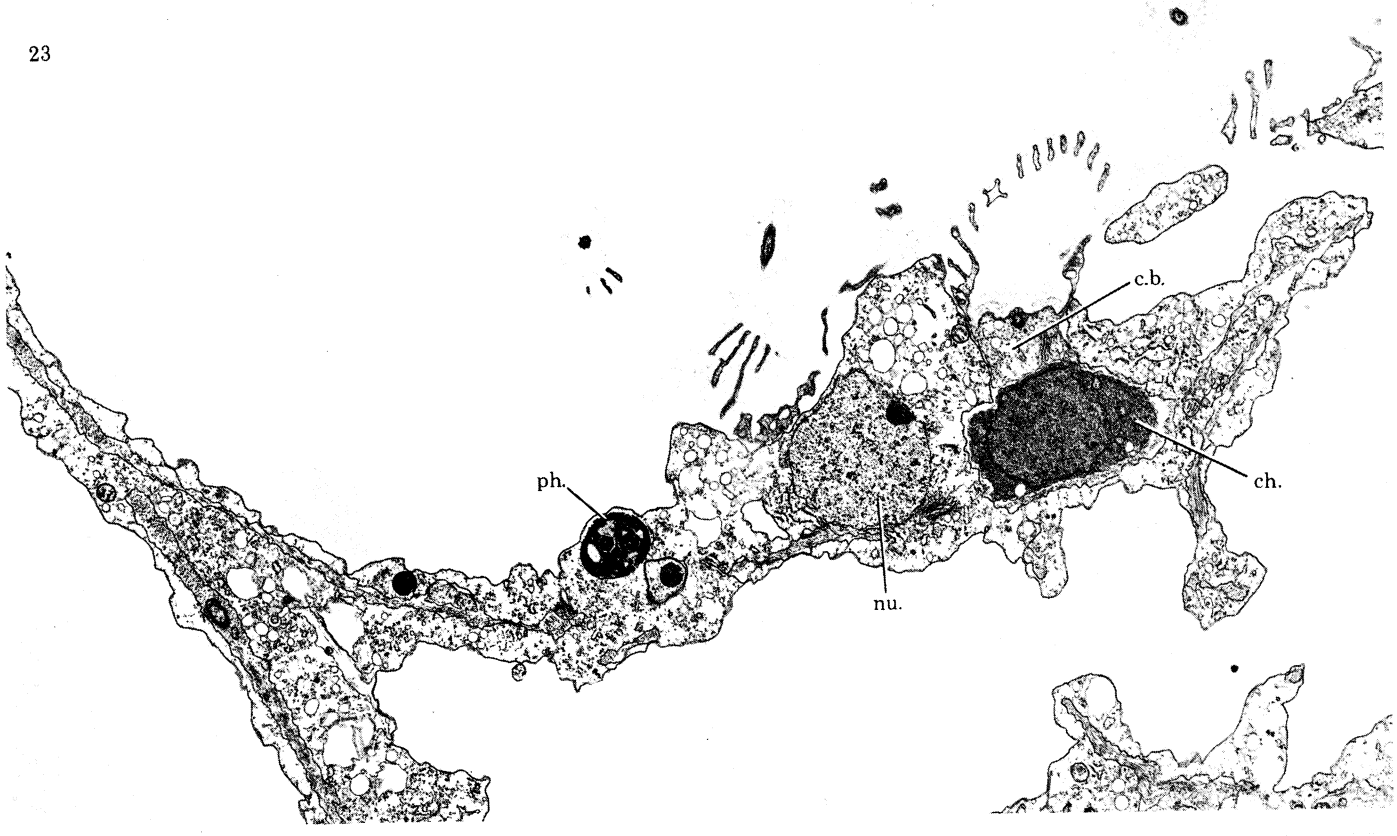

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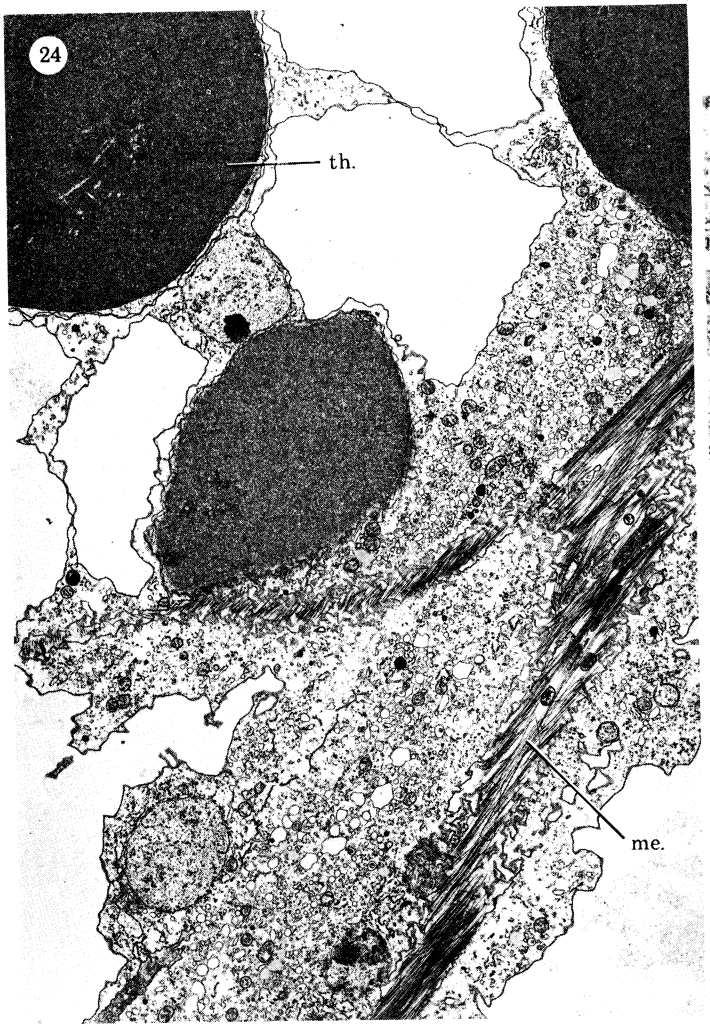

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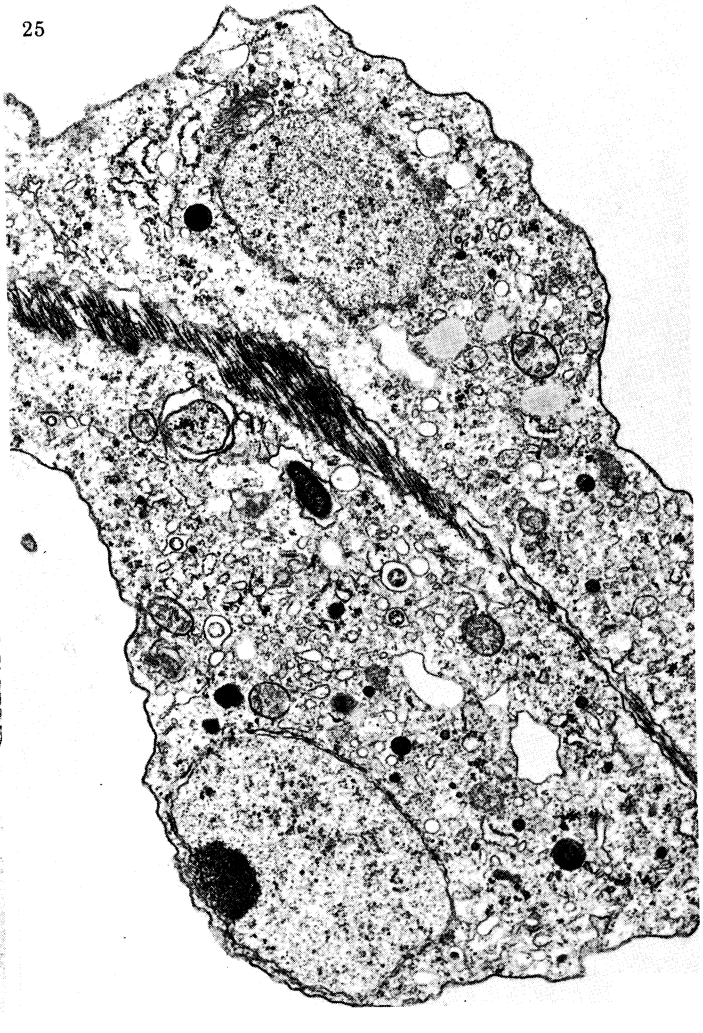
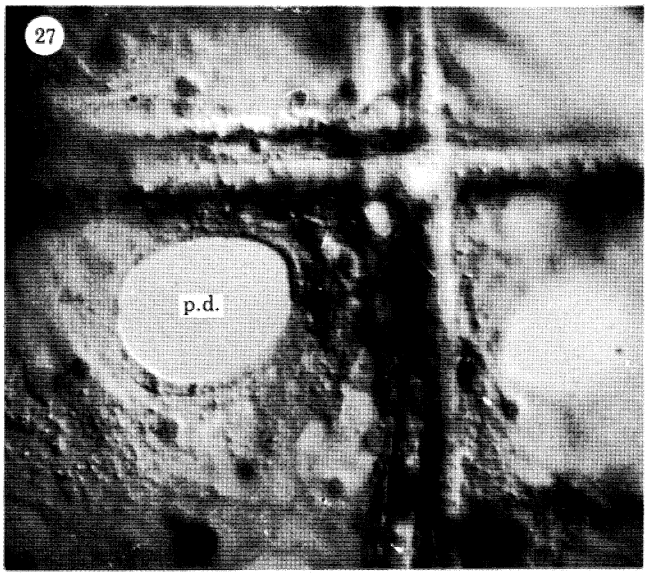
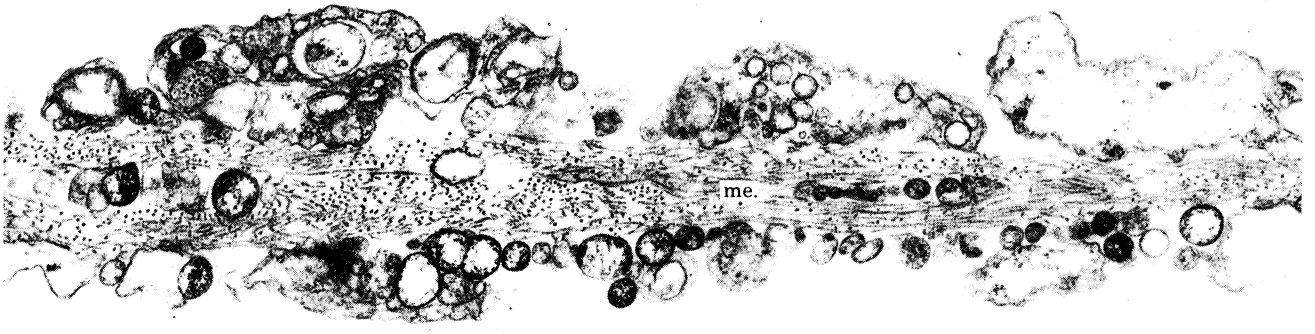
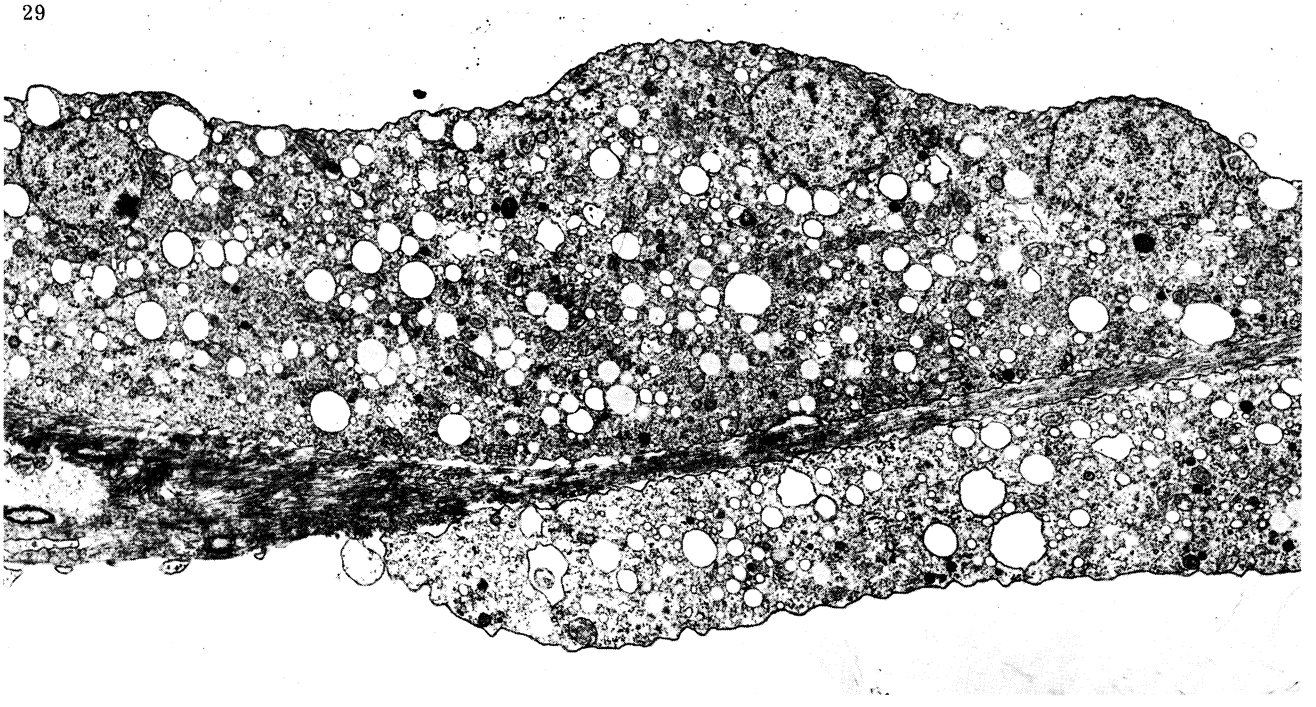


FIGURES 23–25. For description see p. 377.







FIGURES 26–29. For description see opposite.

thick. The inner layer consists of finer, electron dense material and is about 18 nm thick. Between outer and inner layers are 7 nm spaces containing less electron dense material. The whole structure is about 55 nm in cross section. The trilaminar appearance is often not resolvable in aldehyde–osmium material, although very thin sections still show it (figure 15). The layers show much more sharply in Dorey-fixed material, owing perhaps to loss of some soluble component (figure 17). Systematic attempts to explore the effects of solvents on the plug material have not been conducted.

There is no unit membrane component to the plug itself. However, membraneous saccules are frequently observed lodged in the plug with their ends protruding on either side (figure 18). As for plant plasmodesmatal desmotubules, it is not clear whether these saccules are permanent fixtures, or if they represent a component in transit across the plug. The saccules vary greatly in size and some project much more on one side than on the other. The pore seen in figure 17 is probably part of such a saccule, the exposed ends of which are blurred, rather than a pore particle. It is possible, but not certain, that saccules are inserted in pore particles, perhaps using them as routes through the plug, but for this to be true it would be necessary to assume that the pore particle can stretch, as the saccule may be much more than 80 nm thick at the point where it inserts into the plug.

An important feature of plugged junctions is that the cytoplasm usually differs markedly on the two sides, lending support to the view that the plug is an effective barrier against movement of cytoplasmic organelles. Figure 19 shows a choanoblast before the stage when the collar body becomes separated from the nucleated portion by a plugged bridge. Mitochondria are concentrated around the nucleus but otherwise the cytoplasm is quite uniform throughout the cell. Polyribosomes are scattered through all parts. The cytoplasm of older collar bodies, which are separated by plugged bridges, shows fewer polyribosomes than the nucleated area. Figure 21 illustrates the striking differences between the cytoplasms on the two sides of plugged junctions.

All examples of plugs described above have been in the choanosyncytium. Figure 22 illustrates plug formation in the general trabecular syncytium. As noted elsewhere (§4(*b*)) this tissue is largely interconnected by open syncytial bridges. Plugs appear to form at interface points with other tissues, though some plugs have been seen joining cytoplasms of similar appearance (see, for example, figure 22). Plugs occur between all the cell types described,

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Description of plate 3

Structure of trabecular syncytium

Figure 23. Wall of flagellated chamber. (Magn. × 9200.)
Figure 24. Trabecular syncytium with thesocytes. (Magn. × 4100.)
Figure 25. Another part, higher magnification. (Magn. × 11400.)

Abbreviations: c.b., collar body; ch., choanoblast; me., mesolamella; nu., nucleus of trabecular syncytium; ph., phagosome; th., thesocyte.

Description of plate 4

Specialized parts of the general trabecular syncytium

Figure 26. Surface view of dermal membrane in a living sponge, by Nomarski interference contrast microscopy. (Magn. × 475.)
Figure 27. Dermal membrane after fixation; Nomarski. (Magn. × 760.)
Figure 28. Cross-section of dermal membrane by electron microscopy. (Magn. × 24200.)
Figure 29. Cord region of trabecular syncytium showing three nuclei in same mass of cytoplasm. (Magn. × 6700.)

Abbreviations: me., mesolamella; p.d., pore in dermal membrane.

with the possible exception of scleroblasts. Possibly the intracellular environment of the scleroblast is so specialized in terms of ionic composition that complete isolation by a continuous membrane barrier is necessary. Other authors have noted that the extracellular environment in the area where a spicule develops also apparently requires isolation (by septate junctions) from the surrounding extracellular spaces (Ledger 1975).

(iii) *Specialized membraneous junctions*

Gap junctions (nexuses) have not been observed in our material and probably do not occur in any of the Porifera (Green & Bergquist 1979) despite one report of electrical coupling (Loewenstein 1967) and some suggestive but inconclusive electron microscope observations (Gaino & Sarà 1976). Septate junctions were described convincingly in *Sycon* (Ledger 1975), where they occur between sclerocytes, and in *Clathrina* (Green & Bergquist 1979), where they occur between choanocytes. They are postulated to serve an occluding function like their counterparts in other invertebrates (Staehelin 1974). In *Rhabdocalyptus*, adjacent cell membranes are quite frequently observed to run in parallel, separated by a regular 12 nm cleft, and in some sections where the cutting angle is favourable it is possible to resolve fine septa crossing the cleft. The septa are about 15 nm apart, and are arranged in a very regular way. There can be little doubt that we are dealing with septate junctions of the type described in *Sycon* and *Clathrina*. The dimensions are all very similar. Where septa are not visible, it may be that the junctional organization is simpler, or that resolution was imperfect. These junctions occur between processes of the trabecular syncytium, thesocytes, archaeocytes and collar units. The septate junctions joining collar bodies to trabecular membranes differ slightly from those in other regions: they are characterized by the presence of aggregated fibrillar material (presumably tonofilaments) in the cytoplasm on either side of the apposed membranes (figure 32). A junction of this sort would be expected to function for adhesion as well as for occlusion.

(*b*) *Trabecular tissues*

The trabeculae are slender threads or bands of tissue which are draped over, around and in between the spicules. The system reminded Ijima (1901) of an irregular cobweb. It constitutes the greater part of the living tissue in the sponge. Here and there it is specialized into membranes, the best developed being the dermal membrane. Trabecular tissue extends into the walls of the flagellated chambers. Reiswig's (1979) cord syncytia have not been identified with certainty in *Rhabdocalyptus*, but structures possibly representing them are essentially thickened regions of the general trabecular syncytium.

A portion of the general trabecular syncytium merging with the wall of a flagellated chamber is shown in figure 23. The trabecular tissue forms the borders of the prosopyle and surrounds choanodermal elements. In all regions, except the secondary reticulum, trabecular tissues are supported on sheets of collagenous material (mesolamella). Signs of phagocytosis are quite often seen in the cytoplasm (figure 23).

In many places it is possible to trace direct cytoplasmic pathways between nucleated areas in the trabecular syncytium (see, for example, figures 24, 29). Where the tissues seem to consist of cell-like masses, this may be due to subdivision of the syncytium by layers of mesolamella, and if one follows the cellular outlines closely it is usually possible to find openings through the mesolamella, or pathways around it, by which the cytoplasm of adjacent masses is in continuity.

The cytoplasm contains scattered Golgi elements and rather sparse mitochondria. Some

rough endoplasmic reticulum is usually visible, along with many vesicles, some containing electron dense material, ranging from 0.14 to 0.7 μm in diameter (figure 25). Presumably collagen synthesis is a function of the trabecular syncytium, since collagen occurs in many places where no other cellular elements are present. Phagosomes are frequently visible, and the mass of small vesicles probably includes some primary lysosomes, as well as residual bodies etc., related to the phagocytic function of the tissue.

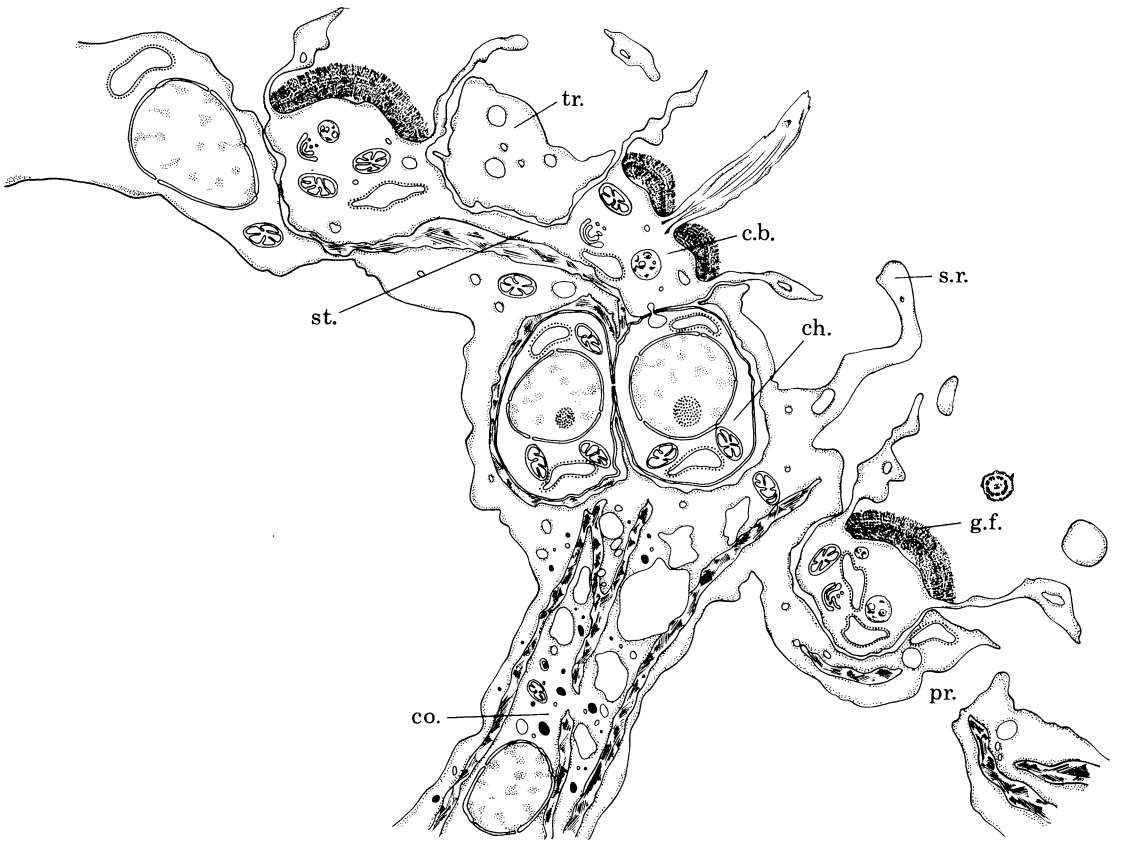


FIGURE 30. Drawing of a portion of the wall of a flagellated chamber.

Abbreviations: c.b., collar body; ch., choanoblast; co., cord region; g.f., glycoprotein filament array; pr., prosopyle; st., stolon; s.r., secondary reticulum; tr., trabecular tissue.

The nuclei are 2.0–4.0 μm in diameter and have small nucleoli. Plugs occur at junctions between the general trabecular syncytium and choanoderm, as well as with most cells.

The dermal membrane forms the outermost layer of living tissue in hexactinellids. There are no pinacocytes. The dermal membrane is a more or less continuous two-dimensional sheet, typically between 1.0 and 2.5 μm thick. It is perforated by numerous pores measuring 4–30 μm ($\bar{X}$ = 11 μm) in diameter which are readily visible in the living sponge by phase contrast or Nomarski interference microscopy (figure 26). The pores in the dermal pinacocyte epithelium of some demosponges are contractile, and can close completely over a period of several minutes (Weissenfels 1980), but there is no indication that this is true in *Rhabdocalyptus*. Living dermal membranes were observed and photographed at intervals over several hours, but no changes in pore diameters were observed, and fixation did not cause closure or contraction (figure 27). Electron microscopy of the cytoplasm in the pore region showed no structural specializations suggestive of contractility, such as characterize contractile cells of other sponges (Vacelet 1966).

Under the electron microscope, the dermal membrane (figure 28) shows features similar to those of the general trabecular syncytium, but the collagenous mesolamella is usually somewhat thickened, with fibres running in several different directions within the horizontal plane. Another feature of the membrane is the vesiculated appearance of the cytoplasm. This however may be a fixation artefact. The layer is generally poorly fixed in our material compared with deeper lying regions.

The cord syncytia of *Aphrocallistes* and *Chonelasma* are described by Reiswig (1979) as cylindrical masses of tissue 5–15 μm in diameter and exceeding 1 mm in length, with densely staining cytoplasm and many nuclei, perhaps numbering in the thousands. The cords are enveloped in collagenous mesolamella with trabecular tissue covering the outer sides. The cords are stated to occur commonly in loose bundles of two to six running for long distances in parallel, but intersecting with other bundles. Somewhat similar structures have been seen in *Rhabdocalyptus*, but the system seems to be developed rather more sparsely and the distinction between cords and trabecular tissue is not well marked, rather it is one of degree. To be sure that we are dealing with the same structures, it would be necessary to examine *Aphrocallistes* material under the electron microscope. In *Rhabdocalyptus*, the cords are multinucleate masses of cytoplasm up to 30 μm thick showing a fairly strong affinity for basic dyes under the light microscope and with a somewhat higher concentration of cytoplasmic vesicles, mitochondria and endoplasmic reticulum than the general trabecular syncytium (figure 29). They are not enveloped by trabecular tissues, but merge into them at their ends through open cytoplasmic bridges (figure 30).

(*c*) *Choanosyncytium*

The tissues composing the choanosyncytium are restricted to the walls of the flagellated chambers. Collar bodies lie at nodal points in the reticulum and lack nuclei (Reiswig 1979; figure 4). Reiswig represents the entire basal reticulum as 'choanosyncytium' but the electron microscope shows it to be a composite of true choanosyncytium and trabecular tissue. The collar bodies are ensconced in pockets of trabecular tissue and are connected to one another in groups by thin stolons and to choanoblasts, which are cells specifically associated with, and apparently giving rise to, groups of collar bodies. The nuclei figured by Reiswig (1979) are probably not those of choanoblasts but the nuclei of the trabecular tissue component. Reiswig's

Description of plate 5

General structure of collar bodies

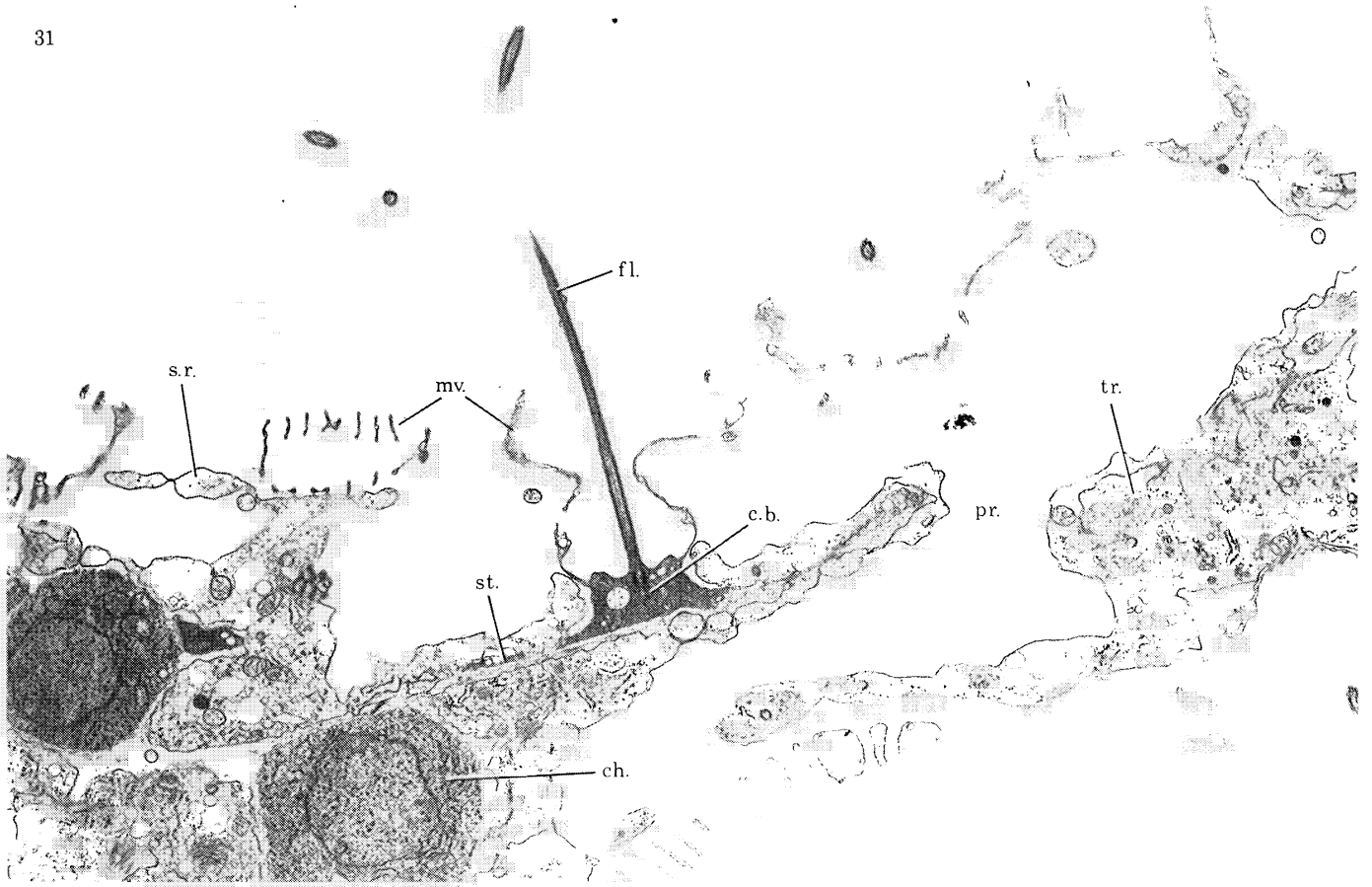
Figure 31. Collar body cut in vertical section. (Magn. × 7600.)
Figure 32. Septate junction between a collar body and adjacent trabecular process. (Magn. × 9400.)
Figure 33. Collars cut transversely. (Magn. × 10300.)
Figure 34. Cytoplasmic organization of a typical collar body. (Magn. × 17100.)

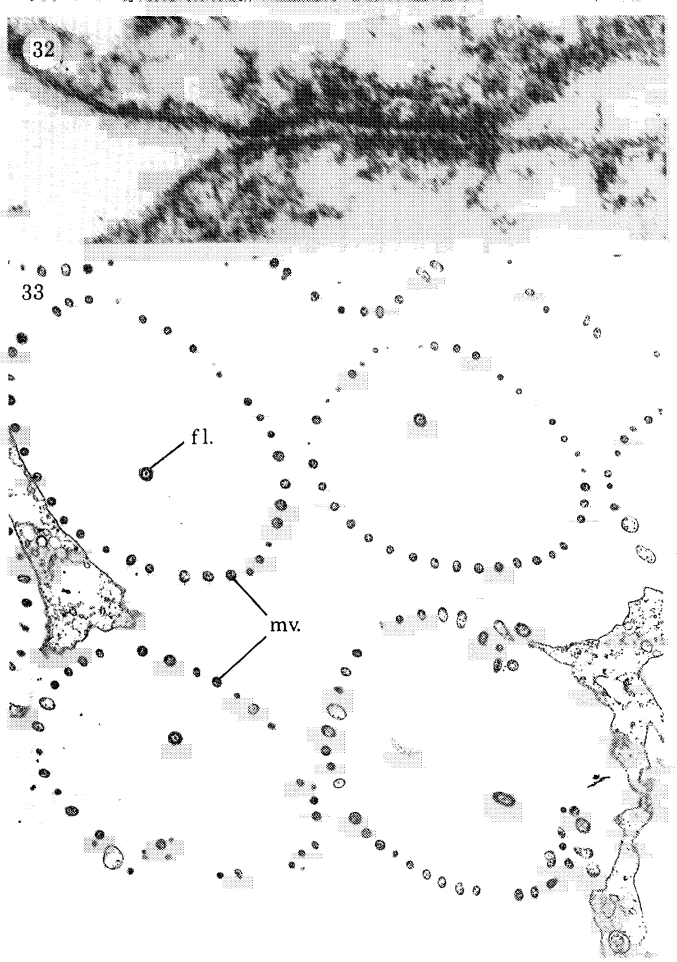
Abbreviations: c.b., collar body; ch., choanoblast; fl., flagellum; Go., Golgi membranes; mv. microvilli; ph., phagosome; pr., prosopyle; st., stolon; s.r., secondary reticulum; tr., trabecular syncytium.,

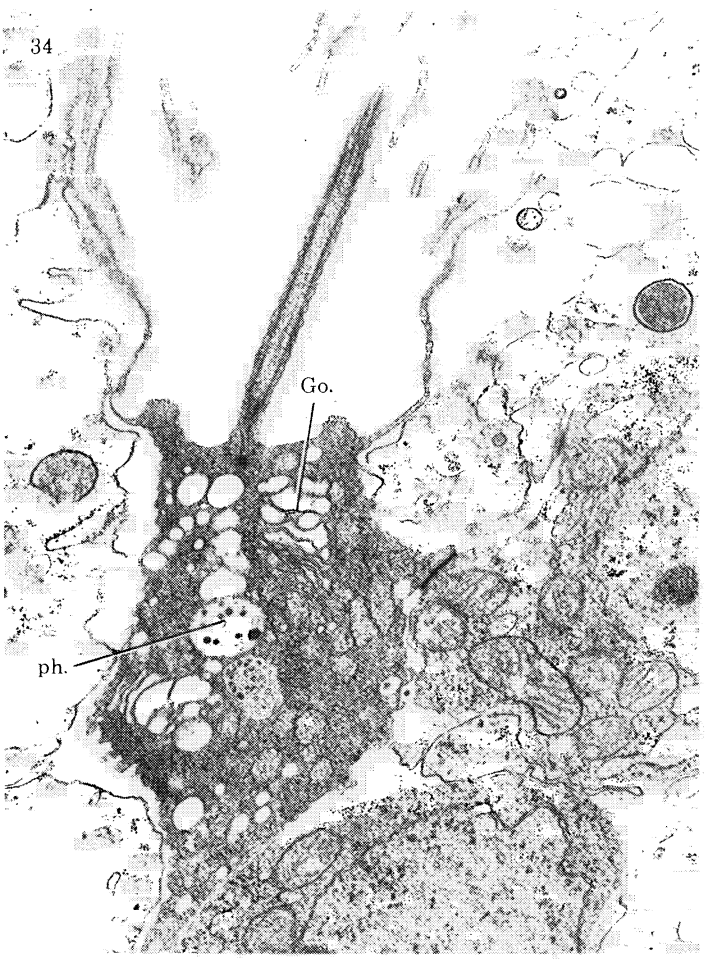
Description of plate 6

Detailed structure of collar components

Figure 35. Bases of collar microvilli, showing microfilament bundles. (Magn. × 55200.)
Figure 36. Base of collar cut in cross section, showing fusion of microvilli and glycoprotein filament mesh. (Magn. × 26400.)
Figure 37. Filament mesh connecting adjacent microvilli. (Magn. × 4400.)
Figure 38. Base of flagellum, showing microtubules and basal body. (Magn. × 33600.)
Figure 39. Tip of flagellum, with terminal dilation. (Magn. × 25600.)







FIGURES 31–34. For description see opposite.

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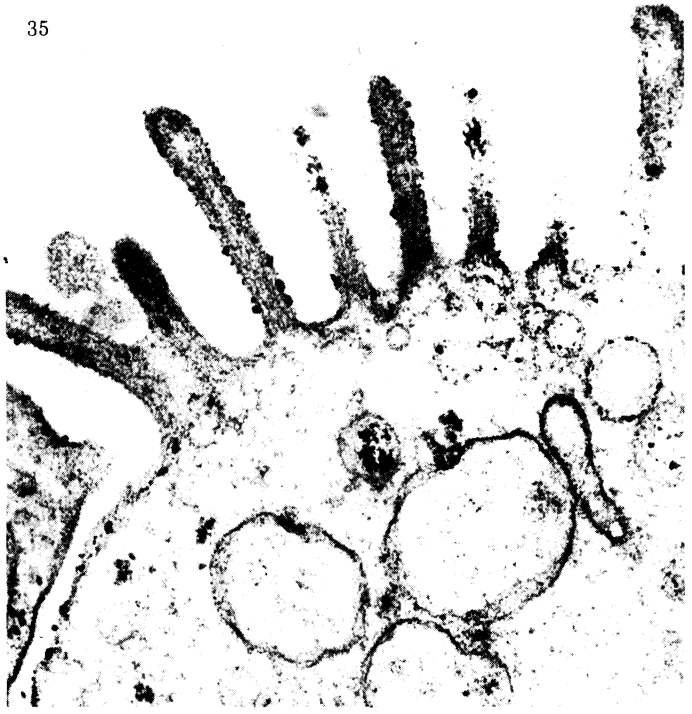
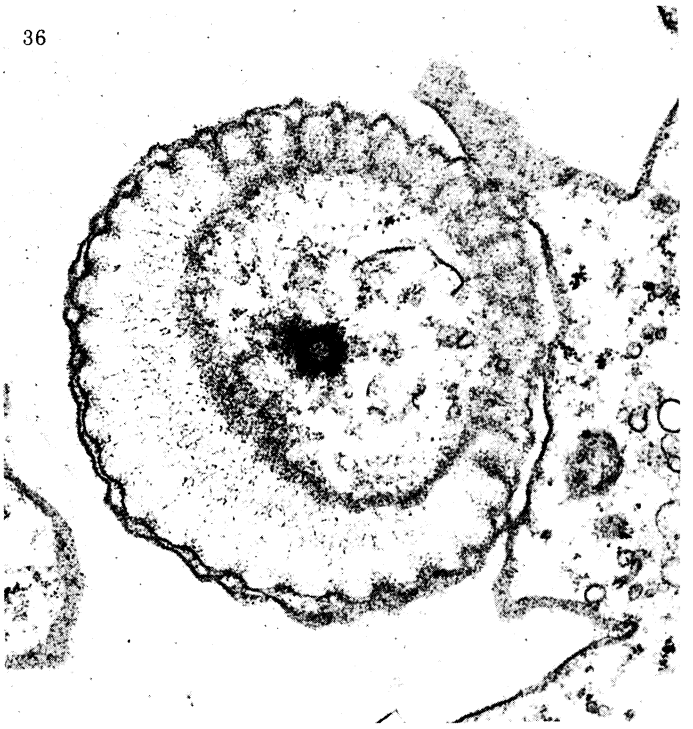
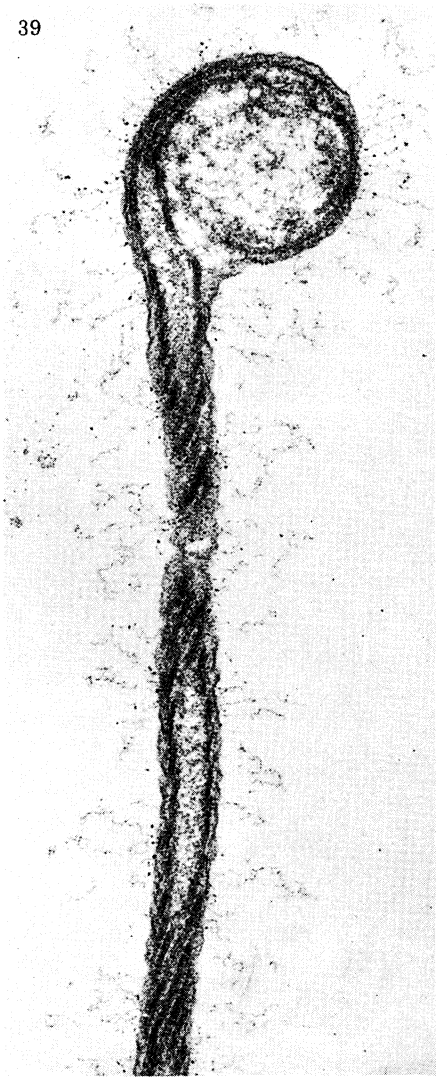
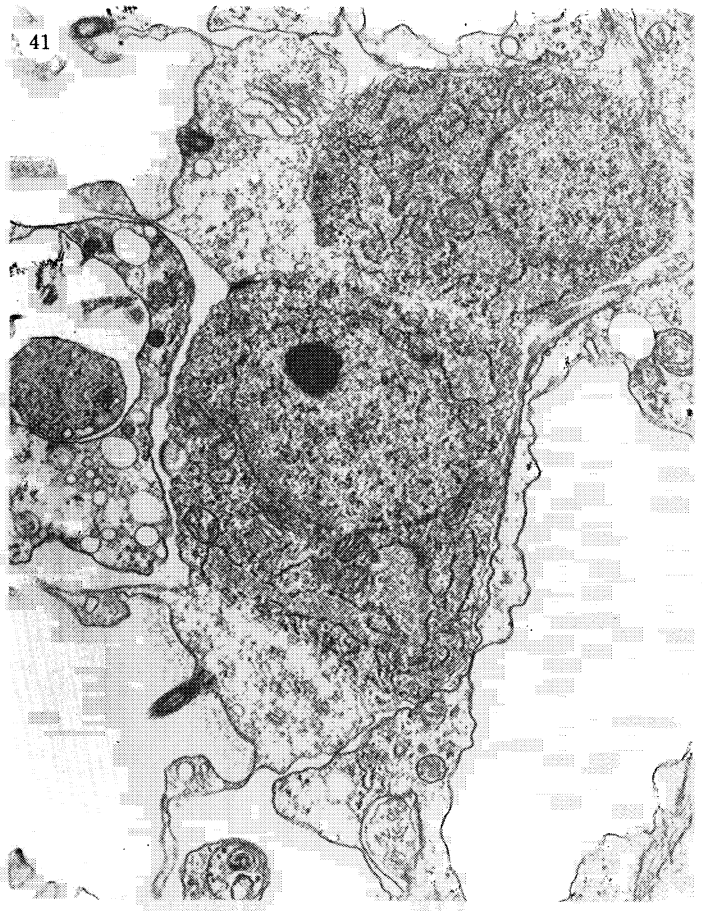
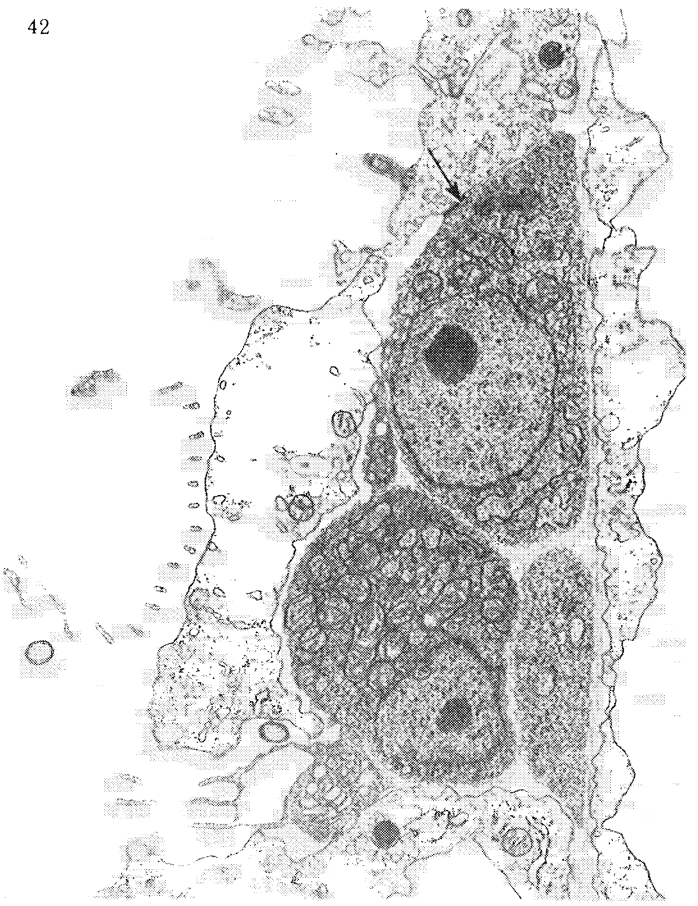


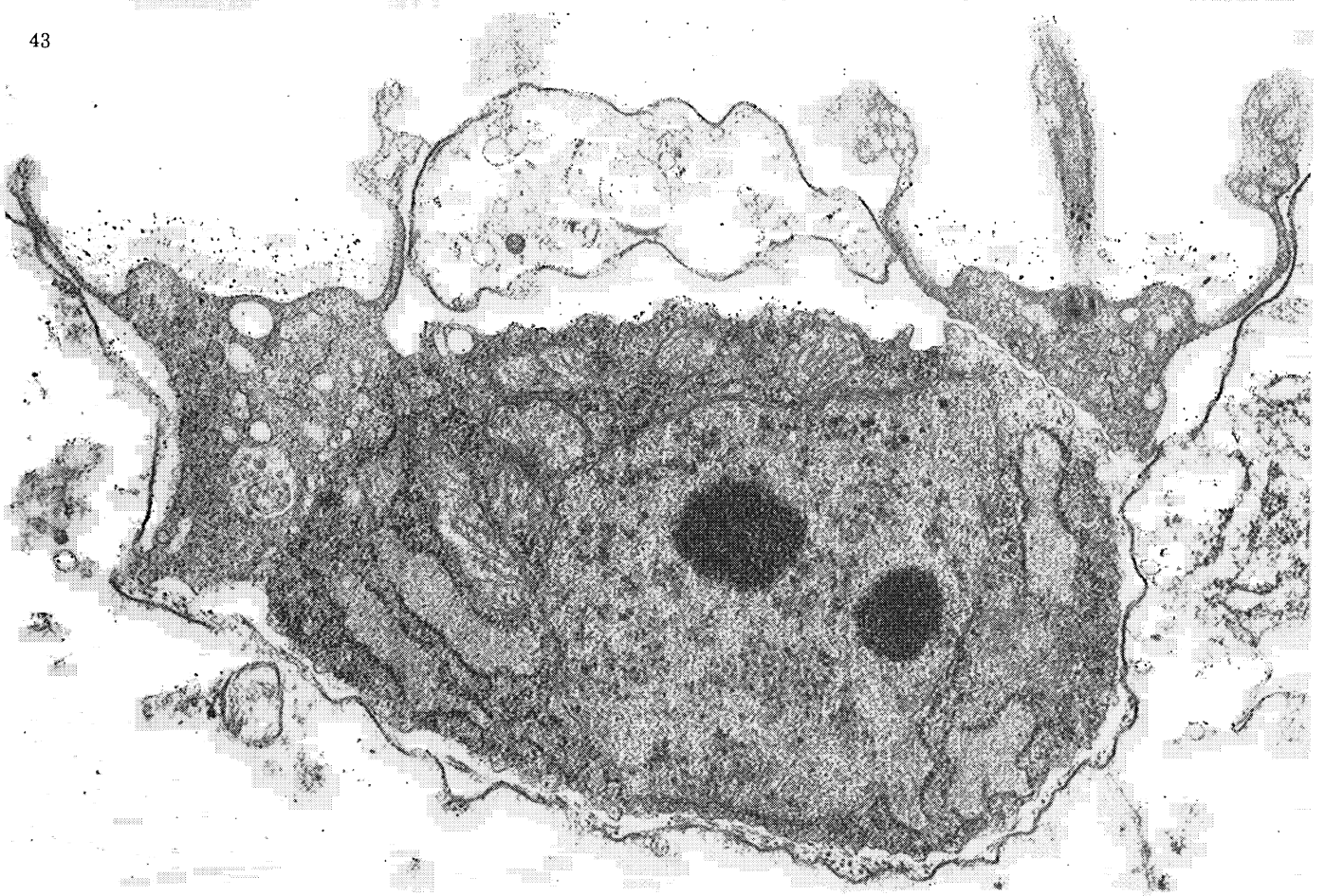




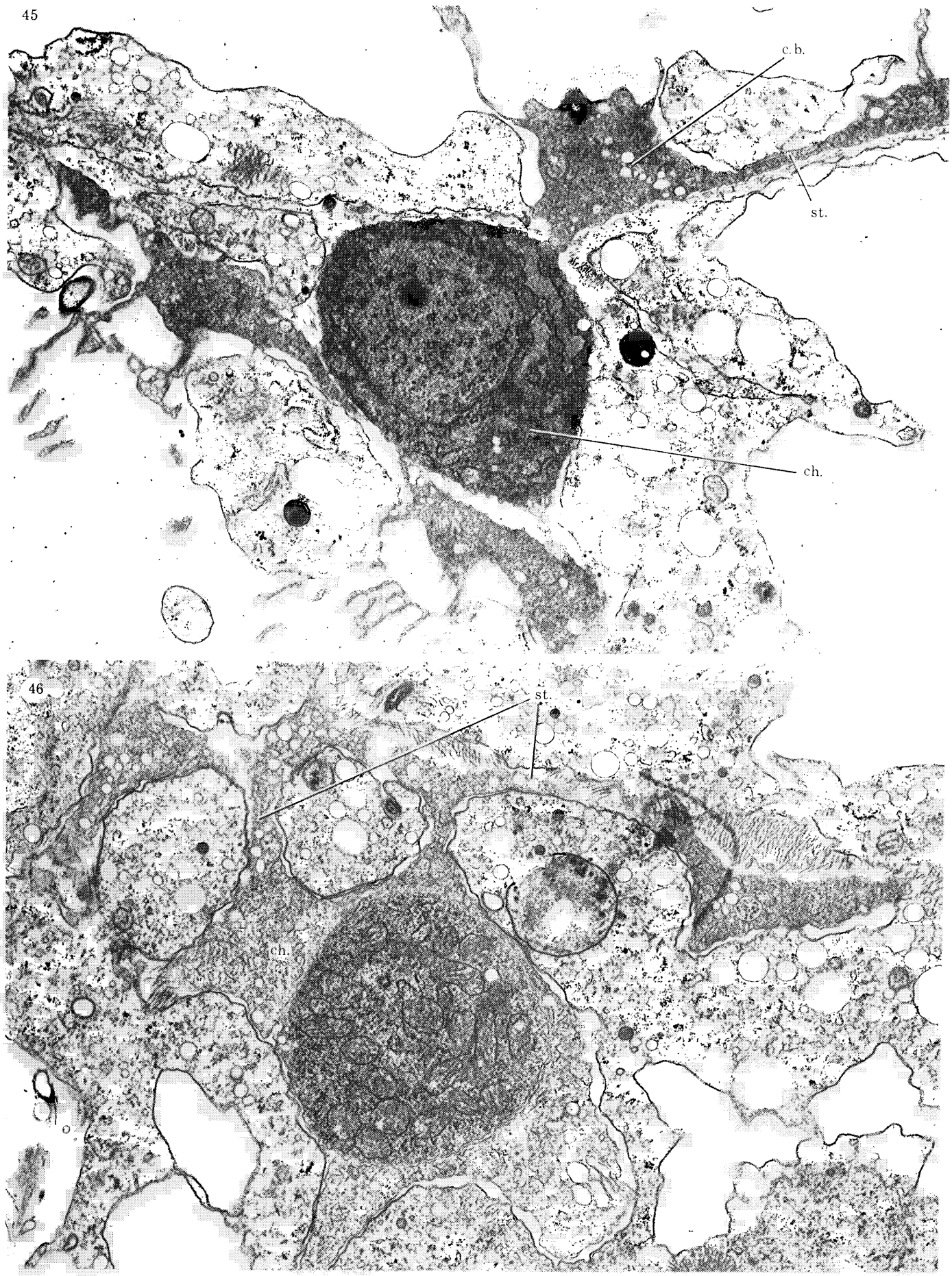
FIGURES 35–39. For description see p. 380.







FIGURES 41–43. For description see p. 381.



FIGURES 45 AND 46. For description see opposite.

secondary reticulum (figure 4) is a derivative of the trabecular tissue, and is not part of the choanosyncytium.

In figure 31 a collar body is cut vertically through the flagellum. Portions of the creeping stolon system can be seen, as well as a choanoblast. The collar bodies, choanoblasts and stolons lie directly on the mesolamella and are surrounded by trabecular tissue. Septate junctions are frequently formed between mature collar body and adjacent trabecular membranes (figure 32). The electron dense cytoplasm and narrow collar microvilli in figure 31 indicate that this is a young collar body. Cut in cross section (figure 33) the collar microvilli are seen as small circular profiles. Collar units are regularly spaced, about 4–6 μm apart. Figure 34 shows details of a collar body at higher magnification, together with an adjoining piece of stolon and an attached choanoblast. Particularly striking in this picture are signs of membrane synthesis in the Golgi region, the presence of phagosomes and the lack of mitochondria in the collar body. The choanoblast, by comparison, has many mitochondria, no phagosomes, conspicuous endoplasmic reticulum spaces filled with granulated material, and an inconspicuous Golgi component. The ultrastructure and development of the collar body will now be considered in more detail.

The collar consists of a ring of from 31 to 38 microvilli similar in form and number to those of demospongiae. The comparable figure for *Ephydatia* is 35 (Brill 1973). Each microvillus is 40–60 nm thick and contains a bundle of microfilaments (figure 35). The microvilli are fused near their bases (figure 36) but are separate over most of their length, lying about 0.2 μm apart in the living state as seen by Nomarski microscopy. Wider gaps may be seen in fixed tissues, and there is a tendency for the microvilli to swell in mature collar bodies, perhaps as an artefact of the preparation method. The microvilli bear filamentous side processes which bridge the gaps between adjacent microvilli (figure 37). The severing of these bridges during fixation would allow the microvilli to spread apart somewhat. In the intact condition, the bridges, which are presumably composed of glycoprotein (Garrone 1978; Garrone *et al.* 1980), lie about 0.05 μm apart, so creating a filter with rectangular meshes 0.05 μm × 0.2 μm. The equivalent values for *Spongilla* (Demospongiae) are 0.03 μm × 0.15 μm, to judge from Fjerdingstad (1961, fig. 4). Glycoprotein filaments also form a complex array at the bottom of the collar cavity (figures 30, 36, 43) and similar filaments bedeck the flagellum (figure 39). In all these respects, collar structure in *Rhabdocalyptus* closely resembles collar structure in other sponges.

The flagellum is 15–20 μm long and shows a conventional basal body and microtubules (figure 38). However, the tips are often swollen (figure 39) and the microtubule array is disordered in the swollen portion. A similar appearance has been noted in the flagella of other

Description of plate 7

Collar body development

Figure 41. Choanosyncytium stage; two choanoblasts in cytoplasmic continuity are both producing collar bodies. (Magn. × 10500.)

Figure 42. Presumed later stage; separated choanoblasts each with a mature collar body, one of which is segregated by a plugged bridge (arrow). (Magn. × 10500.)

Figure 43. Detail of early choanoblast–collar complex. (Magn. × 25600.)

Description of plate 8

Stolon patterns in the choanosyncytium

Figure 45. Developing collars sectioned vertically. (Magn. × 14000.)

Figure 46. A similar complex sectioned in the plane of the stolon system. (Magn. × 14000.)

Abbreviations: c.b., collar body; ch., choanoblast; st., stolon.

sponges (Bergquist *et al.* 1977) and in some other invertebrates, e.g. certain polychaetes (Heimler 1978). M. Pavans de Ceccatty (personal communication) has observed flagella with dilated tips in suspensions of living cells. He suggests that dilation occurs as a result of disproportionate growth: the microtubules grow more rapidly than the cell membrane. Spiralization of the axoneme is also evident.

The cytoplasm of the collar body frequently shows food vacuoles, typically 0.3–0.7 μm in diameter, Golgi bodies, mitochondria and numerous small vesicles. Watanabe (1978) gives evidence that the collar microvilli in *Tetilla* (Demospongiae) grow by addition of membrane at the base, the membrane being derived from vesicles of Golgi origin. She suggests that the vesicles contain filamentous mucous material which is externalized to form the filter strands when the vesicles fuse with the plasmalemma. These suggestions are quite compatible with current ideas about membrane synthesis and production of glycoprotein coatings in intestinal mucosa cells (Leblond & Bennett 1976) and would apply equally well to *Rhabdocalyptus*.

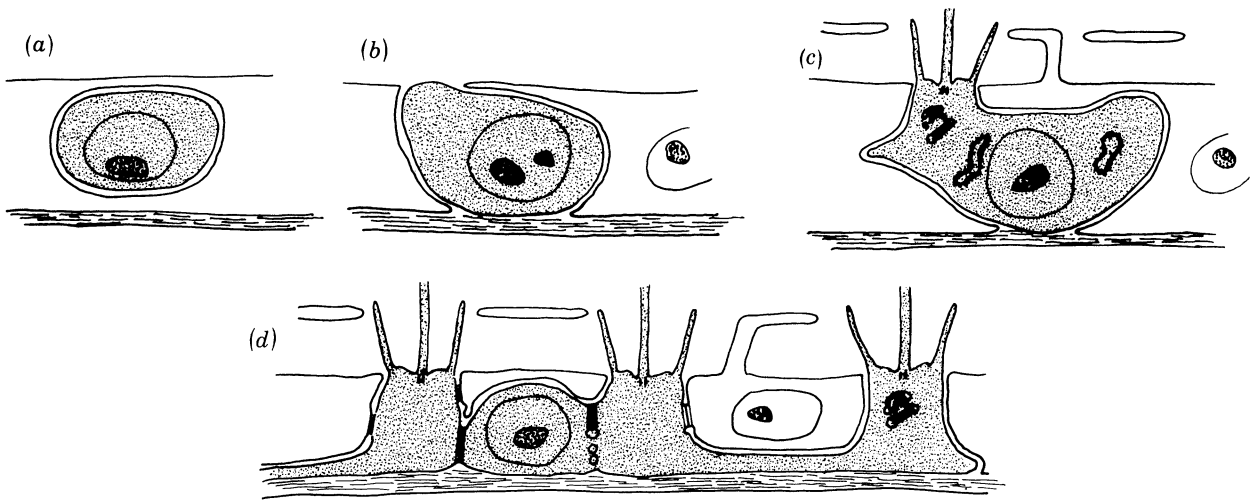


FIGURE 40. Suggested steps in the production of a collar complex. An archaeocyte (*a*) transforms to a choanoblast, putting out a process to the surface (*b*) which becomes a collar body (*c*); stolons grow out, sprouting other collar bodies which eventually become segregated by plugged bridges (*d*).

In what we regard as its mature state the choanosyncytium is organized as an extended reticulum with collar bodies arising from it. These portions are enucleate, but nuclei are present in associated mother cells (choanoblasts). Steps in the production of such an enucleate syncytium have been reconstructed from study of numerous sections, including some serial sections. A simplified interpretation of the early steps is given in figure 40.

The collar bodies arise from cells here termed choanoblasts, which are distinguished from archaeocytes by their expanded form, less electron dense cytoplasm and well developed rough endoplasmic reticulum containing fine granular material. These differences are shown to an unusually marked degree in figure 53. We envisage archaeocytes as undergoing division forming small groups of choanoblasts which are initially interconnected by open cytoplasmic bridges. Production of collar units is already apparent and may be far advanced while the tissue is still in this primary syncytial stage. It is indeed a true choanosyncytium at this point (figure 41). Later (figure 42) the choanoblasts become separate from one another but continue to sprout new collar units from stolons that creep out into the trabecular tissue. Eventually

the bridges connecting the choanoblasts with the collar unit complex become plugged. The choanoblast does not then immediately revert to an archaeocyte, but continues to show an engorged rough endoplsamic reticulum etc., which indicates that its metabolic association with the collar complex is continuing. The process of sprouting a new collar unit is shown at higher magnification in figure 43. The collar body on the left is still in extensive continuity with the choanoblast cytoplasm, but a row of vesicles is forming at the presumed future line of division. On the right a collar body is seen that either has recently separated or is still attached through a narrow bridge not shown in the section. It is presumed that division occurs by fusion of the row of vesicles with each other and with the cell membrane, as suggested diagrammatically in figure 44. A narrow strand remains, usually on one side of the original wide bridge, and this becomes plugged as described earlier (figure 6).

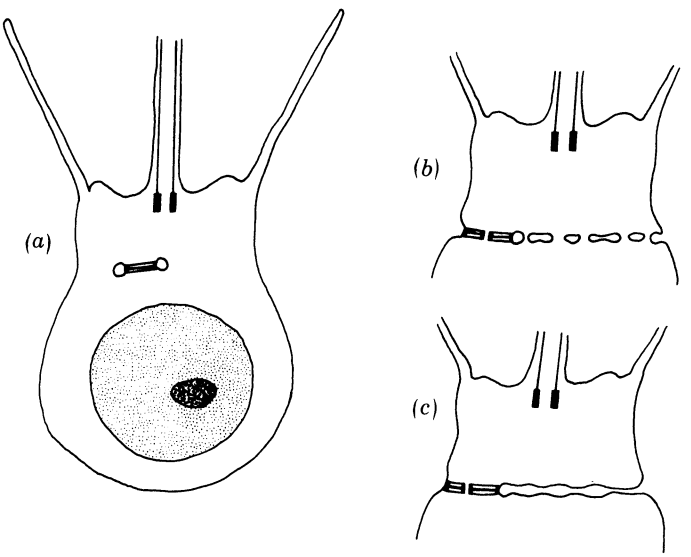


FIGURE 44. Diagrammatic representation of the separation of a collar body from its choanoblast. A plug forms in the cytoplasm (*a*). Plug then attaches to cell membrane of presumptive bridge region and vesicles form along line of future separation (*b*). Vesicles fuse with cell membrane, leading to enclosure of plug within the narrow remaining cytoplasmic connection (*c*).

Figures 45 and 46 show choanoblasts still attached by tenuous strands to the stolon system from which project collar bodies, some of them now being located at a considerable distance from the mother cell. Presumably stolon production and sprouting of collar units goes on for some time and the stolons grow out like fungal hyphae within the trabecular tissues which form the structural component of the wall of the flagellated chamber. These relationships are summarized diagrammatically in figure 47.

Formation of septate junctions with trabecular tissue may occur only after the collar units have reached their definitive locations, as in *Hydra*, where septate junctions form between cnidoblasts and epithelial cells only when the former have migrated to their definitive locations (Campbell 1974). Plugged cytoplasmic bridges are also seen between collar bodies and adjacent trabecular tissues (figure 21). As there is no primary connection between these tissues, the junctions must form secondarily. They would provide a possible route for distribution of nutrient materials from the collar bodies to the trabecular tissues.

The choanoblast shown in figure 46 shows four main stolons which ramify forming an anastamosing network. The cytoplasm of the developing stolons and nascent collar bodies is usually

darker and denser than that of the surrounding trabecular tissue, but later, in mature, enucleate collar bodies, no such difference is apparent. The cytoplasmic density may represent structural materials, enzymes, etc., which are gradually used up as the collar body grows and becomes functional.

It seems likely that collar–stolon complexes have limited life spans and are subject to periodic replacement. The main reason for suggesting this is the frequency with which the presumed developmental stages are encountered in sections of otherwise mature sponge tissue, but also

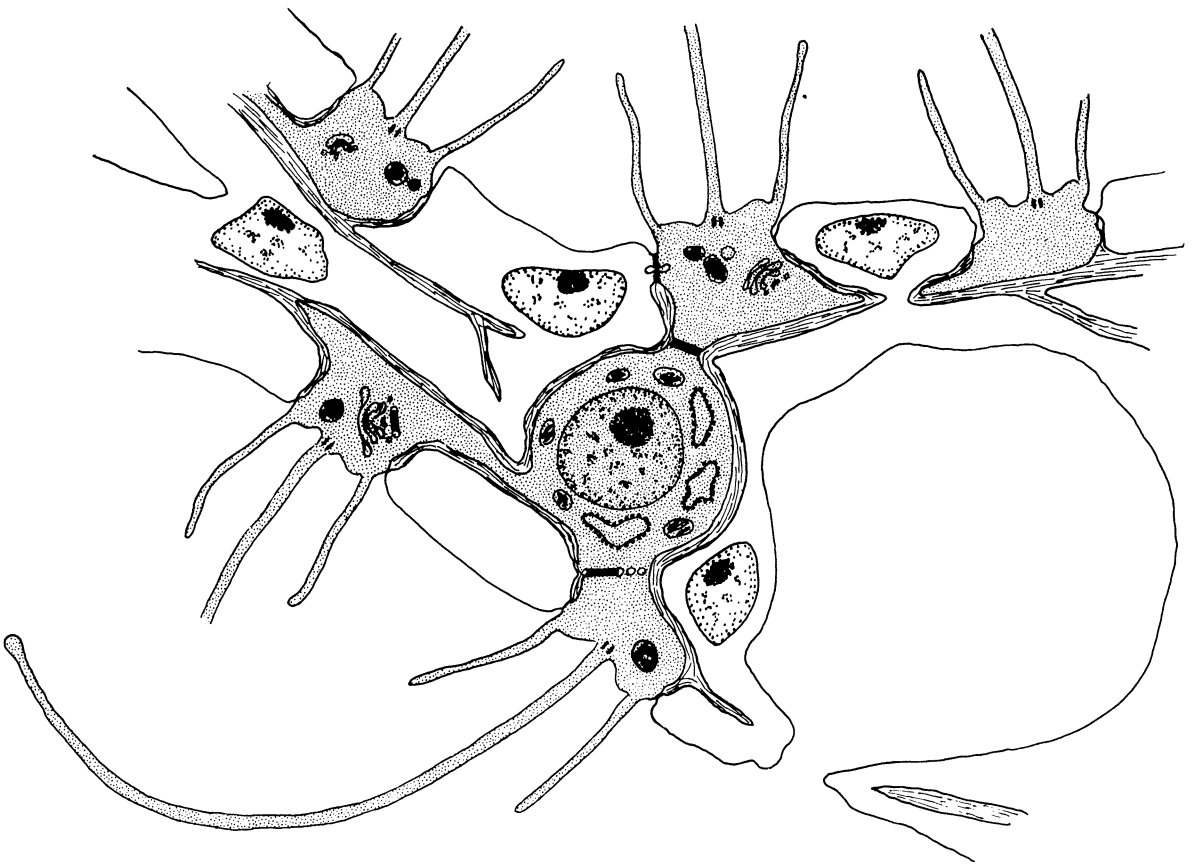


FIGURE 47. Drawing of a choanosyncytial complex similar to that shown in figure 45, simplified by omission of secondary reticulum and other features. Collars belonging to the central complex project into two different flagellated chambers. Bridge formation and plugging are in progress.

on theoretical grounds it would be expected that, in the absence of nuclei, the collar–stolon complex would eventually run out of enzymes and informational molecules, and would then die, as do red blood corpuscles. The extreme scarcity of mitochondria in the tissue also suggests expendability. If this is true we might expect to see signs of absorption of collars and flagella, lysosomal breakdown of tissue components, etc. Such signs are not apparent. We therefore conclude that worn out collar bodies and stolons probably round up and are shed into the flagellated chambers and are carried away in the effluent water stream. The choanoblasts, charged with nutrients obtained from the dehiscent portions before their detachment, would perhaps revert to the status of archaeocytes and become available for other purposes. This sequence of events, however, requires verification.

(*d*) *Cells*

The cells described in this section may be connected to other cells and to syncytial tissues by plugged cytoplasmic bridges and are therefore not as completely enveloped by plasmalemma as are cells in other animals. Nevertheless there is no reason to doubt that they are effectively segregated, and in some cases they closely resemble cells in other sponges. It is therefore appropriate to refer to them as cells.

(i) *Archaeocytes*

Single archaeocytes or groups of two to ten lie scattered through the tissues of the sponge. They are compact cells 3–5 μm in diameter. The cytoplasmic ground substance contains an abundance of electron-scattering particulate material (figure 48). There are few vacuoles, there is little endoplasmic reticulum and the Golgi complex is not conspicuous, but there are many mitochondria. The nucleolus is fairly prominent. The nests of archaeocytes frequently seen may represent mitotic progeny. Mitotic figures are occasionally seen. The cells do not necessarily undergo complete cytokinesis: plugged junctions are often seen between them. Indeed the possibility cannot be excluded that this is the rule rather than the exception where the cells in question lie side by side.

The archaeocytes in *Rhabdocalyptus* are typically compact and well rounded cells. As noted by Reiswig (1979), they lack the elongated shape and pseudopodial outgrowths associated with cells undergoing active migration, and it is therefore doubtful if they are as mobile as their counterparts in other sponges. They lie subepithelially and are probably not exposed to external food sources. Phagosomes are rarely seen and where present might be autophagosomes. It would seem likely that archaeocytes obtain their nutrients by absorption from the extracellular space or from the collar units to which they are attached, when functioning as choanoblasts.

As in other sponges, so in hexactinellids archaeocytes appear to function as stem cells giving rise to various other cell types. Okada (1928) reported that spermatozoa and ova derive from archaeocytes. Thesocytes (Ijima 1901) and choanocytes (Borojević 1966) are said to have a similar origin. Harrison & Simpson (1976) discuss problems of terminology involving archaeocytes and suggest that functional designations be applied only where differentiation has progressed to a point where the functional attributes are demonstrable, or can be confidently inferred. Thus, in *Rhabdocalyptus*, we have given the name choanoblast to what is presumed to be an archaeocyte in the process of generating or maintaining a choanosyncytial complex. Figure 53 shows the cytological differences between the two cell types.

(ii) *Thesocytes*

These cells are found throughout the hexactinellids and have been described by Schulze (1899; as *knollige Körper*, or *Knollen*), Ijima (1901) and Reiswig (1979). They bear little resemblance to the thesocytes found in spongillid gemmules (Brien 1973) and there is no direct evidence that they are concerned with storage as the Greek roots of the word would imply.

The cells are very large (up to 17 μm in diameter) and contain a single massive inclusion which appears as homogeneous granular material under the electron microscope. Within this material, crystals are sometimes formed (figure 49). The cytoplasm is restricted to a narrow sheath around these large bodies, with the nucleus pushed over to one corner. The nature of

the stored material is unknown. It has at various times been likened to fat, starch and glycogen, but the appearance under the electron microscope is compatible with none of these. Crystalline inclusions in animal cells are usually assumed to be proteinaceous (Fawcett 1966), which suggests that the dispersed granular material within which the crystals lie is also a protein.

Ijima describes stages in the maturation and breakdown of the cells and states that they derive from archaeocytes. In the mature state they occur in groups of up to 30 cells. They lie either enveloped by processes of the trabecular syncytium (figure 50) or attached to the outside of it. Their boundaries are usually quite distinct (figure 54) but they are attached to trabecular tissue by plugged cytoplasmic bridges or, more rarely, by open bridges (figure 51). They are most abundant in the incurrent and excurrent trabecular lacunae, but they may occur in the walls of the flagellated chambers.

(iii) *Granulated cells*

Smaller than most thesocytes (5–8 μm diameter), granulated cells have a very densely staining cytoplasmic ground substance and contain numerous 1–2 μm, electron dense inclusions (figures 52, 54, 55). Despite their granular inclusions there is no good reason for supposing that they transform into thesocytes, or *vice versa*. They are distributed throughout the sponge but are most abundant in the subdermal and subatrial regions, especially the latter. They may be enveloped by trabecular processes, but more commonly they are attached in only a limited region, usually by a plugged cytoplasmic bridge.

The cytoplasm shows many small vesicles of about 15 nm diameter in addition to the larger inclusions noted above. There is a fairly abundant rough endoplasmic reticulum, but few mitochondria are seen.

In figure 54 a granulated cell is shown connected to trabecular tissue by an open cytoplasmic bridge partially closed in one place by cell membrane in which a plugged channel is visible. This stage (a unique example) may represent absorption of a granulated cell into the general trabecular syncytium. This may mean that the granulated cell is a storage device which is isolated from the trabecular syncytium only while serving its storage function.

These cells superficially resemble the 'grey cells' of demosponges (Bergquist 1978) which are apparently one of several cell types concerned with glycogen synthesis (Boury-Esnault & Doumenc 1979) but until histochemical tests are done it would be premature to refer them to any of the established cell categories.

Description of plate 9

Discrete cells

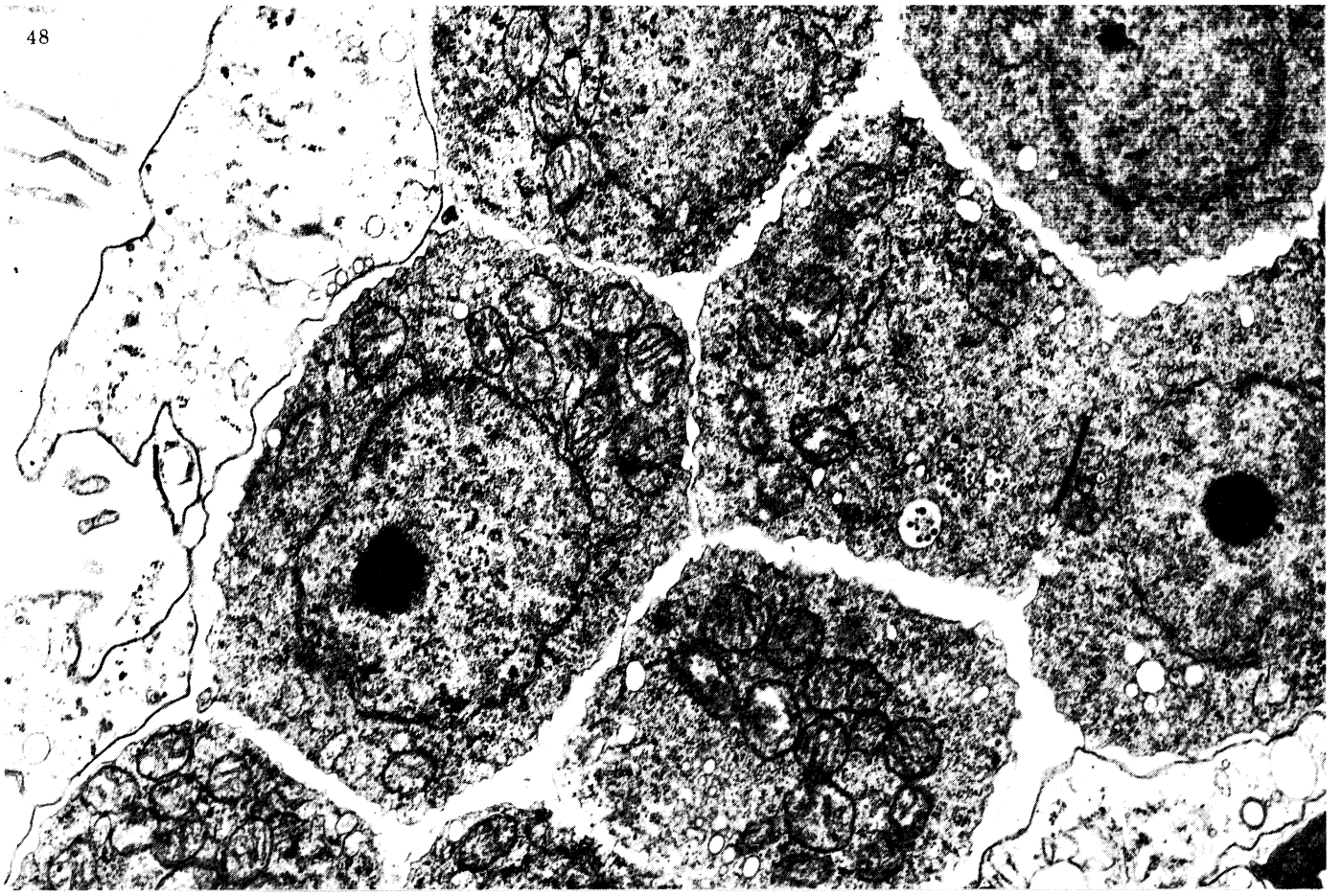
Figure 48. A group of archaeocytes. (Magn. × 18300.)
Figure 49. Thesocytes, one containing crystalline inclusions. (Magn. × 5100.)

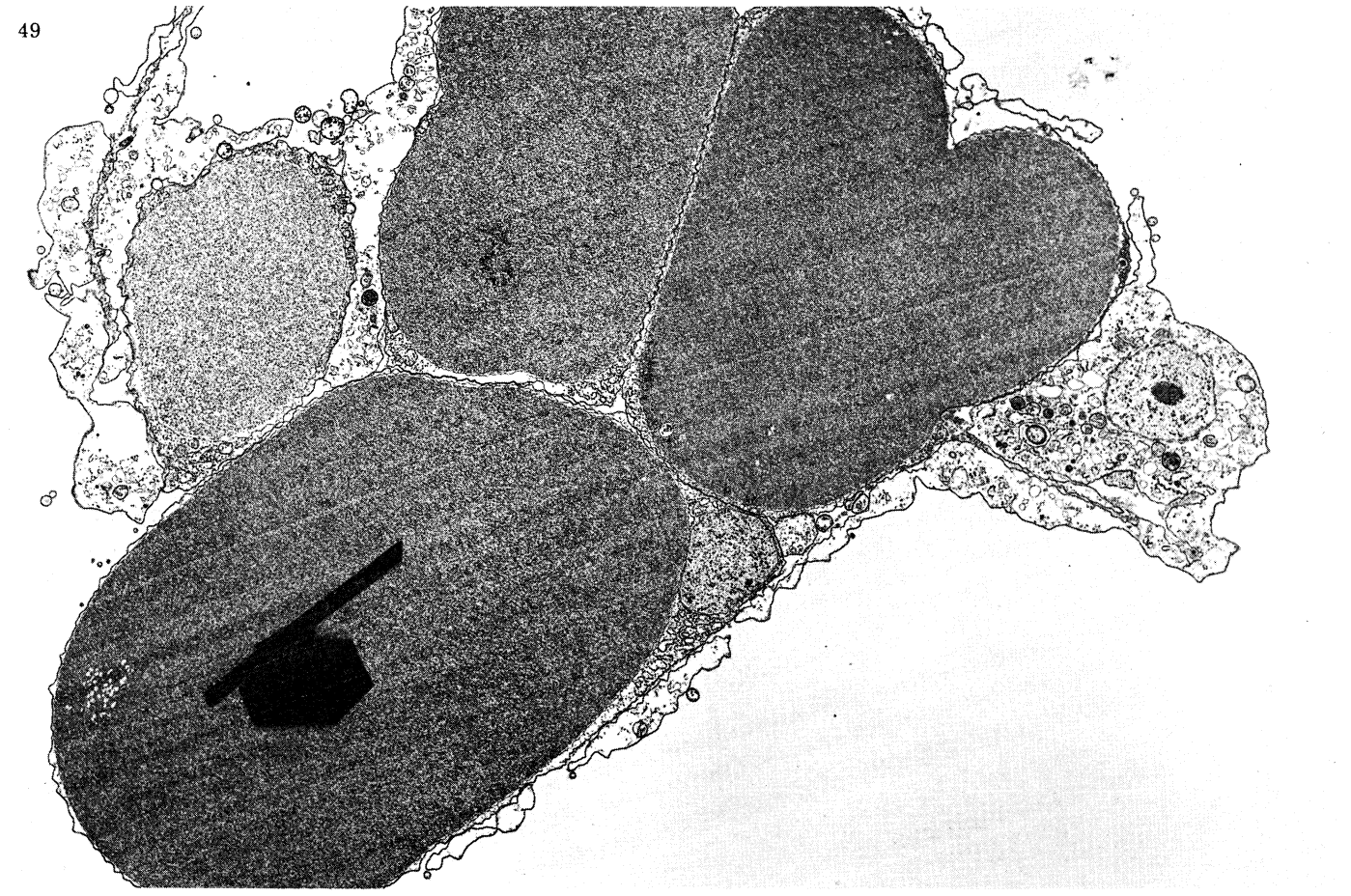
Description of plate 10

Discrete cells

Figure 51. An unusual case of a thesocyte syncytially incorporated into trabecular syncytium. (Magn. × 14100.)
Figure 52. Granulated cell. (Magn. × 13000.)
Figure 53. An archaeocyte, with a choanoblast for comparison. (Magn. × 14300.)
Figure 54. A granulated cell apparently becoming incorporated into the trabecular syncytium. Arrow shows plug in disintegrating membrane. (Magn. × 8700.)

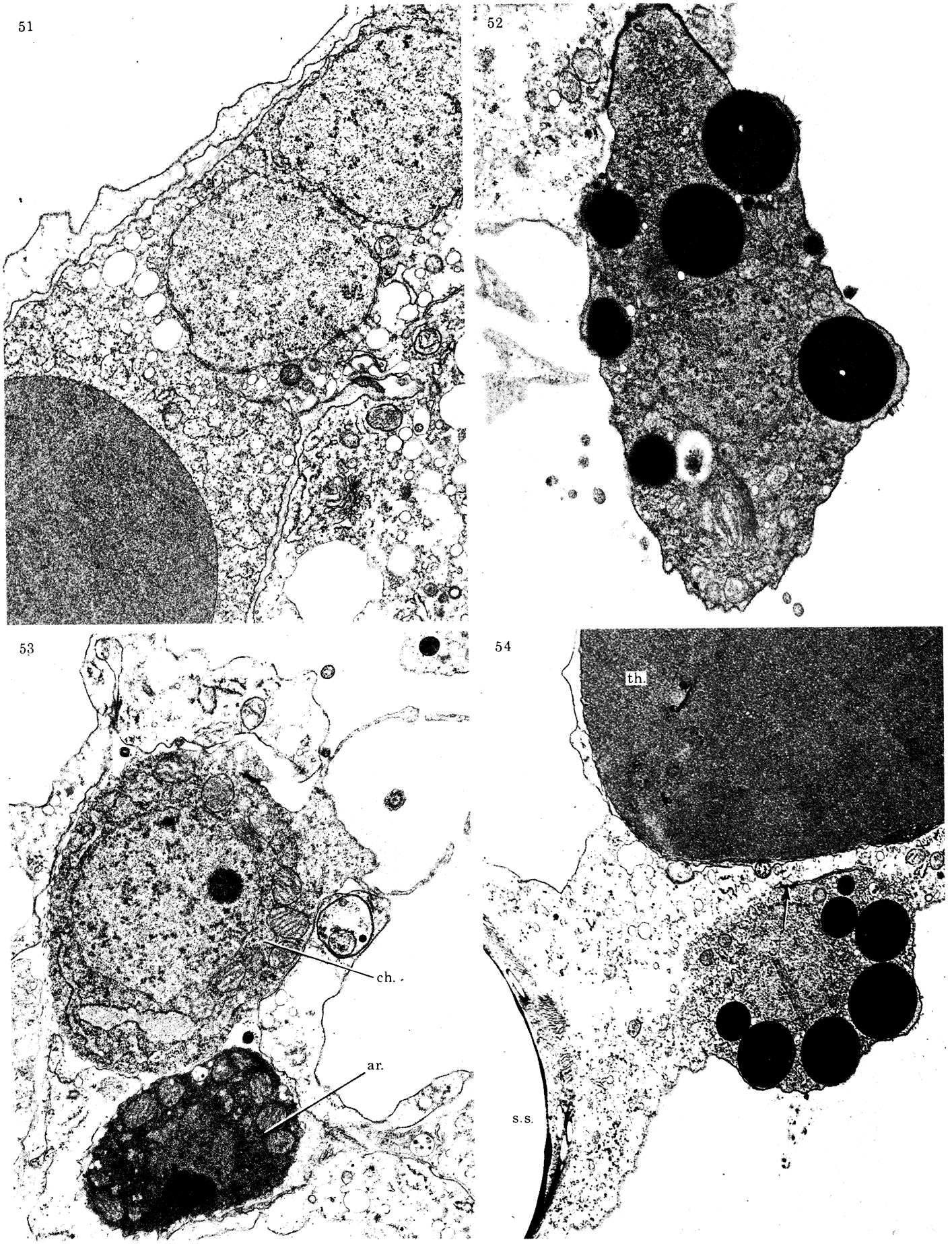
Abbreviations: ar., archaeocyte; ch., choanoblast; s.s., spicule space; th., thesocyte.



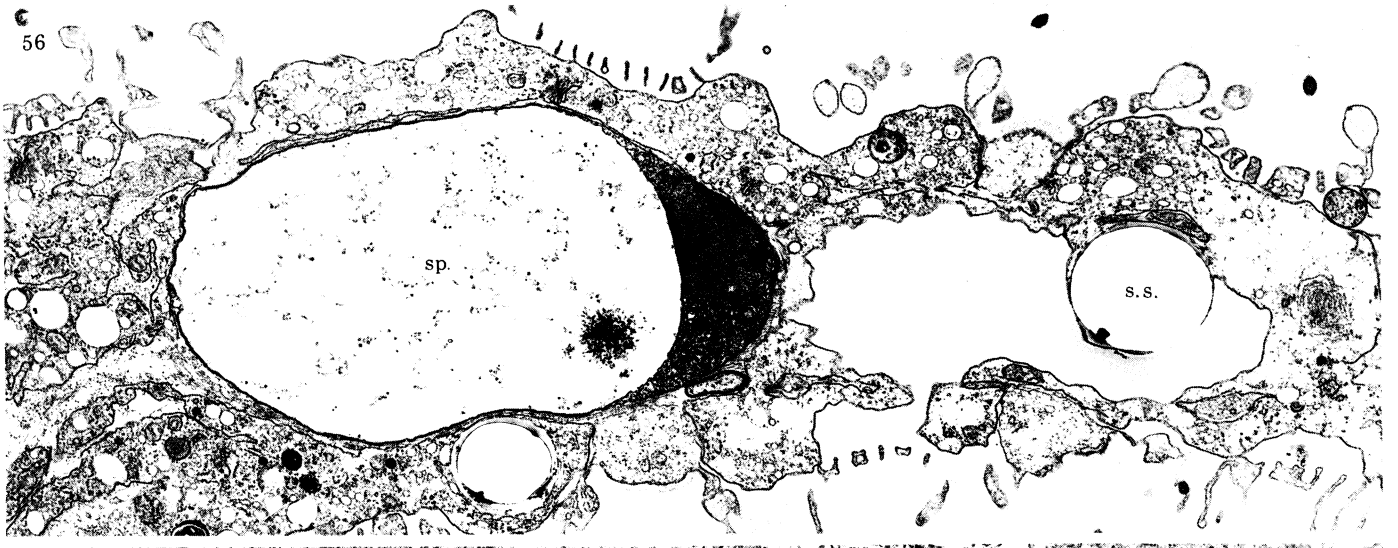


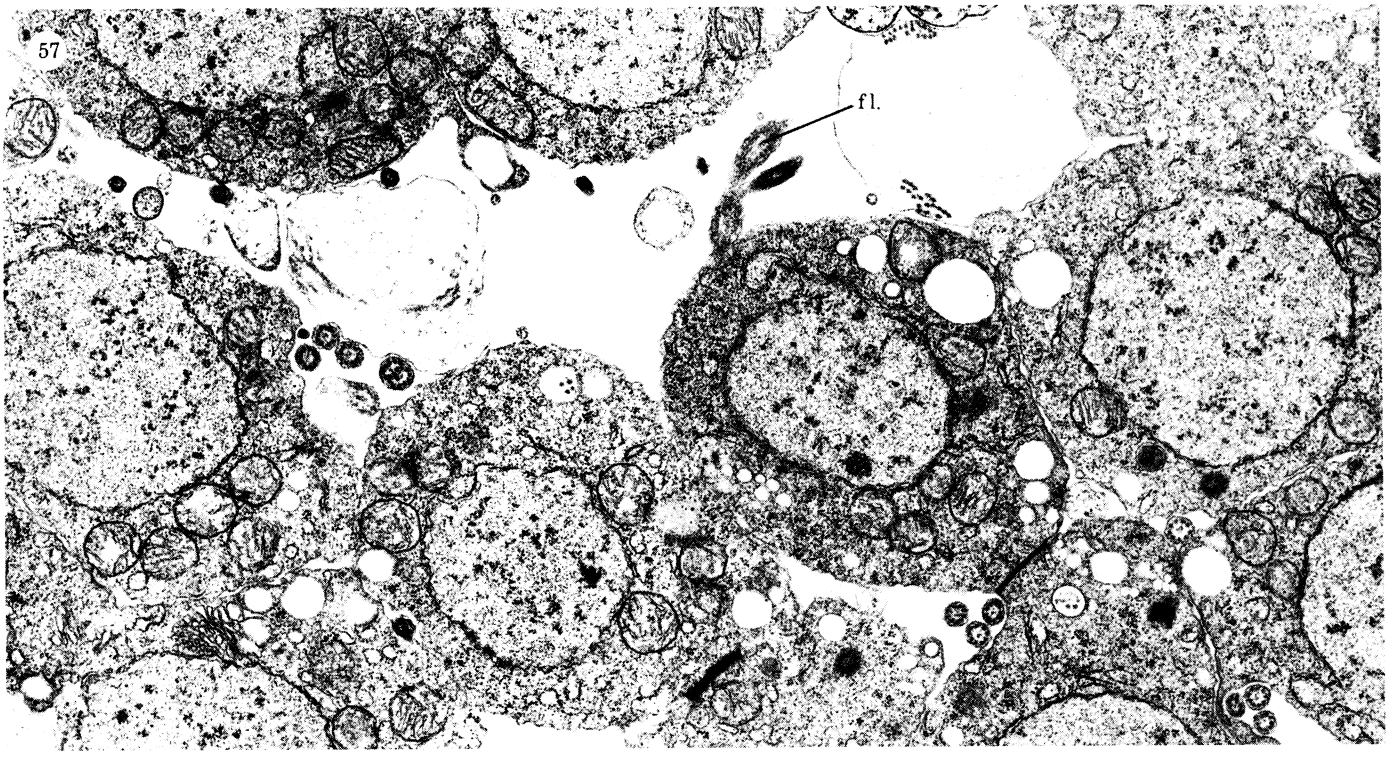
FIGURES 48 AND 49. For description see opposite.

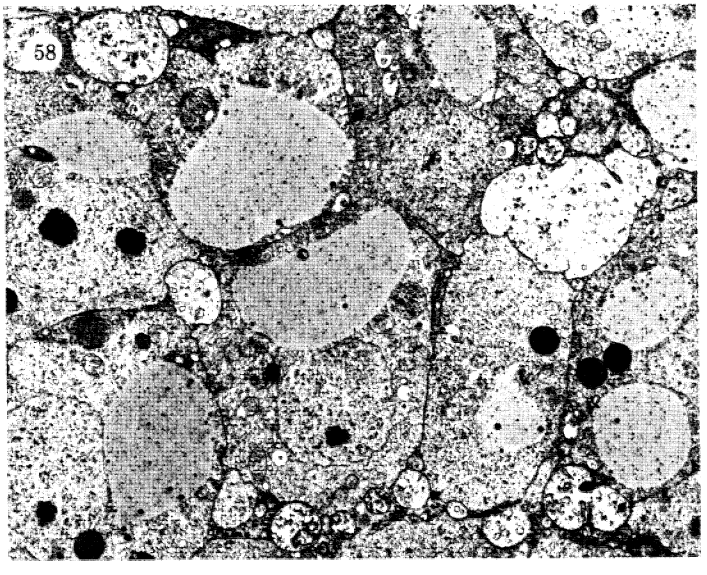
(*Facing p.* 386)

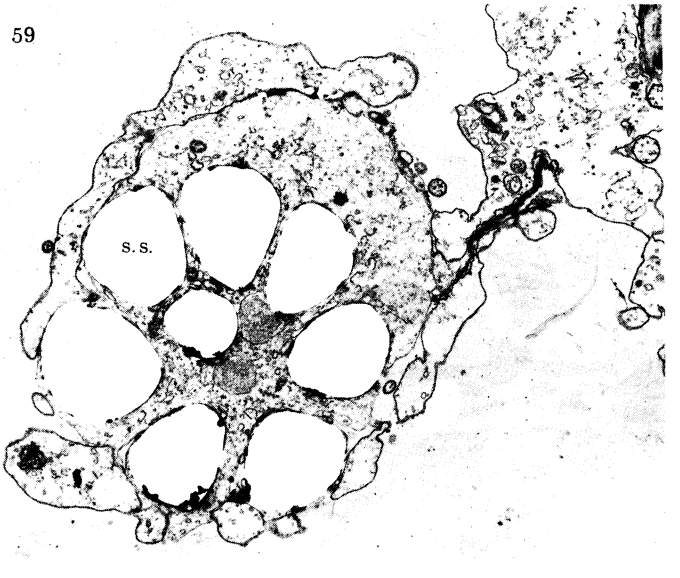


FIGURES 51–54. For description see p. 386.

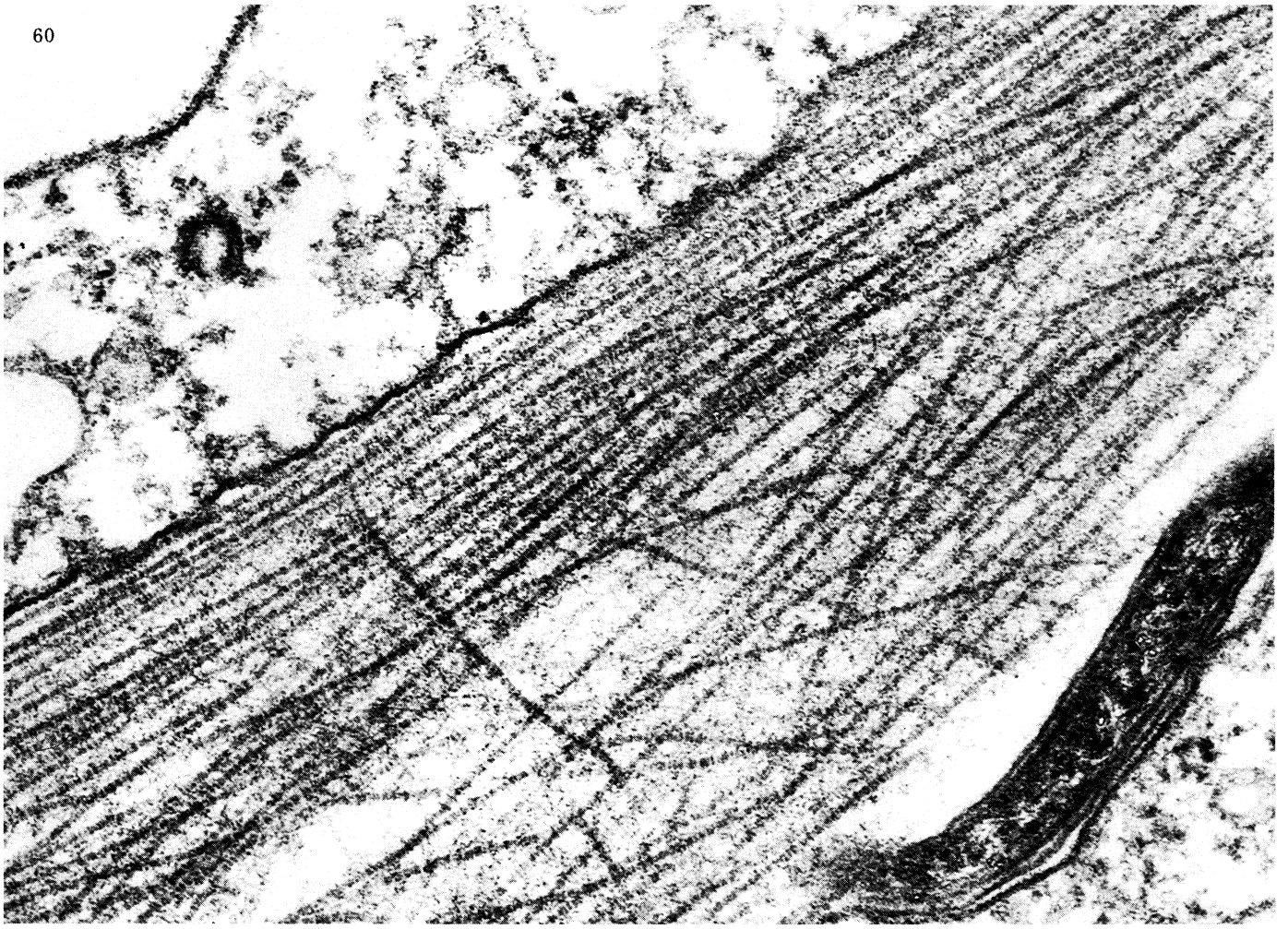


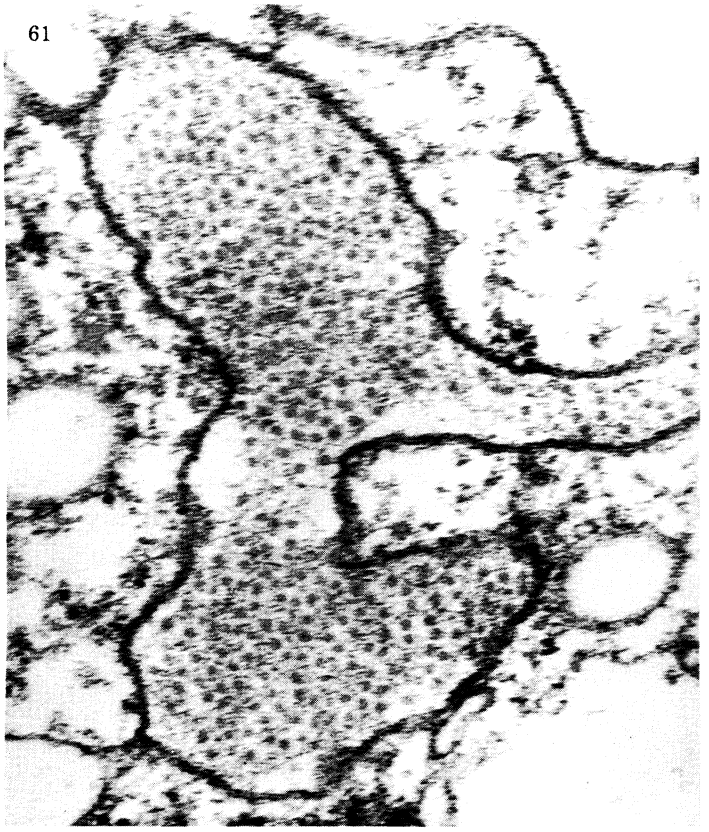


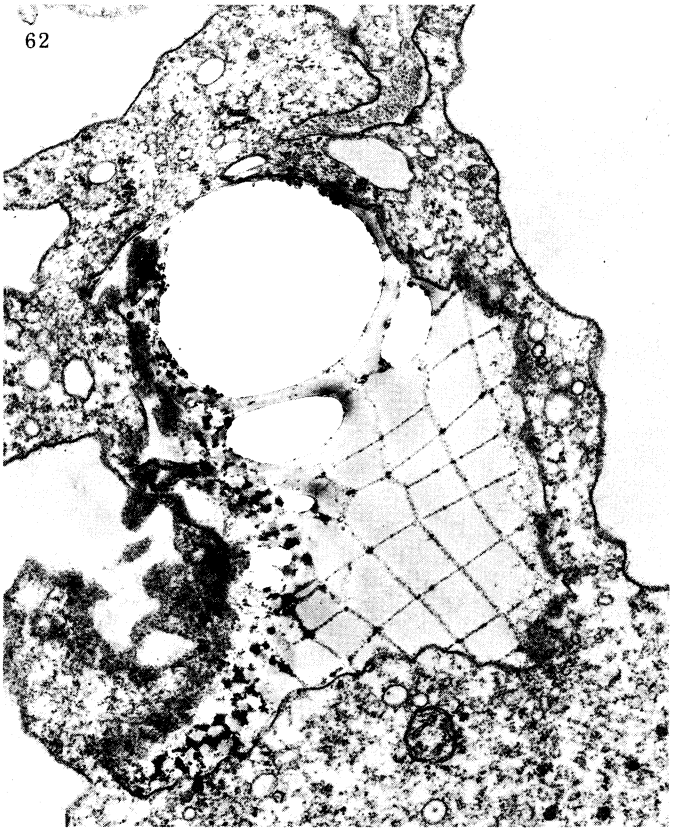




FIGURES 56–59. For description see p. 387.







FIGURES 60–62. For description see opposite.

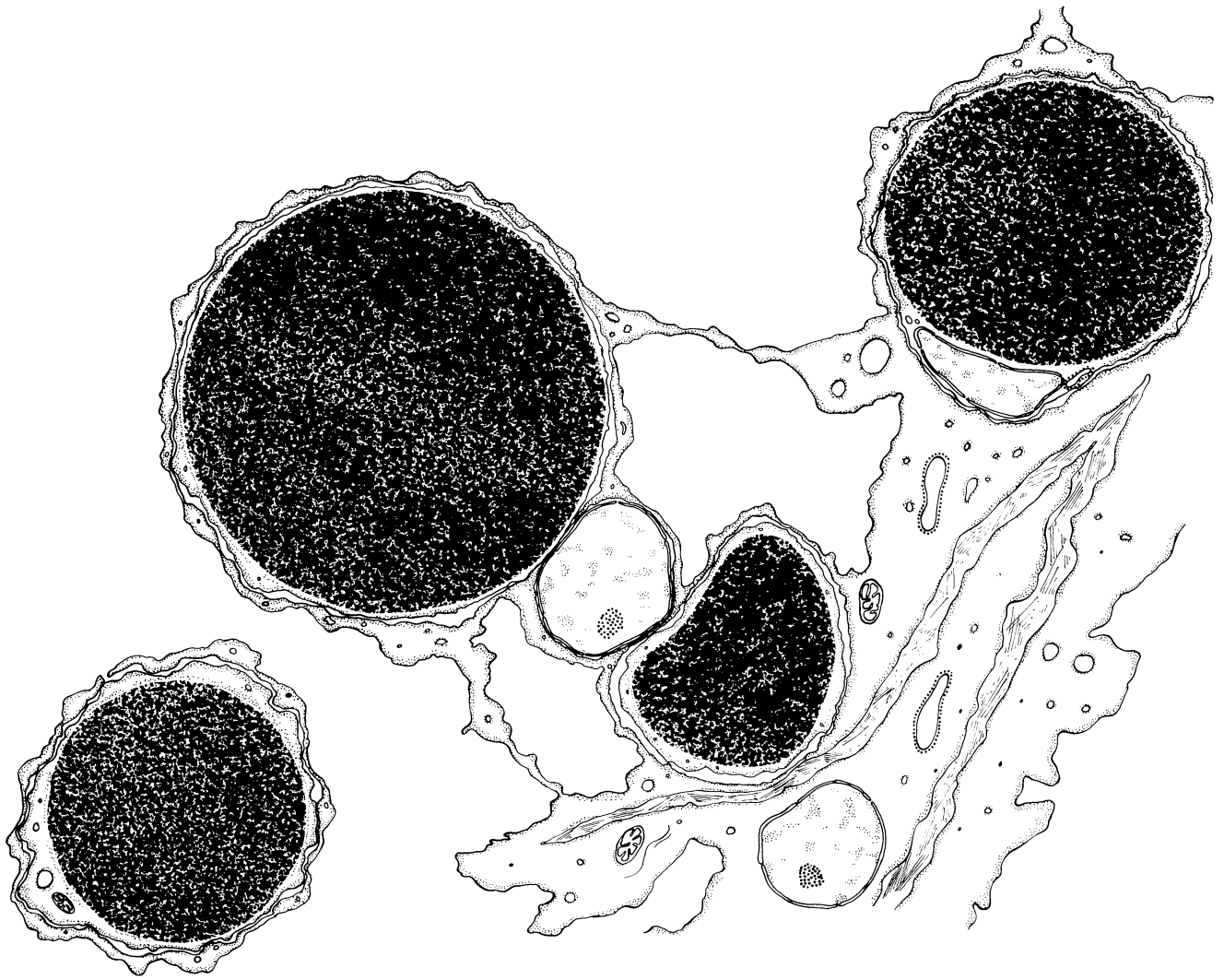


FIGURE 50. Drawing of an area of the trabecular syncytium with thesocytes.

DESCRIPTION OF PLATE 11

Discrete cells

FIGURE 56. A spherulous cell. (Magn. × 6400.)
FIGURE 57. Spermatocytes. (Magn. × 14000.)
FIGURE 58. Spermatogonia, showing large clear vesicles. Spottiness in the picture is due to contamination of the preparation. (Magn. × 4900.)
FIGURE 59. Sclerocyte, with intracellular spaces from which silica has been removed. (Magn. × 6300.)

Abbreviations: fl., flagellum; sp., spherulous cell; s.s., spicule space.

DESCRIPTION OF PLATE 12

Extracellular components

FIGURE 60. Mesolamella, showing collagen cut longitudinally and a presumed bacterial symbiont. (Magn. × 75100.)
FIGURE 61. Mesolamella, with collagen cut transversely. (Magn. × 71300.)
FIGURE 62. Mucus net spanning a lacuna in the trabecular net. (Magn. × 21900.)

(iv) *Spherulous cells*

These are round or ovoid cells, 5–7 μm in diameter, characterized by a large vacuole containing a small amount of thinly dispersed flocculent matter, often concentrated into one or two distinct masses, as in figure 56. The cytoplasm often contains a prominent Golgi component. In some cases several vacuoles, or spherules, are present and their membranes are directly apposed, with virtually complete elimination of cytoplasm in between. Such cells markedly resemble certain types of mucous cell in other animals. Lacking evidence regarding the composition of the secretion product, we here adopt the neutral designation 'spherulous', following Levi (1956).

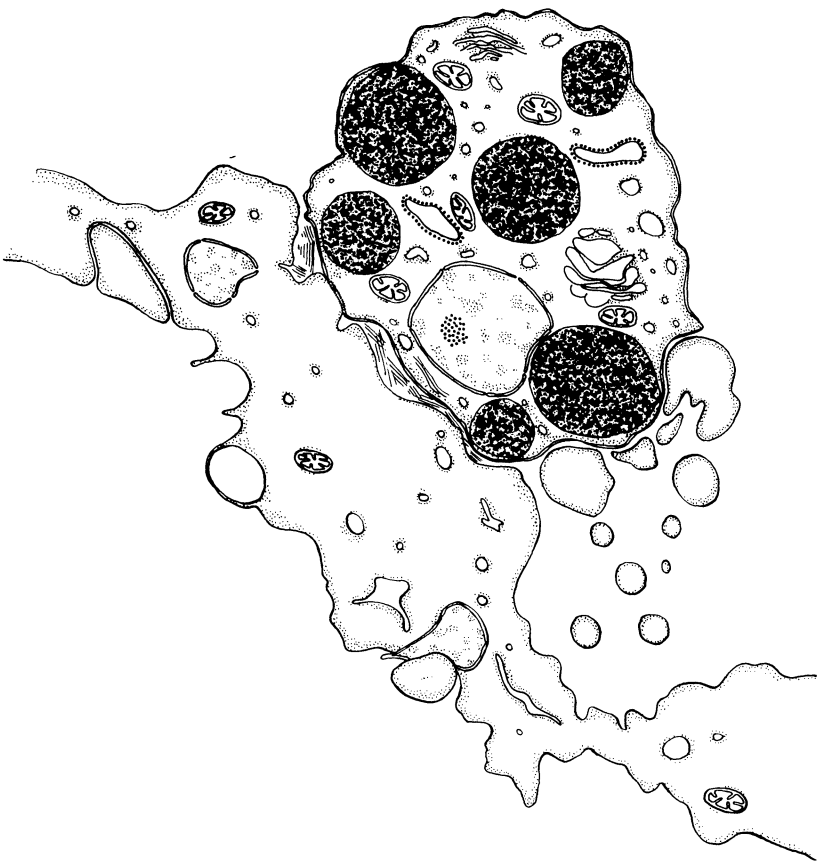


FIGURE 55. Drawing of a granulated cell.

The cells are distributed throughout the general trabecular syncytium and are always enveloped by trabecular tissue, to which they may be connected by plugged cytoplasmic bridges.

(v) *Germ-line cells*

Spermatogenesis has been described most recently by Okada (1928) for *Farrea*, extending earlier observations. Differentiation proceeds from archaeocytes. The cells occur in masses (spermatic cysts) 2.5–3.5 μm in diameter within the trabecular syncytium.

Two stages have been observed in our sections, a relatively early stage preceding the formation of the flagellum, and a later flagellated stage. The cells in the earlier stage are 6–7 μm

in diameter and have an excentric nucleus about 3 μm in diameter, with one or two nucleoli, a large number of mitochondria and cytoplasmic vesicles containing electron dense material. The most striking feature, however, is the large, clear vesicle 2.5–3.5 μm in diameter (figure 58). These cells bear some resemblance to spermatogonial mother cells in *Reniera* (Tuzet 1932, reproduced in Brien 1973, figure 255).

The later cells (spermatocytes) have a flagellum (figure 57). The cell body is now 3.5 μm in diameter and the nucleus 2–2.5 μm, and a single nucleolus is present. The large clear vesicle is absent.

In any given cyst, the cells are all seen to be developing synchronously, as described for other sponges (Chen 1976). Plugged cytoplasmic bridges interconnect the cells until an advanced stage of differentiation, when they finally separate completely. Some cells resembling oocytes have been seen but information is at present too scanty to be worth reporting.

(vi) *Sclerocytes*

According to Okada (1928), hexactinellid hexasters are produced by mononucleate scleroblasts that become multinucleate before forming the spicule in their cytoplasm. In our electron micrographs, multinucleate sclerocytes with intracellular spaces representing spicules from which the silica has been removed are frequently seen, and the cells appear to be sufficiently well differentiated from the trabecular tissues to be considered as discrete. Neither open cytoplasmic bridges nor plugged bridges have been seen between sclerocytes and other cells or tissues.

After desilication, lumps of electron dense material often remain adhering to the wall of the spicule cavity. They are usually concentrated on one side of the cavity, as if they had fallen there by gravity. We assume that they are undissolved particles of mineral matter. In some figures (e.g. figure 59) the spicule cavity shows a densely staining lining which might represent an organic sheath, as reported in calcareous sponges (Jones 1955, 1967; Ledger & Jones 1977). However, the only modern study of the hexactinellid skeleton (Travis *et al.* 1967) suggests the presence of a dispersed amorphous or fibrillar organic component, rather than a sheath, and it is stated that the material shows the X-ray diffraction pattern of cellulose. This finding needs verification. The same investigators found collagen in the material surrounding the desilicated skeleton of *Euplectella*, which would agree with our observations. The mesolamella is usually quite conspicuous around sclerocytes, and around spicules lying within the trabecular syncytium.

In *Sycon* the creation of a special extracellular environment for calcite secretion is apparently achieved by the formation of septate junctions between the sclerocytes surrounding the spicule primordium (Ledger 1975; Ledger & Jones 1977). The problem of isolation of an extracellular space does not apply in hexactinellids, as the spicules are produced intracellularly within multinucleate giant cells. However, these sclerocytes themselves are the only tissue components that we have found that are not connected to other tissues by open or plugged cytoplasmic bridges. This may indicate that the intracellular environment for spicule formation is sufficiently distinctive to require isolation of the sclerocyte cytoplasm by means of a complete plasmalemmal envelope.

Spicules are seen within the general trabecular syncytium as well as within sclerocytes (figures 54, 56). Whether or not they can be produced there is an open question. Possibly, when a spicule is complete, the sclerocyte dedifferentiates, or transforms to trabecular tissue,

leaving the spicule enveloped in this tissue. However, the spicule cavities shown in figure 56 are surrounded by what appears to be a specialized zone of trabecular cytoplasm, suggestive of active secretion. The whole question of spicule production in hexactinellids is overdue for reinvestigation, and further discussion here would be inappropriate. Most mature spicules probably have no cellular envelope, although cell processes attach to them.

(*e*) *Extracellular organic components*

(i) *Mesolamella*

Throughout the trabecular syncytium and choanosyncytium the cell membranes are attached basally to sheets or strands of fibrous extracellular material (see, for example, figure 60). This mesolamella is 0.05–1.5 μm thick in most places. The fibres are about 15 nm thick and show a regular spacing in cross section (figure 61). They are cross-striated, showing a roughly 20 nm banding pattern, with a less distinct 60 nm periodicity in places, indicative of collagen. Collagen (spongin A) occurs throughout the Porifera in this sort of location. Spongin B is not in evidence. Other material is visible in the mesolamella, consisting of thin filaments and particles, and carbohydrates are presumably present as in other sponges (Junqua *et al.* 1979). The mesolamella is particularly thickly developed in the dermal membrane, with layers of fibres running in several different directions.

(ii) *Bacteria*

Figure 60 shows a portion of a bacterium in the mesolamella. Extracellular bacteria are fairly common in the tissues of *Rhabdocalyptus* occurring in spaces between cells or in the mesolamella. Levi & Porte (1962) recognized such bacteria as normal symbionts in *Oscarella*, distinct alike from bacteria invading degenerative tissue and bacteria ingested as food. Though regularly observed, the bacteria in *Rhabdocalyptus* never achieved densities of the order observed by Bertrand & Vacelet (1971) and Vacelet (1975) in *Verongia*, where bacteria may amount to 38% of the total tissue volume.

Wilkinson *et al.* (1979) show that the symbiotic bacteria have collagenolytic ability and it is suggested that they play a role in construction and recycling of connective tissue proteins.

(iii) *Mucus*

Halichondria larvae have a complex glycocalyx or larval coat (Evans 1977) and ruthenium red shows the presence of mucopolysaccharides in the cell coat of aggregating cells (Evans & Bergquist 1974*a*). Traces of such material are present here and there over the trabecular syncytium but the most conspicuous extracellular coating is the one that covers the microvilli and flagellum of collar units (see above, §4(*c*)). This material is generally considered to be a glycoprotein (Watanabe 1978; Garrone 1978), doubtless representing the exposed portions of integral membrane glycoprotein molecules.

A rare but interesting finding is the presence of what appears to be a mucus net spanning some of the trabecular lacunae (figure 62). The spaces in the net measure about 0.4 μm across. The net appears to be forming by expansion of a preformed assemblage of secretion globules, reminiscent of the house rudiment in *Oikopleura* (Fenaux 1971). It is not certain that the net is produced by the sponge itself, rather than by an endobiont. The former seems much more likely, since endobionts are extremely scarce, but we have no clue as to the sponge cells involved in secretion of the net. The infrequency with which we have observed these structures may

only reflect their delicacy. Mucus nets in ascidians tend to disintegrate during fixation and special techniques are required to reveal them (C. A. Pennachetti, University of Victoria, personal communication). No nets of the type under discussion have been reported in other sponges.

5. Discussion

(*a*) *Physiological implications of the present findings*

(i) *Feeding*

Fjerdingstad (1961) proposed that the choanocyte collar acts as a filter trapping particles with diameters greater than 0.2 μm, the width of the mesh formed of mucus strands bridging adjacent microvilli. By the same reasoning, *Rhabdocalyptus*, with mucus mesh apertures measuring 0.05 μm × 0.2 μm, should be able to remove bacteria in the 0.1–0.2 μm size range or larger. The upper size limit for material entering the sponge is presumably set by the pores in the dermal membrane, whose diameters rarely exceed 30 μm, as observed by interference microscopy in living tissue. A comparable value for the upper size limit in some tropical demosponges is 50 μm for ostial diameters (Reiswig 1971), but pores up to 1.0 mm are known. Bacteria are frequently observed lying free in the canal spaces adjacent to the collar bodies and trabecular strands and it seems likely that they constitute an important source of nutrition in *Rhabdocalyptus*. Some sponges can survive entirely on bacteria (Rasmont 1968; Reiswig 1975).

Material undergoing digestion in vacuoles (phagosomes) is seen frequently in collar bodies and sometimes in trabecular tissues. The material in the vacuoles often resembles bacteria, and can be assumed to consist of ingested bacteria. The existence of such phagosomes in trabecular tissue shows that this tissue must, to some extent, be self-nourishing. The uptake of particulate food by cells other than choanocytes was demonstrated in studies by Pourbaix (1932) some 50 years ago and is now thought to be of common occurrence (reviewed by Frost 1976). It is impossible to guage the extent to which the sponge may benefit nutritionally from absorption of dissolved organic materials, but the capability of sponges to absorb such materials directly from the sea water was demonstrated some time ago (Pavans de Ceccatty 1958; Stephens & Schinske 1961; Efremova 1965) and the process is known to be important in many other invertebrates (for references see Jorgensen 1976). Many species inhabit the 'spicule jungle' lying outside the dermal membrane in *Rhabdocalyptus* and it seems likely that particulate wastes, bacteria and organic solutes deriving from this community all enter the sponge, carried by the feeding current, and constitute a potentially important food source (Boyd 1981). Trapping of particles in the trabecular lacunae might be assisted by mucus nets (§4(*e*)(iii)).

From the frequency with which food vacuoles are seen in the choanosyncytium, and from the fact that the collar appears to be structurally adapted to particle extraction, it may be assumed that the choanosyncytium is the principal food-capturing tissue, and that it may therefore supply nutrients to other tissues. Direct transfer of phagosomes to other tissues (reported to occur in *Ephydatia* by Schmidt (1970)) would involve crossing plugged junctions, as the choansome makes no open syncytial connections with trabecular tissues nor with choanoblasts in the mature state. The existence of vesicles or cisternae lodged in junctional plugs may be significant in this context. These structures cannot be described as phagosomes, as they contain no formed particulate matter, but they might derive from phagosomes, and their contents might include nutrients obtained by phagocytic breakdown.

(ii) *Motility of archaeocytes*

In demosponges, archaeocytes are migratory and ingest food including symbiotic bacteria (Donadey 1979). Schmidt (1970) suggests that, in *Spongilla*, archaeocytes are the main digestive organ. In *Rhabdocalyptus*, however, the form of the cell is rounded, the cytoplasmic ground substance is uniformly dense and phagosomes are rarely seen. Where present, they could be autophagosomes. Thus, there is no evidence at present that hexactinellid archaeocytes are motile or capable of ingesting foreign objects. Even if capable of limited movement, it is hard to envisage them moving freely within the trabecular tissue, as so little extracellular matrix material is available for them to move in. The implications of this state of affairs are discussed below in relation to transport mechanisms.

(iii) *Impulse conduction and arrest of the feeding current*

Rhabdocalyptus is capable of conducting signals rapidly (0.2–0.3 cm s^{-1}) through its tissues. Conduction is an all or none propagative phenomenon, and the system serves to procure coordinated arrest of the feeding currents across the body wall of the sponge in all regions (Lawn *et al.* 1981). The present study clarifies certain points:

First, nerve-like and muscle-like cells are absent; therefore conduction is presumably a property of the general trabecular syncytium, the only tissue distributed in a continuous manner throughout the whole sponge.

Secondly, the syncytial character of this tissue makes it unnecessary to look for specialized low resistance pathways mediating communication between cells, such as gap junctions (nexuses) in metazoan conducting epithelia, myocardium etc. Gap junctions have not been observed in *Rhabdocalyptus*.

The nature of the propagative event in *Rhabdocalyptus* is still not definitely known, but if it is assumed to be a depolarization of the cell membrane, longitudinal current flow within the trabecular strands would be unimpeded by membrane barriers. Tortuous routes may be assumed, given the structure of the trabecular mesh and the irregular pattern of cytoplasmic bridge connections within the syncytial complex, which may mean that the actual conduction velocity through the tissues is higher than the values obtained for whole sponges or pieces of body wall. Passage of signals to the choanosyncytium would presumably take place across plugged bridges. The plug is not a membrane barrier and it contains pores (6–8 nm in diameter) which should allow ready movement of small ions acting as current carriers.

Thirdly, close study by electron microscopy of the edges of dermal membrane pores and of prosopyles has failed to reveal any microfilaments, either dispersed or in bundles. Closure of these large openings would be expected to require a substantial concentration of contractile filaments. Further, observation of the pores in living material over a period of several hours has failed to reveal any change in diameter of the pores, and membranes fixed during current arrest were found to contain open pores. It is thus very unlikely that current arrest is due to contraction of pores in this sponge. Current arrest is probably due to inhibition of flagellar beating in the choanosyncytium, as previously suggested (Mackie 1979) and as further discussed in paper II of this series (Mackie *et al.* 1983).

(*b*) *Unique features of hexactinellids*

It has long been recognized that hexactinellids stand somewhat apart from all other sponges, including the recently established Class Sclerospongiae, which resemble the Demospongiae in terms of tissue structure. One prominent specialist says, 'It is reasonable to question whether the Hexactinellida should remain in the same phylum as the other groups' (Bergquist 1978). The lack of information about the physiology and cellular organization of the group has so far precluded a proper evaluation of the relationships.

The present study has confirmed, or revealed, the existence of profound differences in cellular organization between hexactinellids and other sponges, the most important of which may be listed as follows.

hexactinellids	other sponges
no pinacocytes	pinacocytes present
extensive syncytialization	no syncytia
cells and tissues interconnected by cytoplasmic bridges containing plugs	no plugged bridges
archaeocytes show little evidence of motility or phagocytic ability	archaeocytes considered to be mobile and phagocytic
thin mesolamella probably limits cellular mobility and extracellular transport	thick mesohyl serves for cell migration and metabolite diffusion
collar-bearing tissue (choanosyncytium) in the form of numerous collar bodies arising from a ramifying stolon system formed as an outgrowth from one or more choanoblasts which later become isolated by plugged junctions; mature choanosyncytium is enucleate	collar bodies are parts of individual, nucleated cells (choanocytes)
thesocytes of distinctive form always present†	thesocytes, where present, unlike those of hexactinellids†
spicules secreted intracellularly in multinucleate giant cells	spicules secreted extracellularly (Calcarea) or intracellularly in mononucleate sclerocytes (Demospongiae)
no myocytes	myocytes frequently present
body wall rigid, no contractile pores dissociated tissues reaggregate to form a giant syncytial cell (Pavans de Ceccatty 1982)	body wall, ostia and osculum frequently contractile dissociated cells reaggregate as diploid cell clusters

† The distinctions between various 'thesocytes' are based primarily on structural appearance and until histochemical evidence is available some doubt must remain as to the validity of the apparent differences referred to here.

A complete list of the differences between hexactinellids and other sponges would be much longer and would cover the extensive differences in spiculation and gross anatomy, already well known, as well as the recently discovered physiological differences.

The desirability of elevating the hexactinellids to the subphylum level, as suggested by Reiswig (1979), is addressed in paper III of this series (Reiswig & Mackie 1983).

(*c*) *The general significance of the syncytial condition in animals and plants*

The present investigation has shown that, in a hexactinellid sponge, the entire trabecular tissue, including the dermal membranes, portions of the walls of the flagellated chambers and the structures tentatively equated with Reiswig's 'cords' are in all probability one continuous syncytium. Bearing in mind that individual specimens of *Rhabdocalyptus* grow to be over a metre

tall and probably contain many millions of diploid nuclei, these must be the largest syncytia known among existing animals.

It may be useful to consider briefly where else syncytia occur and what functional significance has been ascribed in the condition. The topic is a neglected one at the present time: the whole question of syncytia has attracted less and less interest as the century has advanced and we must look to older papers (e.g. Studnička 1934) for thoughtful reviews. Unfortunately, many of the examples of syncytia given in the older literature are now known to be erroneous. The electron microscope has shown many tissues once thought of as syncytial to be cellular.

Syncytial epithelia are known in a number of animals, for instance turbellarians (Bedini & Papi 1974; Ehlers & Ehlers 1980), rotifers (Clement 1980) and some parasitic helminths (Lee 1977; Lyons 1977; Miller & Dunagan 1976). No agreement has been reached on the significance of the condition. The gut of some (but not all) acoel turbellarians is syncytial (Kozloff 1972; Ivanov & Mamkaev 1977). Smith (1982) reviews this field and argues that the syncytial condition of the gut is secondary rather than being an indication of primitiveness. Its function may be to provide a 'cell' large enough to phagocytize large food objects, digestion in these creatures being entirely intracellular.

Syncytial connections occur in developing germ-line cells in animals at all phylogenetic levels from coelenterates to birds and mammals (Fawcett *et al.* 1959). It is generally assumed that the existence of cytoplasmic bridges between germ cells is a way of ensuring synchronous development. The same argument would apply to developing cnidoblasts in hydrozoans.

Although in some cases cytoplasmic bridges can apparently form by fusion of cells (see, for example, Andreucetti *et al.* 1978), the most common mode of formation is simply the failure of the cells to separate during mitotic division. The resulting cytoplasmic bridges often contain a bundle of microtubules representing the remnant of the mitotic spingle (the 'fusome' of Hirschler (1942)). Later, the microtubules may disassemble leaving an open bridge. In *Loligo* blastoderm the bridges remain open until a subsequent mitotic division when they are closed by a multilayered membraneous plug (Arnold 1974). While this process is reminiscent of the situation in *Rhabdocalyptus*, the structure of the plug is quite different. Nowhere, indeed, in animals have we found accounts of plugs of the hexactinellid type, with the possible exception of the interesting but enigmatic *Trichoplax adhaerens* (phylum Placozoa), where plug-like connecting structures have been described between the contractile 'fibre cells' (Grell & Benwitz 1974). These appear to be true intracellular plugs. Their describers suggest that they are the forerunners of synapses, but this seems unlikely, since synapses are specialized regions of contact between independent cells, not bridges between cells.

In many animals, the oocytes are connected to associated nutritive cells (nurse cells, follicle cells) by direct cytoplasmic connections (see, for example: Telfer 1975; Andreucetti *et al.* 1978) and probably receive materials from them via these bridges. Thus follicle cells may donate ribosomes to oocytes in the lizard ovary (Taddei 1972). Woodruff & Telfer (1974) suggest that transport from follicle cells to ovum occurs along an inward electrical gradient by a process analogous to electrophoresis. The advantages of syncytial connections in these cases are reasonably clear. They permit direct, cell to cell transport of relatively large intracellular organelles, nutritive and regulatory substances, etc. The whole interconnected system corresponds to the 'symplast' of Münch (1930) or the 'symplasm' of Studnička (1934).

Excitable tissues of animals are sometimes syncytial, e.g. conducting epithelia in the siphonophore *Hippopodius* (Mackie 1965), striated muscle in vertebrates and giant neurons in squid

(Young 1939). The functional significance is clear in the last case: pooling of the cytoplasm of several diploid neurons gives a polyploid unit with the large cross-sectional area required for low internal electrical resistance and rapid conduction velocity (Hodgkin 1954).

It will be clear from this brief review that the syncytial condition probably evolved repeatedly in animals and for a variety of reasons, and is not in itself an indication of primitiveness. The most common function is one of enhancing intercellular transport. The same function is served by gap junctions in cellular systems, and these junctions also allow electrical current to flow between cells. Thus the distinction between a syncytium and an epithelium whose cells communicate via gap junctions is really only one of degree: the gap junction particle has a central channel *ca.* 1.5 nm in diameter and molecules larger than about relative molecular mass 1200–1900 cannot pass (Lowenstein 1977). Syncytial connections would allow transport of larger molecules and organelles.

The situation in plants is somewhat different. Plants lack gap junctions and their cells are generally linked by protoplasmic connecting threads, together constituting 'something approaching a true syncytial structure' (Robards 1976). Transport is largely symplastic in plants; cells remote from sources of nutrient can be nourished by flow of materials via the intercellular connections. Extracellular ('apoplastic') routes also exist, but not to the same extent as in animals where blood, lymph etc. provide the major transport media. 'In short, the major long distance pathways of solute transport in the animal body are apoplastic, whereas plants have generated a symplast, and used it to cope with problems of long distance transport as well as of short distance' (Gunning 1976).

Direct cell to cell connections occur at all levels of the plant kingdom. In fungi they are termed perforate septa, pores 0.05–0.5 μm in diameter (in ascomycetes), which mediate protoplasmic streaming and even migration of nuclei (Bracker 1967). The pores can be plugged by Woronin bodies, which are electron dense membrane bounded spheres located near the junction. This occurs following injury in a manner reminiscent of the closing off of gap junctions in injured animal epithelia. In basidiomycetes the pores may be capped by thickened membranes derived from the endoplasmic reticulum.

The pit connections of red algae are of special interest in the present context. Like animal fusomes they are primary connections, and the plasmalemma remains continuous between the connected cells, as clearly shown in electron micrographs by Aghajanian & Hommersand (1978). However, the bridge becomes plugged with dense material and associated membranes. The plug consists of protein, bounded in some cases by a capping layer containing mucopolysaccharide (Pueschel 1980*a*). The resemblances between these structures and fungal septal pores have prompted suggestions that the red algae and the fungi are phylogenetically linked, but Pueschel (1977) shows that these resemblances are superficial. Wetherbee (1980*a*, *b*) distinguishes a special type of pit connection which he terms a 'transfer connection'. These occur in situations where there is circumstantial evidence of large scale transport of materials across the junction. According to Wetherbee, there is no membrane partition in the transfer connection. The reported absence of a membrane barrier requires confirmation by freeze–etch study and there had been no experimental demonstration of translocation across these junctions. While it may be premature to claim a special transfer function for them (Pueschel 1980*b*; Pueschel & Cole 1982), if the interpretation of Wetherbee is correct we have here a remarkably close counterpart to the plugged junctions of hexactinellids.

In the green alga *Volvox* the cells are connected by unplugged, open cytoplasmic bridges.

It has been suggested that these may be the routes whereby signalling events responsible for coordination of flagellar activity spread from cell to cell (Dolzmann & Dolzmann 1964). If so, the arrangement would present an obvious analogy with what is here proposed for *Rhabdocalyptus*.

In higher plants direct cell to cell connections exist in the form of plasmodesmata, which are specialized and often complex structures mediating intercellular symplastic transport. Though not plugged in the usual sense, these connections often contain desmotubules, which are best regarded as structures continuous with the endoplasmic reticulum and connecting the endoplasmic reticulum cisternae of the connected cells (Robards 1976). Such structures recall the endoplasmic reticulum cisternae seen lodged in plugged bridges in *Rhabdocalyptus*.

A final point concerns the potentiality offered by direct cytoplasmic connections in plants for transmission of electrical signals. In excitable plants such as *Mimosa* or *Dionaea* impulse conduction is assumed to occur by local circuit flow of action currents from cell to cell via the plasmodesmata (Sibaoka 1966; Spanswick & Costerton 1967). Thus the intercellular bridges in these cases presumably fulfil the same role as postulated here for hexactinellids.

(*d*) *Significance of the syncytial condition and of plugged cell bridges in hexactinellids*

The unique histological organization of hexactinellids calls for some attempt at an explanation. Briefly, we would propose that the syncytial condition is significant as a means of facilitating transport of nutrients and regulatory metabolites within the sponge. Extracellular transport routes are probably much more limited in hexactinellids than in other sponges: instead of a spacious mesohyl, they have a thin densely collagenous mesolamella. Transport by migratory cells is probably limited for the same reason. The archaeocytes show no signs of motility.

Having achieved an essentially symplastic transport system, the sponge immediately confronts another problem; the open syncytial connections, while allowing nutrients to move, would also permit widespread dispersion of informational molecules and enzymes, even of fairly large cytoplasmic organelles. It is difficult to see how specialized cytoplasmic domains can arise and be maintained in such an open system. Here we suggest that the junctional plug was a crucial development: we envisage that it is a filtering device that would allow certain molecules to pass but would restrict the passage of most larger molecules and organelles. The 6.0–8.0 nm pore in the pore particles might be expected to limit translocation to molecules of relative molecular mass up to about 15000. If the membrane-bounded saccules seen embedded in some plugs are indeed in the process of migration across the barrier, they would provide an alternative route for transport, as desmotubules are thought to do in plant plasmodesmata. Possibly food vacuoles become migratory after breakdown of their contents and move across plugged junctions to other cytoplasmic domains. In sum, hexactinellids have evolved in a rather similar way to plants, and their junctions are more plant-like than animal-like.

In most metazoans, transport from cell to cell in epithelia involves gap junctions, which are arrays of membrane particles (connexons) each of which is a complex of specific protein (connexin) subunits arranged to form a cylinder, whose central space is *ca.* 1.5 nm in diameter (Unwin & Zampighi 1980). Only small molecules (relative molecular mass less than 2000) can pass. The channel can be opened or closed by ionic regulation of the local cytoplasmic environment. No more striking case of specialized membrane substructure exists. It is quite conceivable that the invention of the connexon was a key factor in metazoan evolution and

that the absence of a comparable membrane particle in plants and sponges was a limiting factor in their histological evolution. Both plants and hexactinellid sponges have evolved restrictive intercellular junctions of other (seemingly cruder) kinds. Non-hexactinellid sponges not only appear to lack gap junctions but may possess no alternative intercellular bridging structures. Their capacity for cell to cell translocation must be very limited. Communication via surface reactions may be more important, and nutrient transport is presumably largely apoplastic.

We have suggested an explanation for the histological peculiarities of hexactinellids primarily in relation to metabolite transfer and homeostasis, but this is not to play down the importance of another advantage of the syncytial condition (which again is shared with systems interconnected by gap junctions): the provision of pathways for intercellular ion flow and hence for propagation of electrical impulses. Low impedence pathways of some sort are essential for impulse transmission in all excitable non-nervous tissues (Anderson 1980). The syncytial bridges connecting the cells in plants and hexactinellids may have evolved for metabolic functions but, where the cells concerned have excitable membranes, the bridges would automatically allow direct flow of action currents and impulse propagation from cell to cell. Only the hexactinellids among known sponges appear capable of the rapid conduction of behaviourally meaningful signals, and these are probably electrical impulses. The trabecular syncytium would appear to be the primary conducting tissue and most of the connections in this system are open, unplugged bridges. The existence of plugs between trabecular and choanosome components would not be expected to block conduction, as pores exist in the plugs. The apparent inability of sponges of other groups to conduct impulses is doubtless associated with the fact that, as they lack gap junctions or equivalent structures, the requisite degree of electrical coupling is usually unobtainable. There is one report of coupling between aggregating sponge cells (Loewenstein 1967) but the experiments were done under rather artificial conditions and the coupling reported could be attributed to several possible causes, not all of which would be expected to apply in the normal intact tissue. An electron microscope study of aggregating cells shows no specialized junctional structure at the membrane level (Evans & Bergquist 1974*b*). While new electrophysiological studies are clearly required, it seems best to assume at the present time that sponge cells are generally coupled poorly or not at all. The hexactinellid solution (syncytialization) may have been the only available way of achieving a through-conducting tissue.

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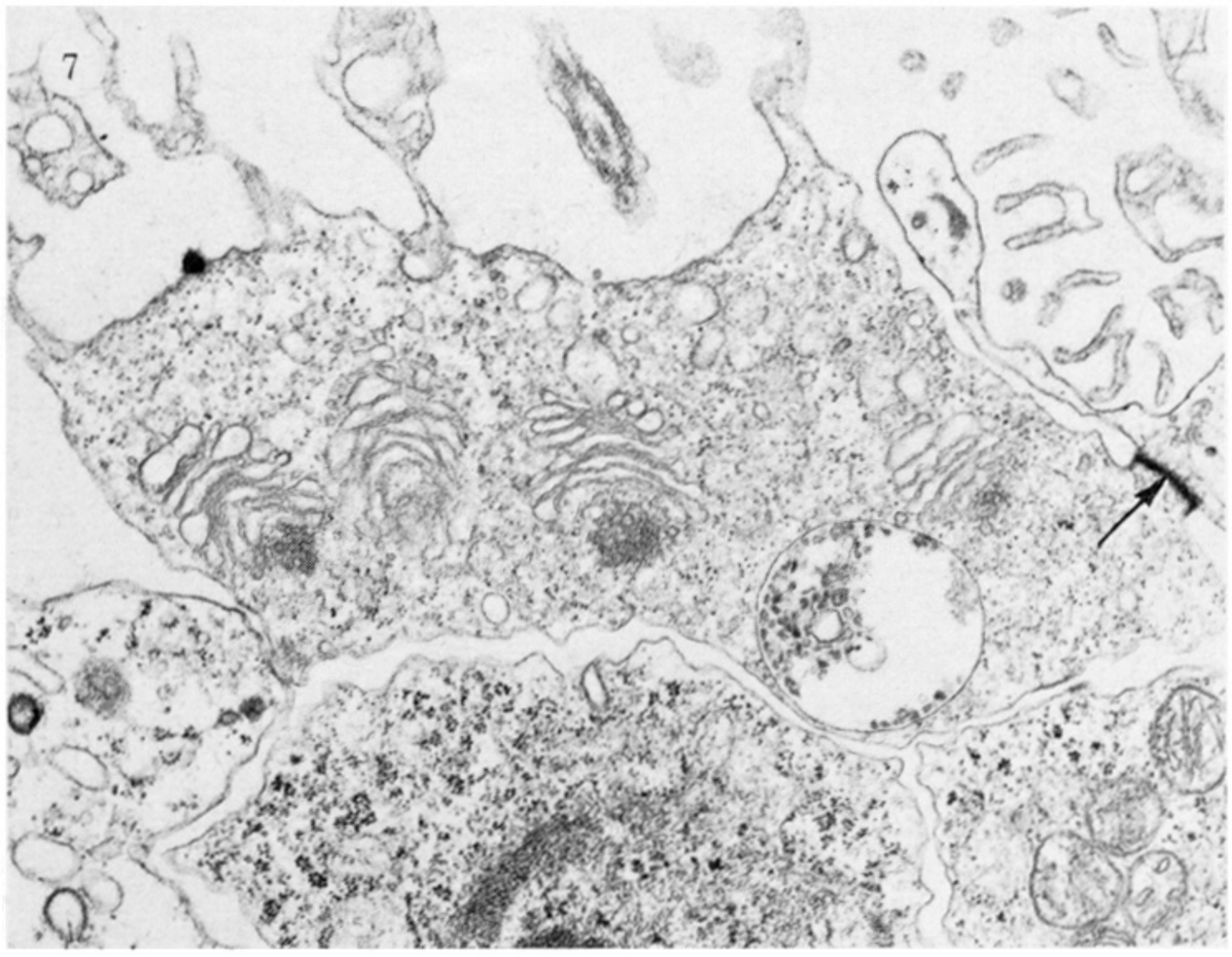
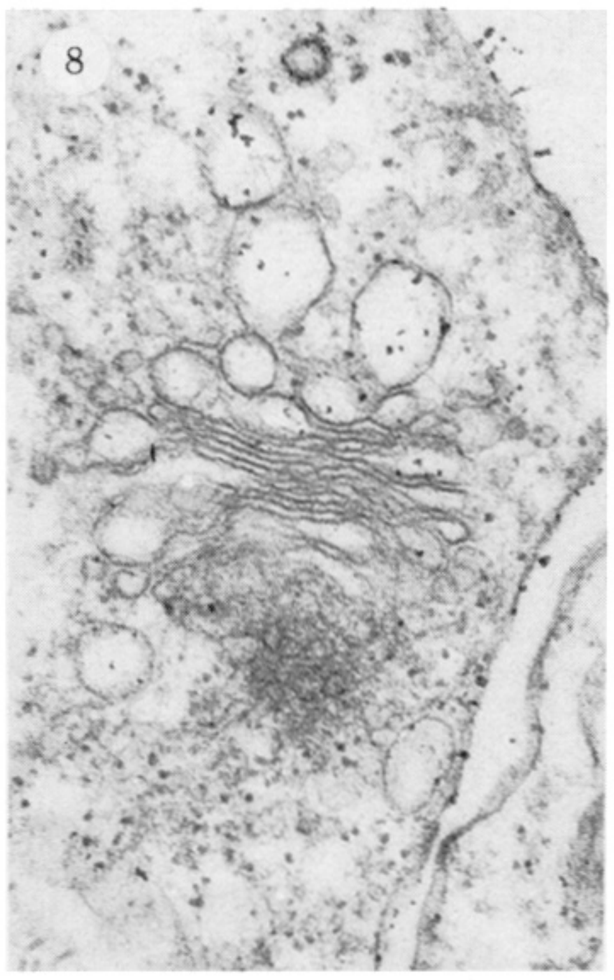
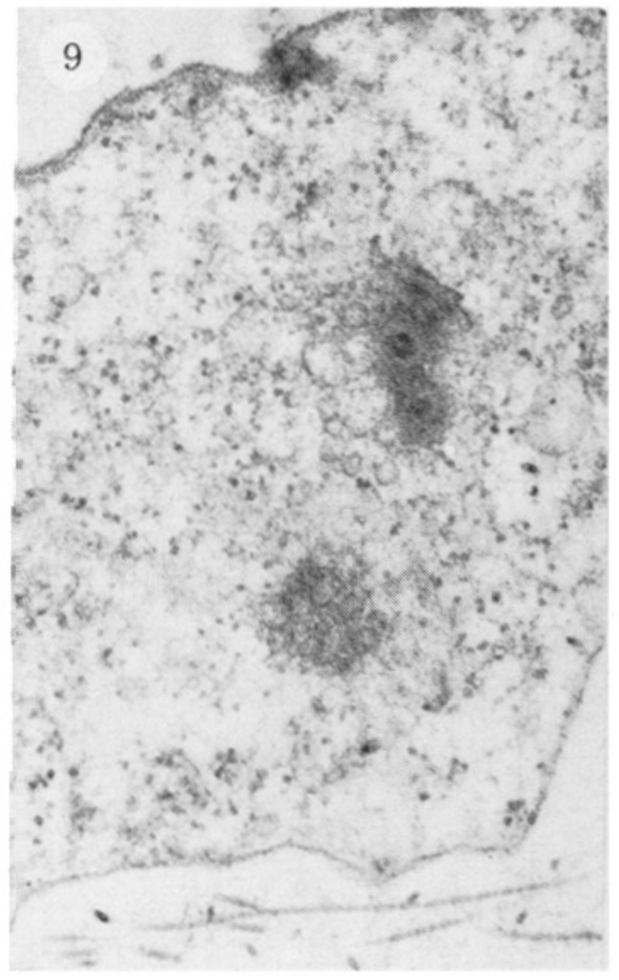
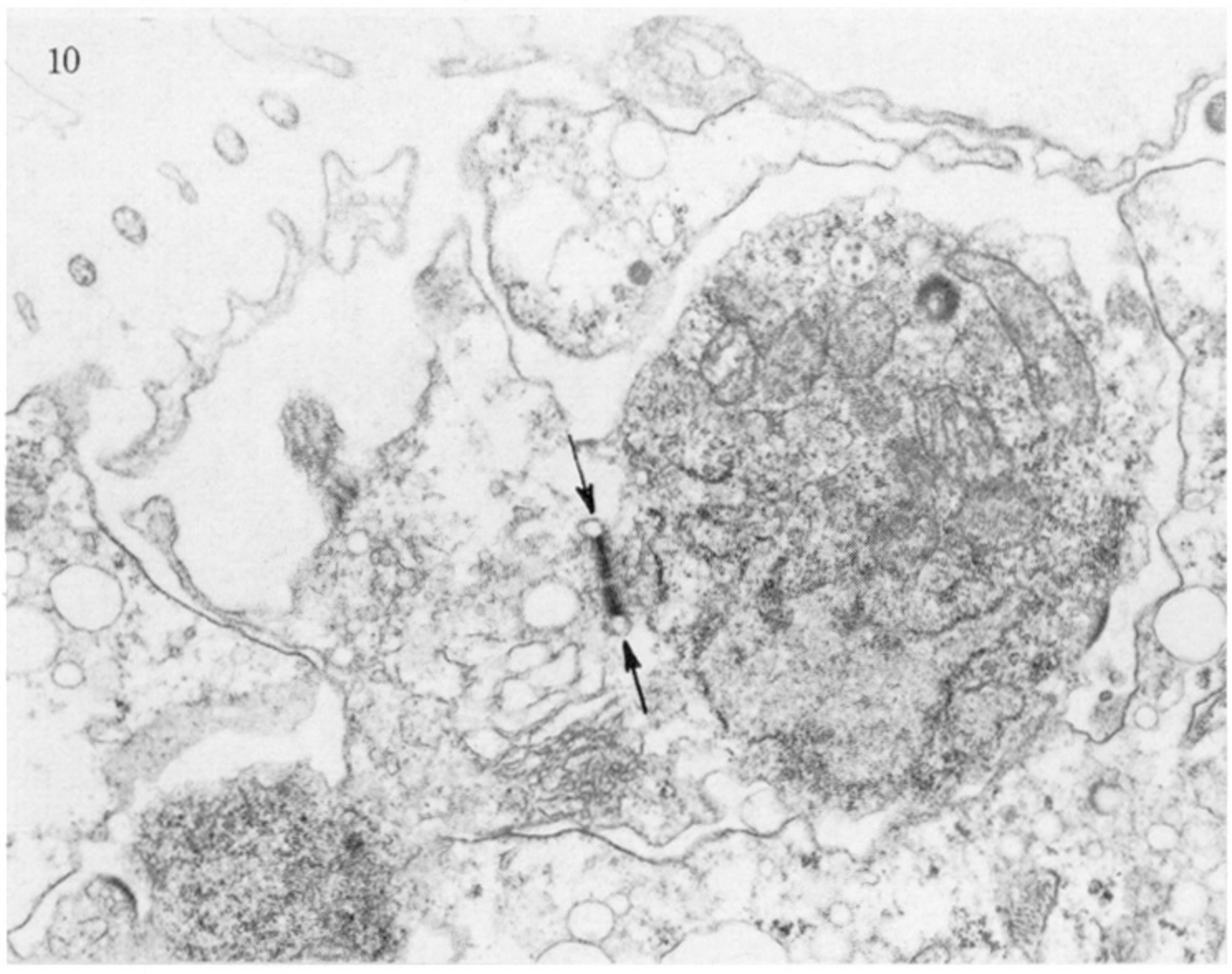
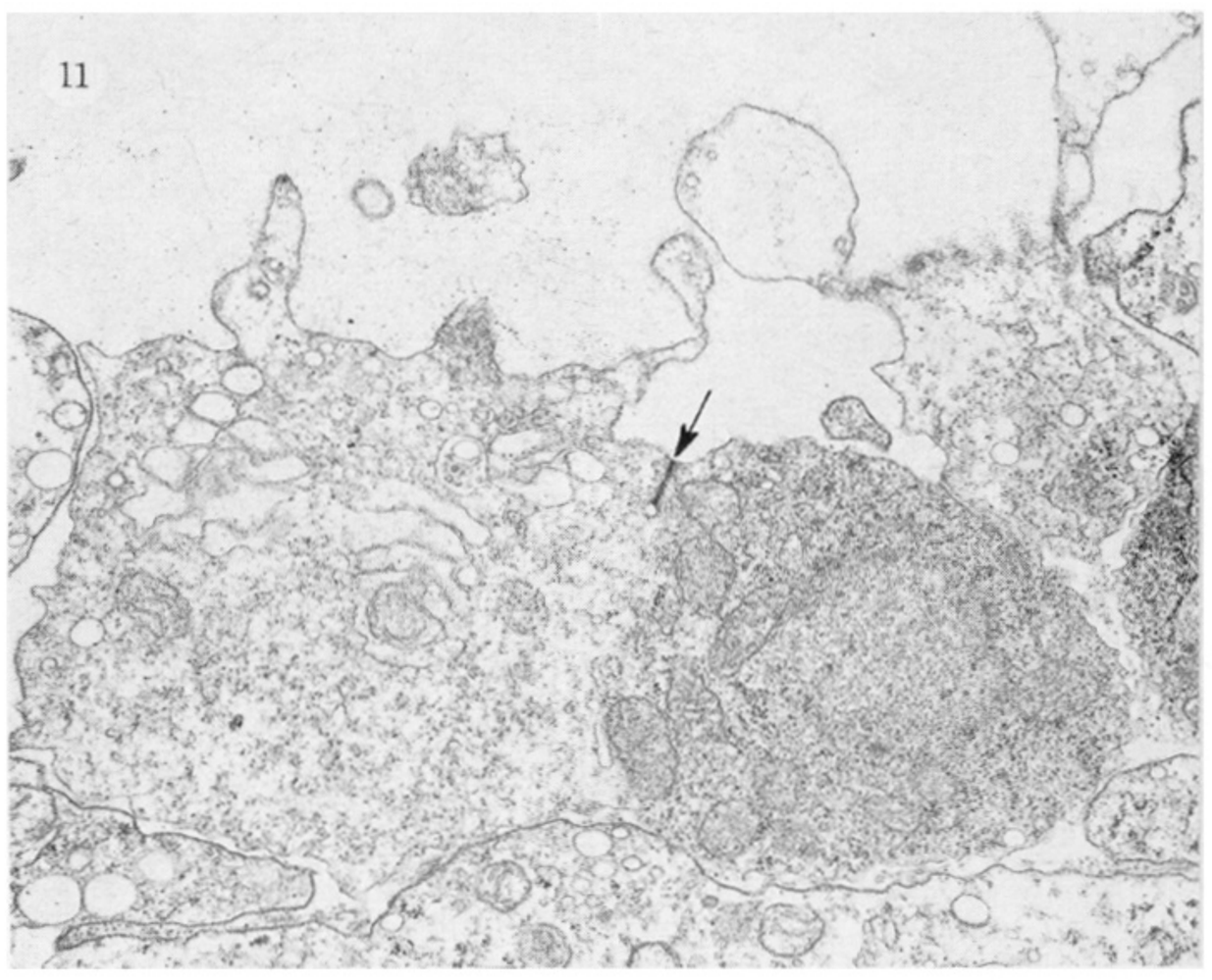
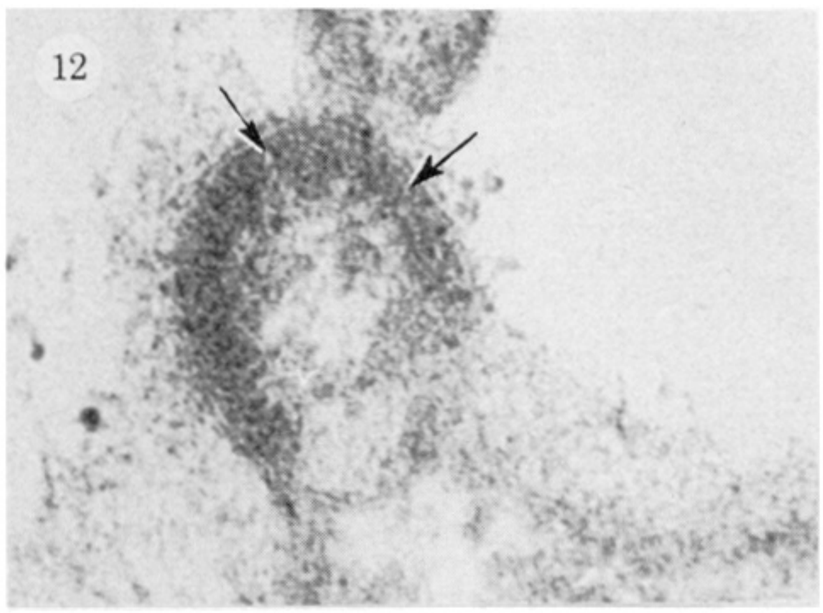


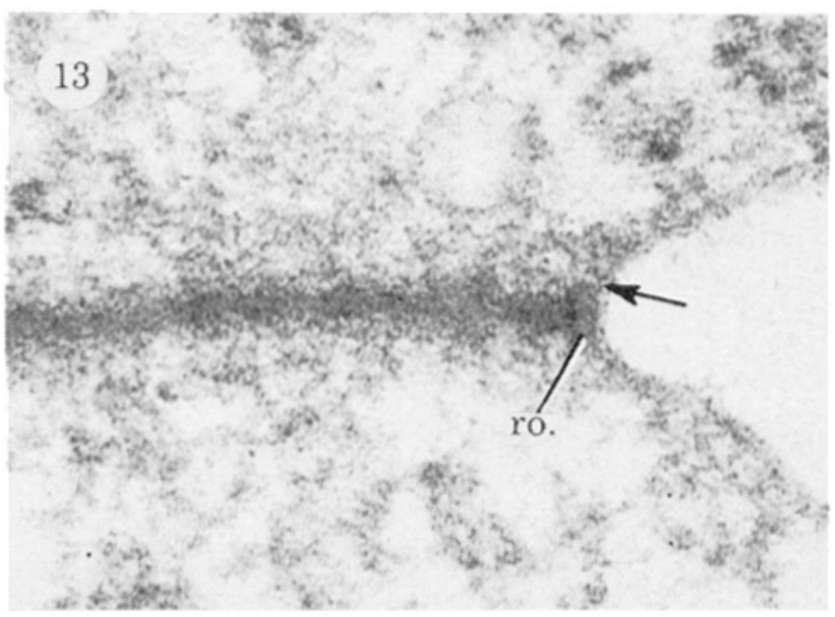


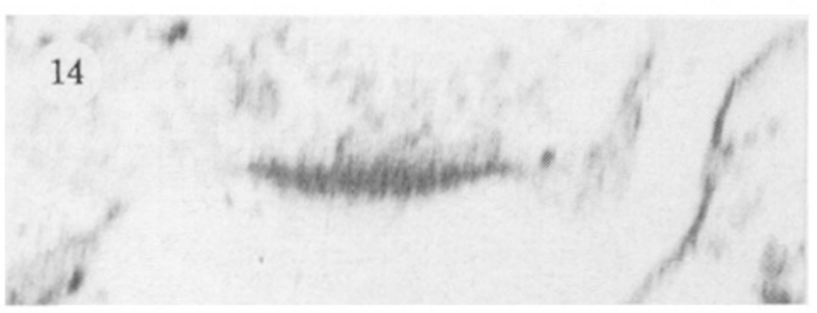


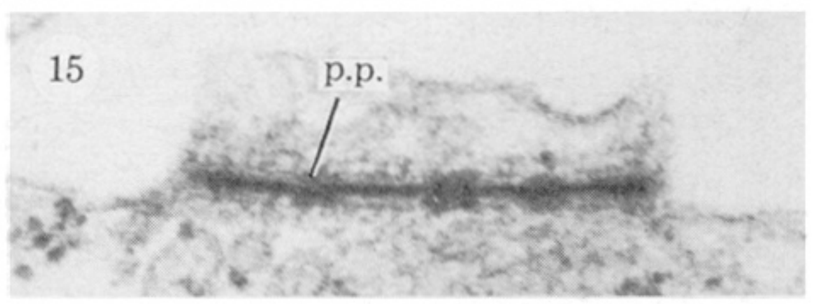


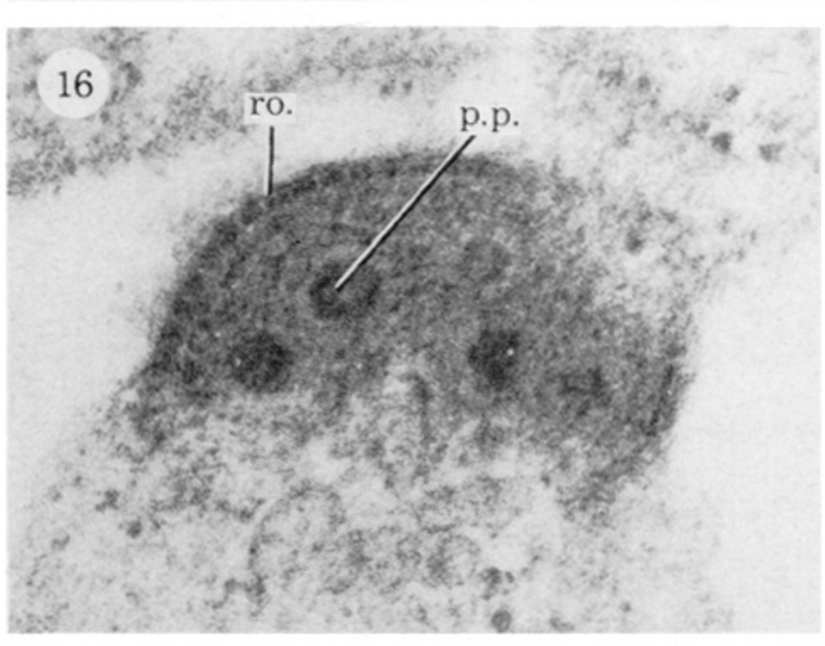


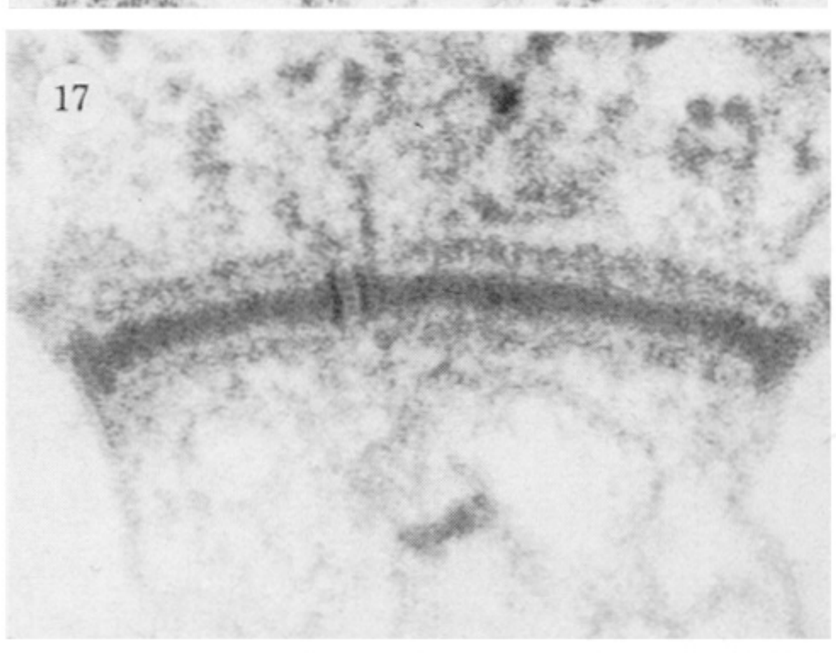


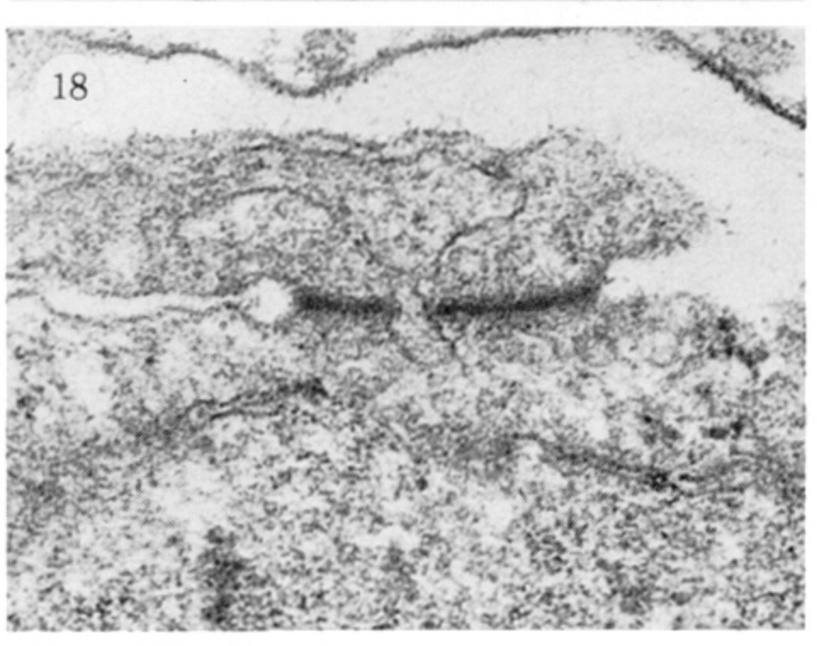




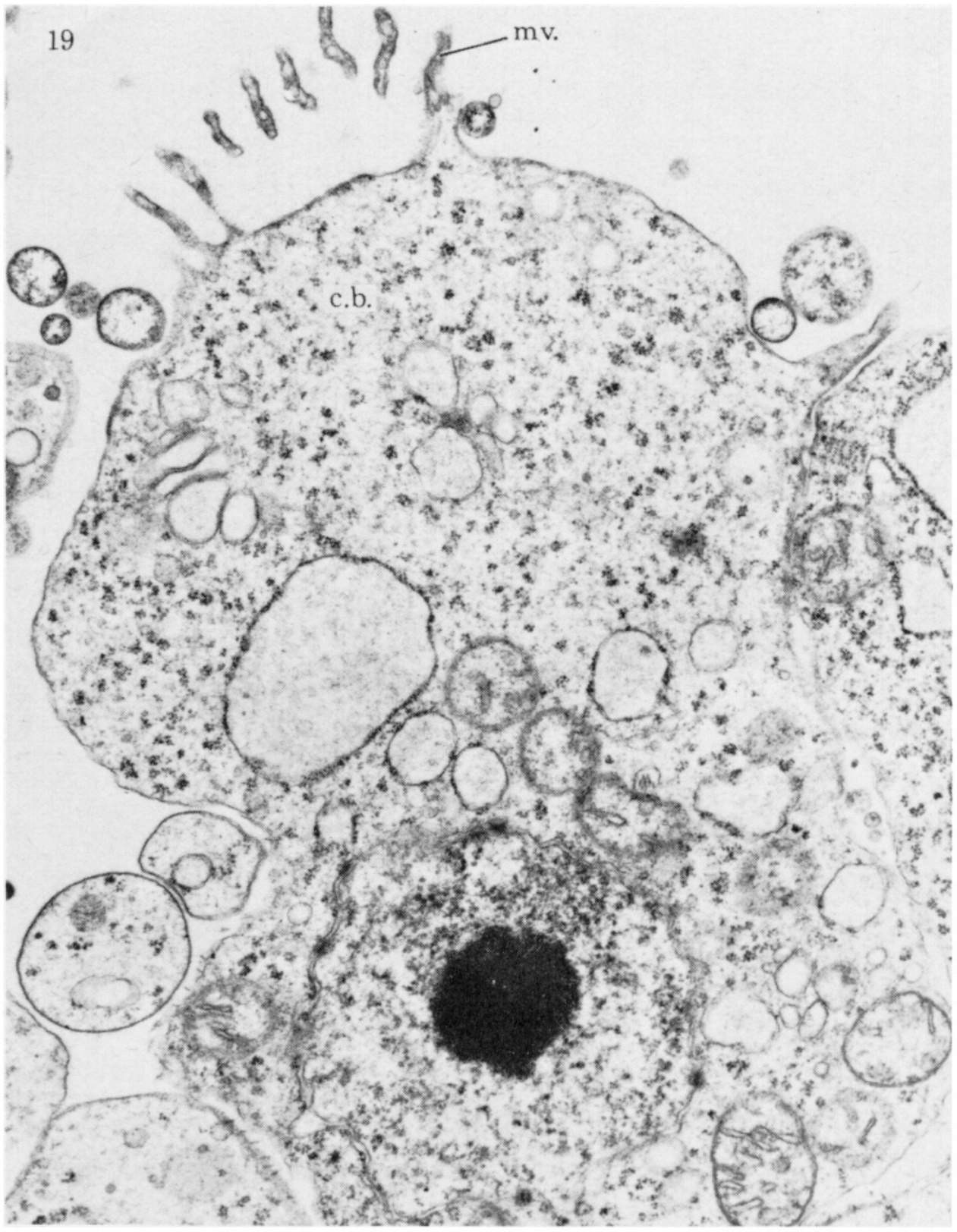


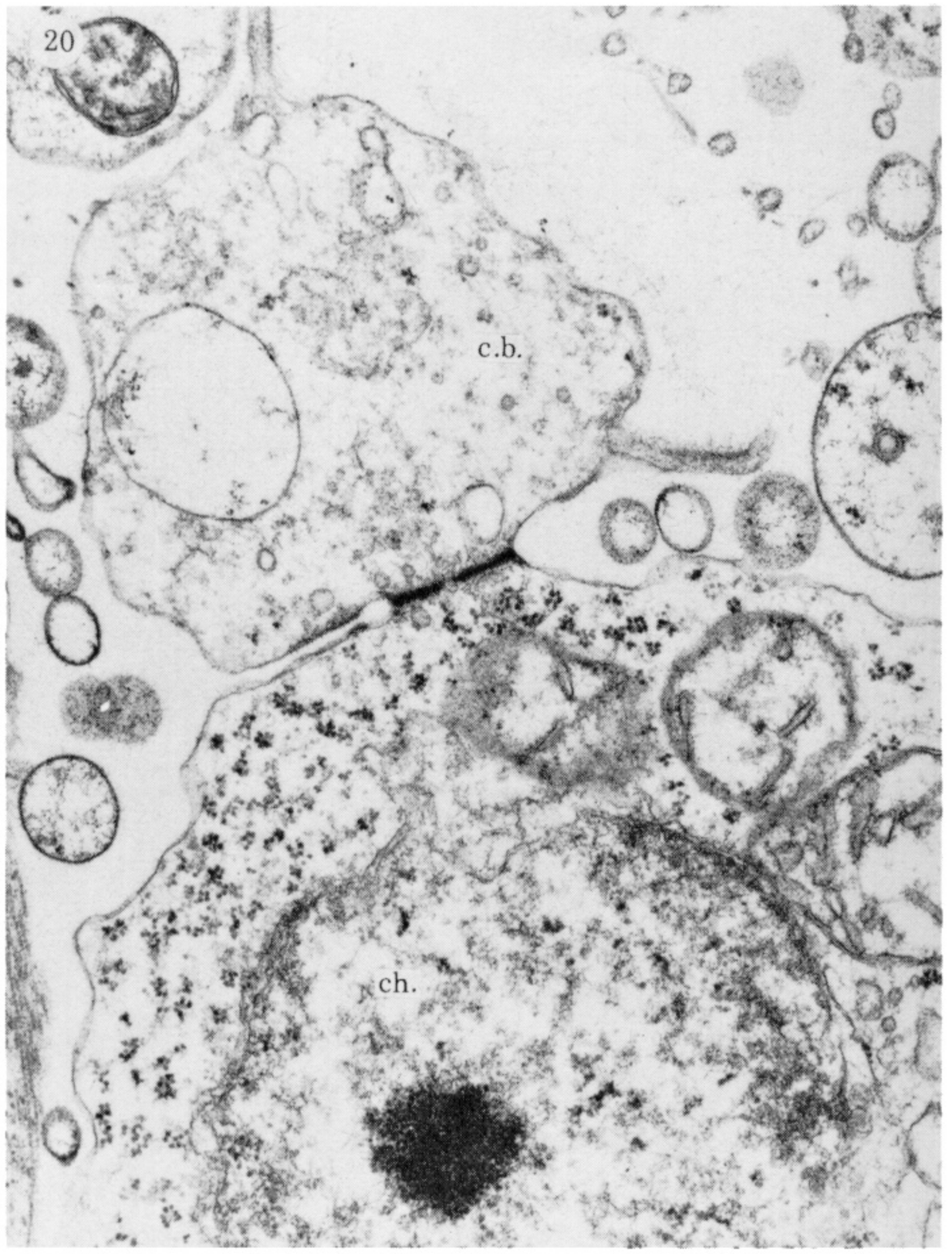


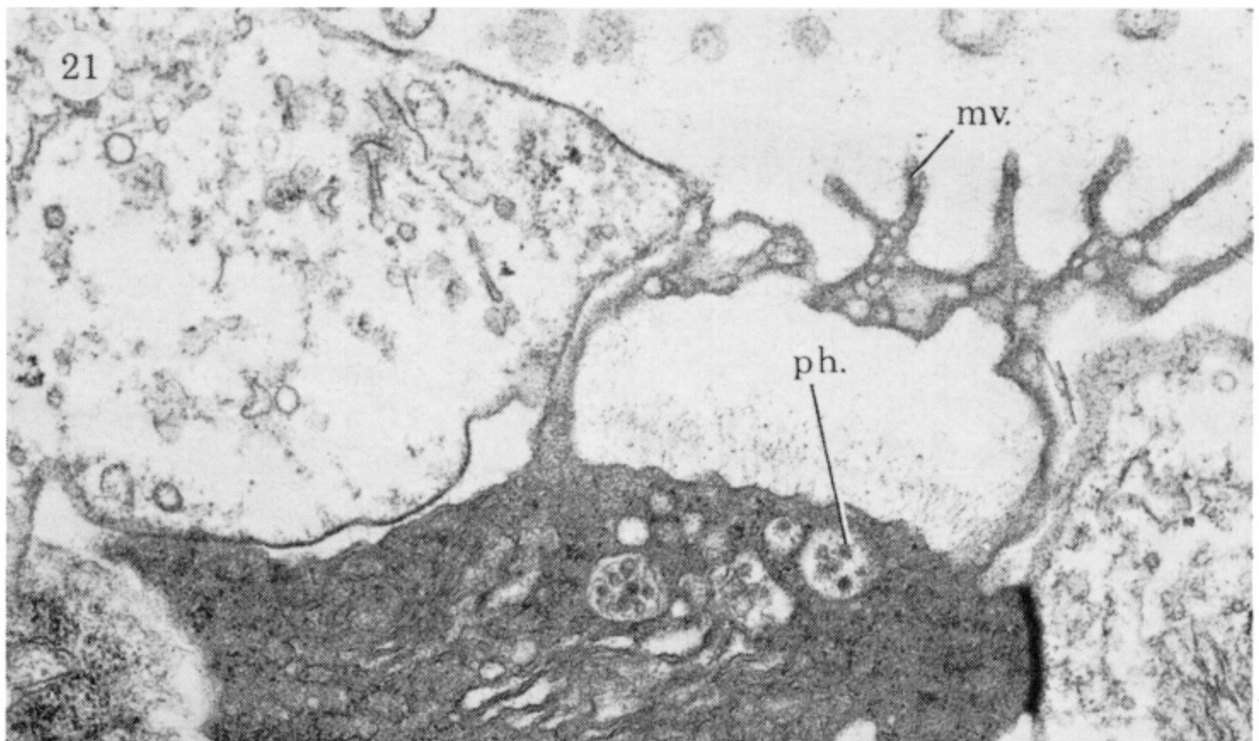


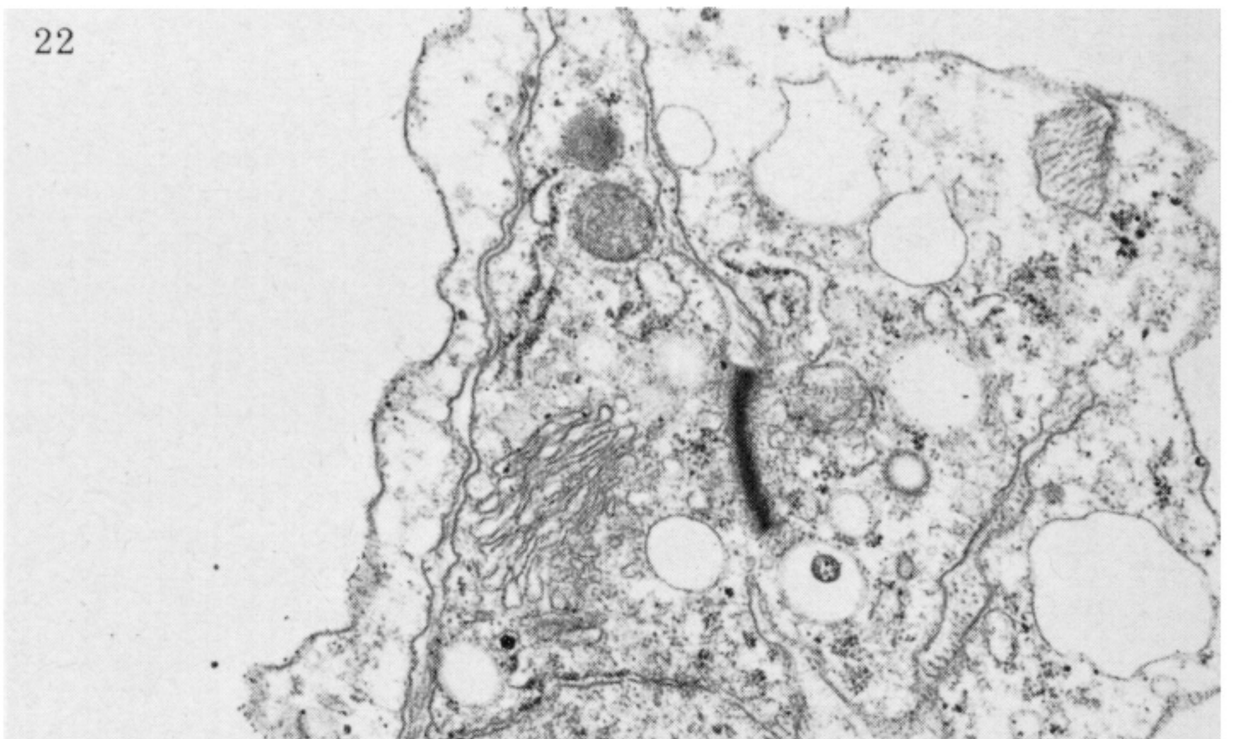


FIGURES 7–18. For description see opposite.



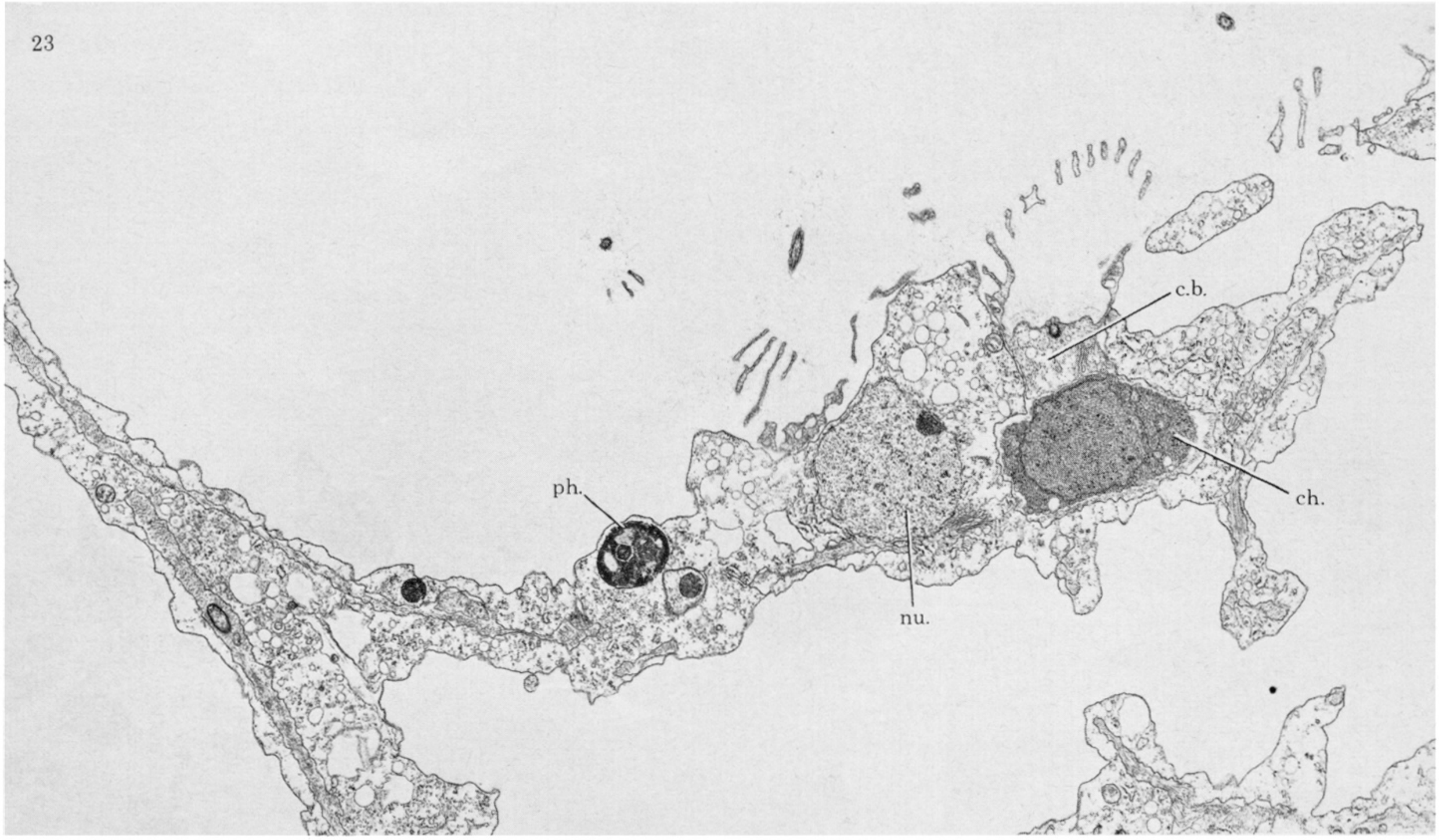






FIGURES 19–22. For description see p. 376.

23



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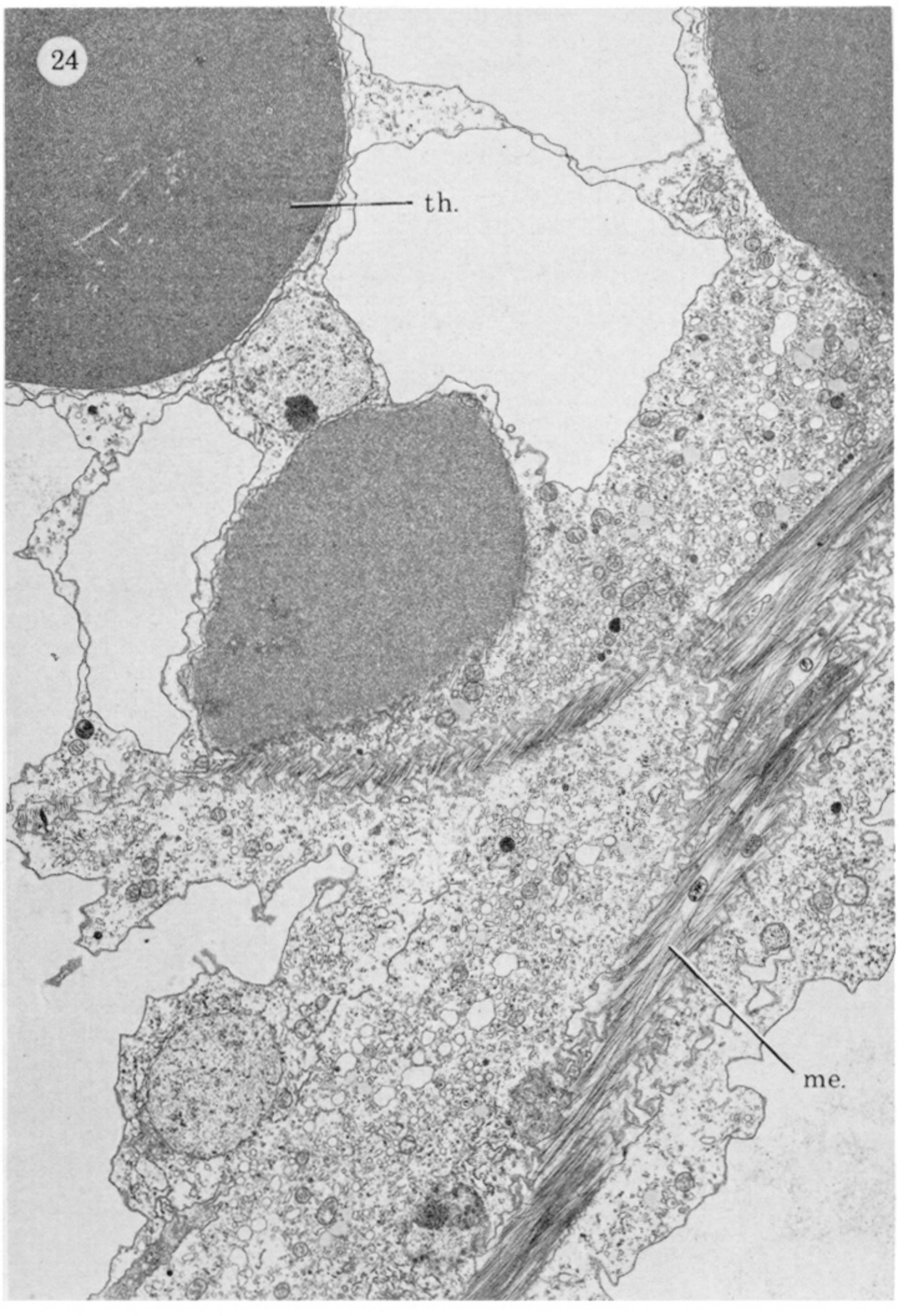

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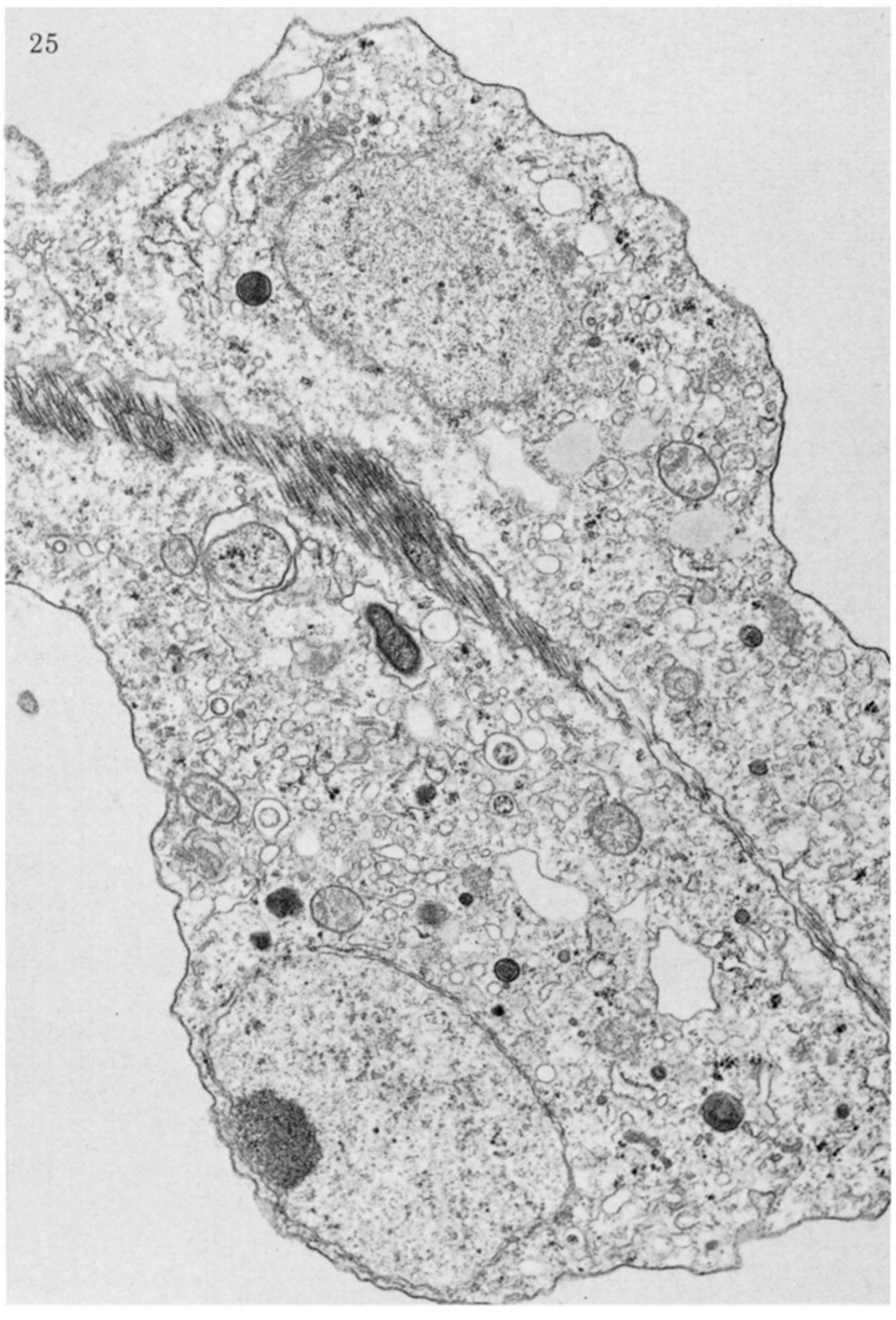
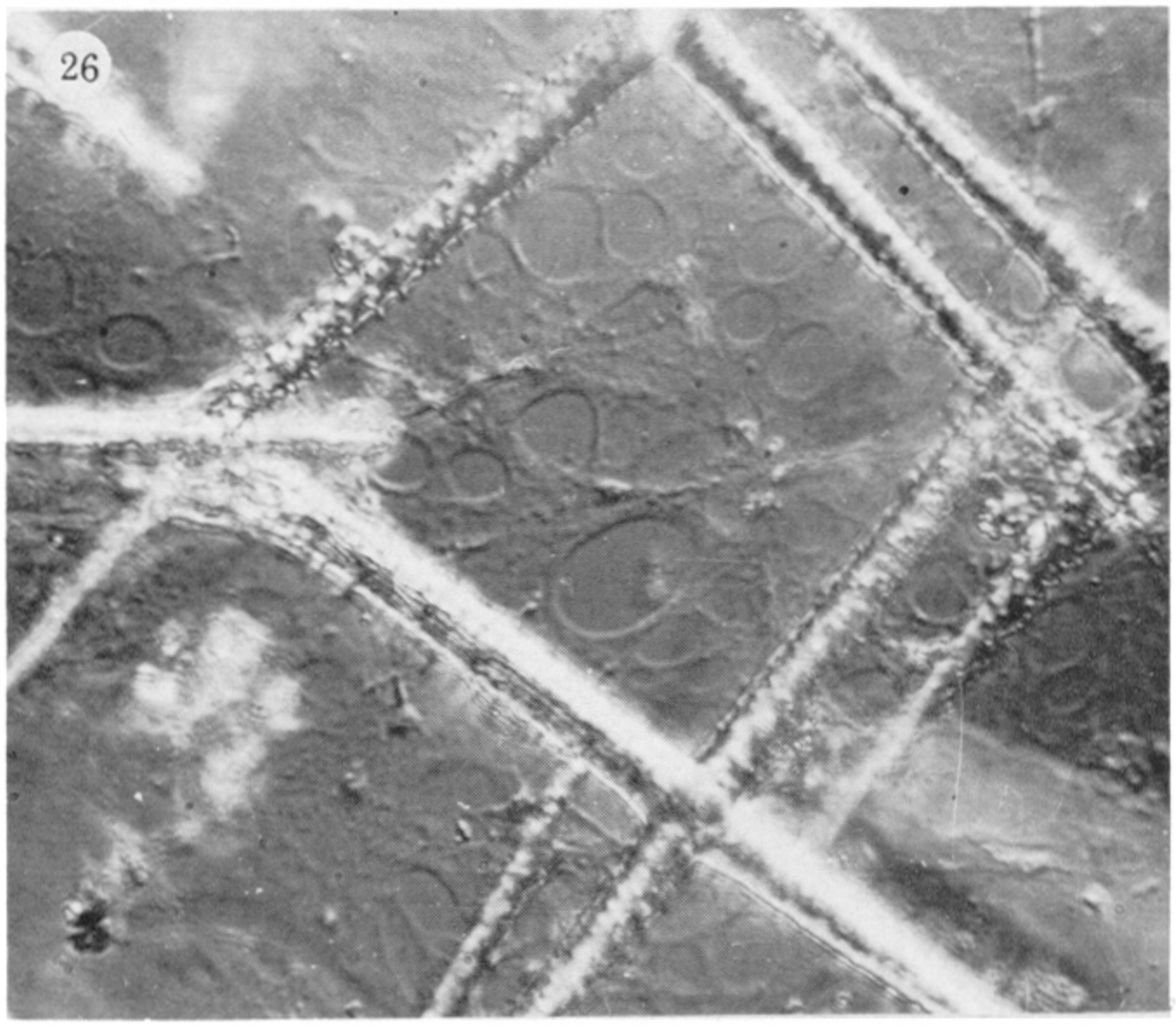
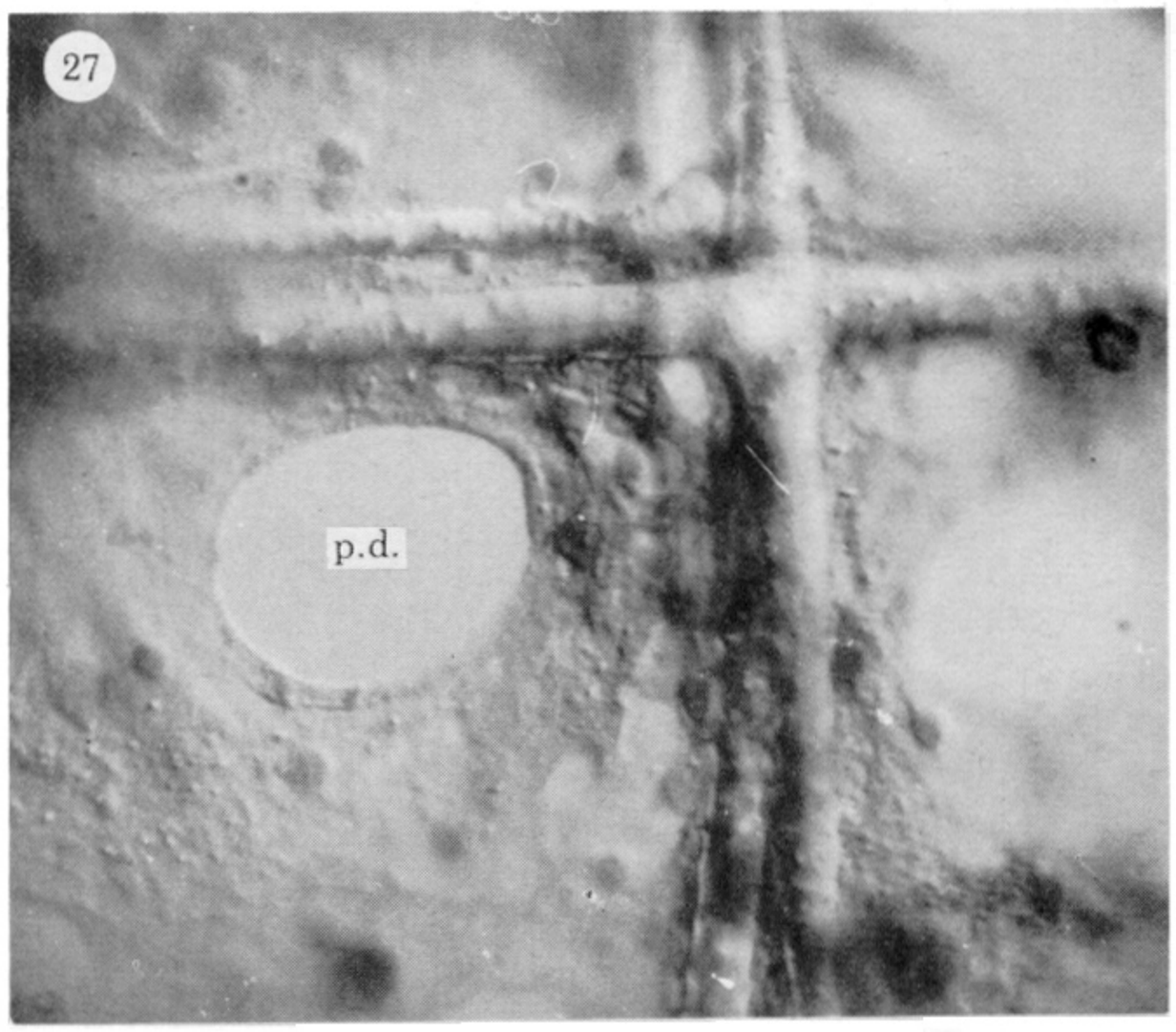
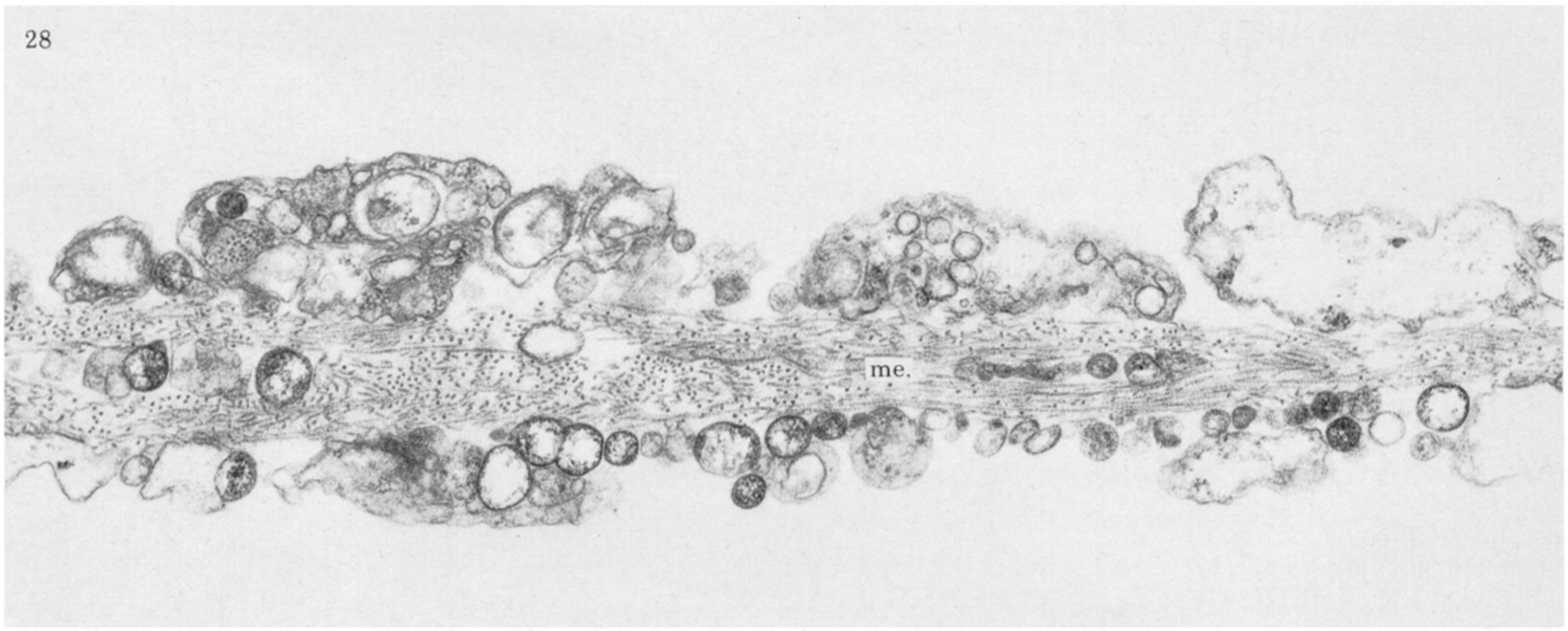
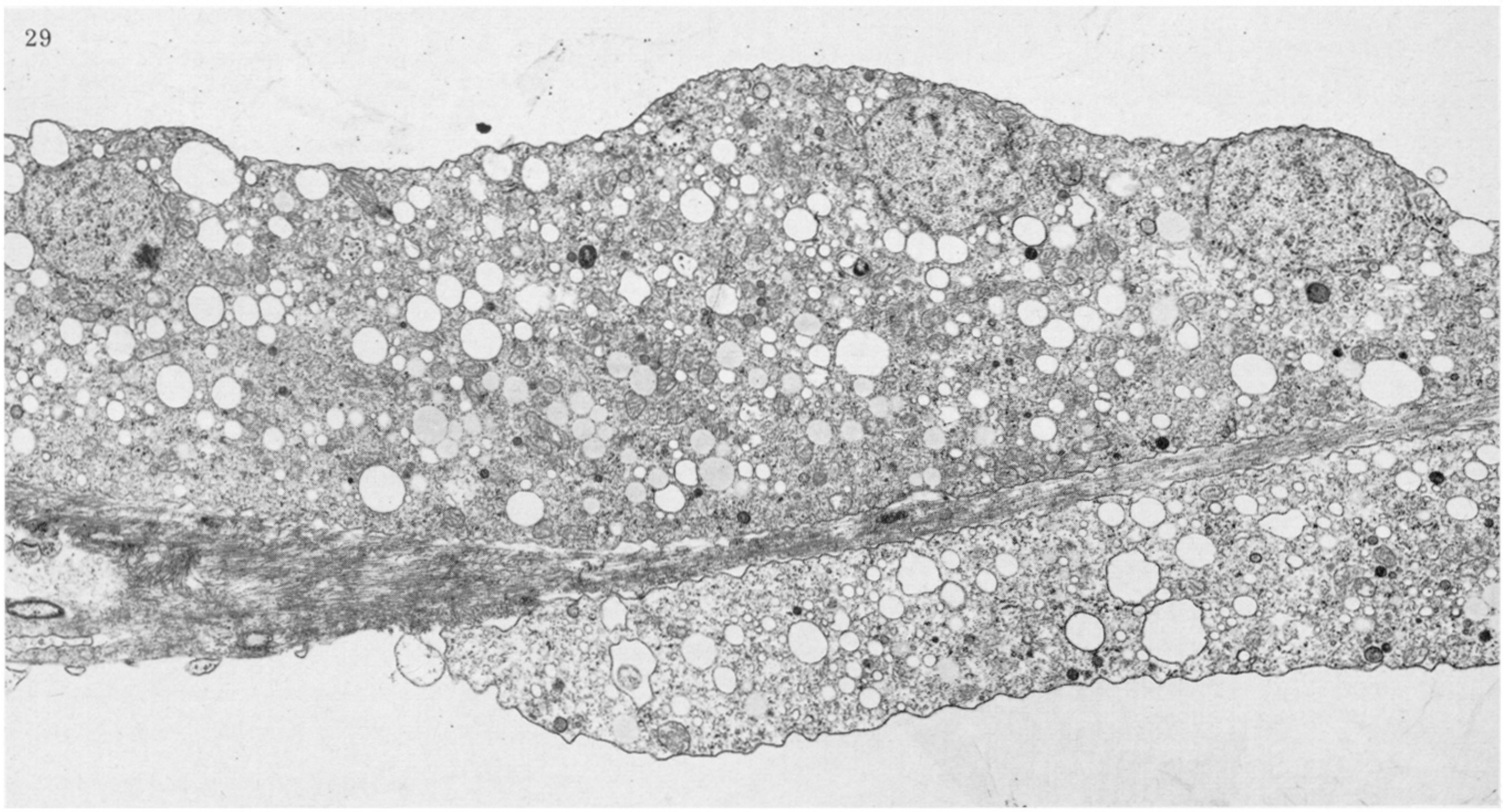


FIGURES 23–25. For description see p. 377.



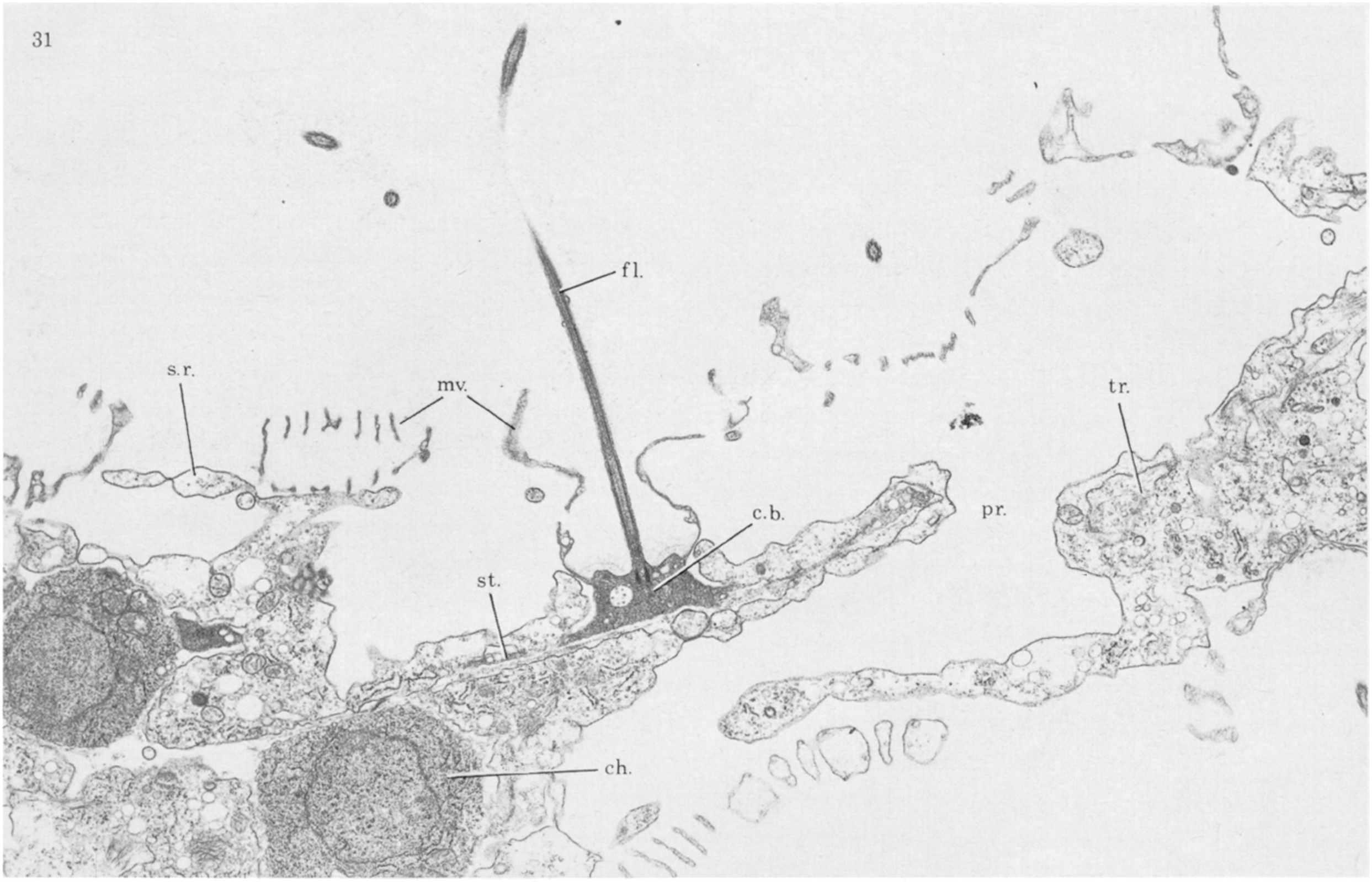






FIGURES 26–29. For description see opposite.

31



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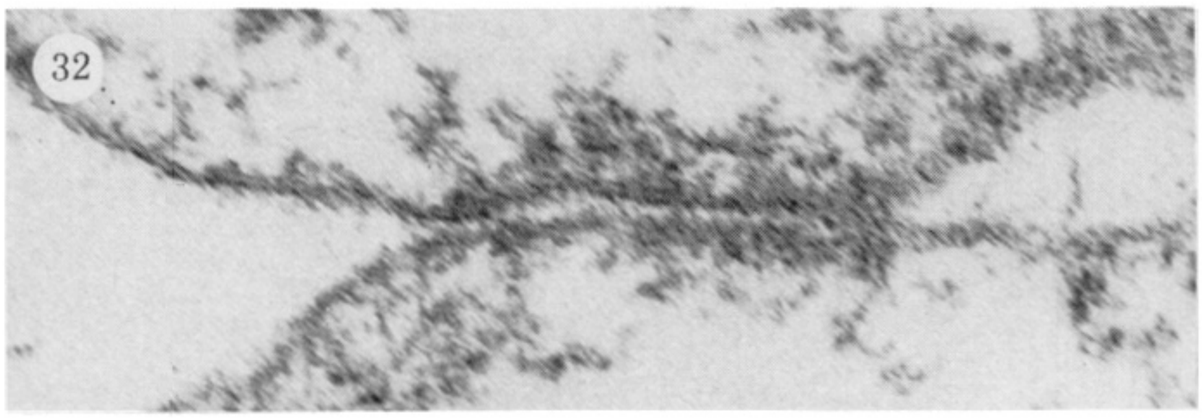

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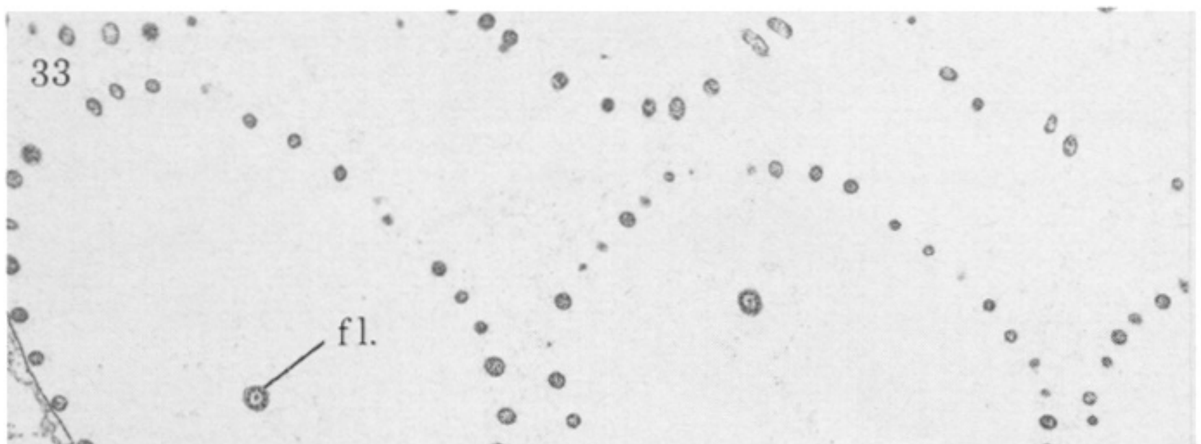

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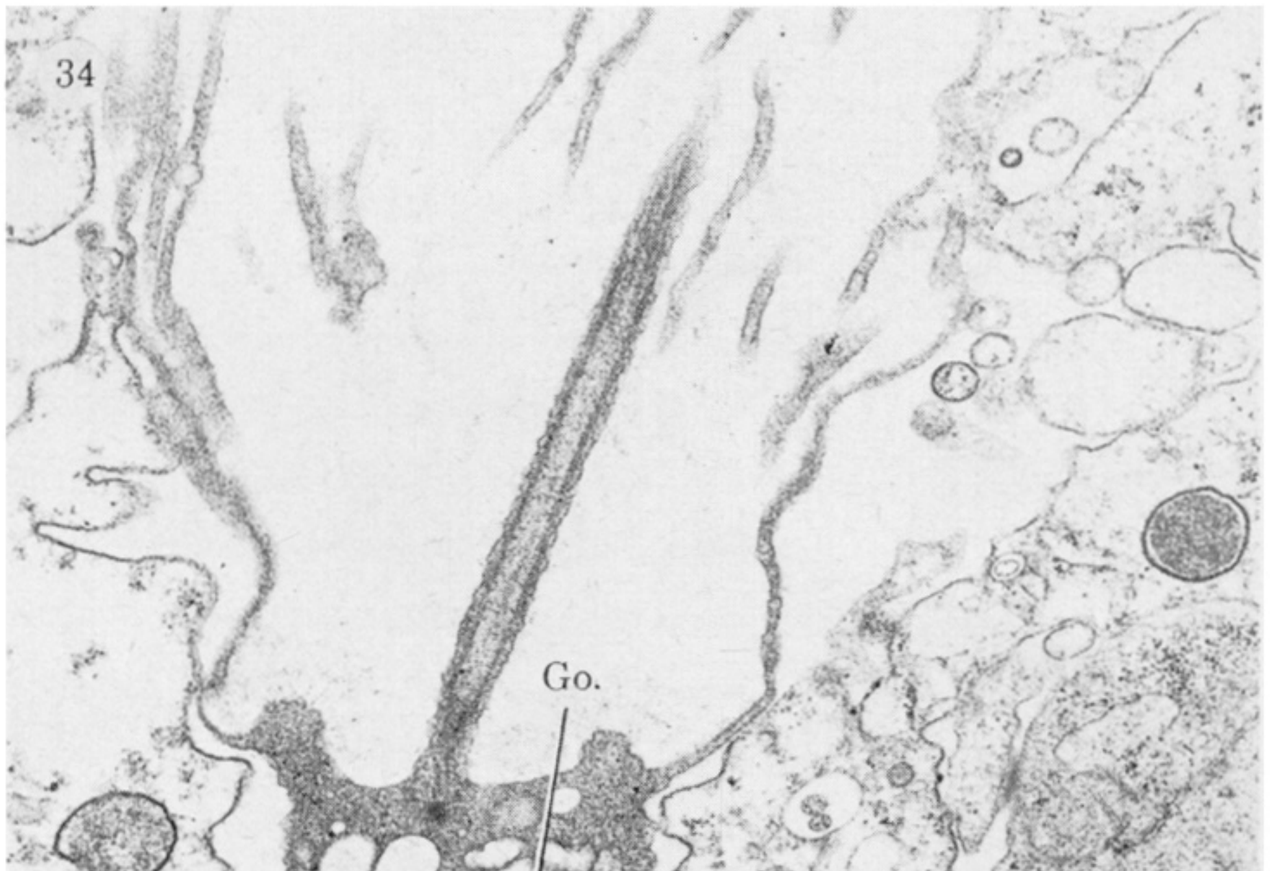

FIGURES 31–34. For description see opposite.

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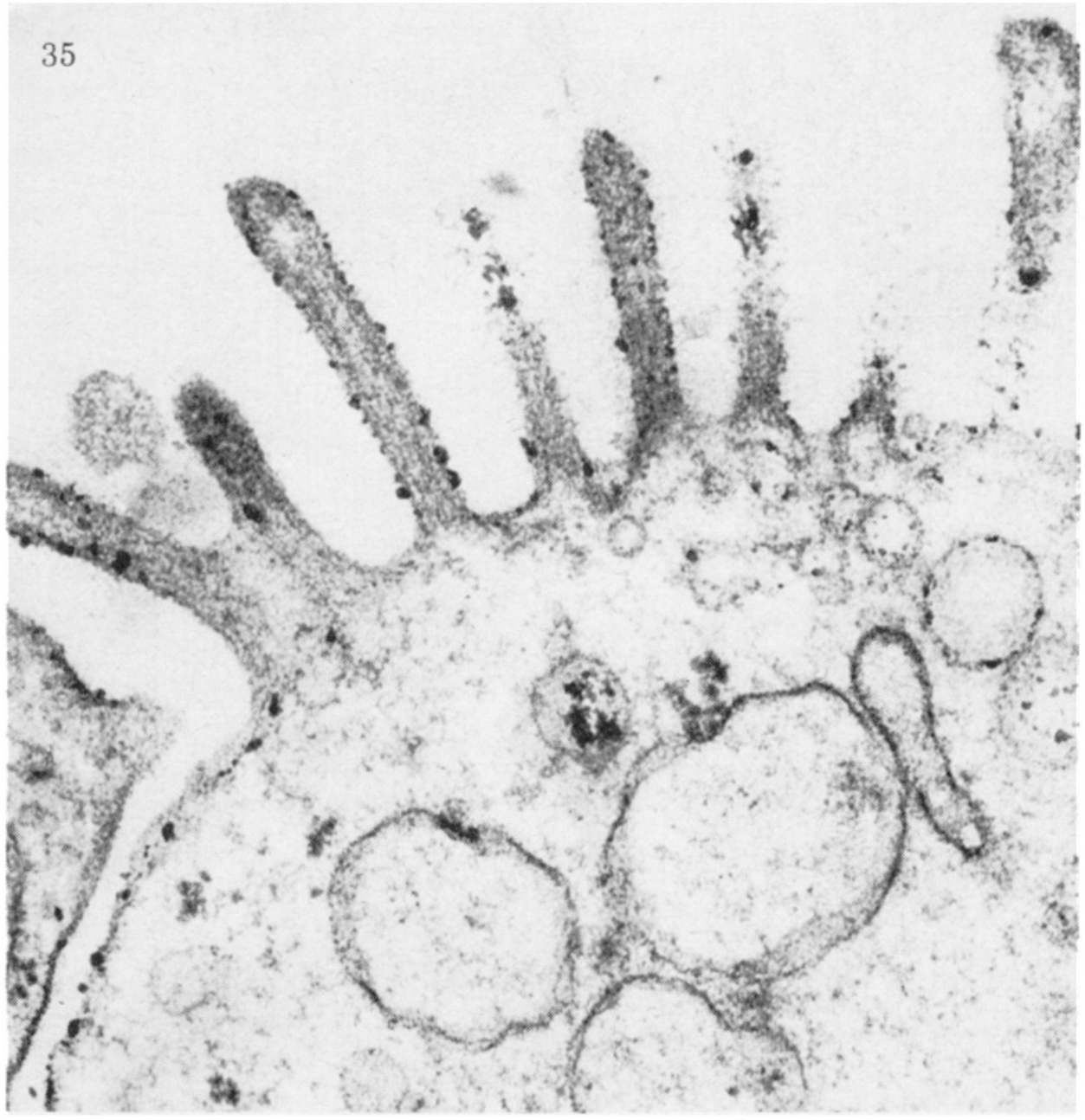

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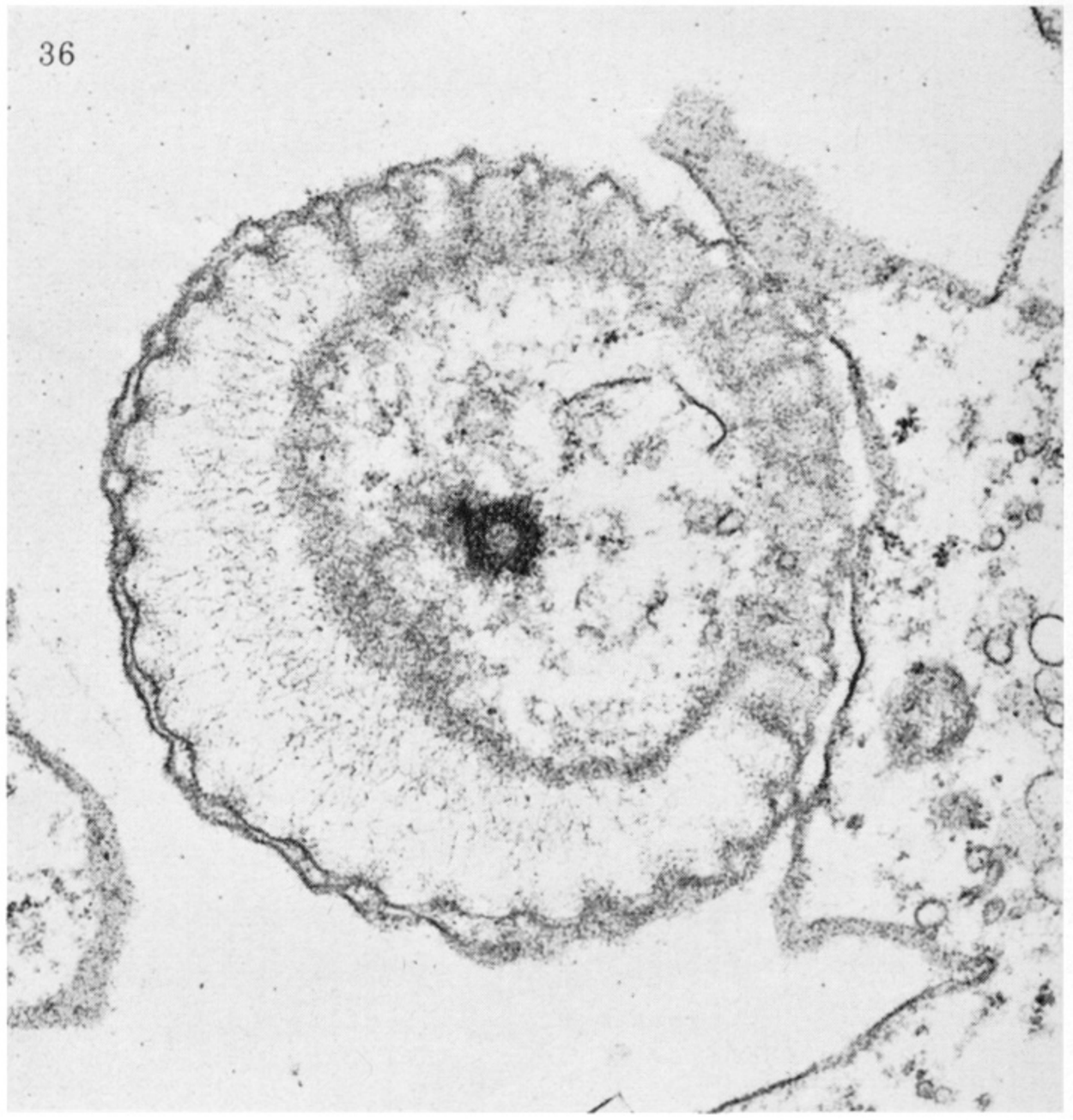

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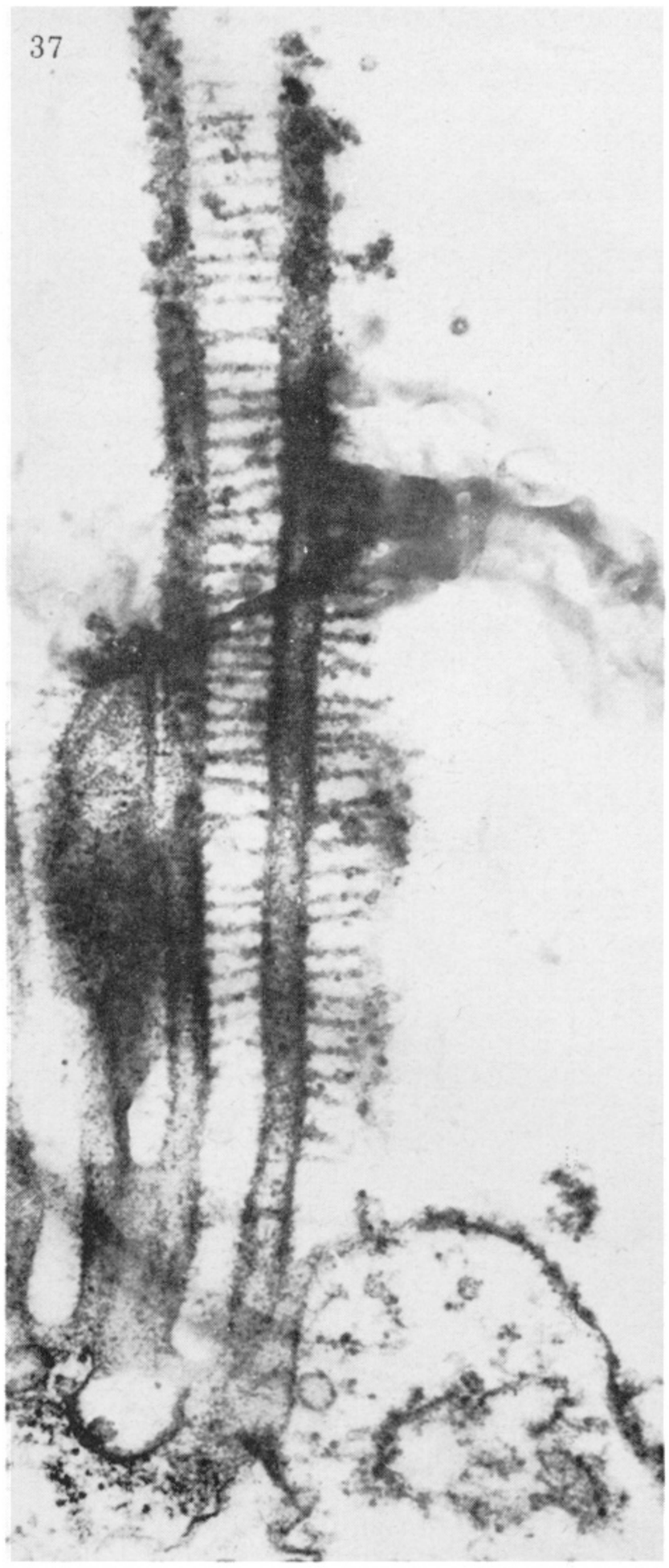

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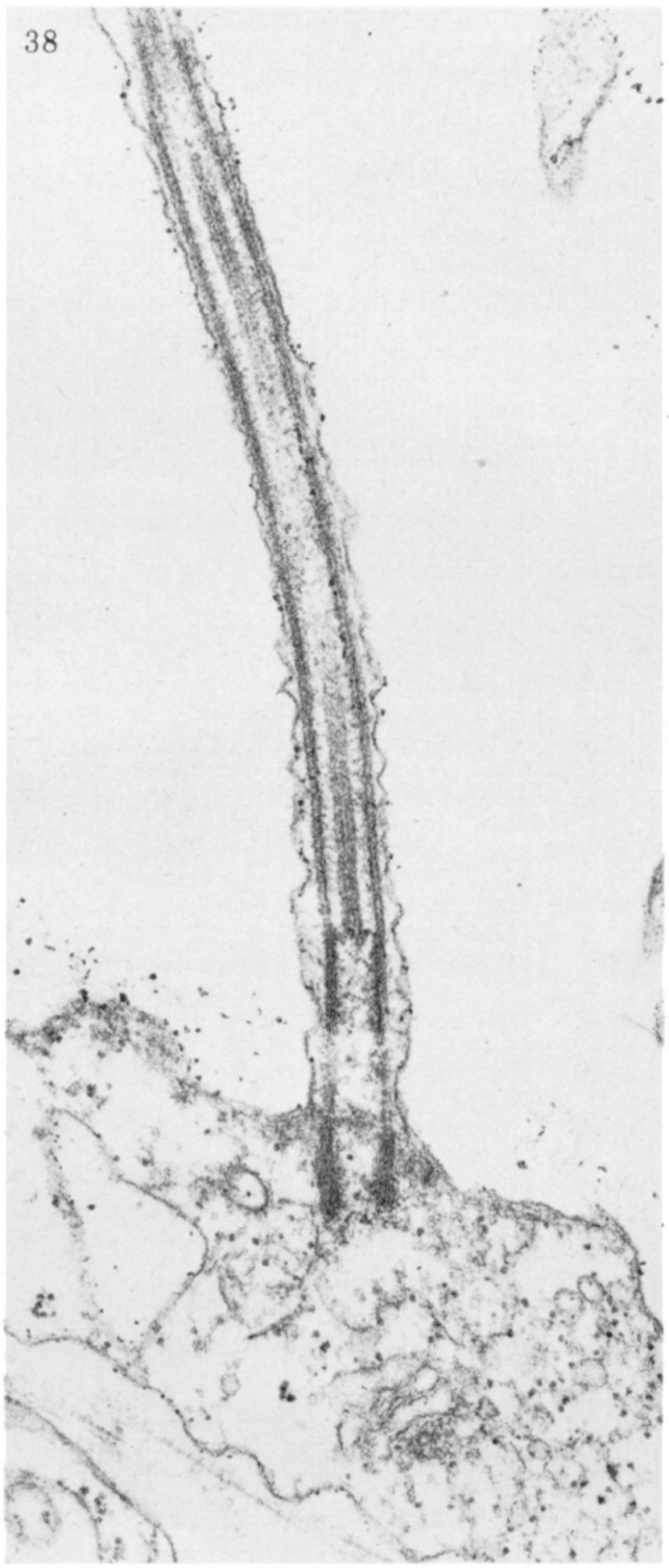

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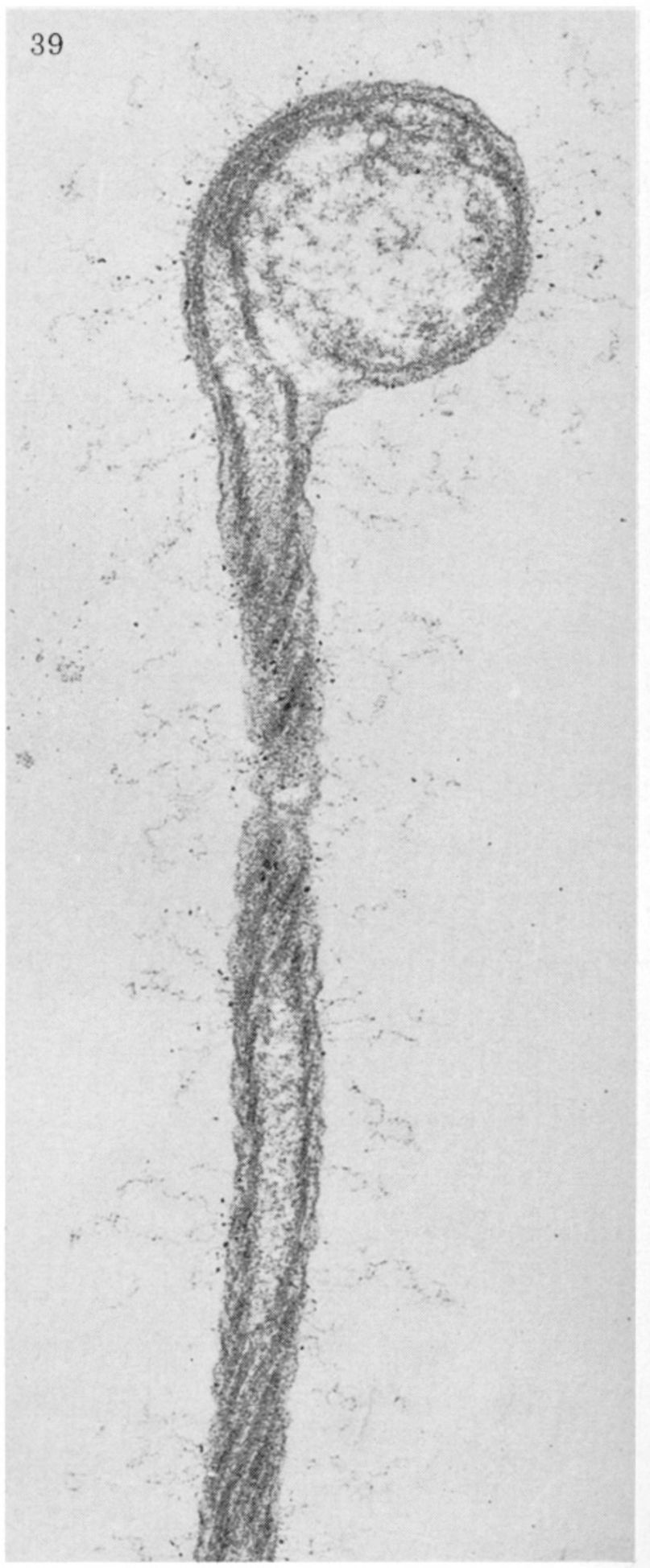

FIGURES 35–39. For description see p. 380.

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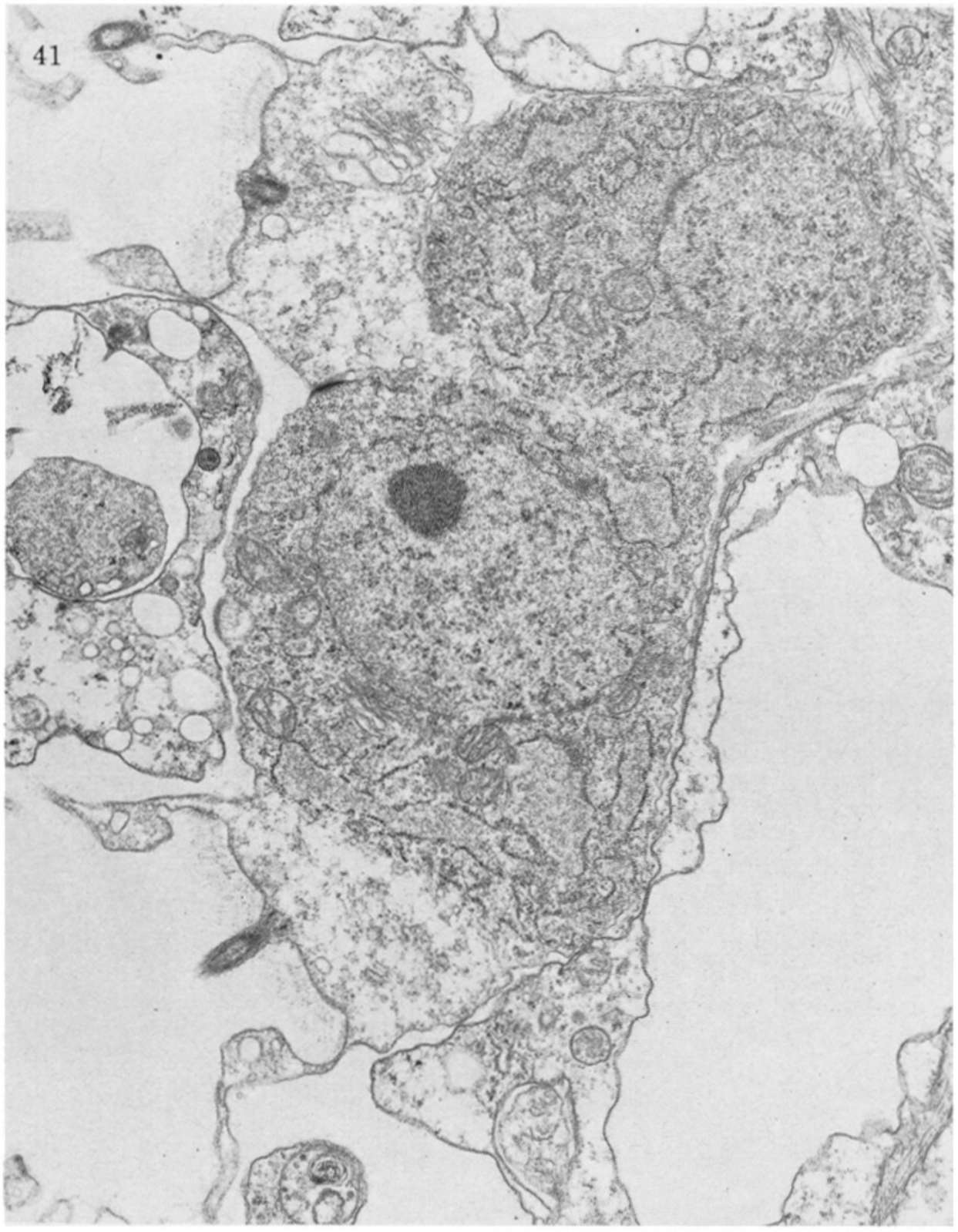

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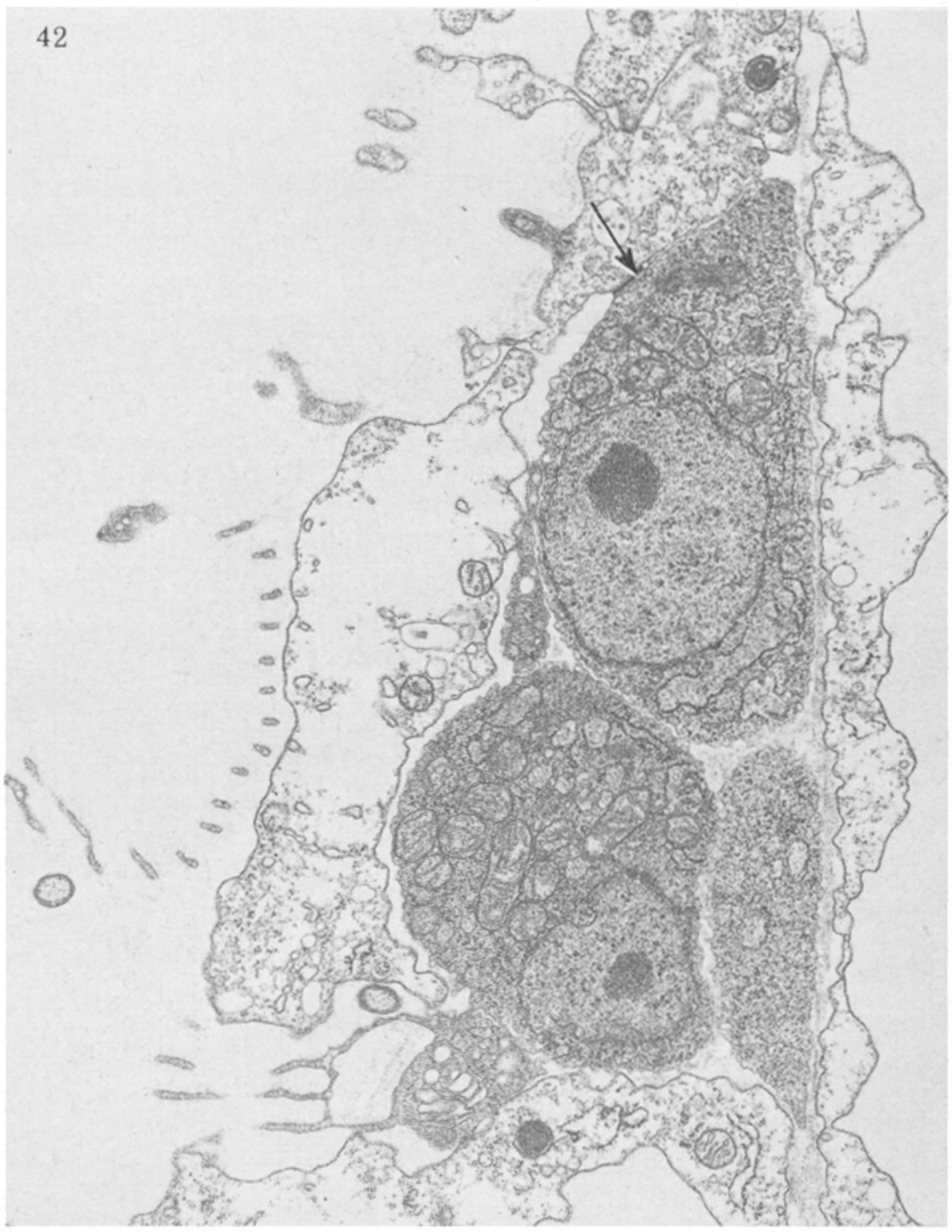

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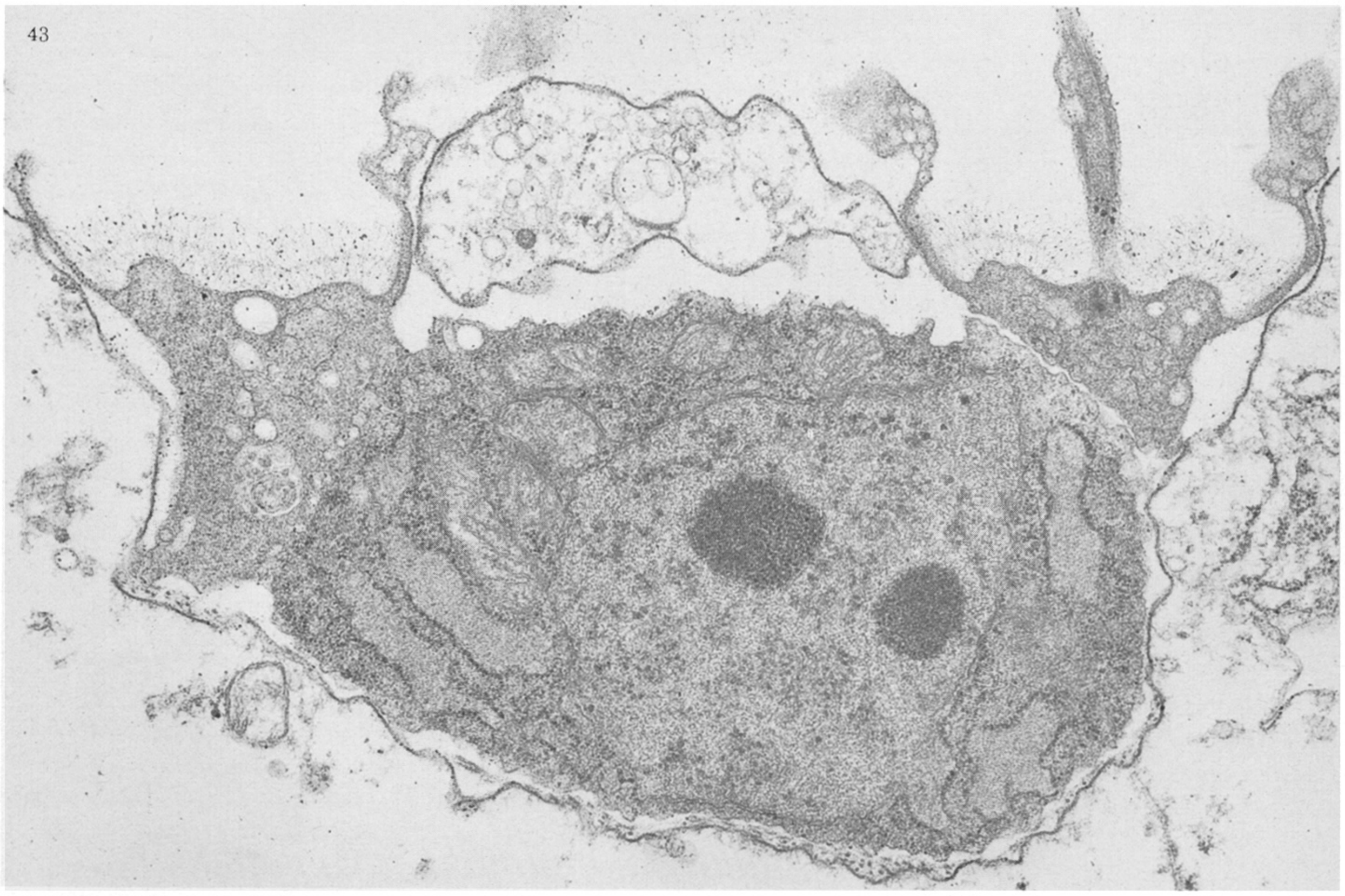

FIGURES 41–43. For description see p. 381.

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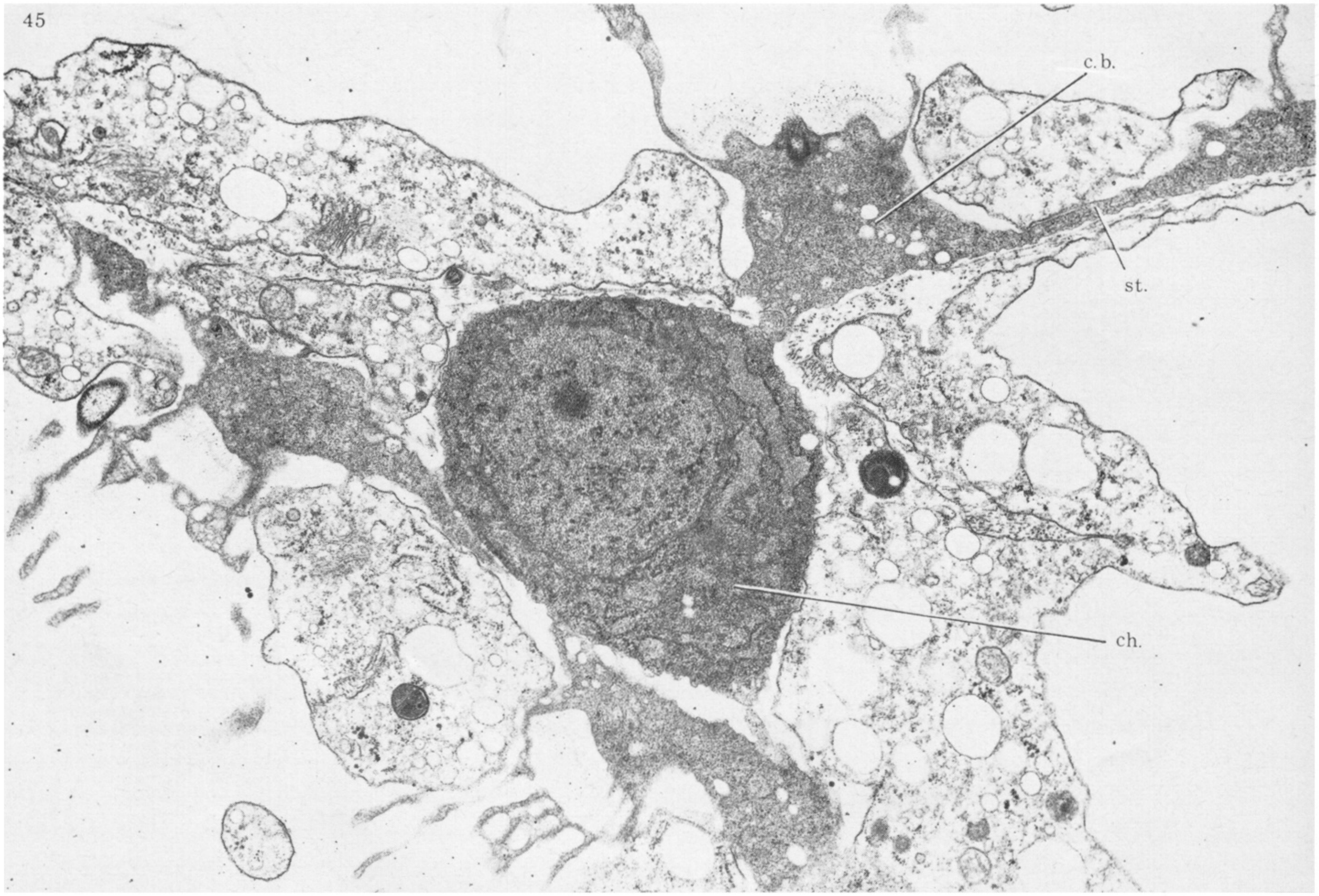

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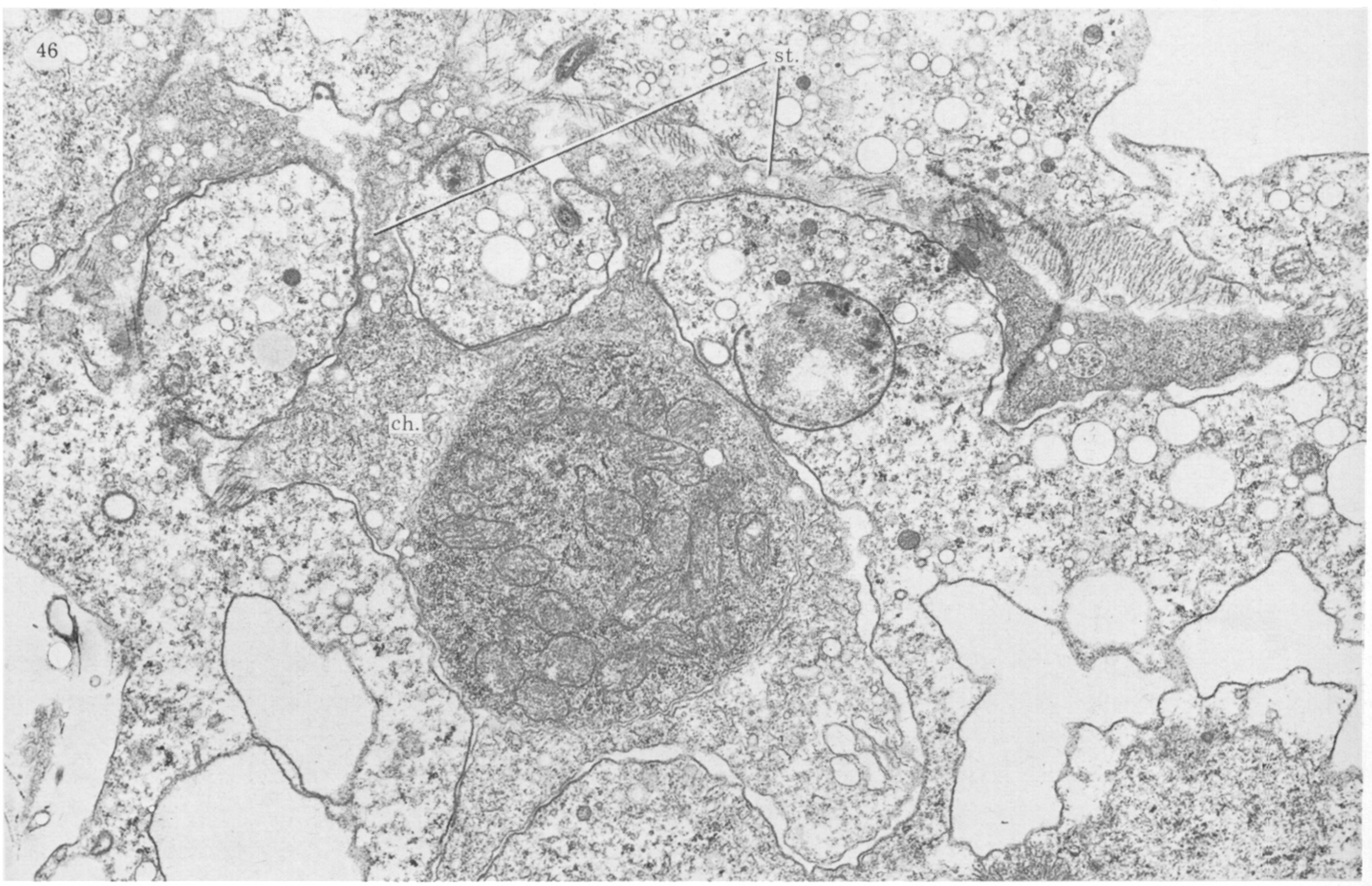

FIGURES 45 AND 46. For description see opposite.

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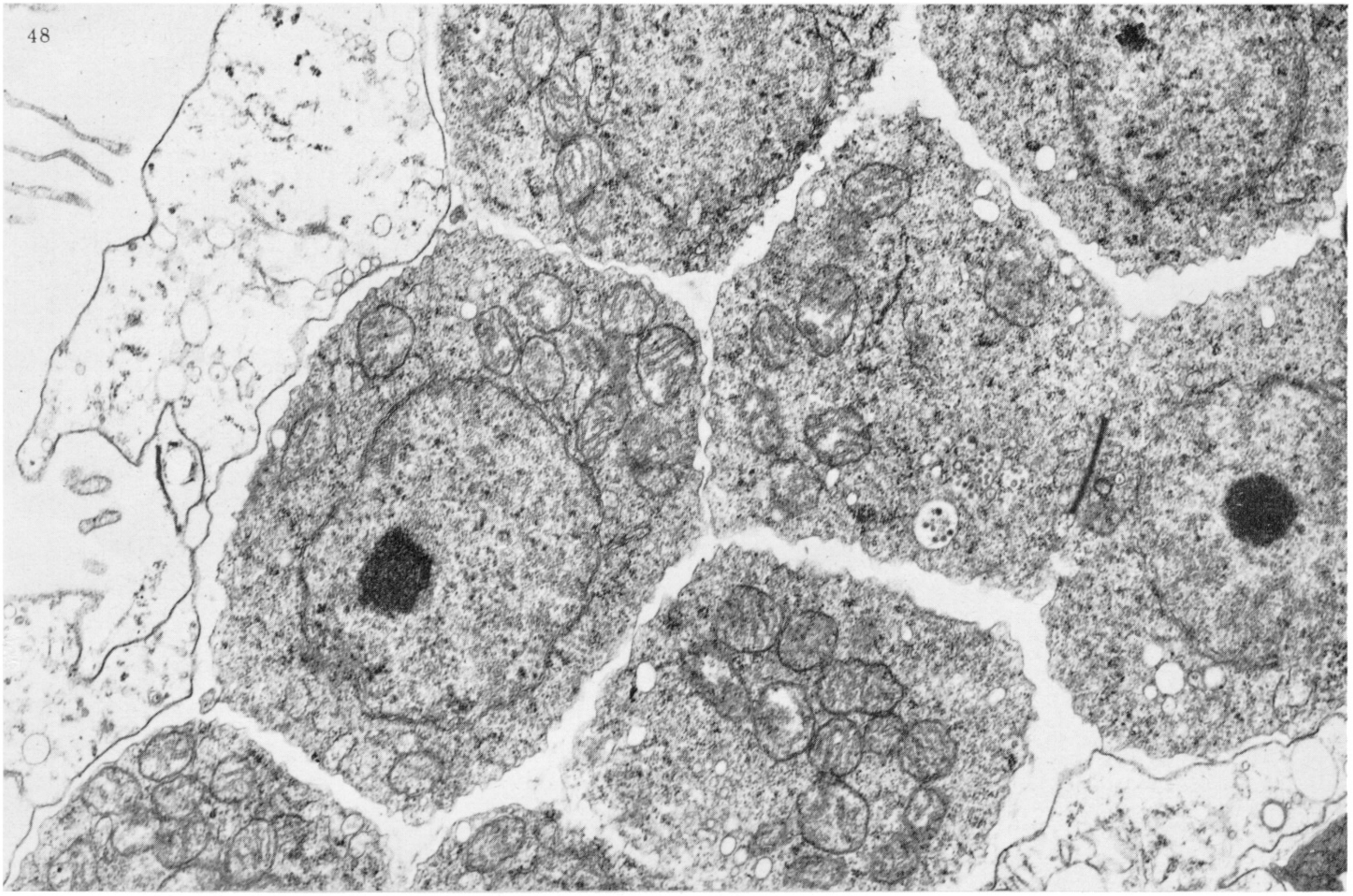

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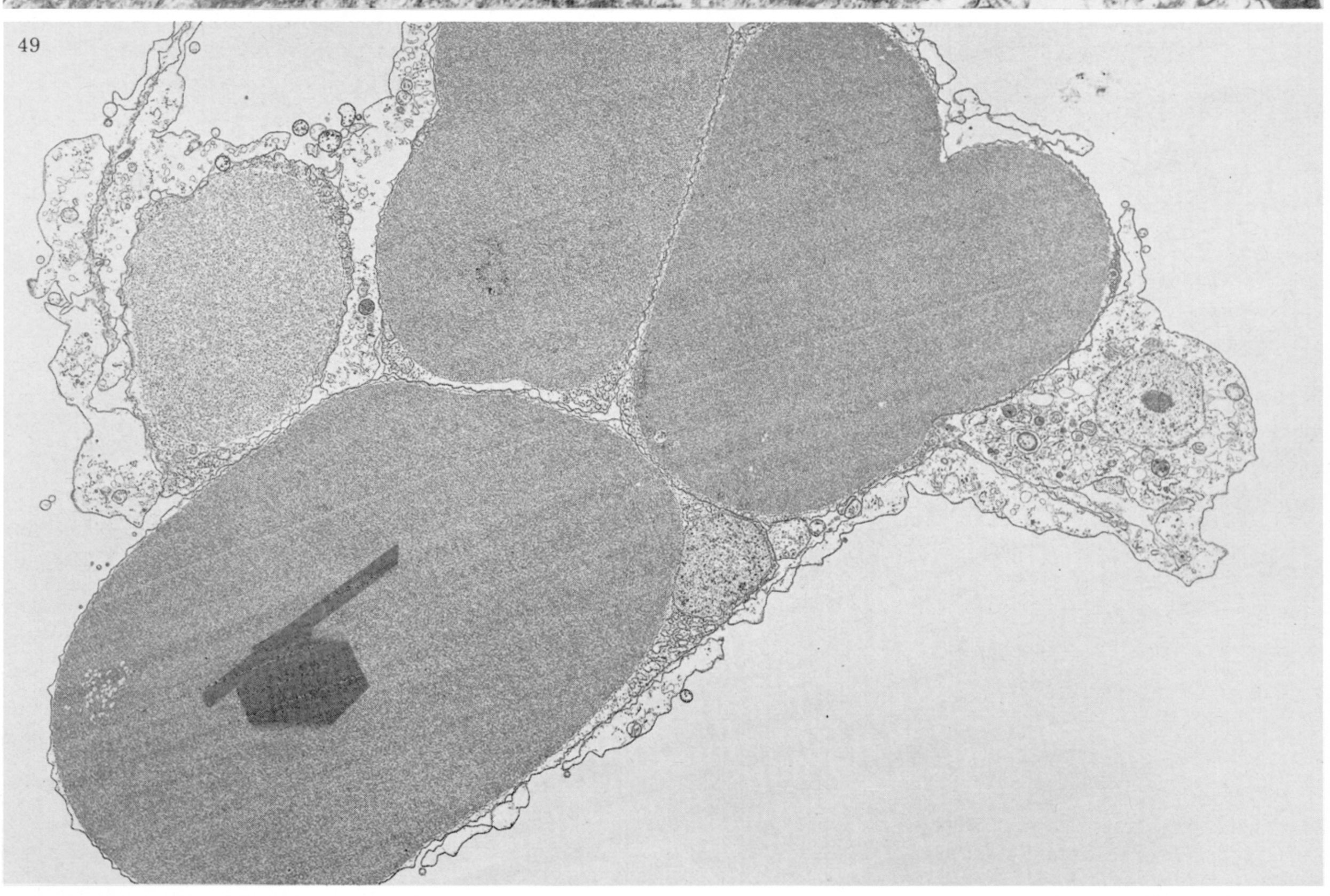

FIGURES 48 AND 49. For description see opposite.

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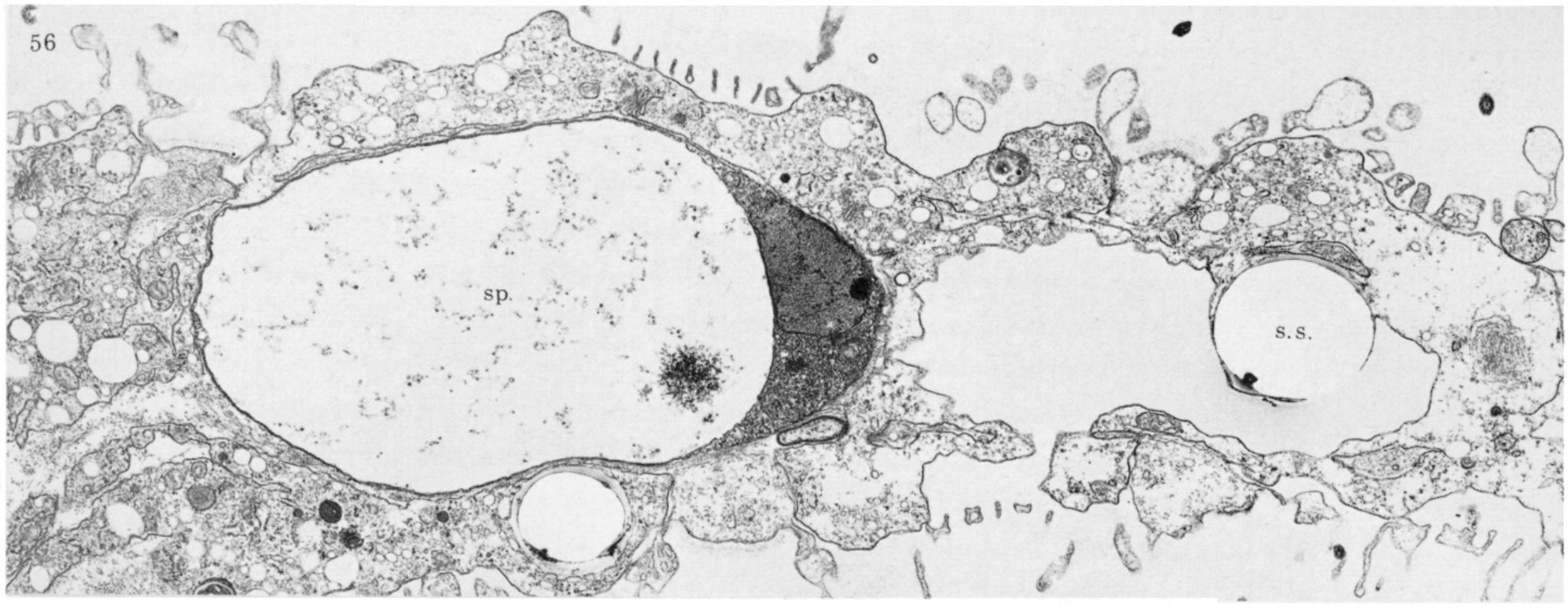
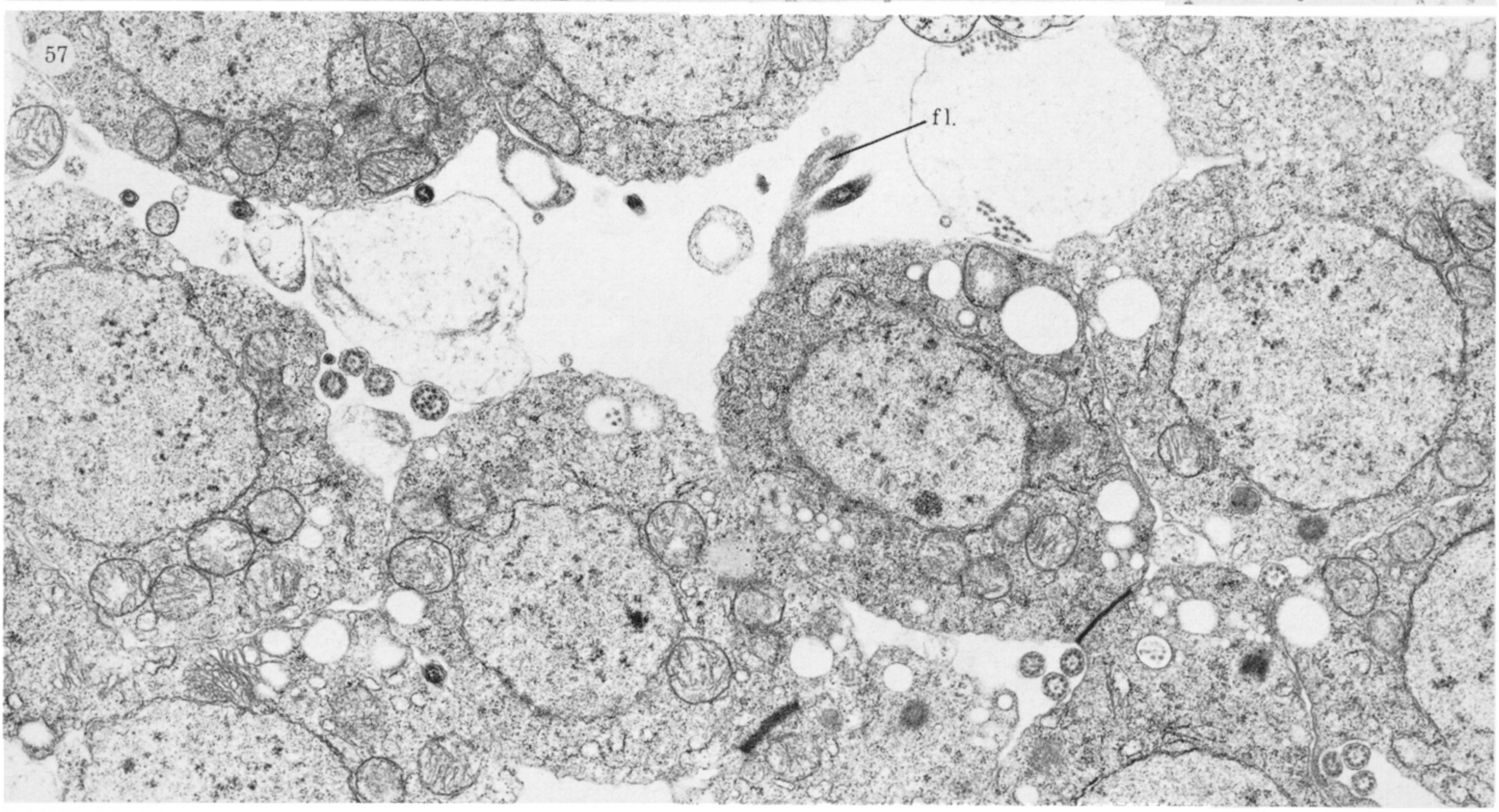
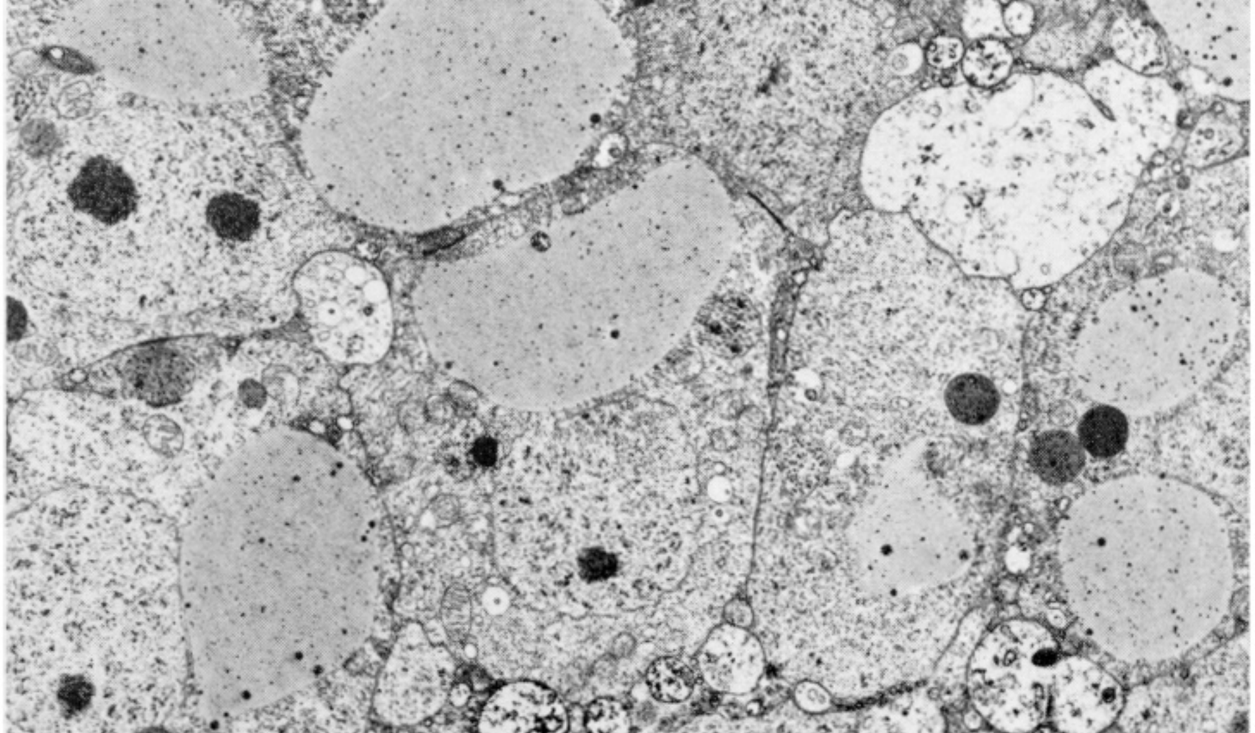
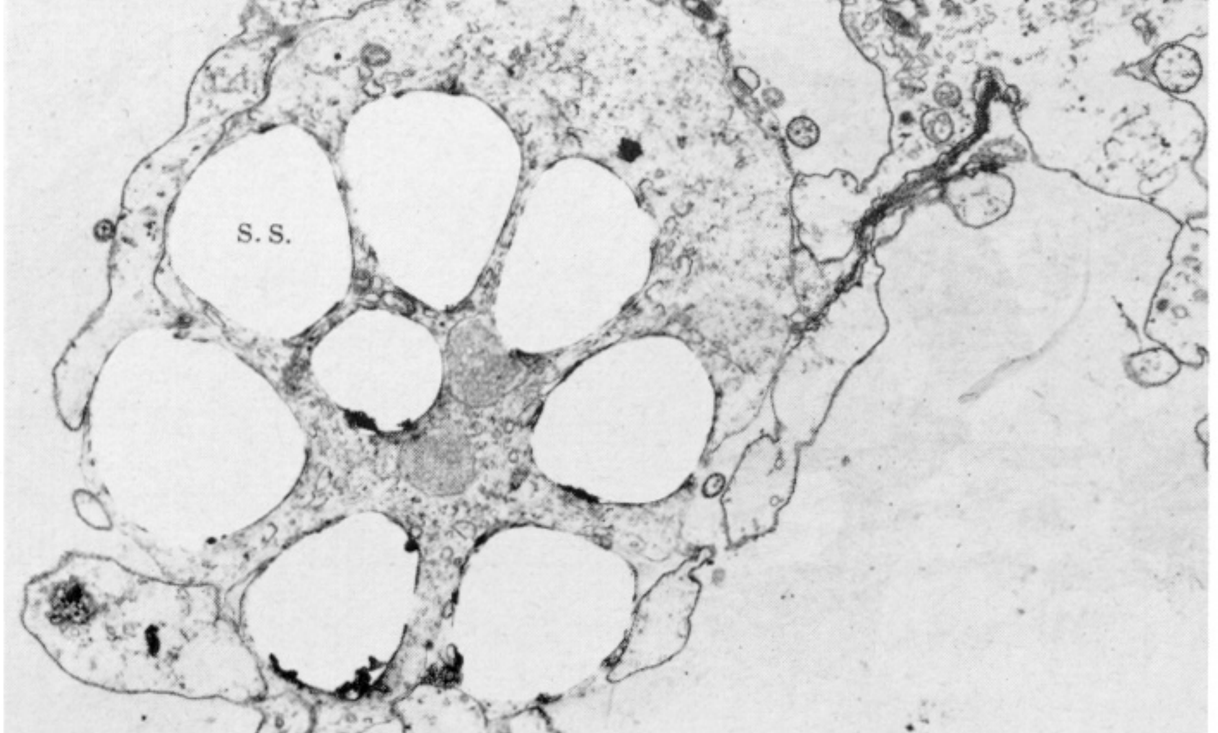
FIGURES 51–54. For description see p. 386.









FIGURES 56–59. For description see p. 387.

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FIGURES 60–62. For description see opposite.