THE ULTRASTRUCTURE OF *GYMNOSPHAERA ALBIDA* SASSAKI, A MARINE AXOPODIATE PROTOZOON

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Gymnosphaera albida has been found on the sponge *Sycon ciliatum* in the Menai Straits, North Wales, during the months of May to December. It commonly adopts a sedentary mode of life when cultured, settling with its body in contact with the substratum and its axopodia radiating upwards and outwards all round. At times it floats freely. When sessile it can displace itself, but not by rolling. It is a voracious carnivore. The largest seen had a body size of 510 μm × 320 μm.

The body of *Gymnosphaera* is divided into three zones: a central medulla, a cortex and a superficial reticulated pseudopodial layer. The medulla is finely vacuolated and contains an axoplast at its centre. The cortical cytoplasm contains many nuclei, Golgi bodies, polysomes, mitochondria, osmiophilic globules, lipoid spherules and vacuoles of various kinds, but no zooxanthellae. The superficial reticulated pseuopodial layer contains osmiophilic globules and occasional mitochondria. Axonemes radiate from the axoplast to the axopodia, along which osmiophilic globules are generally in motion. In between the cortex and the reticulated pseudopodial layer there is a narrow, extracytoplasmic capsular wall (Sassaki's line), consisting of a microfibrillar coagulum. The wall is a labile structure, perforating locally to allow the passage of food vacuoles or faeces and vanishing completely in certain conditions. It is evaginated to form a sleeve around the base of each axopodium. The cortex is completely penetrated by a system of clefts, the lumen of which opens here and there into the space containing the capsular wall. The clefts are distinct from the endoplasmic reticulum, cisternae of which are commonly found near the surface of the cytoplasmic tracts. Some of the cortical vacuoles contain organic refractive crystals. The crystals have the shape of crossed rodlets, each rodlet having a thermostable component ensheathing a thermolabile component. Their properties are described. The nuclei are enveloped in a thin layer of cytoplasm, connected by narrow bridges to the adjacent cytoplasmic strands. They generally contain several peripherally arranged nucleoli, each bearing a number of nucleolar organizers. Near the centre of the nucleoplasm there is usually a 'central chromatin body'. The vacuoles of the medulla are of two kinds, one equipped with a fibrous coat. In the vicinity of an axoneme the coat commonly connects with the microtubules and their cross-bridges.

The axoplast has a central 'hyalosphere' exhibiting a fibrogranular matrix. No tripartite organelle is present therein. The axoplast shell consists of the proximal ends of the axonemes, each enveloped by a fibrous sheath, the fibres coursing around adjacent axonemes, binding them together. The shell thickness is a constant fraction (1/2.5) of the axoplast diameter. The axonemes consist of bundles of parallel microtubules arranged in transverse section in a pattern of alternating rows of hexagons, the microtubules being joined together by 12.3 nm long cross-bridges. The cross-bridges are absent at the proximal tips of the axonemes and their number diminishes distally from the axoplast to the axopodia; the microtubule arrangement, however, is retained throughout.

The capsular wall of *Gymnosphaera* is not essentially different from the tenuous capsular membrane of certain Acantharia and Radiolaria. The reticular organization of the cortex has its counterparts in other groups. The osmiophilic globules are believed to be responsible for the adhesive properties of the axopodia. The refractive crystals have properties like those of the concretions of some Acantharia and the crystals of *Wagnerella*. The definitions of the terms 'axoplast' and 'centroplast' are considered and the neutral term 'centriaster' is suggested to include either. Finally the classification of *Gymnosphaera* is considered to be unsatisfactory; it has features in common with the Acantharia and with *Hedraiophrys*, but more information on the ultrastructure and life histories of axopodiate species is required before one can improve the classification of the Actinopoda.

Introduction

Sassaki (1894) found *Gymnosphaera albida* at the Munich Zoological Institute in 1890, in seawater derived from Rovigno on the Northern Adriatic coast. Since then it has been recorded only once, namely at Banyuls by Caullery (1911), where it was found on *Peyssonelia*, a species of alga.* There is some doubt about the latter record, because Caullery stated that his specimens reached a maximum size of 0.5 mm and contained nuclei varying in number from 1 to 30, whereas Sassaki gave the maximum size as 140 μm and the maximum number of nuclei as

* See also Febvre-Chevalier, C. 1975 *Protistologica* **11**, 331–344 (issued 1976).

several hundred. Also Caullery observed that some of his specimens had ingested large numbers of holothurian and sponge spicules, and that they could undergo temporary encystment.

I can now report that *G. albida* occurred during the years 1970 to 1975 inclusive on specimens of the calcareous sponge *Sycon ciliatum* which were collected from the floating landing stage at Menai Bridge, Anglesey (SH 559720). The protozoon was particularly abundant towards the end of June and in July, when the sponge was releasing larvae. It has also been obtained from the same source in the months of May, September, October and even December. There is no doubt about the identification. While initially only electron microscopic sections of the protozoon were available (it had intruded into suspensions of sponge larvae which were subsequently fixed and sectioned for e.m. studies), the acquisition of living specimens in 1972 enabled me to confirm Sassaki's description, based on optical microscopy, in every respect.

Both Sassaki and Caullery regarded *Gymnosphaera* as a member of the Heliozoa, affinities with the Centrohelidia being indicated by the presence of axopodia supported by organic rods (axonemes) radiating from a central sphere. However, the studies reported below now demonstrate that *Gymnosphaera* exhibits features that are characteristic of the Acantharia and some Radiolaria. The rediscovery of *Gymnosphaera* is thus of considerable interest and could well play a significant part in establishing a more natural classification of the Actinopoda, the group comprising the Heliozoa, Acantharia and Radiolaria.

Materials and methods

Although *Gymnosphaera* occurs abundantly on some specimens of *Sycon ciliatum*, a good source also is the detritus and sediment which settles at the bottom of the collecting jar. If this sediment, or slices of the sponge, are transferred to plastic petri dishes, the individuals of *Gymnosphaera* soon attach themselves to the bottom and one need only remove the surplus sediment or slices, and change the seawater once per week, in order to obtain a thriving population. The dishes were kept at 12 °C when not being examined, but the protozoon can survive temperatures of 22 °C or more for several days. *Gymnosphaera* is a voracious carnivore. Few small organisms seem able to attack it.

Initially suspensions of the sponge larvae and *Gymnosphaera* were treated in a centrifuge tube with 1/5 the volume of cold, 2% aqueous osmium tetroxide for about 1 h at 3–4 °C. The organisms were then rinsed twice in tapwater ($\frac{1}{2}$ h) with the aid of a centrifuge, dehydrated with ethanol and embedded in Araldite. Sections were stained with lead citrate and uranyl acetate.

When living gymnosphaerae became available, attached to the bottom of the petri dishes in which they were kept, it was not necessary to use a centrifuge at each stage of the process leading to embedding. The method adopted was to drain most of the seawater from the dish, leaving only about 10 ml. The dish was then left for about $\frac{1}{2}$ h in a refrigerator (3–4 °C), after which about the same volume of an ice-cold mixture of equal parts of 2% osmium tetroxide, 0.15 M sodium cacodylate buffer adjusted to pH 7.4 with 1 M HCl, and 6% glutaraldehyde in the buffer solution, was added gently. After $\frac{1}{2}$ h the solution was drained from the dish and the attached organisms were rinsed for 5 min with the cacodylate buffer. They were then treated with 5% uranyl acetate for 45 min, washed again with the buffer twice for 10 min and allowed to warm up to room temperature, after which they were dehydrated up the ethanol

series. After three changes of absolute ethanol some Araldite was poured into the dish and then replaced by fresh Araldite after a few minutes. The organisms were dislodged using a fine scalpel and pipetted into gelatine capsules already half-filled with Araldite. After hardening and sectioning the sections were stained with lead citrate. At each level of sectioning a 1 μm thick section was also cut and stained with toluidine blue for examination by light microscopy. Two electron microscopes were employed, namely an A.E.I. EM 6M and an A.E.I. Corinth 275.

Cold solutions of the mixed fixative were used in order to avoid the darkening which rapidly occurs at room temperature. The presence of the OsO_4 largely prevented the depolymerization of the microtubules at the low temperature. When cooled 5% glutaraldehyde was used, followed by osmication, the microtubules were not preserved and the cytoplasm tended to break up into separate spherical globules of varying size.

When observing the living animal under the optical microscope a raised coverslip was usually not used. It was found that the evaporation of the seawater caused the unsupported coverslip to compress the specimen gradually so that the contents of its cytoplasm became progressively more visible.

For the experiments concerning the nature of the crystals found in *Gymnosphaera*, and to obtain suitably thin material for staining and for examination by optical microscopy, specimens were gently dislodged with a fine scalpel and pipetted upon the surface of a coverslip. After leaving for a few minutes to allow the axopodia to extend once more, the surplus seawater was removed almost completely, so that the organism flattened against the coverslip. This was then either inverted over the open neck of a bottle containing 3% osmium tetroxide for 15 s, or 90% alcohol was added, before immersing the specimen in water to rinse away the salt. The attached gynmosphaera could then be stained, or, by resting the inverted coverslip on props, be observed under the microscope while treating it with various reagents.

Observations

(*a*) *Mode of life of* G. albida

Gymnosphaera suspends itself by means of its axopodia between the projecting spicules at the apices of the radial diverticula when it occurs on *Sycon ciliatum*. *In situ* it resembles a spider in its web and no doubt it functions in much the same way, feeding on small organisms and on particles that are drawn towards the surface of the sponge by the inhalent water currents. It does not occur within the tissues or cavities of the sponge, nor is it confined to the surface, for it readily adopts a free floating existence. Probably it is brought to the surface of the sponge by the inhalent currents, because when floating it responds to any slight movement of the water, sometimes rolling over very slowly as it drifts along. Presumably when it is floating it gains buoyancy from the presence of oil droplets in its cytoplasm; there are no projecting spicules as in the Acantharia, only innumerable, long, radiating axopodia.

In the laboratory *Gymnosphaera* is usually sedentary, attaching itself to the plastic or glass bottom of the culture vessel, or to pieces of detritus. When attached it may be either resting on the axopodia directed towards the attachment area, or partly flattened against the substratum, in which case the cytoplasm may spread somewhat over the latter. When the body is in direct contact with the substratum the animal remains more or less stationary, with its axopodia radiating upwards and outwards from the free surface. At times quite large wanderings are

observed, however. For example, one animal moved a distance of 15.1 mm in 48 h, at an average speed of 0.315 mm/h. Speeds of 0.1 mm/h are quite common. The actual mechanism of locomotion in such cases has not yet been investigated. It is known that certain heliozoa can roll along on the tips of their axopodia, at speeds of as much as 125 μm in 30 s (15 mm/h) (Trégouboff 1953*c*). Watters (1968) found that *Actinosphaerium eichhorni* and *Actinophrys sol* moved at velocities of 5–100 μm/min (0.3–6 mm/h); tension in the leading axopodia was of particular significance and rolling occurred primarily when the attached axopodia were extended in directions that were not strictly radial to the organisms. Several times I have seen the bodies of gymnosphaerae recoil when the axopodia on one side were cut. It is thus possible that tension generated in certain axopodia could pull the body over. However, when the two daughters of a binary fission separate, they do not roll away from one another, judging from the unchanging relative positions of prey attached to their surfaces. The two daughter organisms have been observed to separate to a distance of 533 μm in 52½ min, at an average speed of 0.24 mm/h, which is faster than some average rates recorded above. There is thus no reason to think that *Gymnosphaera* necessarily progresses over the substratum by rolling. Amoeboid or gliding displacement is more likely when it is flattened against the latter. This is not to say that, during binary fission, forces generated by the axopodia do not participate in the separation of the daughters. The axopodia remain extended during division, like those of *Actinophrys sol*, concerning which there is good evidence that the axopodia play a major rôle in the separation (Ockleford 1974). I have seen free-floating, paired gymnosphaerae, suggesting that cell separation can occur in the absence of attachment to a substratum, just as is the case with *Actinophrys*. For the Acantharia it has been stated that the rolling motion is mediated by streaming movements of the rheoplasm in the axopodia situated at the tips of the spicules, the axopodia pointing against the direction of rotation of the animal (Cachon & Cachon-Enjumet 1964). However, the axopodia of *Gymnosphaera* always radiate uniformly and straight in all unobstructed directions from the centre, excepting when, immediately after agitation of the seawater, the axopodia are crumpled.

(*b*) *General structure*

Sassaki (1894) recognized three regions comprising the body of *G. albida*, a thin, outer, granular enveloping layer (*Hüllschicht*), a granular and vacuolated cortex (*Rindenschicht*) and an inner, clearer medulla (*Markschicht*). A distinct boundary line separated the enveloping layer from the cortex, and because no structure could be distinguished, he regarded the line as representing merely a change of phase. Food could penetrate through the boundary, which I shall refer to below as 'Sassaki's line'. The cortex and medulla were not so sharply demarcated from one another. At the centre of the medulla occurred a small, hyaline or finely granular sphere, from which the axonemes radiated. The axonemes were labile structures and in fixed and stained preparations only the central body and the proximal ends of the axonemes (together termed *das Ausstrahlungscentrum*) remained distinguishable. A convenient term for this spherical structure would be 'centriaster' (Jones 1975).

By reflected light *Gymnosphaera* is white. By transmitted light its appearance varies from a greyish or brownish-grey translucence to a very dark turbidity caused by the presence of refringent inclusions. Between these two extremes one commonly finds in optical section a dark band around the lighter centre, caused by the accumulation of inclusions in the innermost parts of the cortex. Sometimes the periphery of the body is sharply discernible as a thin dark

streak. Within the cytoplasm may be present some coloured globules. The colours seen have included black, dark brown, reddish brown, deep red, red, light brown, amber, pale orange, yellow and green. Several differently coloured vacuoles may be observed in a given individual. Some (for example, green and amber) represent unicellular algae in digestive vacuoles. Others are probably coloured by carotenoids or animal pigments. Some possibly are oil droplets, though usually some structure is visible within the coloured vacuole. The coloured globules do not persist. For example, one specimen exhibited one red globule on one day, then four on the next day and none at all on the next day after. Another had a green vacuole on one day which became distinctly paler by the next. Clearly these coloured inclusions are a reflection of the nutritional activity of the animal. *Gymnosphaera* is a voracious carnivore, capturing small rotifers, nematodes, organic detritus, radiolaria and other unicellular organisms, sometimes of a size almost equal to its own volume. The axopodia are sticky and adhere readily to objects brought into contact. The captured prey is engulfed by the cytoplasm of the enveloping layer and either passes inwards into the body or, if the prey is large, persists between the axopodia for some days before the remains are liberated as faeces. At times one also observes amongst the axopodia a number of small, hollow vesicles which subsequently decrease in number and apparently disperse, indicating perhaps a process of excretion.

The populations of *Gymnosphaera* which were maintained in petri dishes and bowls in the laboratory consisted throughout the year of individuals of different sizes. The largest seen in these populations measured 161 μm × 174 μm in diameters (26 March). The smallest had a body diameter of 35 μm (22 March). Sometimes the dimensions of the individuals fluctuated from day to day, probably as a result of the animal sometimes rising on the tips of its axopodia, or settling down with its body in contact and spreading on the substratum. In its natural habitat *Gymnosphaera* can attain a much greater size than in the laboratory. One *Sycon* collected on 20 September was particularly revealing in this respect, for by the 21st it had shed a number of very large individuals. One specimen measured 510 μm × 320 μm, another, 240 μm × 160 μm and a third, 188 μm × 148 μm. The largest thus approximated to Caullery's maximum size of 0.5 mm (1911). All three specimens were equipped with radiating axopodia.

When *Gymnosphaera* is spherical and there is nothing in contact with its surface, it is usually difficult to discern the enveloping layer, consisting as it does of a very narrow zone of translucent, colourless cytoplasm (figure 1, plate 1). Sassaki's line likewise is not easily detectable. These regions only become distinct when the organism is partially squashed beneath a coverslip, a process which occurs automatically and progressively as the water evaporates from the slide, or is removed with the aid of filter paper. The appearance of a partially squashed specimen can be seen in figure 2, plate 1. Sassaki's line is visible and consists of a hyaline band with

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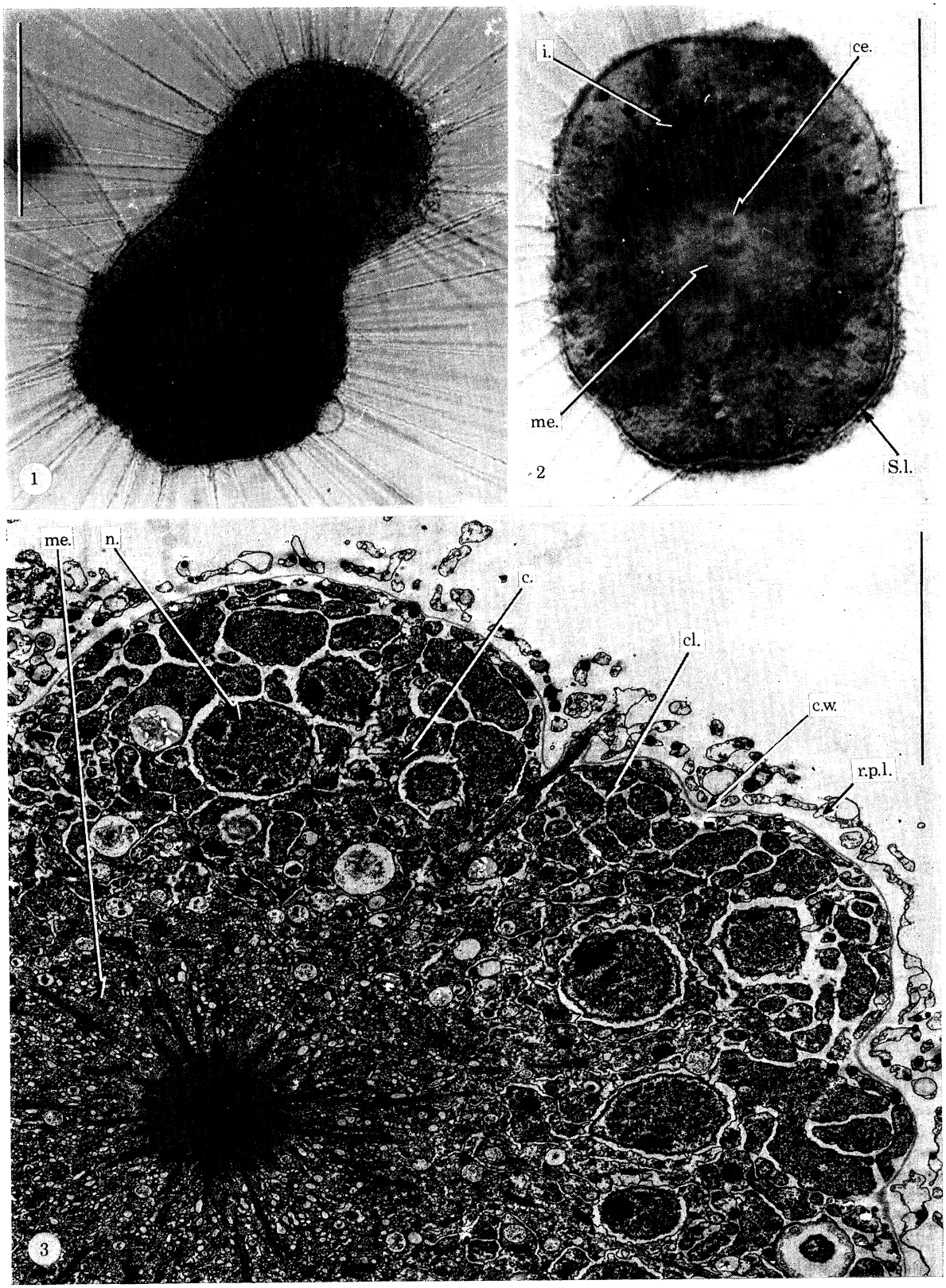
Description of plate 1

Figure 1. *Gymnosphaera albida* in the course of binary fission (slightly compressed). (Scale: 100 μm.)

Figure 2. *G. albida*, more compressed. Sassaki's line (S. l.) is visible as a thin hyaline layer all round the periphery. The centriaster (ce.) is at the centre of the clear medulla (me.). Inclusions (i.) are aggregated in a zone in the mid-region of the cortex. (Scale: 50 μm.)

Figure 3. E.m. section of *G. albida*. Sassaki's line is represented by a thin capsular wall (c.w.). The three cytoplasmic zones (me., medulla; c., cortex; r.p.l., reticulated pseudopodial layer) are easily distinguishable. Note the system of clefts (cl.) in the cortex. The numerous nuclei (n.) occur in a zone within the cortex. (Scale: 10 μm.)



FIGURES 1–3. For description see opposite.

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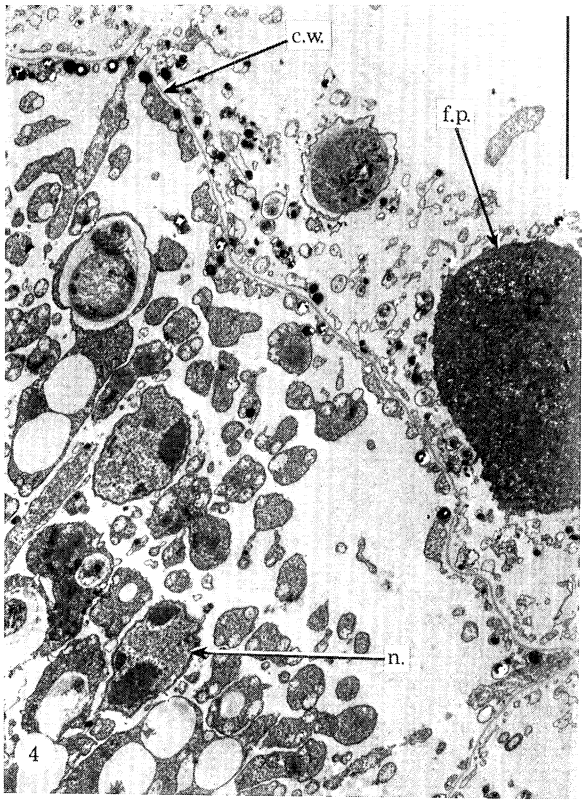

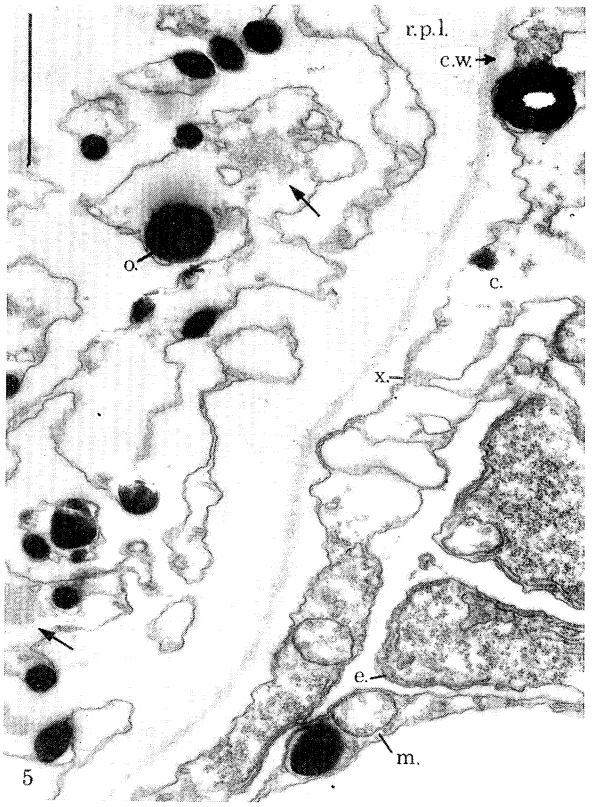

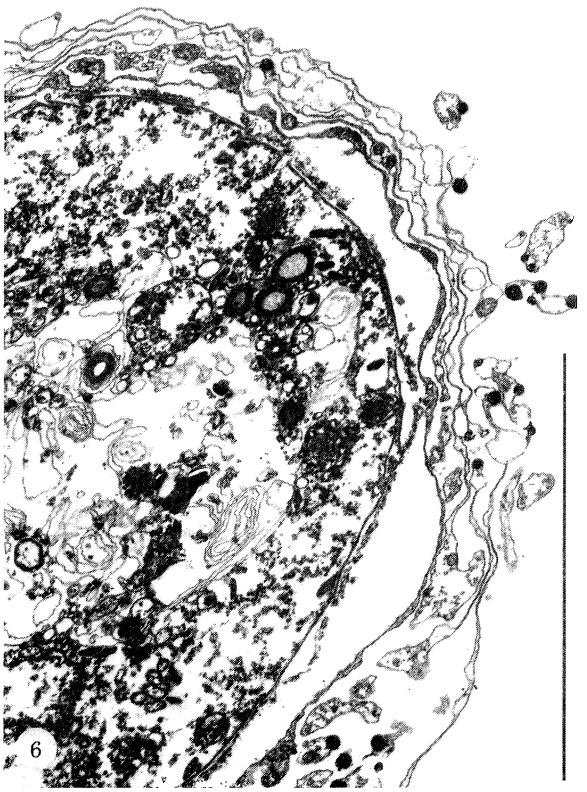

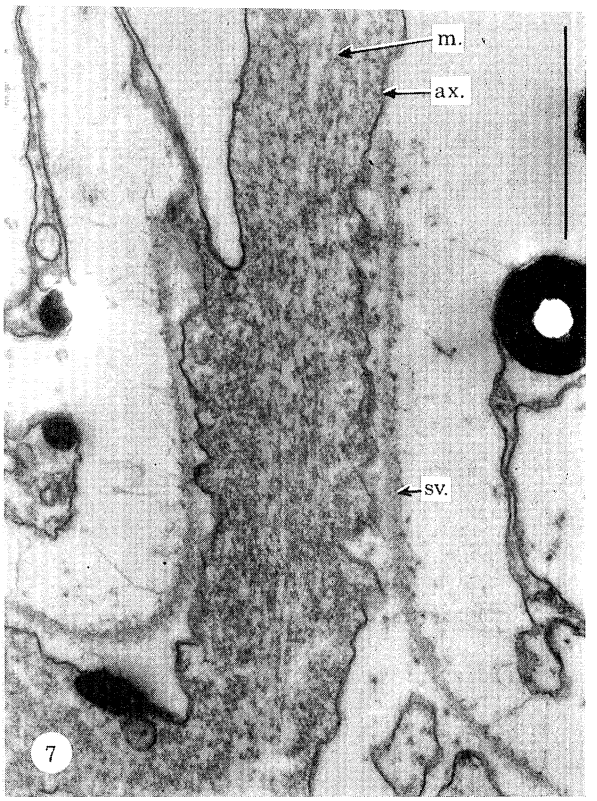

FIGURES 4–7. For description see opposite.

a dark streak along the inner border, caused by the refringency of the material and the curvature, somewhat enhanced by the flattening. One can also see the medulla and centriaster and the dark zone caused by the accumulation of inclusions near the innermost parts of the cortex. At higher magnification Sassaki's line seems quite structureless and homogeneous, of uniform thickness (approximately 1 μm).

Electron microscope sections of *Gymnosphaera* usually have a roughly spherical or oval, occasionally polygonal outline (figure 3, plate 1). Commonly the border is unevenly scalloped, possibly as a result of partial retraction of the axopodia during the process of fixation. The three zones distinguished by Sassaki are clearly recognizable.

(*c*) *The enveloping (reticulated pseudopodial) layer and axopodia*

The electron micrographs (figures 3–5, plates 1 and 2) reveal that the enveloping layer is not a continuous mass of cytoplasm, but consists of cytoplasmic strands cut in all kinds of directions and clearly constituting a three dimensional reticulum. This layer will be termed the 'reticulated pseudopodial layer', because food inclusions can be enveloped in it (figure 6, plate 2). The term 'reticulated pseudopodia' is conventional for the superficial ectoplasm of the Acantharia and Radiolaria (Trégouboff 1953*a*, *b*). The expression 'lacunar ectoplasm', which has been used for the cytoplasm covering the shell of foraminifera (Febvre-Chevalier 1971), would be less appropriate as it does not indicate the pseudopodial function.

The reticulated pseudopodial layer extends outwards for a variable distance (3–35 μm) at the surface of the organism, the extent depending upon whether or not the part is in contact with the substratum, or in the vicinity of a large food vacuole or material about to be defaecated. The innermost strands of cytoplasm are sometimes better described as ribbons or layers, because in sections they often extend for a considerable distance in the circumferential direction. This is not always the case. The cytoplasm of the strands generally appears to be empty, apart from the many electron-dense osmiophilic globules which will be discussed below. One occasionally sees a mitochondrion or some small vacuoles and granules, but no Golgi bodies, ribosomes or lipoid spherules. Much the same has been stated for the superficial ectoplasm of Radiolaria (Hollande, Cachon & Cachon 1970) and Foraminifera (Febvre-Chevalier 1971).

Whether or not a jelly occurs in the meshes of the pseudopodial reticulum cannot be decided

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Description of plate 2

Figure 4. E.m. section of another *G. albida*. The outline is polygonal, with the capsular wall (c.w.) extending from axopodium to axopodium, accompanied by cytoplasmic strands on the two sides. The cavity system in the cortex is more extensive than usual, possibly as a result of poor fixation (the nuclei, n., seem distorted). A faecal pellet (f.p.) lies outside the capsule. (Scale: 10 μm.)

Figure 5. The reticulated pseudopodial layer (r.p.l.), capsular wall (c.w.) and outer cytoplasmic strands of the cortex (c.). The arrows indicate the stereoplasm of the two axopodia cut in near transverse section. The cytoplasm outside the capsule contains little other than osmiophilic globules (o.). Some of the cytoplasmic tracts on the inside have sparse contents, but most contain ribosomes, mitochondria (m.) and, near their surface, some cisternae of endoplasmic reticulum (e.), besides osmiophilic globules. The cavity system almost opens into the space beneath the capsular wall at the site marked x. (Scale: 1 μm.)

Figure 6. Section of a food vacuole outside the capsule. Note the layers of cytoplasmic strands, the innermost enlarging here and there to include denser cytoplasm. (Scale: 10 μm.)

Figure 7. Longitudinal section of the base of an axopodium (ax.) and its sleeve (sv.). The microtubules (m.) are only sparsely interconnected by cross-bridges. (Scale: 1 μm.)

from the electron micrographs. The cavities communicate with the external environment and no trace of material is evident within them. However, around the large food vacuoles one often finds several layers of stretched cytoplasm bands (figure 6, plate 2) and it would be easier to explain how these bands remain separate if interposed gelatinous material were present. A superficial gelatinous layer is characteristic of the Acantharia, Radiolaria and Heliozoa (Trégouboff 1953*a*, *b*, *c*).

Strands of the reticulated pseudopodial layer are continuous with the rheoplasm of the axopodia, the cytoplasm surrounding the axonemes (figures 5 and 7, plate 2); there is no obvious difference in structure or content. When viewed by optical microscopy the reticulated pseudopodial layer usually exhibits a well-defined boundary from which curving and anastomosing thin cytoplasmic strands may project. Probably these are the remnants of axopodia which had become tangled during the handling of the specimen. They tend to sway where they freely project, and change pattern where they form a network. Some crumpling and entanglement of the axopodia are inevitable when *Gymnosphaera* is transferred to a slide, but in the course of 10 min or so many of the axopodia resume their normal, stiffly radiating arrangement. Ockleford & Tucker (1973) have recently studied the processes of contraction and extension in the heliozoan *Actinophrys sol*, as has Davidson (1973*a*) in *Heterophrys marina*. In *Gymnosphaera* the tangle seems to sort itself out by the retraction and narrowing of the axopodia distally until they have become free and can re-extend straight outwards once more. Their thickness varies, but commonly it is in the region of 0.8–1.7 μm. Occasionally one finds a particularly thick axopodium (for example, 4.25 μm wide), but one suspects that this has arisen by a fusion of thinner axopodia following entanglement. Under a coverslip the axopodia remain for periods of from ½ to 2 h or more, before they quite suddenly collapse and disappear, leaving only a few long curving strands, about 1 μm thick, criss-crossing in and around the peripheral parts of the squashed organism. No gelatinous material can be seen at any time. The axopodia of undisturbed specimens can be extremely long. One, with a capsular diameter of 85 μm, had axopodia which were 425 μm in length, the axopodia gradually tapering towards their distal ends. However, specimens usually had their axopodia extended for only from half to twice the diameter of the body.

(*d*) *The refractive globules*

A striking feature of the axopodia in the living animal is the occurrence of small refractive globules in the rheoplasm and cytoplasmic strands. They tend to be of uniform size (0.6–0.9 μm, mostly 0.7 μm) and are spherical. On electron micrographs they appear as electron-dense spots, the maximum measured diameter of which was 0.63 μm. Some are clear

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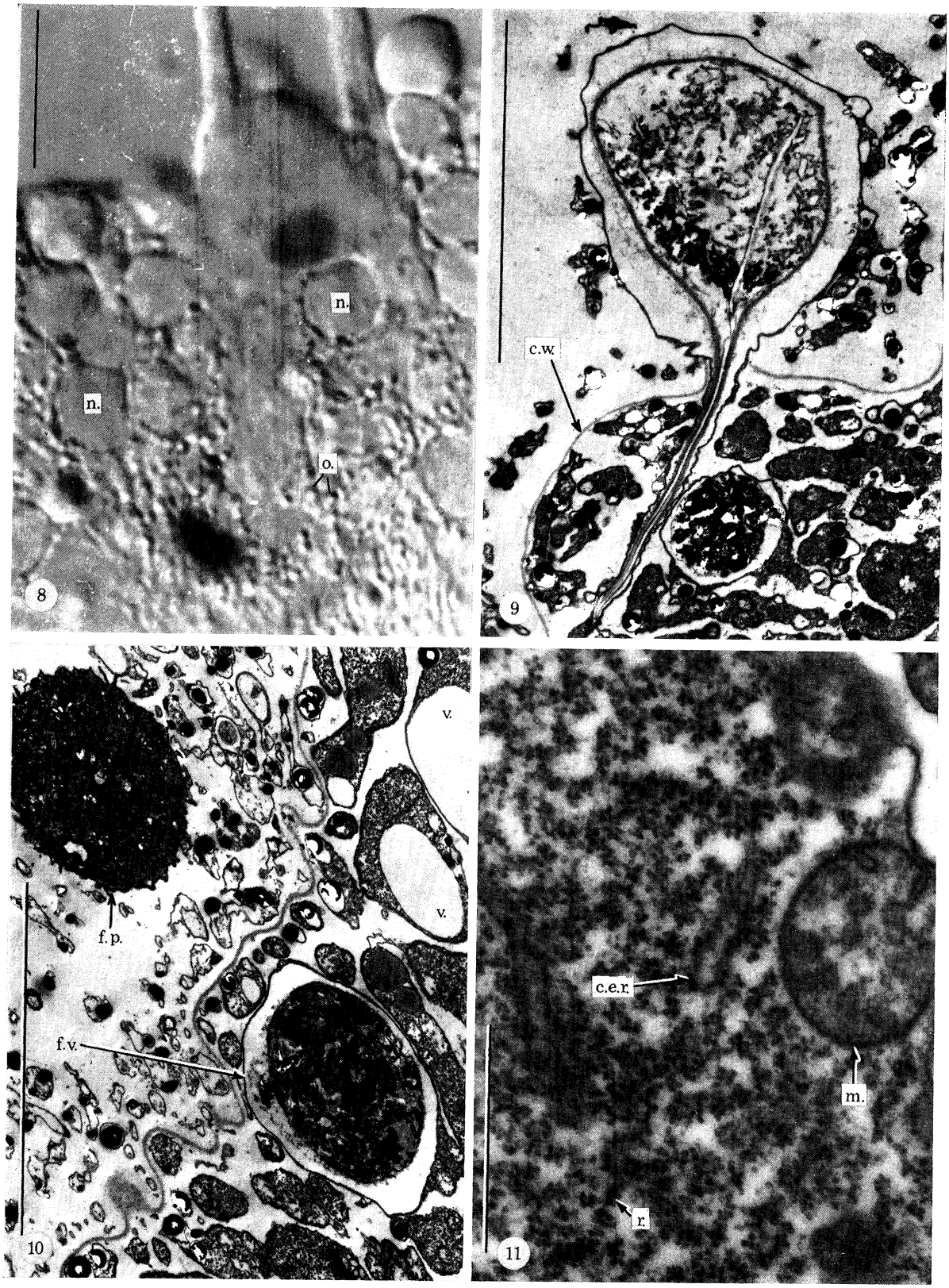
Description of plate 3

Figure 8. Edge of a living specimen of *G. albida* after 2½ h under a coverslip. The capsular wall has vanished. A number of nuclei (n.) can be seen, also innumerable osmiophilic globules (o.). (Scale: 10 μm.)

Figure 9. Food vacuole traversing the capsular wall (c.w.) via a perforation. (Scale: 10 μm.)

Figure 10. Part of the surface of a specimen of *G. albida* showing a faecal pellet (f.p.) outside the capsule and a food vacuole (f.v.) in the cortex within. The pellet is encapsulated by a thin layer of cytoplasm. Two large, empty vacuoles (v.) which may have contained oil or refractive crytals, can also be seen. (Scale: 10 μm.)

Figure 11. Cortical cytoplasm. Note the abundance of polysomes (r.) and the cisternae of coarse-surfaced endoplasmic reticulum (c.e.r.). A mitochondrion (m.) is also present. (Scale: 0.5 μm.)



FIGURES 8–11. For description see opposite.

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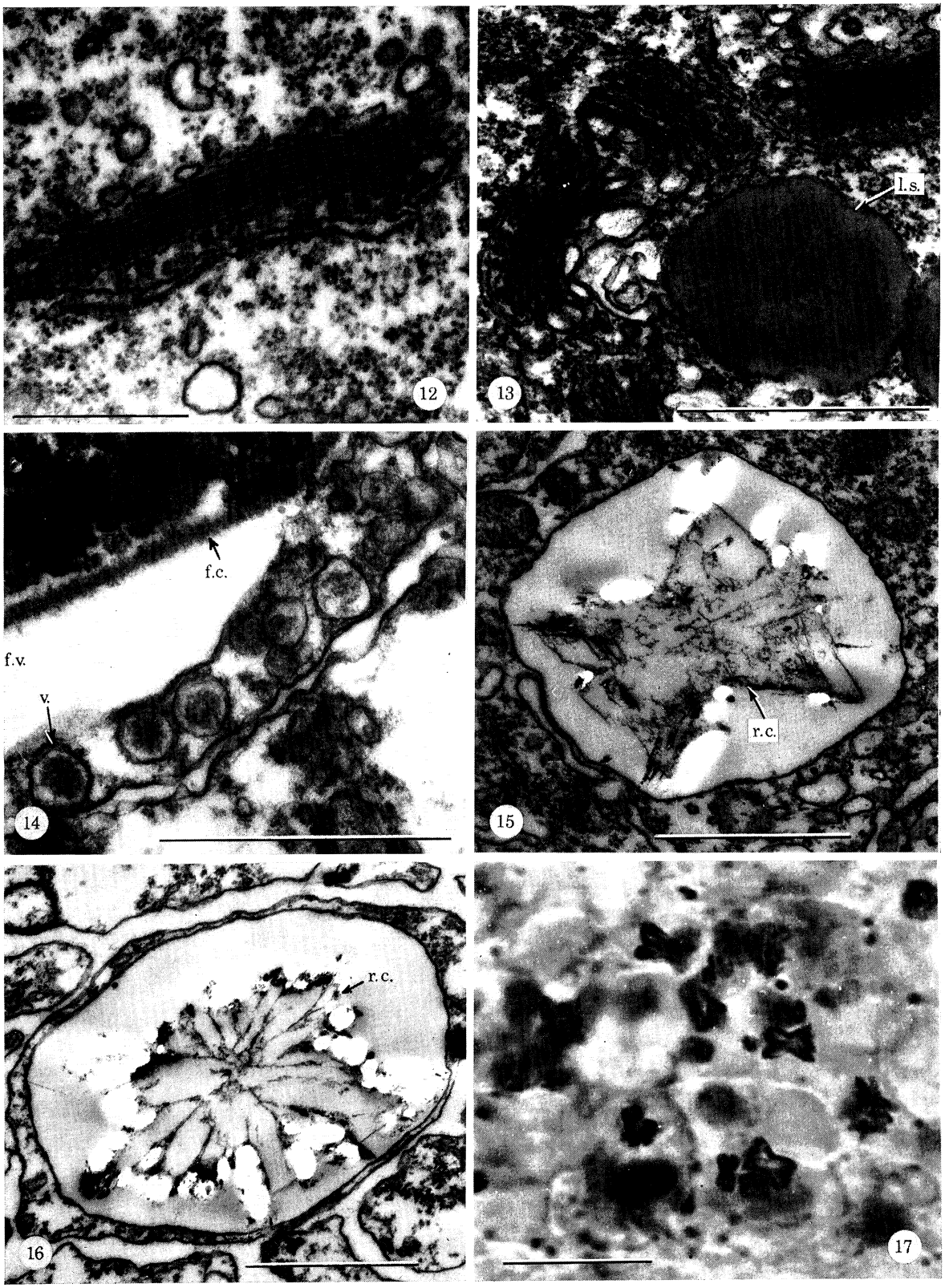

FIGURES 12–17. For description see opposite.

centrally, the osmium tetroxide not having penetrated completely before sectioning and staining took place. Many are enclosed in a somewhat loosely fitting membrane, but usually a membrane cannot be discerned, presumably because of the opacity of the contained material.

The globules make fascinating movements in the living animal. Quite apart from a continual Brownian agitation, they are prone to large displacements, either singly or in trains or clusters, along the axopodia and anastomosing strands of the network interconnecting the axopodia on partially squashed specimens. The movement is variable and seems relatively haphazard. It is rather like that of pedestrians jostling one another in a busy street. At any instant in time globules may be moving in opposite directions along the same axopodium. One may collide with another and be carried back some distance before, as it were, side-stepping and continuing along its original course. Other globules may overtake, or catch up. Some move through long distances while others remain stationary; others pass down between two moving up. Some move to the tip of an axopodium along one path and then return along a different course; others appear to move backwards and forwards along the same course. Even in the narrow strands of the network the globules can move in opposite directions past one another, although commonly they tend to remain like a string of beads. They can move along bent or buckled axopodia. Associated with their movement one sees shallow bulges and constrictions running in either direction along the plasma membrane of the thicker axopodia. One bulge was seen to pass several globules in its path. Bulges have also been seen to collide and ride up over one another. These observations, coupled with the fact that the movement is possible in crumpled axopodia and in cytoplasmic strands, suggest that the axonemes are not directly responsible, but that tensions in the plasma membrane and protoplasmic streaming movements are more likely motivating agencies. Studies of particle movement in the axopodia of *Echinosphaerium nucleofilum* by Edds (1975 *a*, *b*) and Fitzharris & Bloodgood (1972) have indicated that the axoneme is not necessary for the motion. However, it must not be overlooked that in squashed preparations of *Gymnosphaera* particles can be seen moving along the axonemes in the medulla, and that in electron micrographs bridges linking vacuoles to the microtubules are sometimes visible.

The whole surface of the body of *Gymnosphaera* tends to be stippled, somewhat patchily, by the globules. It seems likely that they are responsible for the stickiness of the axopdia, which adhere readily to glass, plastic or metal. The chemical nature of their contents requires to be

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Description of plate 4

Figure 12. Section of a Golgi body of *G. albida*. (Scale: 0.5 μm.)

Figure 13. A lipoid spherule (l.s.) and aggregation of Golgi bodies in the cortex. (Scale: 1 μm.)

Figure 14. Part of the wall of a food vacuole (f.v.) in the cortex. Note the small vacuoles (v.) containing material to be discharged possibly into the vacuolar cavity. The food is enveloped by a fibrous coat (f.c.), possibly a cuticle or cell wall. (Scale: 1 μm.)

Figure 15. Vacuole containing the remnants of a refractive crystal (r.c.). The shape resembles a diabolo wheel in longitudinal section. (Scale: 1 μm.)

Figure 16. Another vacuole containing the remnants of a refractive crystal (r.c.) seen in transverse section. The clear areas are probably the result of gas escaping when the crystal vaporized under the heat of the electron beam. Note the thermostable component ensheathing the crossed rodlets. (Scale: 1 μm).

Figure 17. Refractive crystals of *G. albida* seen by optical microscopy (× 100 oil immersion; Nomarski optics). (Scale: 10 μm.)

investigated. Besides their osmiophilia, I can only state that they are insoluble in ethanol and xylol and that they are refractive.

Kinetocysts (haptocyst-like organelles) such as occur in centrohelidian Heliozoa (Bardele 1969, 1971; Davidson 1972, 1973*a*, *b*) and *Clathrulina elegans* (Bardele 1972) are completely absent from *Gymnosphaera*.

(*e*) *Sassaki's line*

Between the reticulated pseudopodial layer and the cortex, at the level of Sassaki's line, there occurs a space containing a thin layer of finely fibrous material (figures 5 and 7, plate 2). The space usually has a width of about 1 μm (0.3–1.75 μm) and the fibrous layer is 75–100 nm thick. No unit membrane is associated with this fibrous material, the appearance of which suggests a coagulum. The fibres appear to be beaded in places, the beads possibly being other fibres cut in transverse section, or places of contact between fibres, but the detail is too fine to be distinguishable at the highest magnification employed (120000). The fibres tend to run in the tangential directions, while others extend in places from the main layer up towards the cytoplasm on each side, forming a loose network. Where each axopodium protrudes, the fibrous layer is perforated and deflected outwards to form a short sleeve surrounding the base of the axopodium (figure 7, plate 2). The axopodium almost fills the sleeve, but occasionally one finds a cytoplasmic strand running alongside the axopodium within the sleeve. The rheoplasm sends out connections to the cytoplasmic strands above and below the fibrous layer. The length of the sleeve varies from 0.8 to 2.7 μm and the diameter from 0.5 to 1.7 μm. The sleeve is largely cylindrical, but widens at its base like the tube of a trumpet. The orifice at the distal end is not always strictly transverse to the axis of the sleeve. Very occasionally the fibrous layer is simply perforated, a cytoplasmic strand penetrating through it without any deflection of the margin whatsoever.

The fibrous layer often has a wavy course on the sections, possibly because of retraction of the axopodia. However, when the outline of the protozoon is polygonal (figure 4, plate 2), the

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Description of plate 5

Figure 18. Section of an interphase nucleus of *G. albida*. Note the four nucleoli around the perimeter and the 'central chromatin body' (c.c.b.) at the centre. The nucleus is enveloped by a thin layer of cytoplasm which is connected to the cortical strands adjoining by narrow bridges (b.). Two of the nucleoli bear conspicuous 'nucleolar organizers' at their surface facing inwards. (Scale: 1 μm.)

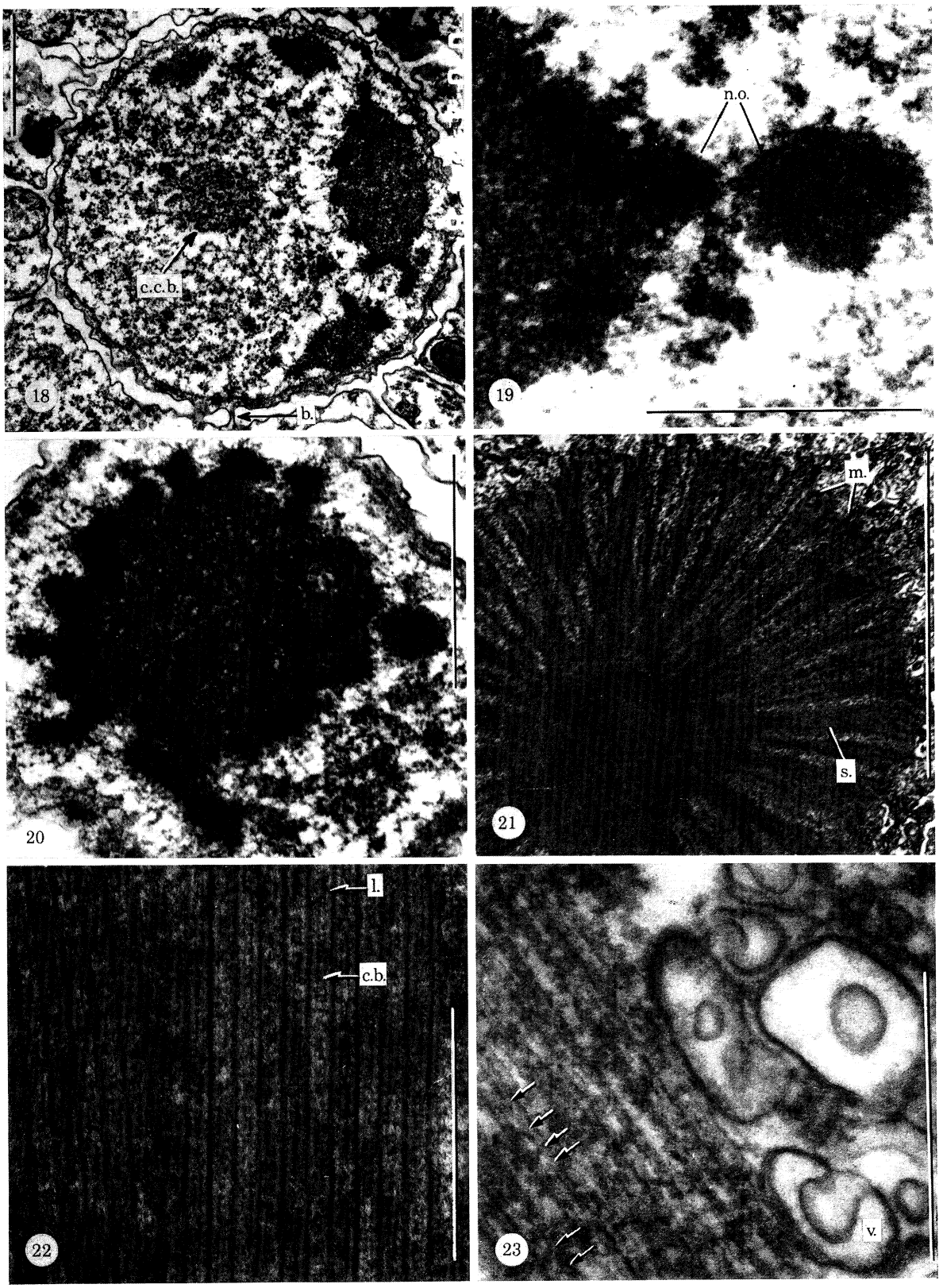
Figure 19. The edge of a nucleolus showing the pars granulosa and two nucleolar organizers (pars fibrosa) (n.o.), one of which is apparently separated from the nucleolus. (Scale: 1 μm.)

Figure 20. Another nucleolus with peripherally distributed nucleolar organizers, some of which appear to have separated and to have been uncoiling and dispersing in the nucleoplasm. (Scale: 1 μm.)

Figure 21. Large axoplast from which the microtubules have dissolved producing patches of electron dense matter at the periphery (m.). The axonemal sheaths (s.) persisted and have a parabolic course, larger ones enveloping several smaller ones in places, suggesting that axonemal branching had occurred. The depolymerization of the microtubules was probably not caused by the method of fixation used, because in other specimens fixed at the same time the microtubules remained intact. (Scale: 1 μm.)

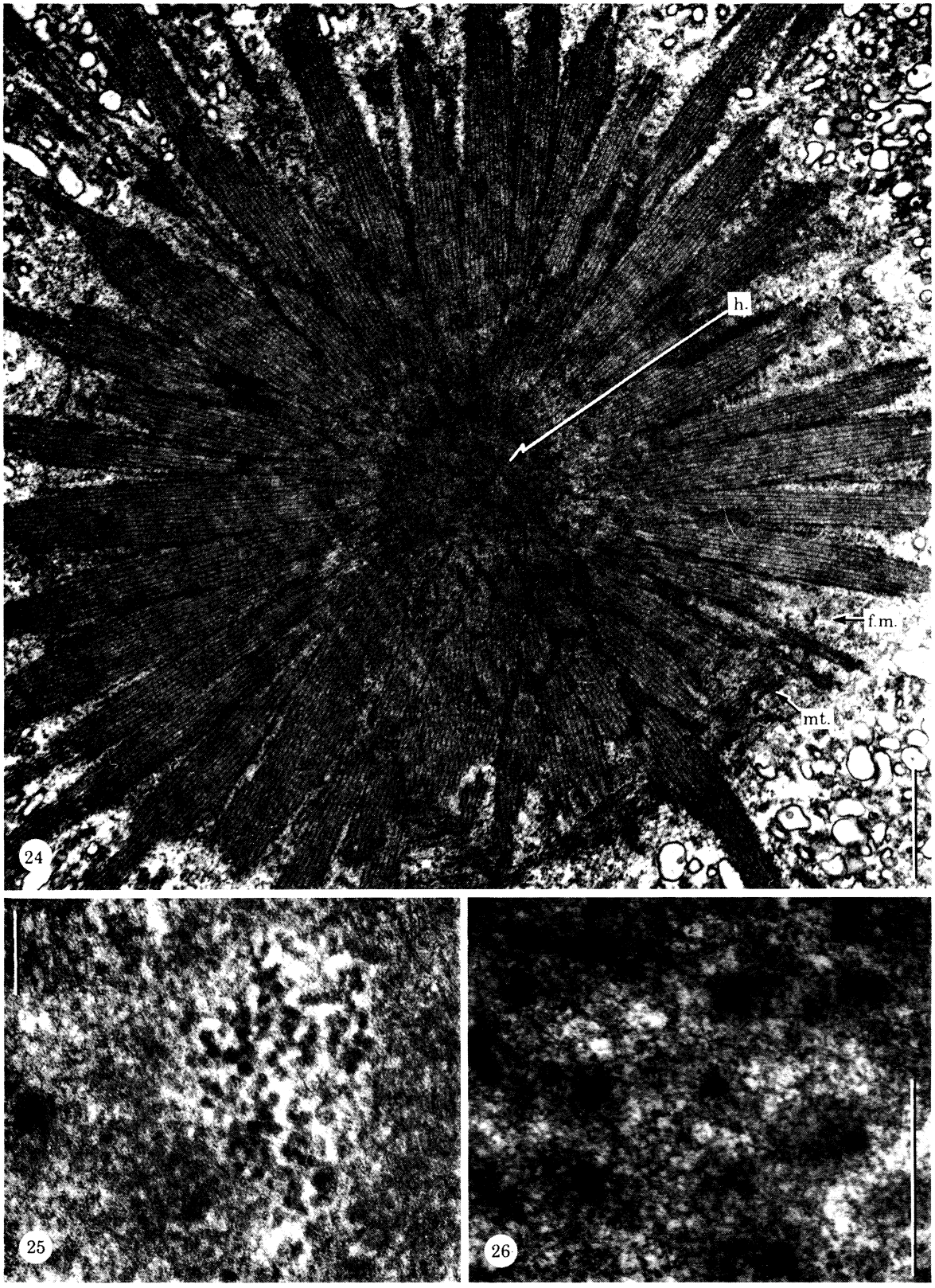
Figure 22. Longitudinal section of two axonemes in the shell region of a centriaster of *G. albida*. Note the ladder-like appearance caused by the regular arrangement of the cross-bridges (c.b.). The parallel electron-dense lines (l.) arise from the partial overlap of microtubules (see figure 33). (Scale: 0.5 μm.)

Figure 23. Longitudinal section of an axoneme situated about one third of the way between the surface of the centriaster and the surface of the organism. Cross-bridges are more spaced apart (see arrows) and the spacing is uneven in places. Vacuoles (v.) with part of their wall invaginated occur in the vicinity of the axoneme. (Scale: 0.5 μm.)



FIGURES 18–23. For description see opposite.

(*Facing p.* 358)



FIGURES 24–26. For description see opposite.

fibrous layer appears to extend more or less directly from axopodium to axopodium. It is always accompanied by cytoplasmic strands, on the inside at least.

The fibrous layer occurs at the level at which one would expect to find a central capsule membrane were *Gymnosphaera* a member of the Acantharia or Radiolaria. However, the absence of a unit membrane at its inner and outer surfaces distinguishes it from the capsular membrane of most of the Radiolaria, in which also the membrane consists of irregularly polygonal plates of up to 1 μm thickness juxtaposed to form a single layer (Hollande *et al.* 1970). To avoid confusion I shall refer to the fibrous layer of *Gymnosphaera* as a 'capsular wall'. It is an extracellular secretion occupying a space that is continuous with the environment, whereas in many of the Radiolaria, if the interpretation of Hollande *et al.* be correct, it is enclosed by a unit membrane and could well be intracellular.

Before squashing the animal to the stage exhibited in figure 2, plate 1, one passes through the condition in which the capsular wall appears as a series of roughly concentric, crinkled lines near the periphery as the focus level is altered. In surface view one gets the impression of branching curved ridges at the appropriate level of focus, the ridges being bordered on each side by a row of refractive globules. The crinkling of the wall is probably caused by tensions developing in the outer cytoplasmic strands as a result of the partial squashing. More complete flattening reveals the wall as a single uniform band all round the periphery of the animal, perforated only for the protrusion of the axopodia. In this stretched condition no sleeves are to be seen round the bases of the axopodia. The capsular wall can remain for 1–2 h in this state before vanishing first from some regions and then completely (figure 8, plate 3). Nuclei and inclusions can be seen to cross the wall in places, again indicating that the material is labile. In the normal animal the wall has to be crossed by food and faeces (figures 9 and 10, plate 3). The lability of the wall is also confirmed by its absence in e.m. sections of some *Gymnosphaera* in the course of multiple fission and of other specimens probably involved in the production of isogametes (to be published). However, the wall also possesses some tensile strength, because there are times when, under a coverslip the reticulated pseudopodial layer becomes stripped off and the wall then retains the capsular contents. Under pressure it can burst in places, allowing the cytoplasm to flow out to some extent. When 75 % seawater is drawn under the coverslip the vacuoles within the capsule swell and the wall appears to become more flexible and extensible. The vacuoles bulge out the wall here and there. However, one must not conclude that water can readily penetrate through the capsular wall; the axopodia also swell in the hypotonic medium indicating that water can enter via the axopodial cytoplasm. When hypertonic media are employed, for example, 0.75 M NaCl, one finds that the capsule shrinks only slightly,

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Description of plate 6

Figure 24. An axoplast of *G. albida*. The section passes almost through the centre. No distinctive structure is present in the central hyalosphere (h.). The proximal ends of the axonemes are embedded in a finely fibrogranular matrix (f.m.). Note the secondary centre of radiation in the lower hemisphere, suggesting that the process of binary fission or budding has been initiated. At the bottom right can be seen some tangentially running microtubules (mt.). The axoplast is embedded in a finely vacuolated medulla. (Scale: 1 μm.)

Figure 25. Portion of the hyalosphere of an axoplast of *G. albida* revealing a patch of ribosome-like granules in the fibrillar matrix. (Scale: 0.1 μm.)

Figure 26. Part of the hyalosphere of another axoplast, showing a number of electron-dense granular bodies in the fibrillar matrix. This axoplast had recently been formed by a process of division from a precursor axoplast. (Scale: 0.5 μm.)

whereas the capsular contents, which become more turbid, seem to shrink more. The cytoplasm appears to separate from the wall, leaving a vaguely defined space in between. Re-application of normal seawater results in the expansion of the central mass of cytoplasm as would be expected. In the hypertonic medium the axopodia rapidly shorten and vanish, demonstrating that it is the wall that resists the enforced shrinkage at the surface, rather than the axonemes.

The capsular wall of *Gymnosphaera* delimits the central capsule, which comprises the cortex and medulla. The terms seem somewhat inappropriate, but they will be retained because they, or rather, their German equivalents, were used by Sassaki and there seem to be no better alternatives. Terms such as 'ectoplasm' and 'endoplasm' could lead to confusion: the capsular membrane of the Radiolaria separates the so-called ectoplasm from the endoplasm (Trégouboff 1953*b*), whereas in the Acantharia it is regarded as occurring within the ectoplasm (Schewiakoff 1926), the cytoplasm just within the membrane being indistinguishable in structure and content from that outside. Likewise in *Gymnosphaera* the cytoplasmic strands just within the wall may be no different from those outside, at least in parts here and there around the periphery of the organism (figure 5).

(*f*) *The cortex*

Like the reticulated pseudopodial layer the cytoplasm just within the capsular wall consists of a tangle of branching and anastomosing strands, some of which may be band-like. Some of the superficial strands contain little in the way of inclusions, apart from osmiophilic globules, which tend to be concentrated in a thin zone just within the capsular wall. Elsewhere the cytoplasm of the cortex contains numerous inclusions and tends to be densely packed with ribosomes, which are strung together in clusters (polysomes; figure 11, plate 3). Single clefts of coarse-surfaced endoplasmic reticulum also occur within the cytoplasm of the strands, and frequently one sees, close to the unit membrane at the surface of the strands, a single cleft of endoplasmic reticulum, 25–33 nm wide, the innermost membrane of which bears attached ribosomes (figure 5, plate 2).

The cytoplasmic network is not confined to the outermost region of the cortex. In fact the whole of the cortex (and possibly part of the medulla as well) is constituted by a network of thick protoplasmic tracts separated from one another by narrow clefts. These clefts are not tubules, because the appearance is the same regardless of the level of the section through the organism. They represent a continuous, three dimensional system of crevices penetrating through the cytoplasm. The nuclei in particular, each encased in a thin envelope of cytoplasm, are generally almost completely surrounded by a narrow gap, which is here and there crossed by a few protoplasmic bridges (figure 18, plate 5). Narrow processes sometimes join one cytoplasmic tract to an adjacent one, and such bridges probably give rise to the curled filaments and apparently isolated material occasionally seen in the gaps. The width and extent of the spaces varies according to the method of fixation employed. With 5% glutaraldehyde in seawater followed by post-osmication the strands appear rounded and widely separated from one another. When the osmotic pressure of the fixative is the same as that of seawater, however, the clefts are narrow and the system opens relatively infrequently around the periphery of the cortex into the space beneath the capsular wall.

Apart from the ribosomes and coarse-surfaced reticulum already mentioned, the cortex contains many mitochondria and Golgi bodies (dictyosomes), lipoid spherules and vacuoles of various sizes and types, but so far no symbiotic zooxanthellae have been observed. The nuclei

occur in a broad zone occupying roughly the mid-region of the cortex. One also sees the axonemes, not separated from the rest of the cytoplasm by a sheath. The osmiophilic globules become much less numerous as one moves inwards from the tracts immediately subjacent to the capsular wall.

The cytoplasm of the cortex usually appears to be metabolizing at a very high rate. The abundance of ribosomes has already been mentioned, while the mitochondria and Golgi bodies are present in high concentration, the latter generally near or within the zone of nuclei. One area of 16 μm^2 was found to contain 8 sections of mitochondria and 7 of dictyosomes. A different area of 100 μm^2 contained 55 sections of mitochondria and 8 of dictyosomes. Golgi bodies may be so close that two share the same cisterna of coarse-surfaced ergastoplasm. The relationship between the latter and the Golgi elements appears to be the same as that found by Friend (1965) in the cells of the Brünner's gland of the mouse and by Jamieson & Palade (1967) for the exocrine cell of the cavy pancreas. A cisterna of ergastoplasm, 25–60 μm wide, bearing ribosomes on the membrane farther from the Golgi body and evaginations on the other, nearer membrane, buds off small vacuoles, 33–55 nm in diameter, which proceed towards, and fuse with, the outermost of the series of flattened, 10 nm wide cisternae that are forming vacuoles peripherally (figures 12 and 13, plate 4). The Golgi cisternae are sometimes flat, sometimes strongly curved with large, sparsely granular (presumably condensing) vacuoles in the concavity. At times it appears as if the whole of the innermost cisterna is expanding to form a condensing vacuole. The Golgi bodies usually comprise eight cisternae and are about 1 μm long in section. Their overall width lies in the range 0.15–0.19 μm. Occasionally much smaller vacuoles with a fuzzy membrane ('coated') occur near the distal (concave) surface of the Golgi bodies. These vacuoles are not unlike the transport vacuoles on the proximal side, although at times they are somewhat larger (figures 12 and 13). They may occur in contact with the innermost (most distal) cisterna. Coated vesicles also occur in the medulla (see later).

The mitochondria are bounded by two approximately 6 nm thick membranes, making a total wall thickness of about 16 nm. The inner membrane projects inwards as tubular invaginations, between which one commonly finds wisps of material. The appearance of the mitochondria is somewhat variable, ranging from those whose cavity is almost filled by long invaginations to those which are practically empty, with only a few short invaginations, and finally to those containing nothing but wisps of material. The largest mitochondrion measured on a section was 2.53 μm × 0.6 μm.

The lipoid spherules (figure 13, plate 4), which presumably constitute the reserve food material, have a diameter reaching 1.15 μm. Usually their boundary stains heavily and then appears finely granular, but a distinct membrane is not exhibited. They can occur at more or less any level in the cortex.

Vacuoles containing food in various stages of digestion, or compacted faecal material, occur in the cortex (figure 10, plate 3). Sometimes the cavity surrounding the ingested organism appears to be empty; at others a diffuse coagulum is present. The vacuoles are enclosed by a wall of cytoplasm that is bounded on the internal and external surfaces by a unit membrane. This wall may be extremely thin, consisting of little more than the two unit membranes closely apposed. Here and there it is thicker and contains the inclusions normally found in the cytoplasmic tracts. Where the vacuole appears to be floating in isolation in the cavity system of the cortex, this is probably the result of the plane of the section missing the adjoining parts of the cytoplasmic tract in which the vacuole is presumed to be embedded. Sometimes a row of small

vacuoles is seen in the cytoplasmic envelope of a food vacuole (figure 14, plate 4). These small vacuoles have a diameter of 0.2 μm and they contain a moderately electron-dense conglomeration surrounded by an electron-lucent halo. They could well contain material which is to be liberated into the vacuole. The faeces (figure 10, plate 3) appear to be encapsulated only by a thin wall of cytoplasm, so that only digestive juices need be secreted into the food vacuole. The fibrous coat visible around the ingested material in the vacuoles shown in figure 14, plate 4, and figure 9, plate 3 is probably the remains of the pellicle of the prey. It can be seen from figure 9 that the whole vacuole, including its cytoplasmic envelope, penetrates through the capsular wall of the gymnosphaera. The vacuole in this case appears to contain a partially digested small vorticellid; I have seen these taken. Whether the vacuole was in the process of entering or leaving the capsule in this case cannot be decided with certainty. The outward curl of the rim of the perforation in the wall suggests the latter, but might well have been caused by shrinkage of the capsular contents during fixation.

Another category of large vacuoles is shown in figures 15 and 16, plate 4, figures 3 and 4, plates 1 and 2, and figure 10, plate 3. These vacuoles usually contain a membranous formation of about 1–2 μm diameter. Sometimes they contain nothing, or just traces of material. Optical studies of living *Gymnosphaera* have revealed the presence of refringent crystals, the properties of which will be discussed in the next section. The membranous formations seen with the e.m. are the remnants of these crystals. When such remnants are out of the plane of the section, the vacuole appears to be empty. However, it is possible that some of the empty vacuoles may have been food vacuoles from which the prey has been completely digested away, or they may have originally enclosed oil droplets. Oil would tend to dissolve in the alcohol used when dehydrating the specimens. However, oil droplets have not been detected in the living animal, and neither have excretory vacuoles. The vacuoles with crystal remnants are usually enveloped by a more extensive layer of cytoplasm than are the food vacuoles, which tend to be much larger.

Finally one commonly finds small vacuoles (roughly 40–120 nm in size) arranged around the axonemes seen in transverse section in the cortex. These will be considered in more detail in the section dealing with the medulla.

The lipoid spherules, food vacuoles and refringent crystals can be easily seen by optical microscopy in flattened specimens of *Gymnosphaera*. Generally these inclusions are surrounded by a thin, hyaline streak, representing the enveloping cytoplasm. The dictyosomes and mitochondria cannot be discerned, nor are any clefts or cavities visible other than those contained in vacuoles. The spaces seen by Sassaki (1894) on fixed preparations are artefacts, as he surmised.

(*g*) *The refringent crystals*

The crystals have the shape of crossed rods or diabolo wheels (figure 17, plate 4). On the electron micrographs some appear to be stellate in form, but these are probably the diabolo wheels seen end-on; examples of the remnants seen in side view and end-on view are shown in figures 15 and 16, plate 4, respectively. These suggest that organic matter envelopes and penetrates into the crystal substance, forming a delicate sheath around parts of this. The crystals vary in size in any given specimen of *Gymnosphaera*. The largest in one was 3.2 μm long × 1.6 μm wide at the maximum width. In another the corresponding measurements for the largest were 2.7 μm × 2.5 μm, but other crystals had dimensions of 2.25 μm × 2 μm, 2.1 μm × 1.6 μm and 1.6 μm × 1.1 μm. The crystals are nearly always plentifully present in individuals of average to large size, but their distribution can vary. Often one finds them concentrated in a zone in the inner-

most parts of the cortex. At other times they seem more uniformly dispersed in the cortex, and at others again, they are concentrated more just beneath the capsular wall, with some occurring at the surface and on some axopodia. Possibly they represent an excretory product which is eliminated at a certain phase or phases, of the life cycle. The crystals are not present in the biflagellated isogametes.

When seen by optical microscopy, the crystals generally occur in spherical vacuoles and display Brownian movement, even after osmication. However, in some specimens the enveloping vacuole and vibratory motion could not be detected. It is known that the heating effect of the microscope lamp, or the pressure of the coverslip, can induce vacuole formation around the crystals occurring in *Amoeba proteus* (Byrne 1963). Vacuoles are always present around the crystals of *Gymnosphaera* when examined by means of the electron microscope. The most one can claim, therefore, is that an enlargement of the vacuoles may occur when living specimens are microscopically examined. In some living specimens the vacuolar diameter was more than twice the length of the contained crystal. Sometimes two crystals were found in the same vacuole. Vacuoles containing crystals have been seen on axopodia. Isolated crystals have also been found at the surface of fixed and stained preparations, supporting the view that the crystals are an excretory product.

When squashed preparations of *Gymnosphaera* are mounted in DPX and examined between crossed nicols, the crystals are found to be birefringent. They behave quite like the straight monact spicules of calcareous sponges, which consist of single crystals of magnesian calcite (Jones 1970). Thus they appear dark when the axis of the diabolo wheel lies in or at right angles to the plane of the polarized light. This axis coincides with the optic axis of the crystalline material therefore. When the analyser nicol is removed, the crystals become indistinct when their axis lies in the plane of polarization, and sharply defined when it is at right angles. The refractive index of the extraordinary ray must thus be close to that of DPX, namely about 1.5. Judging from the Becke line effect, it is probably slightly less. Use of a quartz wedge indicates that the crystals are uniaxial negative. The strong birefringence and shape preclude the possibility that the crystals are of strontium sulphate, crystals of which are stated to occur in certain Radiolaria (Trégouboff 1953*b*), or of carbonyldiurea, of which bipyramidal crystals, of length 2–7 μm, are present in *Amoeba proteus* (Carlstrom & Møller 1961).

That the crystals are not of strontium sulphate was confirmed by immersing a squashed, fixed specimen of *Gymnosphaera* in a saturated solution of strontium sulphate for 2 h at room temperature (22.5 °C). All the crystals had dissolved in this time, whereas they were still present, apparently unaffected, in another specimen treated for the same time with distilled water. The pH of the strontium sulphate solution was 4.6, while that of the water was 5.8.

By using similar preparations it was found that the crystals dissolve rapidly in acids and fairly quickly in the acid alcohol used for the differentiation of the haemotoxylin-stained material. They dissolve in 0.1 M hydrochloric and 0.05 M sulphuric acids and in phosphoric acid within the period of a minute or so and without effervescence, a result which would seem to preclude carbonate. The application of sulphuric acid did not result in the formation of needles of calcium sulphate. The crystals also dissolve in a matter of seconds in 5 % and 10 % sodium hydroxide solutions, without leaving any detectable residue. Pure calcite does not dissolve in caustic soda, but the magnesian calcite of calcareous sponge spicules does dissolve slowly with the formation of an insoluble inorganic sheath (Jones 1955). Saturated ammonium chloride (pH 4.2) dissolved the crystals, but only very slowly. Traces still remained after $4\frac{1}{2}$ h.

Substances such as calcite, calcium hydroxide and calcium hydrophosphate are soluble in ammonium chloride solution. However, these appear to be excluded by the ready solubility of the gymnosphaeran crystals in alkaline solutions. In any case the presence of calcium in the crystals could not be confirmed. Thus the crystals dissolved within seconds in 5% sodium hydroxide saturated with sodium fluoride, without leaving a detectable residue. A more direct test for calcium, namely the GBHA method (Kashiwa & House 1964), also proved unsuccessful; no red calcium chelate precipitated out when the crystals dissolved.

The application of heat until the cytoplasm had turned brown and shrunk somewhat (heating over a bunsen for $\frac{1}{4}$ h) resulted in the complete disappearance of the crystal birefringence. The resulting preparation had a brown medulla and brown axonemes radiating across a paler cortex. The axopodia had vanished, as had the crystals. In place of the latter were now irregular pallid blotches. These were larger than the original crystals, but their distribution and concentration remained the same. Under Nomarski optics they had the appearance of white concretions with rounded off, irregular boundaries, but could well have been gas-filled bubbles. Rounded vacuoles also develop instantaneously in the crystal vacuoles on sections in the e.m. as a result of the heating effect of the electron beam; the crystals are usually visible in the 1 μm control sections cut for optical microscopy. The effect could be due to the loss of carbon dioxide or water of crystallization. When crystals of calcium carbonate were heated in exactly the same way over a bunsen, some developed turbid contents, but their superficial parts retained the original form and birefringence. The turbidity, which is caused by calcination, became progressively more extensive as the heating time was increased to 1 h, after which time some birefringence was still detectable. Even though the calcite crystals employed were larger (4.5–20 μm) than the gymnosphaeran crystals (about 2 μm or less), so that longer heating would be required for the same effect, the absence of turbidity in the remains of the latter after heating would suggest that they are not composed of calcium carbonate.

Attempts to use the electron probe microanalyser on whole, squashed preparations of *Gymnosphaera* have so far proved disappointing. Only phosphorus and traces of sulphur were detected, but the sensitivity and resolution of the apparatus were insufficient to enable one to conclude that these elements were concentrated in areas of a shape and size similar to those of the crystals. Unfortunately the solubility of the crystals in dilute acids and alkalies, together with their small size, make extraction of the crystals from the cytoplasm a difficult, if not impossible task.

All of the evidence points to the conclusion that the crystals are completely organic in composition, with, after fixation, a thermostable component ensheathing and penetrating into a thermolabile component (figures 15 and 16, plate 4). The chemical nature of these components is at present obscure. Uric acid crystals can have the form of dumb-bells, prismatic rosettes and crossed sheaves of slender, curved needles. They are birefringent and soluble in alkalies and their crystalline form and birefringence are heat labile. However, uric acid is not soluble in dilute acid. Clearly, further research is needed on the nature of the crystals of *Gymnosphaera*, but one can state that they do not represent strontium sulphate, calcite, calcium orthophosphate, carbonyldiurea, or uric acid. A comparison between the gymnosphaeran crystals and those of *Wagnerella* and certain Acantharia will be given below (§*d*).

(*h*) *The nuclei*

On partially squashed preparations of living *Gymnosphaera* the nuclei appear as clear transparent vesicles (figure 8, plate 3), in which nucleoli are not readily discernible, even when using Nomarski optics. The diameters of the nuclei in a particular specimen can vary considerably. Thus one squashed specimen had nuclei ranging from 4.5 μm in diameter to 7.6 μm × 5.4 μm. Another had nuclei from 4.75 to 6.6 μm and a third, after Bouin–seawater fixation, nuclei from 4.3 to 6.1 μm. Several hundred nuclei may be present in a single specimen. According to Sassaki (1894) there may be many hundreds, but Caullery (1911) found a maximum per specimen of only 30. The nuclei are similar in size to those of the multinucleated foraminiferan *Gromia oviformis* (6 μm; Hedley & Wakefield 1969) and much smaller than the single nucleus of the marine heliozoan *Hedraiophrys hovassei* (20 μm on average; Febvre-Chevalier 1973*b*).

On sections the nuclei can be seen in the mid-region of the cortex, sometimes forming a single ring around the centriaster, but more usually being scattered more or less evenly across a broad circular band of up to about 4 nuclear diameters in width. Occasionally they occur at the outer surface of the organism, but only when the capsular wall has been ruptured or lost. The wall is incomplete or absent in specimens that are undergoing binary or repeated multiple fission, and in specimens at the surface of which are nuclei apparently participating in the formation of isogametes.

Each nucleus is always enveloped by a thin layer of cytoplasm (40–50 nm thick) which is separated from the remaining cytoplasm of the cortex by a cleft, except where a few cytoplasmic bridges cross. The cleft is continuous with the general cortical system of clefts, which communicates with the space beneath the capsular wall by a number of openings distributed around the circumference. There is the possibility of nucleo-cytoplasmic interchange of at least small molecules via this system in view of the narrowness and small number of cytoplasmic bridges per nucleus. No cell walls occur other than the plasma membrane lining the clefts. The organism is thus a syncytium.

The nuclei have a conventional double membrane with a narrow perinuclear space (8–12 nm wide) between the paired leaflets (each of 4–6 nm width). The perinuclear space has not been seen to communicate with the cavities of the endoplasmic reticulum, which is not surprising in view of the paucity of cytoplasm in immediate contact with the nuclear envelope. Also, as with the nucleus of *Hedraiophrys*, there are no ribosomes to be found on the outer surface of the envelope. However, each nucleus is well equipped with nuclear pores. These are more or less evenly distributed with a spacing from one to the next of 100–130 nm. In surface view they each exhibit an electron-dense 'core' (9 nm in diameter), surrounded by a dark ring, 8 nm thick, of external diameter 75 nm, the ring thickening to form eight spots (diameter 10 nm). Their structure thus resembles that of nuclear pores found in a variety of animal species (Franke & Scheer 1974).

The nucleus of *Gymnosphaera*, in contrast to that of *Hedraiophrys*, is not lined by a lamina densa (figure 18, plate 5) (explanations of the terms used for nuclear structures in this account are given by Bouteille, Laval & Dupuy-Coin 1974). Instead the nucleoplasm usually appears clearer around the periphery than in the centre, an effect probably caused by shrinkage as a result of OsO_4-fixation, because it is much less noticeable with glutaraldehyde followed by osmication.

The appearance of the nuclei can vary considerably from one specimen of *Gymnosphaera* to another, but the majority of the specimens examined had nuclei like that depicted in figure 18,

plate 5. This is assumed to represent the interphase stage in consequence. Several (up to 5) nucleoli are generally included in each nuclear section and these are distributed close to the nuclear envelope. However, Sassaki (1894) found only one nucleolus, sometimes two, per nucleus.

Each nucleolus comprises two components, the 'pars granulosa' and the 'pars fibrosa'. The former consists of a fibro-granular mass in which the granules are attached to filaments and tend to be arranged in parallel rows here and there. The granules generally measure 13.5–20 nm in diameter and are similar in size to the cytoplasmic ribosomes. The filaments are 7–8.5 nm thick. Some filaments at least continue outwards from the nucleolus towards the adjacent nuclear envelope or into the nucleoplasm on the opposite side. In some sections some of the filaments extend each to the core of a nearby nuclear pore, and exhibit a somewhat spiral course. The filaments emerging from a nucleolus have relatively few granules attached to them and these tend to be irregularly dispersed along their length. Embedded in the pars granulosa near its surface are islands of the pars fibrosa. These have a denser texture and consist of closely associated finer fibrils and smaller granules than those of the pars granulosa (figure 19, plate 5). The small granules (4 μm in diameter) resemble ribosomes in being, as it were, strung first on one side, then on the other, of each string. These islands have been interpreted as 'nucleolar organizers' in other species (see, for example, Satir & Dirksen 1971). At a certain stage in the nuclear cycle they protrude markedly from the surface of the pars granulosa and appear to be in process of either separation or aggregation. Similar bodies, but seemingly unattached to the nucleolus, can occur in the vicinity, and others, farther off, can be seen in a more dispersed state (figure 20, plate 5). The nucleolus doubtless 'fragments' and disperses in preparation for nuclear division.

The nucleoli are lenticular bodies reaching 2.4 μm × 0.9 μm in size. The 'nucleolar organizers' (up to 0.23 μm) generally occur near or on the surface facing inwards (figure 18, plate 5). They are distributed all round the periphery of the nucleolus on sections that have included the equatorial plane of the nucleolus (sections almost tangential to the nucleus). Organizers that are apparently separate from the nucleolus may be somewhat larger (for example, 0.32 μm × 0.25 μm and 0.48 μm × 0.15 μm) than those still attached, reinforcing the view that dispersion or aggregation was occurring. As many as 14 nucleolar organizers have been counted on a single section of a nucleolus. These must all be parts of the same chromosome, because when synaptinemal complexes are present within the nuclei, there is never more than one per nucleolus (Jones, to be published). Synaptinemal complexes are diagnostic for the prophase stage of meiosis. Such complexes have been seen in nuclei containing nucleoli with protruding nucleolar organizers, and even in some such nucleoli.

No 'fibrillar centres' (namely clearer areas consisting of protein fibres only) have been seen in the nuclei of *Gymnosphaera*. Very occasionally granules of the pars granulosa may not be evenly distributed so that somewhat clearer areas occur, but these cannot be regarded as fibrillar centres. The zebra-striped effect found in the nucleus of *Hedraiophrys* (Febvre-Chevalier 1973*b*) has never been observed in *Gymnosphaera*. Likewise distinct nucleonemata have not been detected.

Another feature of the interphase nucleus is the presence of a body near the centre (figure 18, plate 5). The largest measured was 1.06 μm × 0.81 μm, but commonly the cross section ranges from about 0.5 to 0.8 μm. Sometimes more than one of these bodies, of differing sizes, may be present in the same nucleus. Thus, one had two such bodies of 1.06 μm × 0.81 μm and 0.47 μm

× 0.65 μm respectively. The texture of these bodies differs from that of the pars granulosa of the nucleoli. They are fibrogranular, with the fibres intercrossing to form a mat and extending far outside of the body, in some cases apparently up to the nuclear envelope. The fibres are 3.5–6 nm thick. The granules (7.5–11 nm) are less concentrated than in the nucleoli. The mat gives the impression of dispersing at prophase. These bodies will be termed the 'central chromatin bodies' until a more precise knowledge of their function and relation to the chromosomes and nucleoli has been determined. They are not to be confused with the central body (interzonal material) left at the centre of the dividing nucleus of the slime mould *Physarum polycephalum* after anaphase (Goodman & Ritter 1969; Guttes, Guttes & Ellis 1968); the latter consists of fibres and amorphous material and disappears after telophase. The gymnosphaeran central bodies are perhaps better equated with the 'endosome' of spongy chromatin occurring in the macronucleus of the ciliate *Chilodonella cucullus* (Radzikowski 1973).

The remaining nucleoplasm of the gymnosphaeran interphase nucleus exhibits 7.5–11 nm thick fibrils bearing granules of 13.5–20 nm diameter, the fibres being concentrated here and there at random to form small mat-like chromatin bodies of variable size (for example, 125 nm, 78 nm × 152 nm, 210 nm). The fibres presumably represent parts of chromosomes. In parts of some nuclei several (commonly 2, sometimes 4) fibrils have been seen running in parallel for some distance.

The interpretation of the nuclear components would be facilitated by the use of RNA and DNA staining techniques, and studies of this kind are currently in progress. Observations which have so far been made concerning nuclear division and the occurrence in some nuclei of structures not reported above will be published at a later date.

TABLE 1. MEASUREMENTS MADE ON E.M. SECTIONS OF *GYMNOSPHAERA*

diameter of specimen/μm (*a*)	67	86	100	122
diameter of hyalosphere/μm (*b*)	1.2	1.7	2 × 2.4	3 × 4.8
quotient (*a*/*b*)	55.8	50.6	45.5	31.3
diameter of axoplast/μm (*c*)	7.6	8.7	12.2	16 × 18.8
quotient (*c*/*b*)	6.3	5.1	5.5	4.5
quotient (*a*/*c*)	8.8	9.9	8.2	7.0
shell thickness/μm (*d*)	3.2	3.52	5.0	6.75
quotient (*c*/*d*)	2.4	2.5	2.4	2.6
quotient (*b*/*d*)	0.37	0.48	0.44	0.58
maximum diameter of axoneme/μm	0.54	0.70	0.66	(0.92)

(*i*) *The centriaster and axonemes*

The most interesting structure in *Gymnosphaera* is undoubtedly the self-replicating centriaster. This is difficult to discern in unsquashed specimens, but readily becomes distinct in partially flattened examples, particularly when Nomarski optics are employed (figure 2, plate 1). It appears as a hyaline sphere enclosed in a thick shell composed of the radially arranged, tapering proximal ends of the axonemes. Very occasionally these are set at an angle to the radii, giving the appearance of a catherine-wheel in optical section, an effect also noticed by Sassaki (1894). The centriaster is a stable structure, persisting for some time after the parts of the axonemes distal to the shell have completely disappeared. Centriasters vary in size. In two living, partially flattened specimens of *Gymnosphaera* the diameters of the hyaline sphere ('hyalosphere' would be a convenient term) and the whole centriaster respectively were 3.6 and 17.5 μm for the first and 5.5 and 22 μm for the second. The ratios of the former to the latter diameters were thus 1:5

and 1:4 respectively. Corresponding measurements, made on e.m. sections passing through the centres of four gymnosphaerae, are given in table 1. They indicate that both the hyalosphere and the centriaster tend to increase in size more or less in proportion to the diameter of the whole organism. The ratio of the two diameters ranges from 1:31.3 to 1:55.8 for the hyalosphere, but allowance should be made for the approximation of the measurements; the organisms were not exactly spherical. One might have expected the ratio to remain constant, because binary fission probably involves a halving of the volumes of both hyalosphere and whole organism, so that the ratio of their diameters in each daughter cell would be the same as in the parent. However, this is not the case. The ratios of hyalosphere diameter to centriaster diameter, and of centriaster diameter to diameter of the organism, vary respectively from 1:4.5 to 1:6.3 and 1:7 to 1:9.9. These one would not have expected to remain constant, because after binary fission the shell thickness in the daughters could well equal that in the parent, the centriaster simply having separated into two after passing through a figure of eight configuration. Moreover Sassaki (1894) noted a change in the proportion of the hyalosphere to the shell size, at least in a gymnosphaera that appeared to be about to divide. He also stated that in very young specimens, while the shell is present, the central body (hyalosphere) is lacking. The relation between the sizes of hyalosphere, shell and whole centriaster is clearly not simple. Data in table 1 indicate that the shell thickness in fact increases with increasing diameter of the organism, and that the quotient c/d, but not b/d, remains constant more or less, at least between the size limits measured. Thus the shell thickness is a constant fraction of the centriaster diameter, but is not closely correlated with the diameter of the hyalosphere. As regards the diameter of the widest axoneme, there would be no reason to expect this to be correlated with the diameter of the organism once the latter had exceeded a certain size. More measurements are needed to clarify

Description of plate 7

Figure 27. Transverse sections of the proximal tips of axonemes in the innermost region of the axoplast shell. The basic bilaterally symmetrical hexagon of the stereoplasmic pattern can be seen. Cross-bridges are absent, or weakly developed. Axonemal sheaths likewise tend to be absent. (Scale: 0.1 μm.)

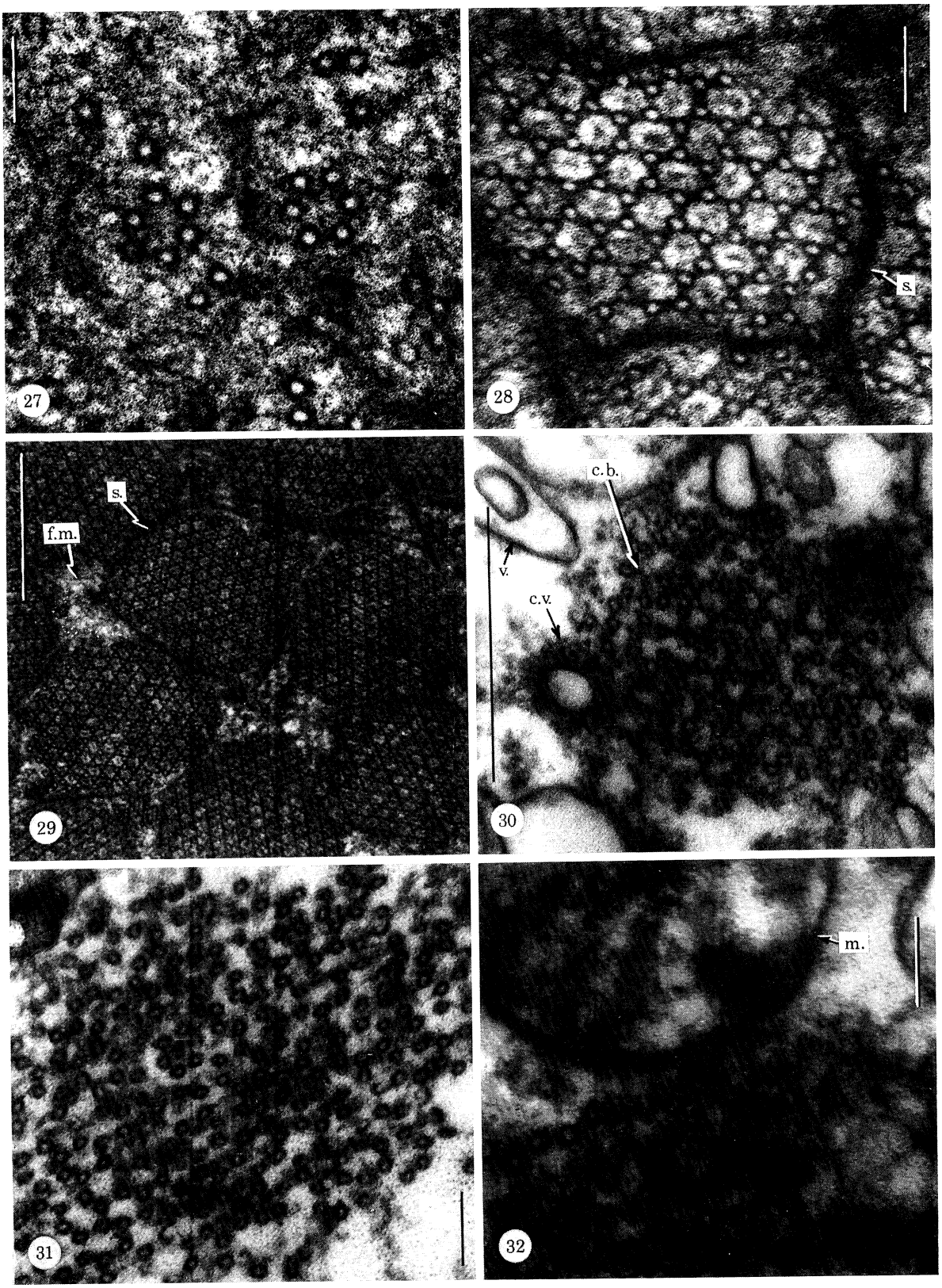
Figure 28. Transverse sections of axonemes from the mid-region of the axoplast shell. Cross-bridges (some double) interconnect the microtubules to form an arrangement of alternating rows of irregular hexagons. The principal transverse direction in the central axoneme runs from top left to bottom right of the figure. The alternating rows are parallel to this. Note the thick, fibrous sheath (s.). (Scale: 0.1 μm.)

Figure 29. Transverse sections of axonemes near the surface of the centriaster. In between the axonemes can be seen the microfibrillar matrix (f.m.) containing some electron-dense patches and, here and there, some small groups of microtubules. These presumably represent the tips of additional axonemes situated more distally. Axonemal sheaths (s.) in general only partially envelope the axonemes and sometimes course round several, suggesting that branching has occurred. The principal transverse directions of adjacent axonemes have different orientations. (Scale: 0.5 μm.)

Figure 30. Transverse section of an axoneme in the medulla outside the centriaster. No axonemal sheath can be seen and cross-bridges (c.b.) are only visible between some of the microtubules. The central dark area seen in the hexagons in sections at more proximal levels is generally absent, but the alternating rows of hexagons can still be seen. Note the two kinds of vacuoles, one coated with fibrillar material (c.v.) and the other commonly with a partly invaginated wall (v.). Coated vacuoles in the vicinity of the axoneme generally appear to be attached to them. (Scale: 0.5 μm.)

Figure 31. Transverse section of an axoneme in the cortex. The pattern of alternating rows of hexagons can be made out, but cross-bridges are absent between many of the microtubules. The central dark area likewise is absent in most hexagons. (Scale: 0.1 μm.)

Figure 32. Section near the outer edge of the medulla showing a mitochondrion (m.) connected by filaments to an axoneme. The filaments join on to the cross-bridges as well as microtubules. (Scale: 0.1 μm.)



FIGURES 27–32. For description see opposite.

(*Facing p.* 368)

this relationship, particularly as the axonemal diameters of the largest specimen measured were only approximate, the microtubules having disorganized prior to fixation (figure 21, plate 5).

The centriaster stains with eosin and toluidine blue, but never exhibits a conspicuous, densely staining granule within its central hyaline sphere. One is thus correct in designating the centriaster an 'axoplast' in the sense of Cachon & Cachon (1972*a*) and not a 'centroplast', a term first used by Dobell (1917) for the minute sphere containing at its centre a densely staining granule in the heliozoan *Oxnerella maritima*. The electron microscope has recently revealed that this granule in other centrohelidian Heliozoa consists of three parallel, electron-dense disks (Bardele 1971; Davidson 1972), but nothing comparable has been seen in *Gymnosphaera* by myself. Instead the hyalosphere exhibits a fibrillar texture (figures 25 and 26, plate 6), the fibrils tending to run in parallel in some places at least. Occasionally one finds patches of ribosome-like granules (10–12 nm in diameter; figure 25). Microtubules also may be seen here and there. Sometimes some areas stain more intensely than the rest, without there being any obvious difference in structure at high magnification. In the Radiolaria the axoplast likewise consists of an entanglement of microfibrils (6 nm thick), but in some groups these are embedded in a granulum (Cachon & Cachon 1971, 1972*a*, *b*).

The shell surrounding the hyalosphere in *Gymnosphaera* consists of the proximal ends of the axonemes, each of which is enveloped by a densely staining fibrillar sheath (figure 28, plate 7). The sheath is absent at the proximal tip of each axoneme so that the matrix of the shell is confluent with that of the hyalosphere (figure 24, plate 6, and figure 27, plate 7). As with the axoplast of the Radiolaria Nassellaria (Cachon & Cachon 1971) there is no membrane bounding the axoplast of *Gymnosphaera*. Likewise the centroplasts of *Raphidiophrys* (Bardele 1971) and *Heterophrys* (Bardele 1975) are not enclosed by a membrane. The suggestion of Dobell (1917) that the axonemes in *Oxnerella* terminate in minute granules on a delicate membrane can be rejected. Besides being absent at the proximal tips of the axonemes, the sheath in *Gymnosphaera* also peters out towards the surface of the axoplast. Sections at this level show axonemes only incompletely surrounded by a sheath, if indeed any occurs at all (figure 29, plate 7). Generally the sheath reaches 20–40 nm in thickness. Its function is undoubtedly to bind the ends of the axonemes firmly together. The interlacing fibrils of the sheath have a sinuous course running round one axoneme to the next. Sheaths are never seen in the cortex, axopodia or vacuolated part of the medulla (figures 30 and 31, plate 7).

Enclosed by the axonemal sheaths and between the separating axonemes near the surface of the centriaster occurs the general matrix of the shell (figure 24, plate 6, and figure 29, plate 7). Like the hyalosphere this has a fibrogranular texture, likened to cotton wool by Tilney (1971) for the heliozoan *Raphidiophrys*. It stains less intensely than the hyaline sphere (figure 24), even though the two matrices are confluent. The matrix which embeds the microtubules within the axonemal sheaths is no different from that in between the axonemes; the two are continuous where a sheath is lacking. However, the inter-axonemal matrix may contain bundles of fibrils running from one axoneme to the next (figure 24, extreme left) where the sheaths are sparse, and even some microtubules oriented more or less transversely to the direction of the axonemes (figure 24, bottom right), particularly when the centriaster is preparing to divide or is a product of a recent division. The axonemes in figure 24, plate 6, are not all strictly radial; a secondary focus of radiation is present in the bottom right quadrant, which suggests that the process of binary fission was under way. The methods by which centriasters replicate will be described in another publication. Also occurring in the matrix may be patches of electron-dense

granular matter, either outside, or just within, the axonemal sheaths (figure 26, plate 6). This, or similar, matter is abundantly present when the microtubules have disorganized and vanished (figure 21, plate 5). In such cases the axonemal sheaths remain. Masses of granular material also occur in the medulla, cortex and axopodia in the vicinity of recently disorganized axonemes. It is well known (see, for example, Tilney 1968) that colchicine-induced breakdown of microtubules involves the formation of filamentous material and then amorphous material.

The axonemes comprise bundles of parallel microtubules (figures 22 and 23, plate 5), each of which is joined to its neighbours by cross-linkages. The linkages are particularly obvious near the proximal ends of the axonemes (figures 28 and 29, plate 7), but farther out their number per unit length diminishes. In the region of the cortex (figure 31, plate 7) and in the axopodia, they occur only sparsely, so that transverse sections of the axonemes there hardly display them. They are still plentiful in the medullary region, however, far beyond the level at which the axonemal sheath has petered out (figure 30, plate 7).

Where the cross-linkages are abundant, the microtubules have the appearance of a row of grains on a maize cob, with roughly transverse lines at slightly constricted levels along their length (figure 22, plate 5). The effect could well be a fixation artefact. The cross-bridges generally occur at the level of the transverse lines, but the relationship has not been observed with complete clarity because of the thickness of the sections and probably imperfect fixation. Measurements of the width of the linkages range from 5 to 13 nm on transverse sections of axonemes and from 5 to 15.6 on longitudinal sections. Cross-bridges in other organisms are generally 3.5–7 nm in thickness (McIntosh 1974). Paired cross-linkages, 6–7 nm in thickness, have been observed in certain Nassellaria (Cachon & Cachon 1971) and occasionally one sees double bridges on transverse sections of gymnosphaeran axonemes (Jones 1975). The lengths of the cross-bridges in *Gymnosphaera* range from 11.5 to 17.5 nm. In the Centroaxoplastididae they are 15 nm long (Cachon & Cachon 1972*a*). The spacing likewise is variable in *Gymnosphaera*, intervals of 15.3, 17.2, 18.5, 21, 31 and 38 nm having been measured, even in parts near the proximal ends of the axonemes. One would expect the interval to be a multiple of either 4 or 8 nm, equivalent to the spacing of the tubulin monomers or dimers respectively, but distortion during fixation and dehydration and small differences in orientation, would doubtless induce some irregularity from one section to another. The interval in the Nassellaria is given as 20 nm by Cachon & Cachon (1971).

Cross-bridges are usually absent between adjacent microtubules at the extreme proximal tips of the axonemes, where the number of microtubules in a transverse section is very small (figure 27). Serial sections in this region have indicated that the number of microtubules increase as the level of sectioning moves distally. Cross-bridges correspondingly appear between some of the microtubules. Some microtubules curve into the appropriate relationship with their neighbours to give the characteristic arrangement seen in figures 27 and 28, plate 7, but others appear to arise already in the appropriate positions. The cross-linkages become more conspicuous, like the microtubules themselves, at more distal levels of section. Sometimes a cross-bridge is seen projecting from one microtubule before an adjacent microtubule has appeared.

The characteristic pattern made by the microtubules and their cross-bridges in transverse sections of the axonemes has already been described in detail (Jones 1975). Each microtubule is joined to three neighbours by cross-bridges in such a way that the microtubules are arranged at the corners of usually asymmetrical hexagons, which are always of two types, rows of one kind alternating with rows of the other across the section (figure 28). At the centre of each

hexagon one can usually discern a vaguely defined dark area (figure 28), which may represent the spiral element mentioned by Davidson (1974) as occurring at the centre of the hexagons of *Heterophrys marina*. Similarly situated dark areas occur in the hexagons of the aberrant radiolarian *Sticholonche zanclea* (Hollande, Cachon, Cachon & Valentin 1967, figure 5) and of *Hedraiophrys hovassei* (Febvre-Chevalier 1973*a*, figure 8). The two types of hexagons vary greatly from one axonemal section to another, because the sections are usually oblique to the axonemal axes. However, I have demonstrated mathematically that all the observed arrangements are derivable from the same basic pattern which would be seen on transverse sections of the axonemes. This pattern consists of alternating rows of bilaterally symmetrical hexagons, all of the same type but occurring in one of two possible orientations (figure 33). The angles of this type of hexagon are 138° 28′ and 110° 46′, there being two of the former and four of the latter arranged as paired opposites. Because the three angles between the cross-linkages at each microtubule are in the ratio of 5:4:4 I concluded that there were 13 protofilaments in the wall of the microtubule, having assumed that the orientation of the cross-linkages was related to the ultrastructure of the wall. Partial confirmation of the correctness of the basic pattern is afforded by the section in figure 27, plate 7, which shows an almost perfectly symmetrical hexagon. The

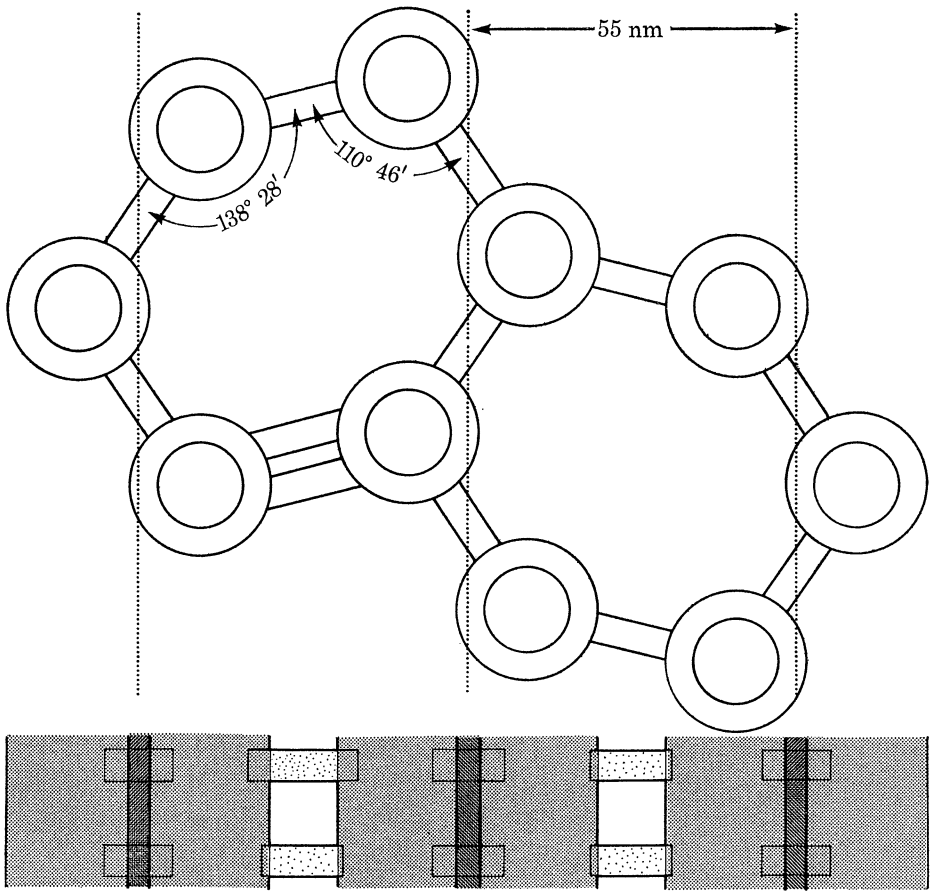


FIGURE 33. The basic pattern of the axonemes of *G. albida* (i.e. the pattern seen in a strictly transverse section). There is only one type of bilaterally symmetrical hexagon and the hexagons are oriented in one of two interlocking ways to form alternating rows. The dotted lines indicate the principal transverse direction. When a longitudinal section of an axoneme is viewed along this direction, the appearance is as shown in the lower diagram (compare figure 22, plate 5). Evenly spaced electron-dense lines are seen as a result of the overlapping of microtubule walls.

angles of this are 137.75°, 109.5°, 115°, 129.25°, 118.25° and 111°. The average length of the six sides is 37.6 nm. The tannic acid fixation method (to enable the number of protofilaments to be counted directly) and the use of a tilting stage (to set the section for viewing along an axonemal axis) are currently being undertaken.

The pattern of alternating rows of hexagons persists along the length of the axoneme even though the cross-bridges become much less frequent. Figure 31, plate 7, shows a section of an axoneme at the level of the cortex, and a similar arrangement has been seen just beneath the capsular wall.

In transverse sections near the proximal end of the axonemes the cross-bridges are always present and conspicuous, the sections not being thin enough to pass in between two adjacent cross-bridges. That true bridges and not partition walls are involved can only be determined by examining longitudinal sections. The appearance obtained with these depends upon the orientation of the section in relation to the axonemal axis. The most striking effect is obtained when the plane of the section is parallel to the axis and at right angles to the principal longitudinal planes of the axoneme (Jones 1975). These planes are evenly spaced and correspond to the microtubule rows set in the 'principal transverse direction', namely the direction of two-step zig-zagging (figure 33). The effect is of a series of ladders arranged side by side (figure 22, plate 5). The ladders overlap somewhat at their edges in such sections, increasing the electron opacity, so that the section exhibits a series of evenly spaced dense lines. The width of these lines fell within the range of 4.2–8.5 nm on the examples measured. E.m. sections are usually about 70 nm in thickness, ensuring that there is always an overlap of microtubules to produce the electron dense lines, as shown in figure 33 (lower diagram). With other types of orientation of the plane of the section different effects are obtained. Thus one may observe the microtubules evenly spaced apart, with no electron-dense lines, when the line of sight is inclined by about 30° to that concerned in figure 33. In some sections the pattern of parallel dense lines displaces itself laterally by approximately the width of a microtubule at intervals along the axoneme, as microtubules successively reach the surface of the section. The spacing between the parallel dense lines varied somewhat from axoneme to axoneme, depending upon the orientation of the section and possibly differences in degree of shrinkage as a result of fixation. Measured values were 50, 52, 55 and 60 nm. The approximate average of 55 nm has been used in the diagram in figure 33, particularly as this value was measured on a perfectly oriented example. From this value one can calculate the length of the sides of the hexagons, namely 35.74 nm, which agrees reasonably well with the average length of 37.6 nm measured for the symmetrical hexagon of figure 27, plate 7. The value is larger than the estimate previously given (Jones 1975), namely 28.4 nm, which was the average of nine calculated values ranging from 23.05 to 32.7 nm. The nine values concerned nine axonemes for which the angle and direction of tilt relative to the line of sight were unknown, but taken to be those giving the least range of variation in side length calculated from ten trigonometrical equations and measurements of sides and angles made on the oblique sections. It was realized, however, that this criterion for estimating the angle and direction of tilt would give reliable results only if the electron micrographs were completely free from distortion other than that produced by the tilt of the axonemes. That this was not so is shown by the inconsistent values obtained for the side length of the basic hexagon of the nine axonemes. Neglecting these values, the arrangement shown in figure 33 represents the basic axonemal pattern as precisely and consistently as it can be obtained at present. In this the microtubules have an external diameter of 23.4 nm and a wall thickness of 5 nm. The wall

comprises 13 protofilaments spaced at 5 nm intervals. The length of the cross-bridges is 12.3 nm. According to McIntosh (1974) cross-bridges between microtubules generally have lengths of from 10 to 25 nm.

On some longitudinal sections of axonemes one gets the impression in places of transverse lines continuing across several adjoining 'ladders'. These are caused by the cross-bridges being situated at the same levels. Sometimes the lines can be traced into adjoining, diverging axonemes, suggesting that branching has occurred, unless the effect be coincidental. Evidence in support of branching has already been presented (Jones 1975); several axonemes with similarly oriented arrangements of microtubules may occur within the matrix bounded by a single sheath, without there being any granular matter in between to indicate that dissolution of microtubules has occurred. Supporting evidence may also be gleaned from figure 21, plate 5, in which sheaths of smaller axonemes are in places enclosed within the sheaths of larger ones. However, the axonemal diameter generally becomes uniform before reaching the surface of the centriaster, so that in between the diverging axonemes one finds areas of matrix in which microtubules doubtless arise *de novo*. The tips of several apparently newly forming axonemes can be seen between the larger axonemes in figure 29, plate 7.

(*j*) *The medulla*

The medulla is quite distinct from the cortex but the two regions merge with one another so that it is not possible to decide exactly where one begins and the other ends. The cortical cavity system penetrates but little into the medulla; some narrow clefts may be seen extending inwards, but they soon peter out. The medullary cytoplasm contains innumerable small vacuoles and some localized patches of fibrillar material, but, apart from the centriaster and axonemes, no other cell organelles are present. Mitochondria have been found near the outer boundary of the medulla of an organism which had just been produced by multiple fission, and possibly the mitochondria were being transported outwards to their more usual place in the cortex while the cytoplasm was undergoing reorganization. However, Golgi bodies, nuclei and polysomes have never been seen in the medulla of normal individuals. The medulla contrasts strongly with the 'zone of exculsion' surrounding the centroplast of *Heterophrys marina*, which does contain ribosomes and is not vacuolated (Bardele 1975); also with the central cytoplasm of *Hedraiophrys hovassei*, in which mitochondria, Golgi bodies, coarse endoplasmic reticulum and small vacuoles all occur (Febvre-Chevalier 1973*b*).

The vacuoles in the medulla of *Gymnosphaera* are mainly of two types (figure 30, plate 7). Some, of diameter 80–200 nm, are enveloped by a fuzzy coat, from 20–70 nm in thickness, attached to the vacuolar membrane. Sometimes these coated vesicles are elongated, for example 490 μm × 55 nm. The other type of vacuole has an uncoated membrane and tends to reach a larger diameter, for example 453 μm × 327 nm. Frequently these vacuoles have part of their unit membrane invaginated, so that in profile they appear chelate, while in the section at right angles one sees a smaller circle within a larger concentric one (figure 23, plate 5). Sometimes the invaginated part is dimpled so that three concentric circles are seen. Sometimes again the section is tangential to the invaginated pocket so that the vacuole appears to have electron-dense contents at its centre. In addition to these two types one occasionally finds larger vacuoles containing products of digestion (figure 3, plate 1).

The vacuoles are usually concentrated around the axonemes, which are unsheathed in the medulla and cortex. The vacuoles in close contact with the axonemes appear to be connected

to the outer microtubules and cross-linkages by fibrillar material (figure 30, plate 7). Likewise, when mitochondria occur close to an axoneme, they are often connected by fibrils (figure 32, plate 7). It seems likely that the vacuoles and mitochondria are transported by such connections. Certainly particles have been seen moving in either direction along the axonemes

he medulla. I have recorded their movement on ciné film. Cachon & Cachon (1975) have recently published photographs showing the occurrence of projecting arms on microtubules near the extremities of regenerating axopodia of the radiolarian *Thalassicolla nucleata*; the arms bear a swelling distally and they are set in parallel, except in the vicinity of small vesicles in the rheoplasm where they tend to be curved. The two authors postulate that movements of the arms mediate the centripetal current in the rheoplasm. On the other hand, in certain heliozoan axopodia the movement of granules does not require the presence of axonemes (Tilney 1968; Fitzharris & Bloodgood 1972; Edds 1975*a*, *b*; Troyer 1975). In so far as *Gymnosphaera* is concerned, the axonemes are connected to vacuoles and mitochondria by fibrillar material and not by discrete microtubular arms; the fibrils link with the axonemal cross-bridges as well as with the microtubules (figure 32, plate 7). No linkages have been detected between the axonemes and the refractive globules in the axopodia.

The diameter of the medulla is usually about half that of the whole capsule.

Discussion

Certain aspects of the structure of *Gymnosphaera* merit particular comment and discussion, namely the capsular wall, the reticular organization of the cortex, the osmiophilic globules, the refringent crystals and the centriaster. Finally there is the problem of classification.

(*a*) *The capsular wall*

The wall in *Gymnosphaera* occurs at the level of the central capsule membrane in the Acantharia and Radiolaria. It is an extracellular secretion occupying a space that is continuous with the external milieu (figure 34*a*). The wall is a microfibrillar coagulum. It does not consist of juxtaposed plates and it is not bounded by a unit membrane as in many of the Radiolaria. Moreover it is not perforated in the way that the true radiolarian capsular membrane is perforated. The only orifices in the wall are those through which the axopodia pass, excepting the temporary openings serving for the passage of food material and faeces. In the absence of distinct pores and of a limiting membrane the gymnosphaeran wall corresponds closely to the capsular membrane of the Acantharia, the structure of which has recently been elucidated by Febvre (1974) (figure 34*b*). It is composed of several microfibrillar sheets, each of which is perforated by extremely fine micropores. Larger openings, believed to be temporary, allow for the removal of waste products from the intracapsulum and presumably for the passage of axonemes (microtubules were not preserved by the fixatives employed). In its pluristratification, microperforation and openings for spicules it differs from the gymnosphaeran wall. It is perhaps significant that not all of the Acantharia reveal a distinct capsular membrane when viewed under the optical microscope. As many as one third of the species apparently lack the membrane (Schewiakoff 1926). However, it seems not impossible that a structure similar to the wall of *Gymnosphaera*, which appears merely as a thin hyaline gap at the highest optical magnification, would be revealed in some of these species by the electron microscope. Even in the Radiolaria there is considerable variation in the structure and distinctness of the capsular membrane. In *Thalas-*

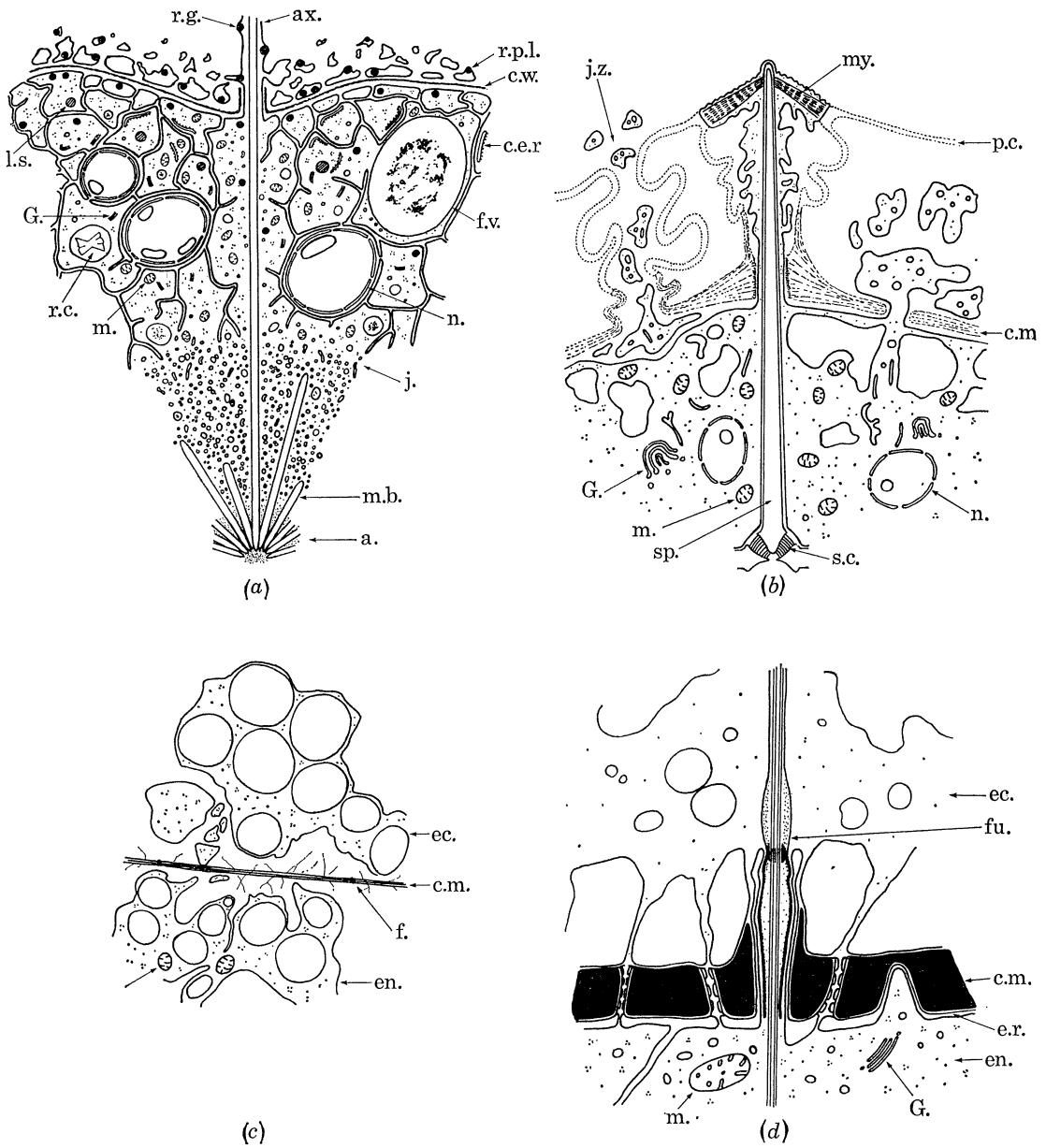


FIGURE 34. (*a*) *Gymnosphaera albida*. (*b*) *Stauracantha orthostaura*, a species of the Acantharia, after Febvre (1974). Microtubule bundles are not shown because the method of fixation was unsuitable to reveal them, but the axopodia probably pass through the envelope via the junctional zones (j.z.). (*c*) Part of the capsular membrane and adjoining cytoplasm in the Collozoum stage of *Thalassophysa sanguinolenta*, a member of the Radiolaria, after Hollande *et al.* (1970). The tenuous capsular membrane appears to be extracellular, lying between cytoplasmic tracts which are bounded by unit membrane. (*d*) Part of the capsular membrane and adjoining cytoplasm of the trophozoite of *Thalassophysa sanguinolenta*, after Hollande *et al.* (1970). The relationship between the cytoplasm and the capsular membrane requires clarification (see text).

Key: a., axoplast; ax., axopodium; c.e.r., coarse-surfaced endoplasmic reticulum; c.m., capsular membrane; c.w., capsular wall; ec., ectoplasm; en., endoplasm; e.r., endoplasmic reticulum; f., fissure; fu., fusule; f.v., food vacuole; G., Golgi body; j., junction between medulla and cortex; j.z., junctional zone; l.s., lipoid spherule; m., mitochondrion; m.b., bundle of microtubules; my., myoneme; n., nucleus; p.c., periplasmic cortex; r.g., refractive globule; r.c., refractive crystal; r.p.l., reticulated pseudopodial layer; sp., spicule and its sheath; s.c., spicular cement.

solampe it is delicate. In the Nassellaria it is homogeneous, in the Phaeodaria fibrous. In the Collodaria Centroaxoplastididae it consists of five or six layers of glycoprotein (Hollande *et al.* 1970). The aberrant *Sticholonche zanclea* exhibits merely a tenuous membrane (Hollande *et al.* 1967). The relationship of the tenuous type of membrane to the more differentiated, enclosed membrane of the perforated Radiolaria requires to be elucidated, but there seems no reason at present why the wall of *Gymnosphaera* should not be equated with the former kind. Certainly the wall seems to be no different in position from that of the capsular membrane of *Thalassophysa* at the Collozoum stage (Hollande *et al.* 1970) (figure 34*c*); the latter also occurs between cytoplasmic strands. Only the presence of the fissures between the capsular plates of *Thalassophysa* would appear to be a significant difference. In the trophozoite of *Thalassophysa*, on the other hand, Hollande *et al.* (1970) claim that the capsular membrane is lined on the inside by a membrane which also bounds the subcapsular endoplasmic reticulum, and on the outside by a cytoplasmic membrane (figure 34*d*). This suggests that the capsular membrane is intracytoplasmic. However, in the fissures between the capsular plates the cytoplasm is separated from the capsular membrane by a boundary linking vacuoles of endoplasmic reticulum together, which suggests that fluid cytoplasm and capsular membrane are not in direct contact. Further study is required to clarify the relationship.

The position and structure of the gymnosphaeran wall are also similar to the decalcified shell of the foraminiferan *Globigerina*, excepting that the latter is three-layered (Febre-Chevalier 1971).

The sleeve around the proximal end of each axopodium on *Gymnosphaera* has its counterparts on the central capsule membrane of the more differentiated radiolaria, but differs from those that have been described in lacking the ancillary apparatus termed the 'fusule' by Cachon & Cachon (1971, 1972*a*, *b*). In effect the orifices serving for the extrusion of axopodia in the Radiolaria are sealed by 'corks' of electron-dense material. A dense ring or muff surrounds the axoneme within the sleeve formed by the capsular membrane, and the microtubules have to pass, either together or individually according to the species, through canaliculi in the diaphragm or plug set across the interior. The size of the fusule in the Nassellaria (1.2–2.5 μm in diameter) is similar to that of the sleeve in *Gymnosphaera*, but electron-dense rings, plugs or diaphragms are absent from the orifices in the latter. Fusules would hardly be necessary in *Gymnosphaera*, because the tenuous wall is a labile structure, readily perforated for the passage in and out of food and waste material, and absent during repeated, multiple fission.

(*b*) *The reticular organization*

A description has been given of the system of clefts which divide the syncytial cytoplasm of *Gymnosphaera* into branching and anastomosing strands. The system could be regarded as an endoplasmic reticulum, as Hollande *et al.* (1970) have done for a somewhat similar feature in certain Radiolaria, but there is an important difference. The radiolarian system is sealed and bounded continuously by a unit membrane which applies itself against the capsular membrane wherever the cavities lie immediately beneath this structure. In *Gymnosphaera* there is no barrier between the cavities and the wall, and the former can communicate, via the sleeves, with the spaces in the reticulated pseudopodial layer, although in life the axopodial cytoplasm probably fills these sleeves. Moreover, the system of cavities in *Gymnosphaera* is quite distinct from the coarse-surfaced endoplasmic reticulum, which in profile has much narrower channels and occurs within the cytoplasmic tracts. The cavity system is similar to the **T**-system of the striated

muscle fibre, disregarding the fact that the spaces are clefts rather than tubules. Possibly it functions as a transport system. A similar lacunar system, containing seawater, penetrates throughout the cytoplasm of the foraminiferan *Allogromia laticollaris* (Wohlfarth-Bottermann 1961; Lengsfeld 1969) and occurs in the outer cytoplasm of *Allogromia flexibilis* (Lena 1972), Likewise in the heliozoan (?) *Hedraiophrys hovassei* the peripheral cytoplasm is highly lacunar, with invaginations of the plasma membrane extending deeply inwards towards the axoplast. However, the cavities in this species differ from those of *Gymnosphaera* in being filled with microfibrillar material (Febvre-Chevalier 1973*b*). In the pseudoheliozoan *Clathrulina elegans* a somewhat different type of lacunar system is present, particularly when the filopodia have been retracted. The cytoplasm then contains aggregates of cisternal cavities, suggesting that their function is to serve as stores of plasma membrane (Bardele 1975).

(*c*) *The refractive globules*

The electron-dense globules concentrated in the cytoplasmic strands on each side of the capsular wall and in the axopodial rheoplasm probably function in the capture and/or digestion of food, or in the defence of the organism. It seems likely that they are the cause of the stickiness of the axopodia, but this needs confirmation; no globule has been seen in the act of discharging its contents, either in life or with the aid of the electron microscope. Until histochemical tests have been carried out the function of the globules will remain obscure.

Many other types of Protista possess organelles resembling in varying degrees the osmiophilic globules of *Gymnosphaera*. The actinophrydian heliozoan *Actinosphaerium nucleofilum* has small dense granules 0.1–0.25 μm in diameter and up to 1 μm in length, each bounded by a unit membrane (Tilney & Porter 1965). Similar opaque bodies, possibly a little smaller, are present in the oral ectoplasm of the foraminiferan *Globigerina bulloides* (Febvre-Chevalier 1971). Small refringent droplets occur in species of the Radiolaria Nassellaria (Cachon & Cachon-Enjumet 1964). So-called muciferous bodies have been found in the chrysomonad *Pedinella hexacosta* (Swale 1969) and 'extrusisomes' occur in the helioflagellate *Ciliophrys* (Bardele 1972; Davidson 1974). While the sizes of these bodies may differ from species to species, and while at times they may contain an aggregation of dense granules (as in *Pedinella*, occasionally in *Actinosphaerium* and rarely in *Gymnosphaera*), these globules may well be equivalent in function and composition. They contrast strongly with the kinetocysts (Bardele 1969) which occur in the pseudoheliozoan *Clathrulina elegans* (Bardele 1972) and the centrohelidian heliozoan *Raphidiophrys*, *Acanthocystis* and *Heterophrys* (Bardele 1969, 1971; Davidson 1972). Kinetocysts are structurally differentiated organelles with a pointed central element which is directed towards the outside. They are attached to the cell membrane (Davidson 1973*a*, *b*) and only move actively on the axopodia when the animal is stimulated by disturbances in the ambient medium (Bardele 1975). They are considered to be extrusible. Different again are the 300 nm bodies found in *Hedraiophrys hovassei*. These consist of an ovoid nucleoid of fibrous appearance enclosed in a unit membrane, excepting those of one individual in which the bodies were more ovoid, with a more osmiophilic matrix (Febvre-Chevalier 1973*b*). The author was unable to observe any saltatory movement of these bodies and suggests that they are a type of microbody (peroxisome). Peroxisomes are usually 0.5 μm in diameter and have finely granular or amorphous contents which are only moderately opaque (de Duve & Baudhuin 1966). The extreme opacity of the globules of *Gymnosphaera* indicates that these are not peroxisomes.

(*d*) *The refractive crystals*

While the attempt to determine the nature of the refringent crystals of *Gymnosphaera* has so far not been completely successful, it is useful to compare their properties with those of the concretions or crystals found in related organisms, notably in certain of the Acantharia and in the marine heliozoan *Wagnerella borealis*.

According to Trégouboff (1953*a*) the concretions found in acantharia consist of calcium orthophosphate. This is incorrect, because when heated on a slide, either *in situ* or after isolation, they turn rapidly brown, then carbonize and finally disappear without trace (Schewiakoff 1926). They consist therefore of an organic substance and not of mineral salts.

Schewiakoff investigated the properties of the concretions of *Pseudolithium compressum* in detail. He found that they are insoluble in alcohol and in a mixture of alcohol and ether. They are not blackened by osmium tetroxide, nor by Sudan stain, nor do they stain with iodine. They dissolve completely in distilled water within 1–1½ h when *in situ*, and instantly in dilute or concentrated mineral acids. In acetic acid, ammonia and corrosive alkalies they dissolve within 1–2 min, the dissolution proceeding uniformly from the periphery inwards and revealing a concentric layering. As dissolution proceeds the concretions lose their birefringence and finally disppear without trace. These reactions, together with the optical properties of the concretions, suggested to Schewiakoff that they were neither lipid, protein, nor carbohydrate, but possibly uric acid, or a salt of uric acid. He obtained a positive result when he tried the murexide test, but, in view of the small amount of test material available, he did not regard this result as conclusive. A further test, namely the attempt to precipitate uric acid by the addition of acetic acid to an alkaline solution of the concretions, proved negative. It is clear that the chemical basis of the acantharian concretions is still undecided, apart from their organic nature. That the concretions only occur in individuals which also contain food vacuoles and lipid reserves, and which are not engaged in gamete formation, supports the view that the concretions represent an excretory organic product to be liberated from the organism at an appropriate stage in the life cycle.

Acantharian concretions are not diagnostic for the group. They occur in relatively few of the species, and while they are always highly refringent and anisotropic, their form varies, according to species, from spherical or irregular granules to long-oval, rod-shaped, or cylindrical structures with rounded ends, or dumb-bell or biscuit-shaped forms. Sometimes they look crystalline, at others they appear as sheaves of fine needles, while the larger examples may exhibit a stratification suggestive of a union of crystals. Their size ranges from 6–16 μm for the rod- or biscuit-shaped forms, and from 2 to 4 μm for the spherical or irregular forms. Clearly the diabolo and crossed fused rod crystals of *Gymnosphaera* could well be included in such a range of forms, while the heat lability, optical properties and solubility in acids and alkalies suggest that their composition could be closely similar to that of the acantharian concretions investigated. The e.m. studies reported above (§*g*) have indicated that a heat-stable component ensheaths and penetrates within a heat labile component. If the same were true of the acantharian concretions, their range of form could be caused by relatively different amounts of the two components. Different amounts would also affect the degree of charring when the concretions are heated, and could well be correlated with the occurrence of growth rings and form birefringence (Shewiakoff 1926).

Wagnerella borealis also exhibits strongly refractile, birefringent crystals, of variable form and

size. Generally they are about 12 μm long and cylindrical, with a swelling in the middle where they are sometimes bent. They dissolve in hot water, dilute acetic and mineral acids, ammonia, 1 % potash and ammonium oxalate. However, they never occur in vacuoles (Zuelzer 1909). Apart from this last property and the fact that Zuelzer was unable to confirm their organic nature (the crystals did not brown with iodine), the crystals of *Wagnerella* closely resemble those of *Gymnosphaera*. Freshwater heliozoa do not exhibit such crystals, presumably because they excrete their waste material in soluble form via the contractile vacuole.

(*e*) '*Axoplast*' *and* '*centroplast*'

The terms 'axoplast' and 'centroplast' require clarification, because both have recently been used to denote structures that are not precisely equivalent to those for which the terms were originally coined. The 'centroplast' was originally defined by Dobell (1917) as a minute, feebly refringent corpuscle (the so-called 'central granule') which occurred at the centre of the heliozoan *Oxnerella maritima*. Within it the radiating axonemes were rooted. The corpuscle was surrounded by a clear area of protoplasm and, in fixed and stained preparations, it appeared as a minute clear sphere containing a densely staining central granule. In *Heterophrys marina*, which differs from *Oxnerella maritima* only in the possession of organic spicules (Bardele 1975), this central granule consists of three disks stacked one upon another (Bardele 1971; Davidson 1972).

The confusing use by Dobell of the expression 'central granule' for two different entities, namely the whole centroplast and the granule within the centroplast, particularly when both are surrounded by clear protoplasm, has resulted, I believe, in Cachon & Cachon (1974) regarding the latter granule as the centroplast and (1972*a*) equating the clear zone which surrounds this tripartite organelle with the axoplast. This usage misled me (Jones 1975) into regarding the hyaline sphere in the radiation-centre of *Gymnosphaera* as an axoplast and coining a new term, 'centriaster', for the sphere and its enveloping shell composed of the proximal ends of the axonemes. However, it is clear from the original use of the term (Hollande & Enjumet 1954, 1960) that the axoplast represents the fundamental substance from and in which the axonemes are formed. Thus the axonemes penetrate into the axoplast, which comprises therefore both the hyaline sphere and its shell. Using the original definitions the axoplast and the centroplast become easily comparable structures; the axonemes originate within both and the essential difference is the absence of the tripartite organelle from the former, neglecting differences in the microtubule patterns.

Because not all types of 'central granules' have yet been investigated with the aid of the electron microscope, and because the centroplast of even *Oxnerella* may at times not exhibit a central granule (Dobell 1917), there would appear to be a need for a neutral term to designate either axoplast or centroplast when it is not certain which is concerned. I propose that the term 'centriaster' be used in such cases. I have already used the term 'hyalosphere' for the hyaline sphere previously called an axoplast by myself. The hyalosphere could well turn out to be an important organelle in its own right. For example, it may possess the ability, at present uncertain, to organize microtubule formation and arrangement. In this connection the recent discovery of an extranuclear 'nucleus-associated body' in the slime mould *Polysphondylium violaceum* (Roos 1975) is of considerable interest. This body lies near the nucleus at interphase and resembles the heliozoan tripartite organelle in having three main electron-opaque disks stacked

together, the middle one being much thicker than the other two. The body bears nodules which are microtubule organizing centres. At mitosis, however, the nucleus-associated body gives rise to spindle pole bodies which appear structureless and yet serve as foci for the radiating spindle microtubules. It remains to be seen whether and how far the axoplast can be equated with the spindle pole body, and the centroplast with the nucleus-associated body, and whether the patches of granular matter seen in some axoplasts of *Gymnosphaera* have their counterparts in the microtubule organizing nodules of *Polysphondylium.*

(*f*) *The problem of classification*

Sassaki included *Gymnosphaera albida* among the Heliozoa, and without the results of e.m. studies few would disagree with this. With its axopodia radiating from a central centriaster it appears to be a typical sun-animalcule belonging to the Order Centrohelidia Kühn 1926 (Trégouboff 1953*c*). Because it lacks a mucilaginous envelope and spicules it was classified in the Sub-Order Aphrothoraca, along with *Oxnerella* and the stalked form, *Actinolophus*, making a quite heterogeneous assembly. However, *Oxnerella* is now believed to be identical to *Heterophrys*, apart from the absence of spicules (Bardele 1975), and the question arises whether *Gymnosphaera* likewise would be more naturally classified with spiculate forms, because it is clear from other groups of animals (for example, the Porifera) that categories of spicules can be lost in the evolution of species, or only sparingly produced in certain individuals. However, when one compares *Gymnosphaera* with members of the two other Sub-Orders, the Chalarothoraca and the Chlamydophora, the differences appear to outweigh by far the similarities. The Centrohelidia Chalarothoraca comprises spiculate forms such as *Heterophrys*, *Raphidiophrys*, *Acanthocystis*, *Wagnerella* and *Cienkowskya*. The remaining genera and those of the aspiculate Chlamydophora do not exhibit a conspicuous centriaster (Trégouboff 1953*c*). Of the five named genera, the first three have a centriaster which is definitely a centroplast (Bardele 1975). *Wagnerella* and *Cienkowskya* have not been examined by electron microscopy, but optical studies have shown that a granule occurs in the inner sphere of their centriaster (Villeneuve 1937; Zuelzer 1909), indicating that the latter also is a centroplast. *Gymnosphaera* has an axoplast. The axonemal pattern of the centrohelidian Heliozoa so far determined is also very different from that of *Gymnosphaera*, each microtubule being associated with a pair of microtubules on either side, forming in all a pattern of equilateral triangles and regular hexagons (Tilney 1971; Bardele 1971). Cross-bridges have been seen on some micrographs of *Raphidiophrys* by Tilney, but in general they are not visible. Kinetocysts, which occur in *Heterophrys*, *Acanthocystis* and *Raphidiophrys* (Bardele 1971; Davidson 1972), and also in the pseudoheliozoan *Clathrulina elegans* (Bardele 1972), are absent from *Gymnosphaera*, which exhibits osmiophilic globules instead. The capsular wall of *Gymnosphaera* is not a heliozoan character, unless it be regarded as a vestige of the mucilaginous envelope which usually surrounds the body of the Centrohelidia Chalarothoraca and Chlamydophora. Moreover, apart from *Gymnosphaera*, the Heliozoa Centrohelidia are uninucleated during the vegetative phase of the life cycle, a difference of possibly minor significance in view of the occurrence of multinucleated species in the Heliozoa Actinophrydia and multinucleated stages in the life cycles of other groups (for example, the Foraminifera and slime moulds). Regarding the mode of sexual reproduction a comparison is hampered by lack of information. *Gymnosphaera* liberates biflagellated isogametes which have been seen to fuse together in pairs (Jones, unpublished). *Acanthocystis* and *Wagnerella* produce biflagellated isospores. On balance it must be admitted that *Gymnosphaera* stands apart from the other Heliozoa.

While its refringent crystals have similar properties essentially to those of *Wagnerella*, this may be merely a reflection of the marine mode of life of the two species.

The recent discovery of *Hedraiophrys hovassei* by Febvre-Chevalier (1973*b*) has brought to light a possible heliozoon with an axoplast and with an axonemal pattern very similar to that of *Gymnosphaera*. Both species are semi-sedentary, *Hedraiophrys* somewhat more so, having a short stalk and zone of cytoplasm differentiated for attachment. However, *Hedraiophrys* is uninucleated and possesses spicules. There is no capsular wall, but the cavity system is filled with microfibrillar material which may possibly be correlated with the gymnosphaeran wall, or represent the gelatinous envelope of the typical heliozoon. The axopodial organites are not the same as the osmiophilic globules of *Gymnosphaera*, and there is no mention of refringent crystals by Febvre-Chevalier. *Hedraiophrys* appears to have affinities with the uninucleated, stalked, spiculate, brackish *Cienkowskya*, apart from the different type of centriaster. Unfortunately there is no information available on the axonemal pattern of the latter species. It remains to be evaluated how important is the axonemal pattern as a diagnostic feature compared with other characters such as the presence or absence of spicules, the type of centriaster, the number of nuclei and the production of isogametes or isospores.

The Acantharia exhibit certain features in common with *Gymnosphaera*. They are multinucleated, marine, spherical organisms, some species of which have a tenuous capsule membrane and a cytoplasmic zonation comparable to those of *Gymnosphaera*. Some acantharia possess concretions with properties similar to those of the refractive crystals. Acantharia reproduce sexually by means of biflagellated isogametes (Schewiakoff 1926). Moreover, in the species so far examined by electron microscopy the axonemal pattern is basically similar to that of *Gymnosphaera* (Cachon, Cachon, Febvre-Chevalier & Febvre 1973). Acantharia have an axoplast (Hollande & Enjumet 1960; Cachon & Cachon-Enjumet 1964). On the other hand, the main diagnostic feature of the Acantharia is the presence in them of autogenous spicules of strontium sulphate arranged in conformity with Müller's Law (Trégouboff 1953*a*). Associated with the spicules is the so-called hydrostatic apparatus, consisting of intracytoplasmic myonemes and extracytoplasmic cortex (figure 34*b*) (Febvre 1973, 1974). Neither spicules nor ancillary apparatus occur in *Gymnosphaera*, but this is not an insuperable dissimilarity. *Gymnosphaera* is a part-time sessile form occurring commonly between the spiculate diverticula of the sponge *Sycon ciliatum*; spicules and hydrostatic apparatus might well be unnecessary and a disadvantage in such a habitat. Besides, some acantharian species lack myonemes. One other difference, namely the absence of zooxanthellae from *Gymnosphaera*, can be readily dismissed; zooxanthellae do not occur in all species of the Acantharia.

Apart from the occurrence of a tenuous central capsule membrane in certain species, most of the Radiolaria have little in common with *Gymnosphaera*. The radiolarian centriaster is an axoplast (Cachon & Cachon 1972), but no radiolaria have yet been discovered with the same axonemal pattern (Cachon & Cachon 1974). The pattern in the aberrant radiolarian *Sticholonche zanclea* consists of bilaterally symmetrical hexagons, but these are set in a tile-work and not a parquet-floor arrangement (Hollande *et al.* 1967). Radiolaria typically possess a large polyploid nucleus (Hollande, Cachon & Cachon 1969) and the majority of species have a siliceous skeleton. Their intracapsular cavity system is stated to be an endoplasmic reticulum whose lumen is closed off by a unit membrane from the central capsule membrane and from the spaces in the reticular cytoplasm (calymma) outside the capsule (Hollande *et al.* 1970). Radiolaria liberate biflagellated isospores, but their subsequent fate is unknown (Trégouboff

1953*b*; Cachon & Cachon 1969). Certain radiolarian species contain crystals in their endoplasm, but these are composed of strontium sulphate. Albuminoid crystals are said to be present also and to be destined to serve for the nourishment of the spores (Trégouboff 1953*b*), but recently Hollande (1974) and Hollande & Martoja (1974) have stated that the isospores of three species have strontium-containing crystals. Refractive crystals are not present in the isogametes of *Gymnosphaera* (Jones, to be published).

It must be concluded that while the axonemal pattern and axoplast suggest that *Gymnosphaera* is related to *Hedraiophrys* and the Acantharia, more information is required concerning the ultrastructure and life histories of the axopodiate Protista before one can justify the elaboration of a new natural classification of the Actinopoda.

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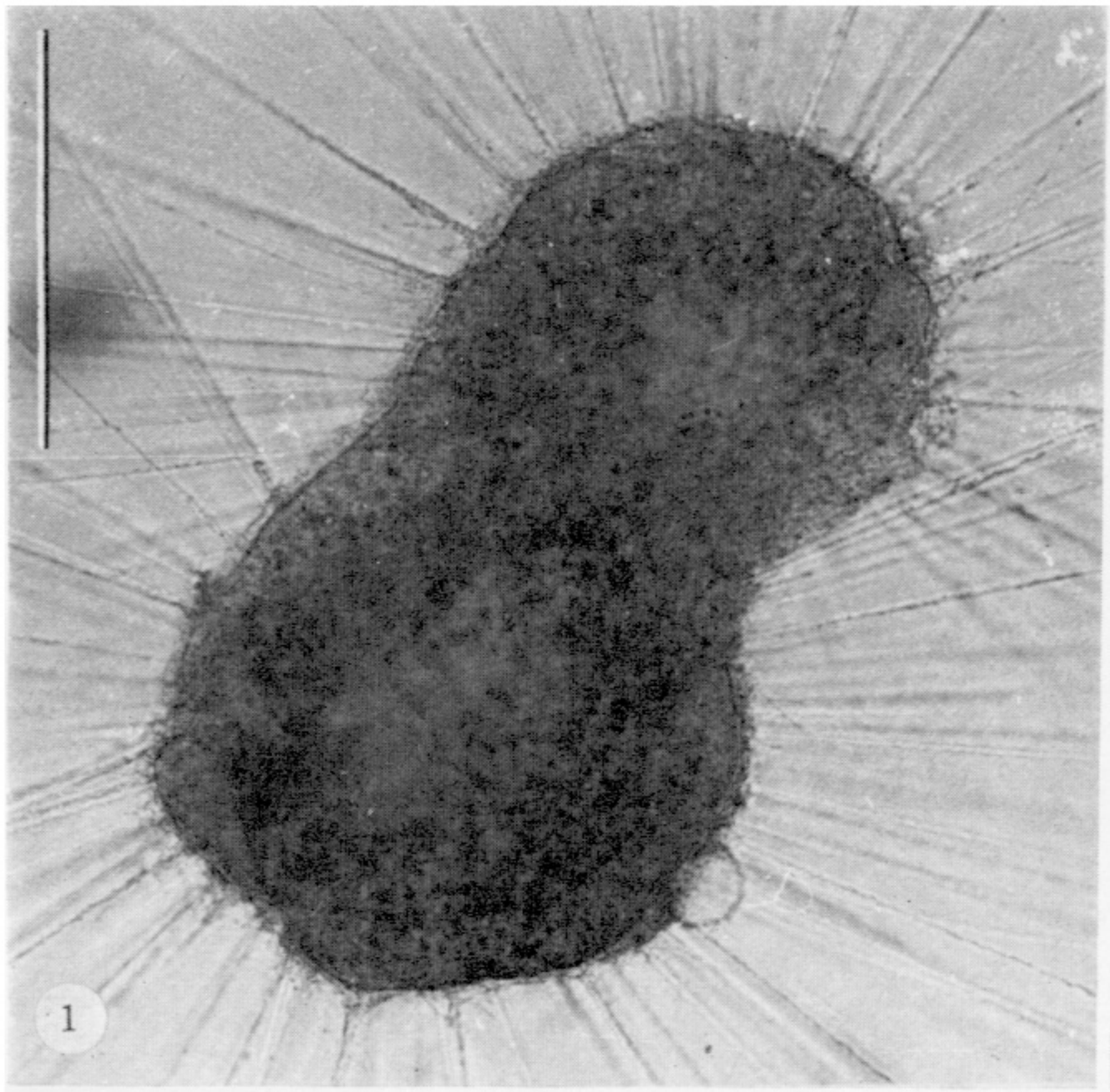

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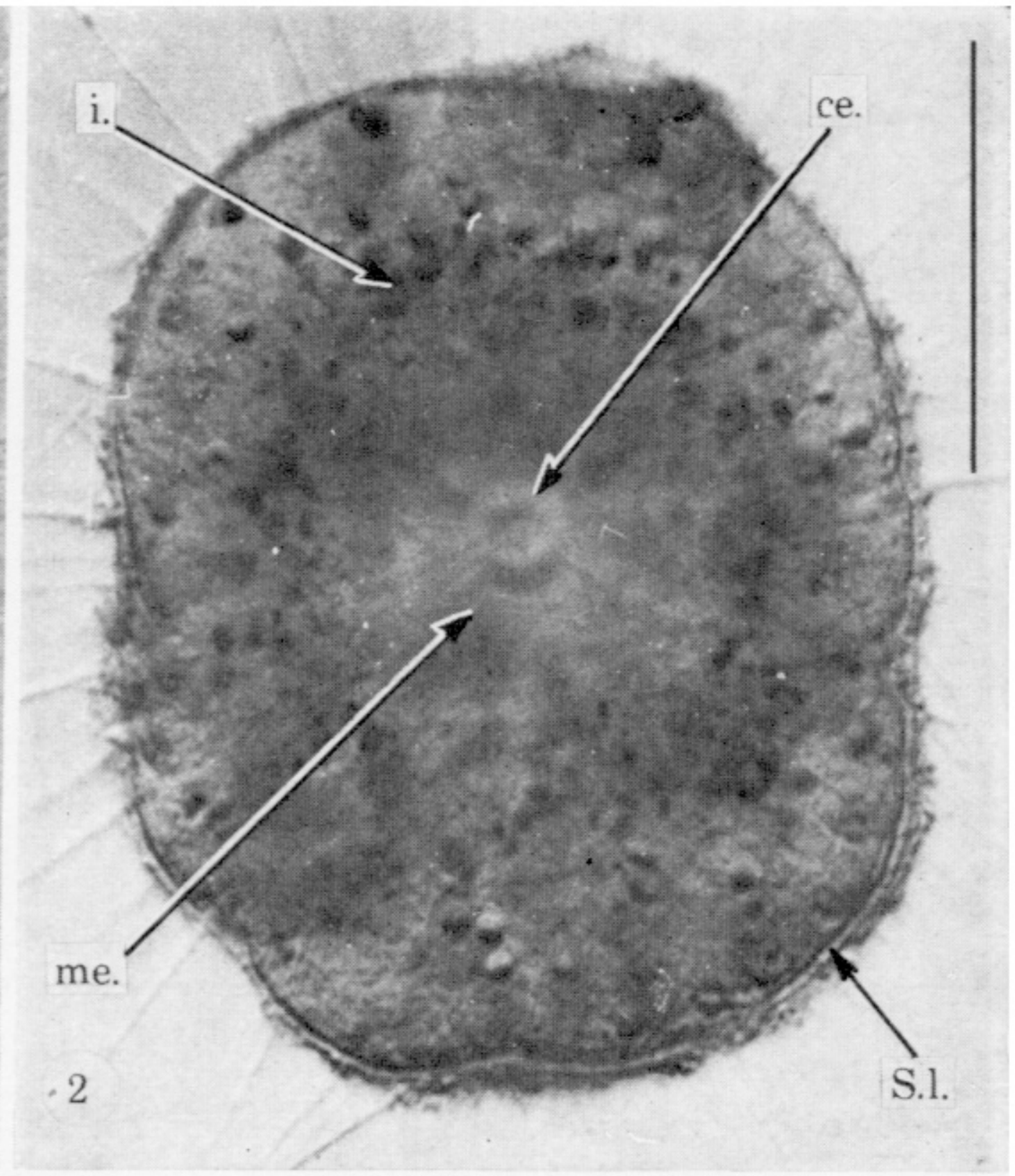

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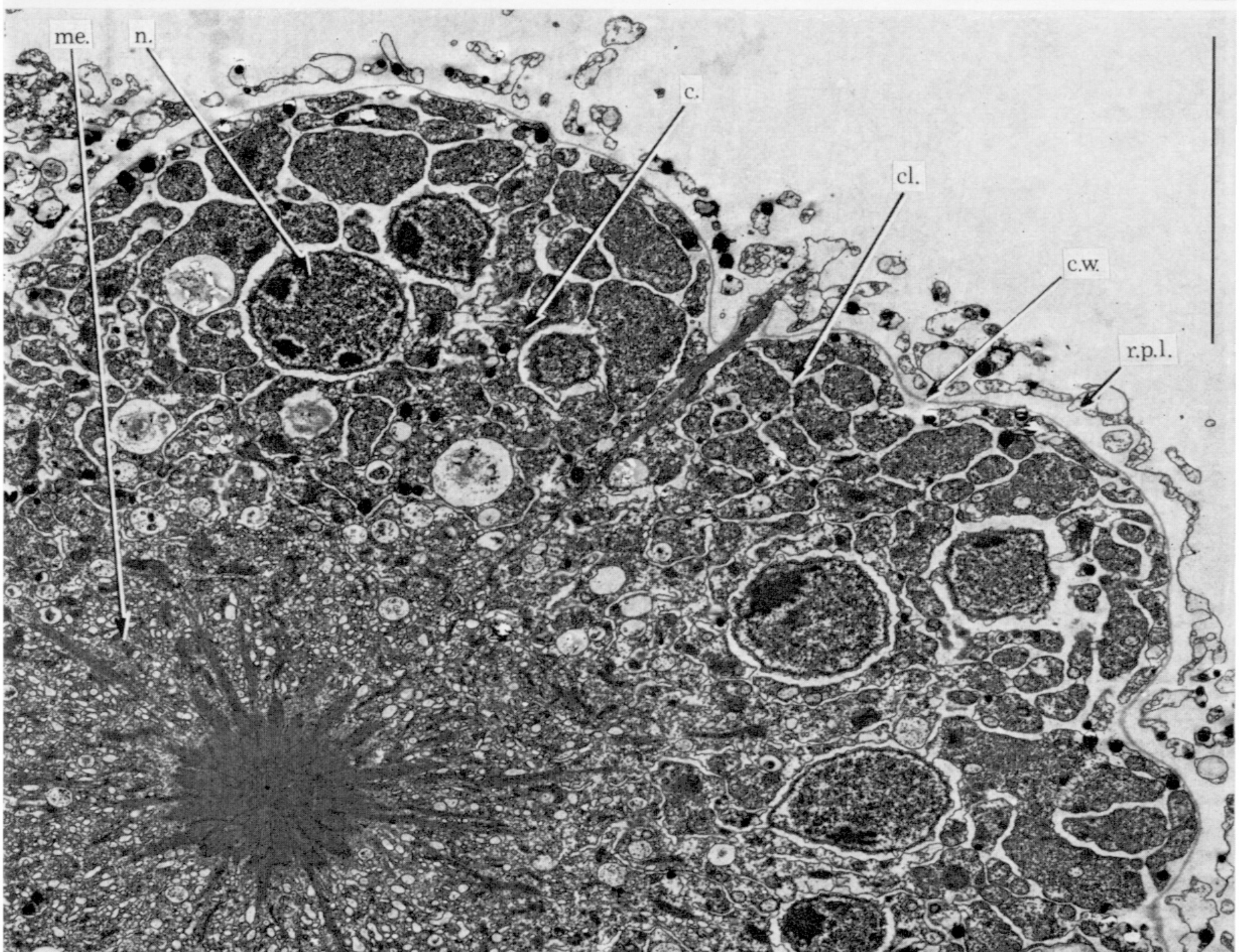

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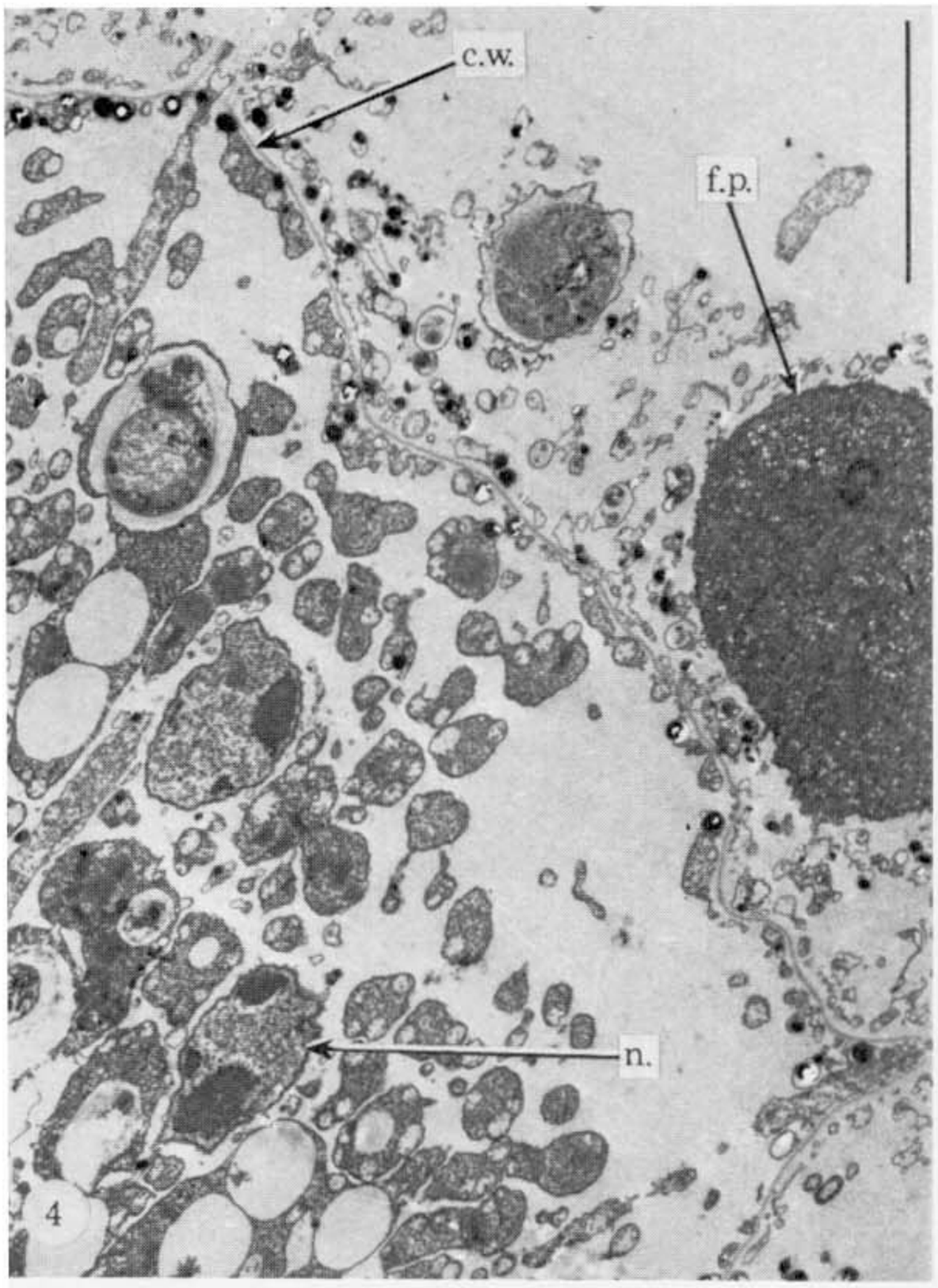

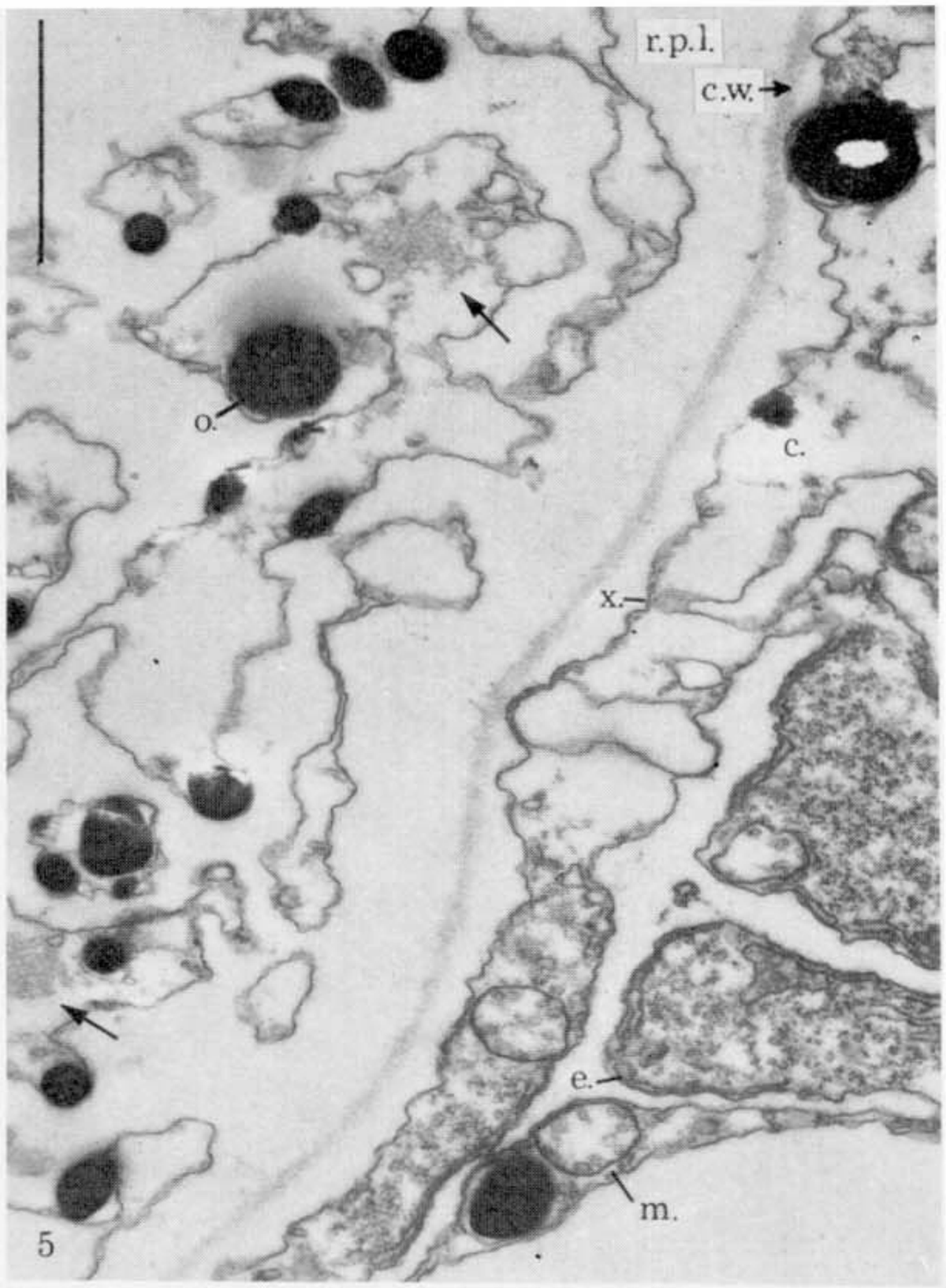

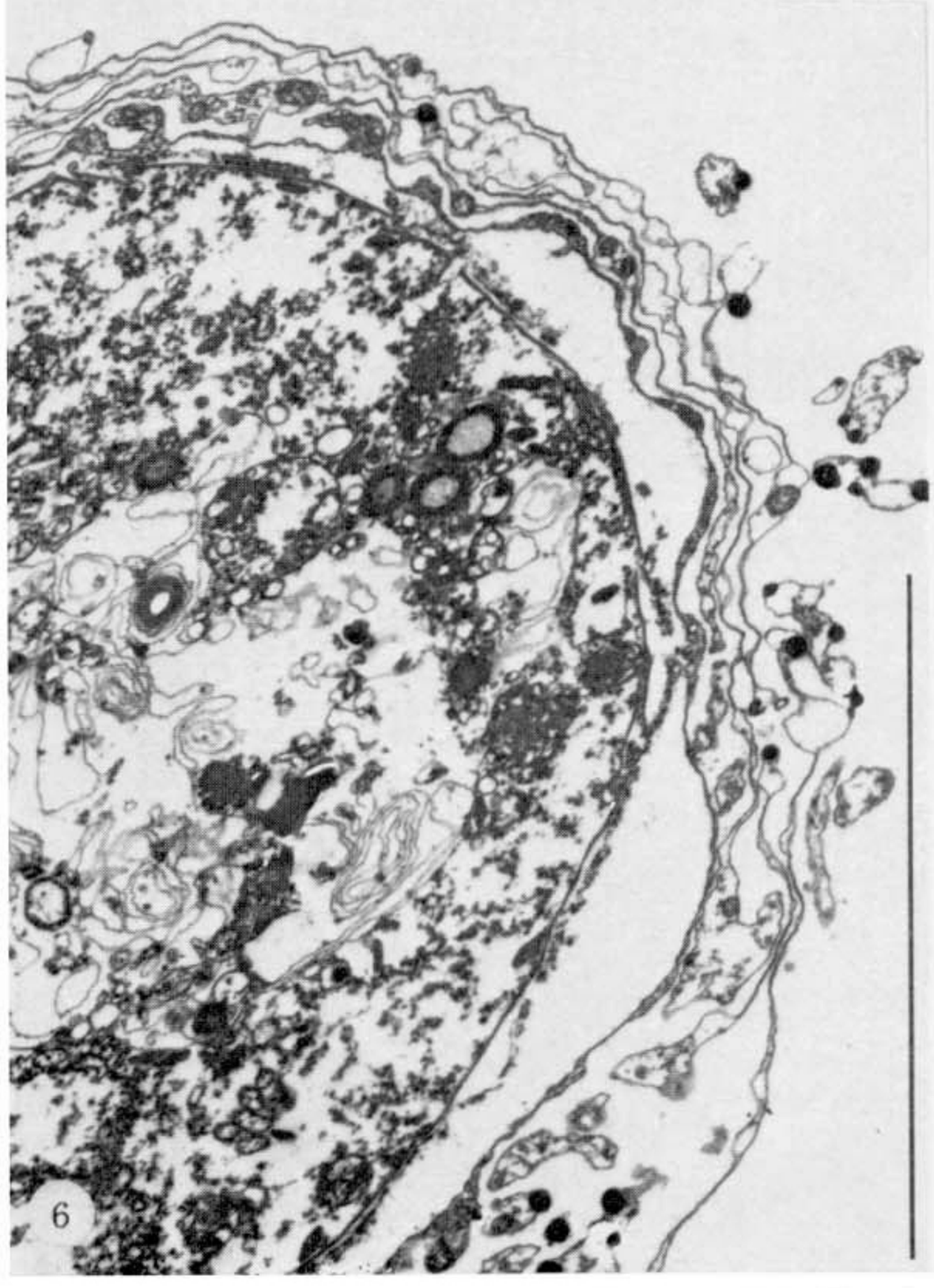

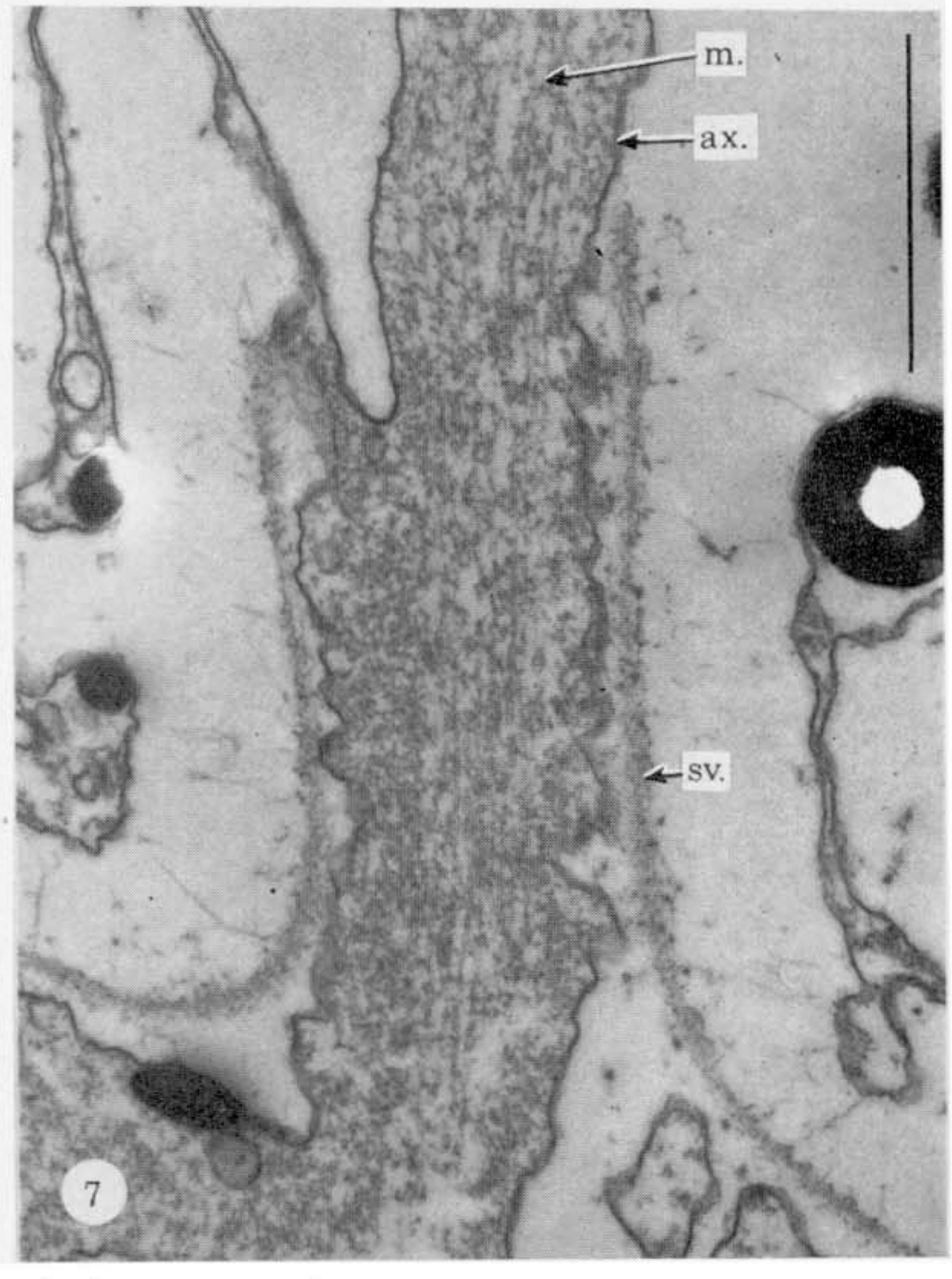

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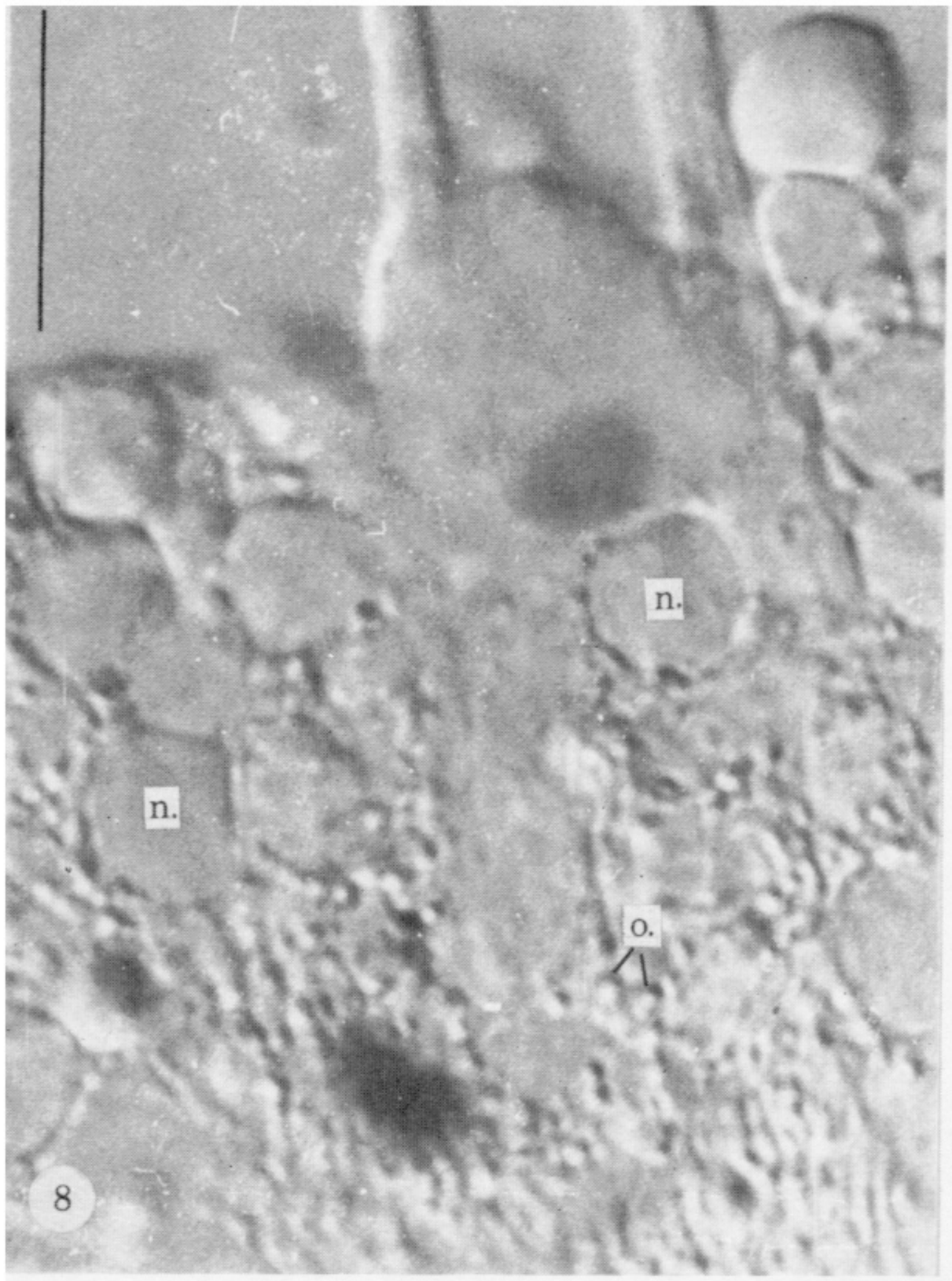

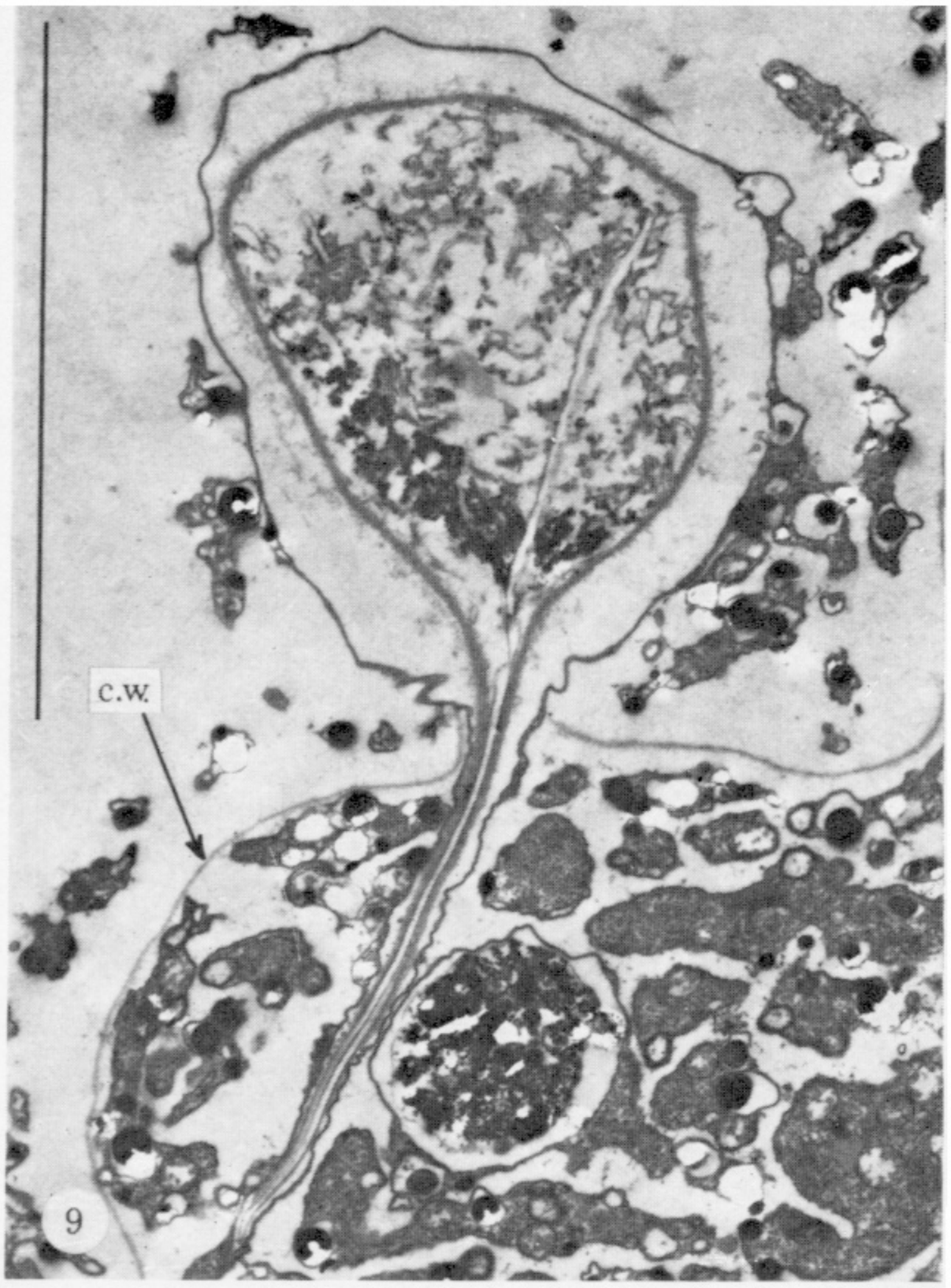

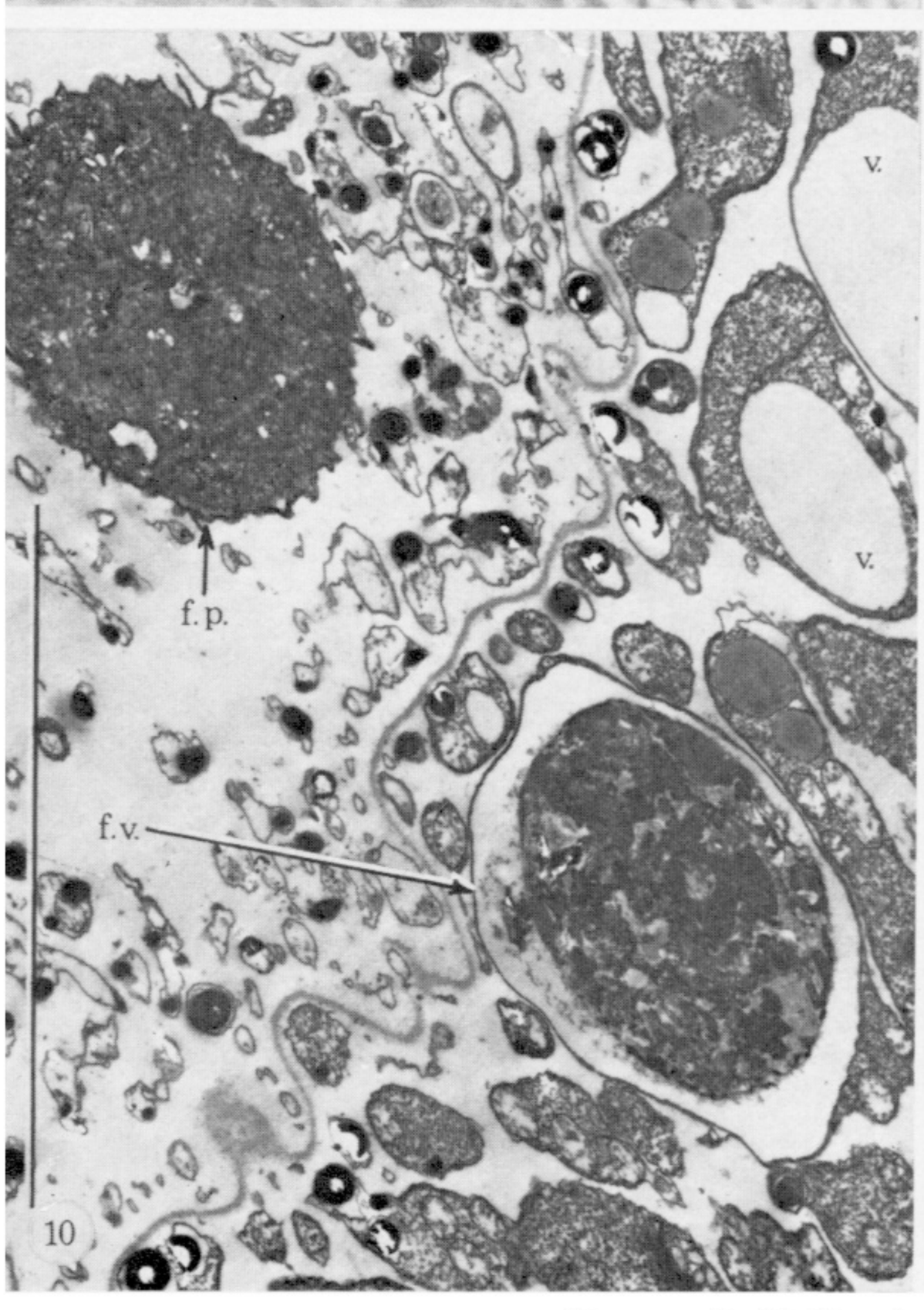

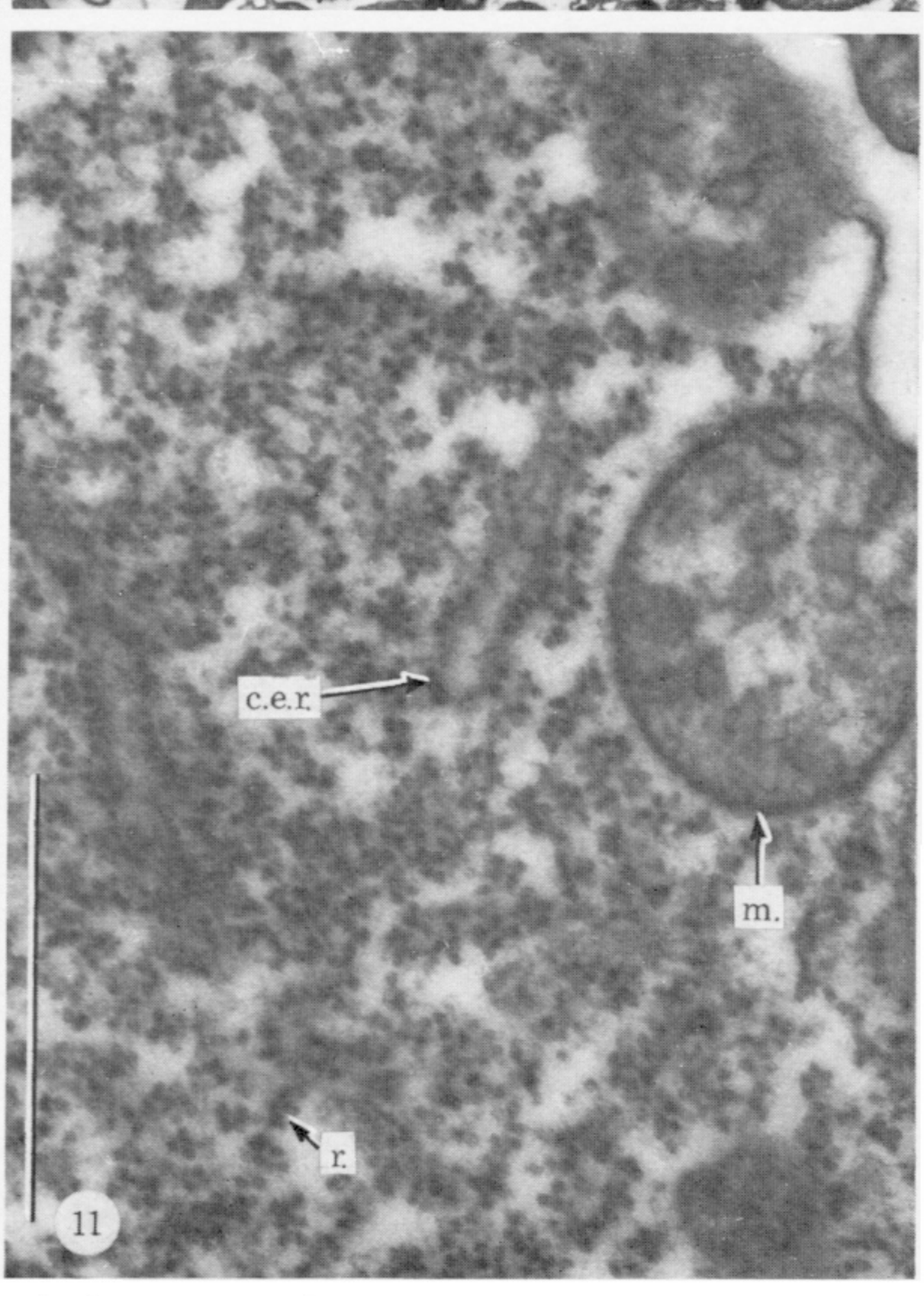

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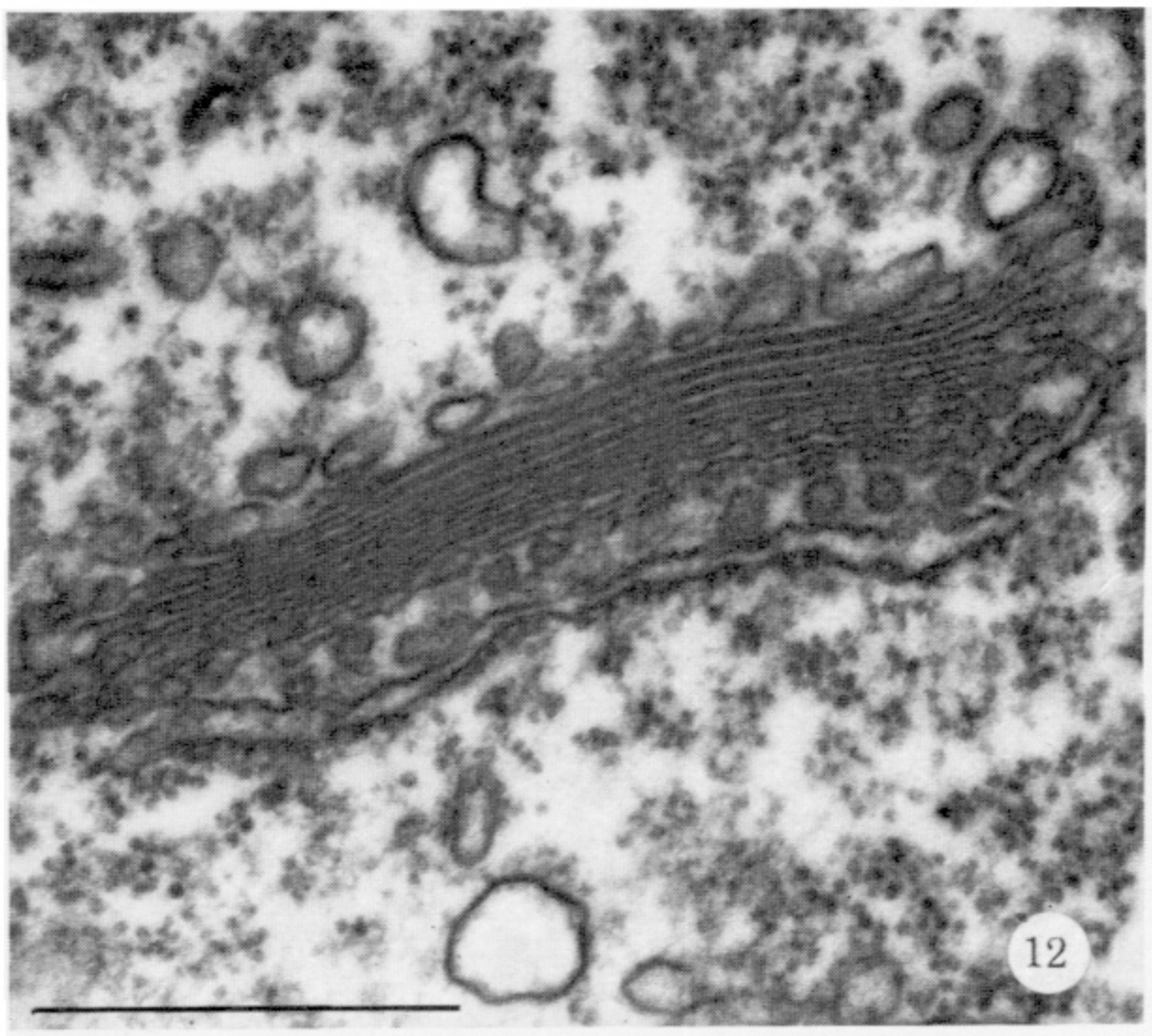

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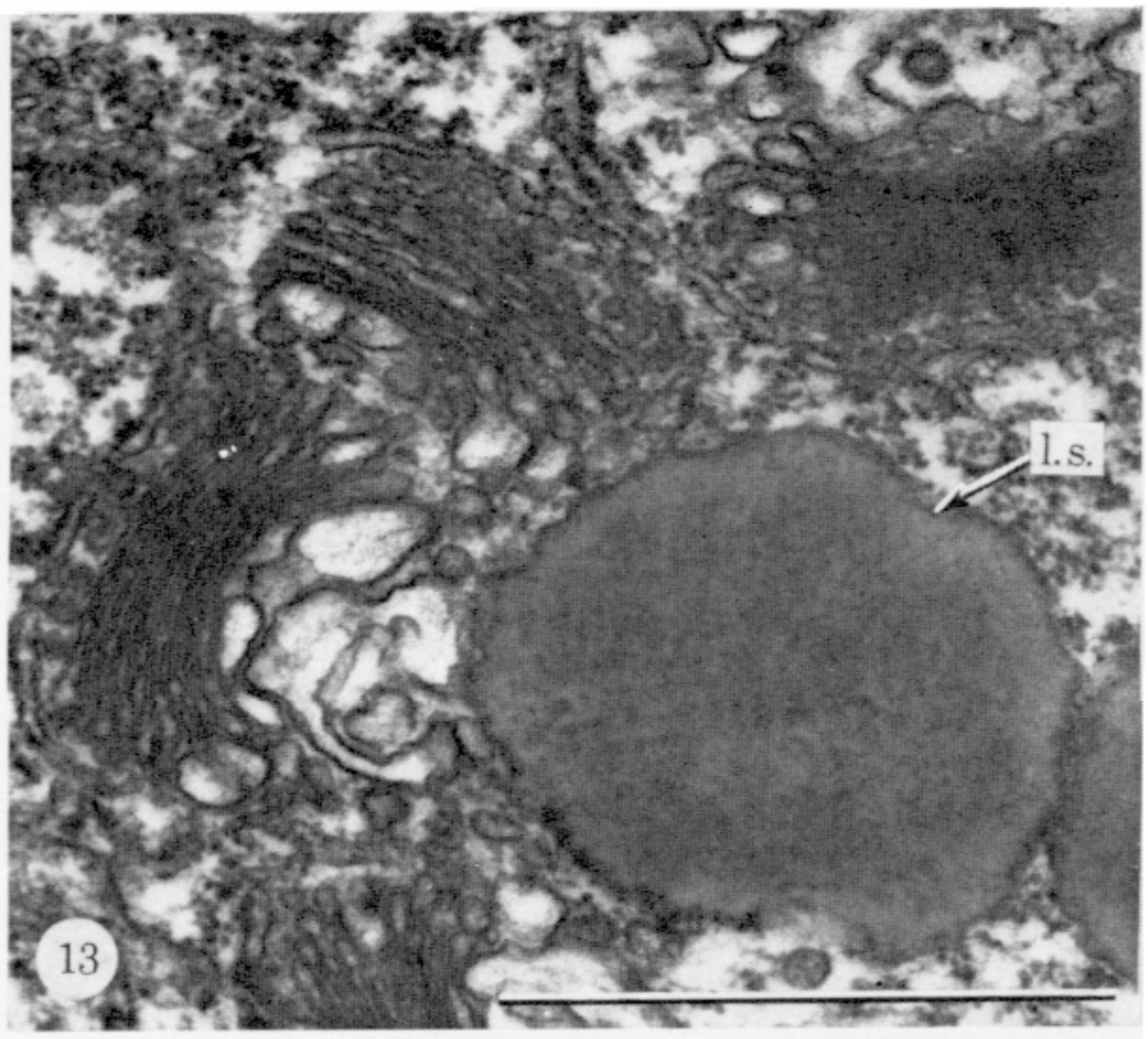

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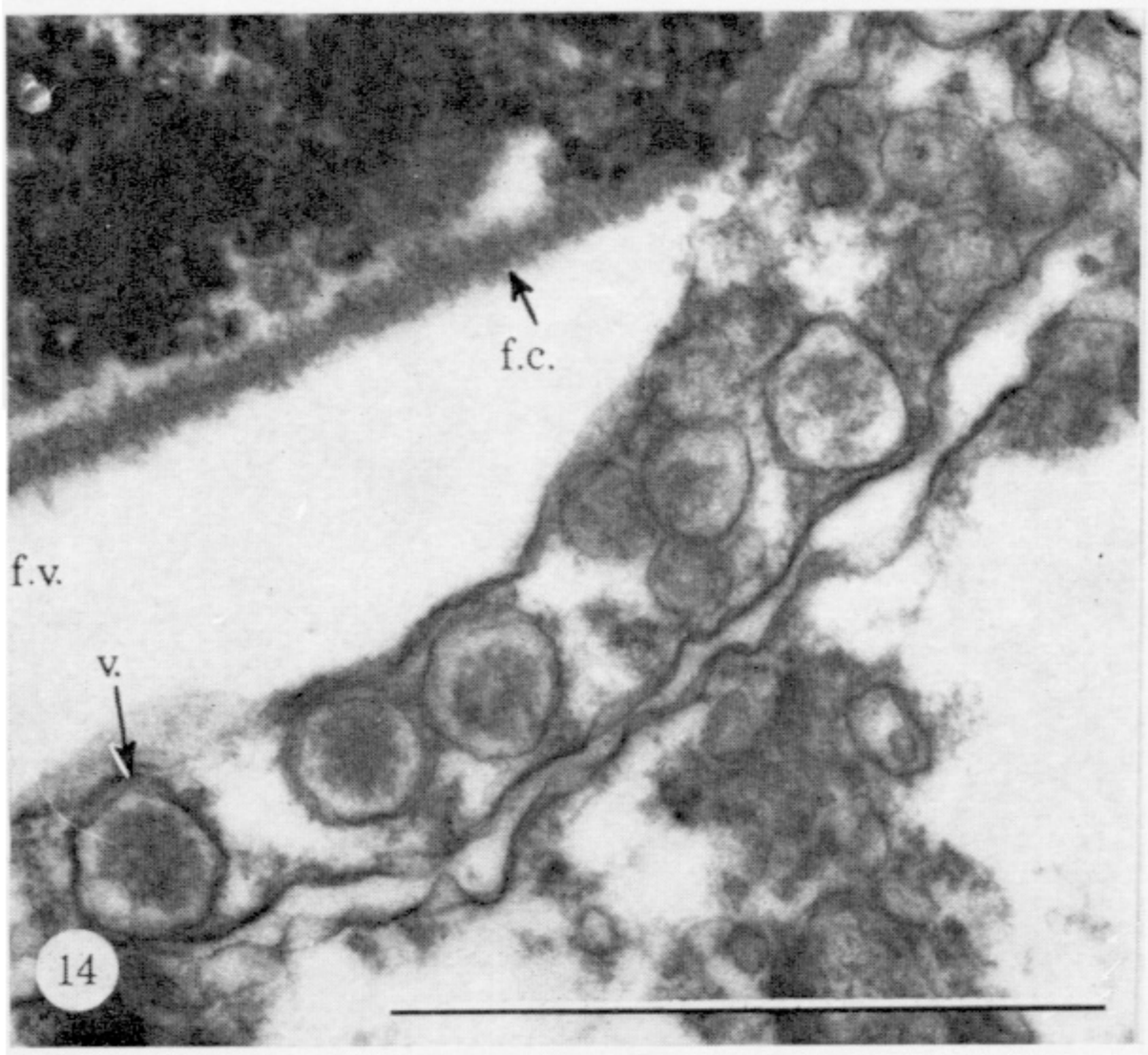

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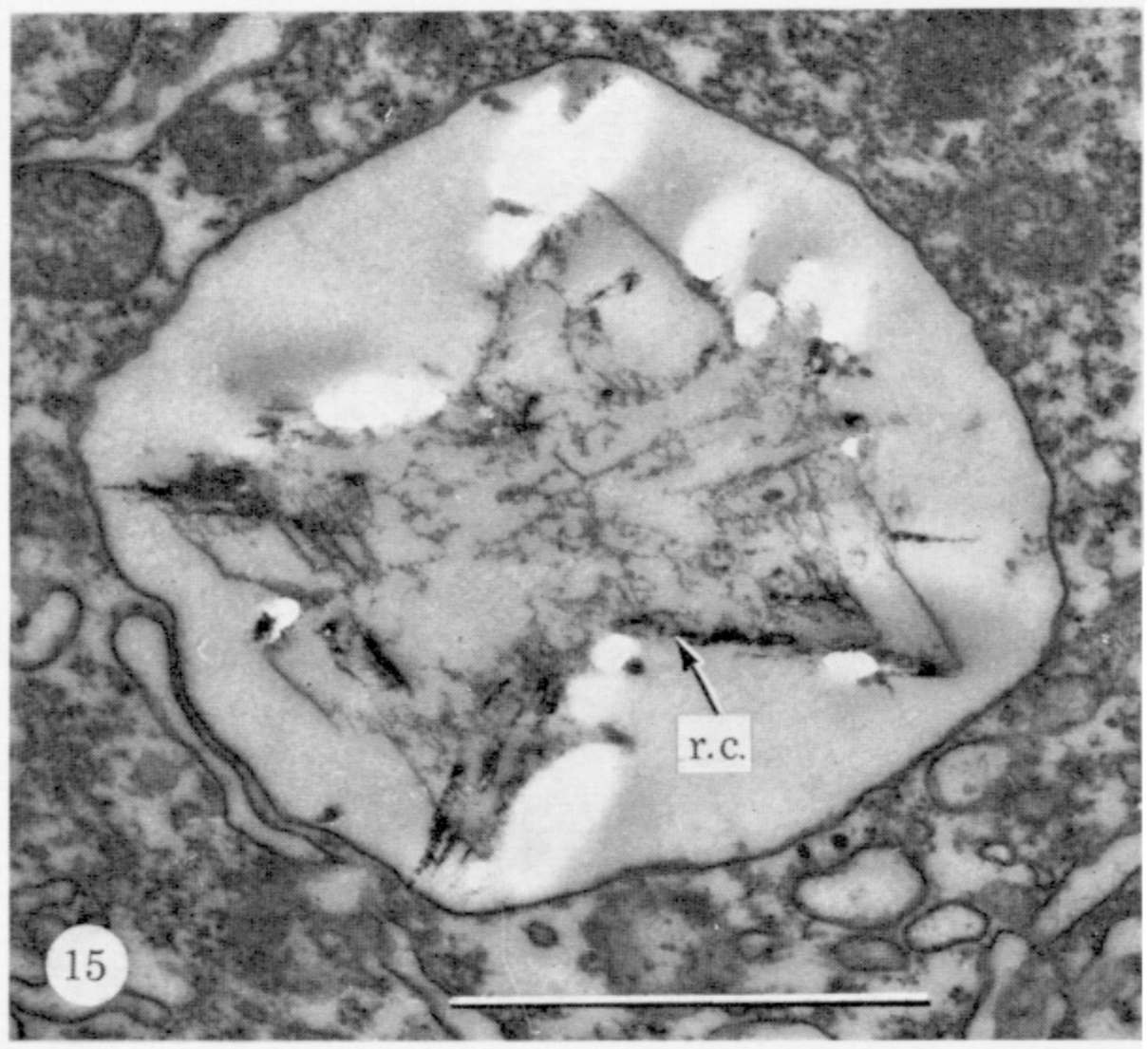

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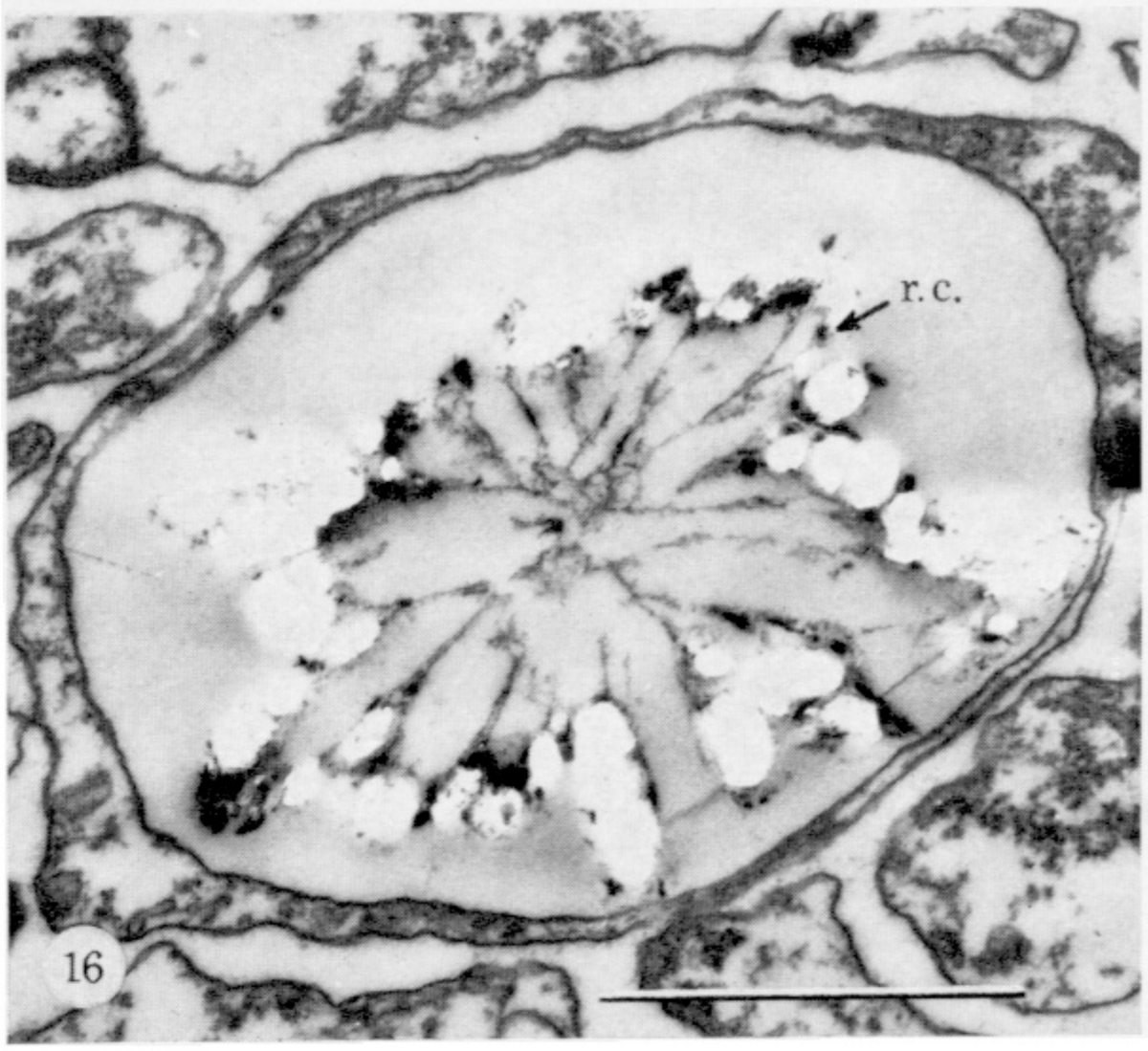

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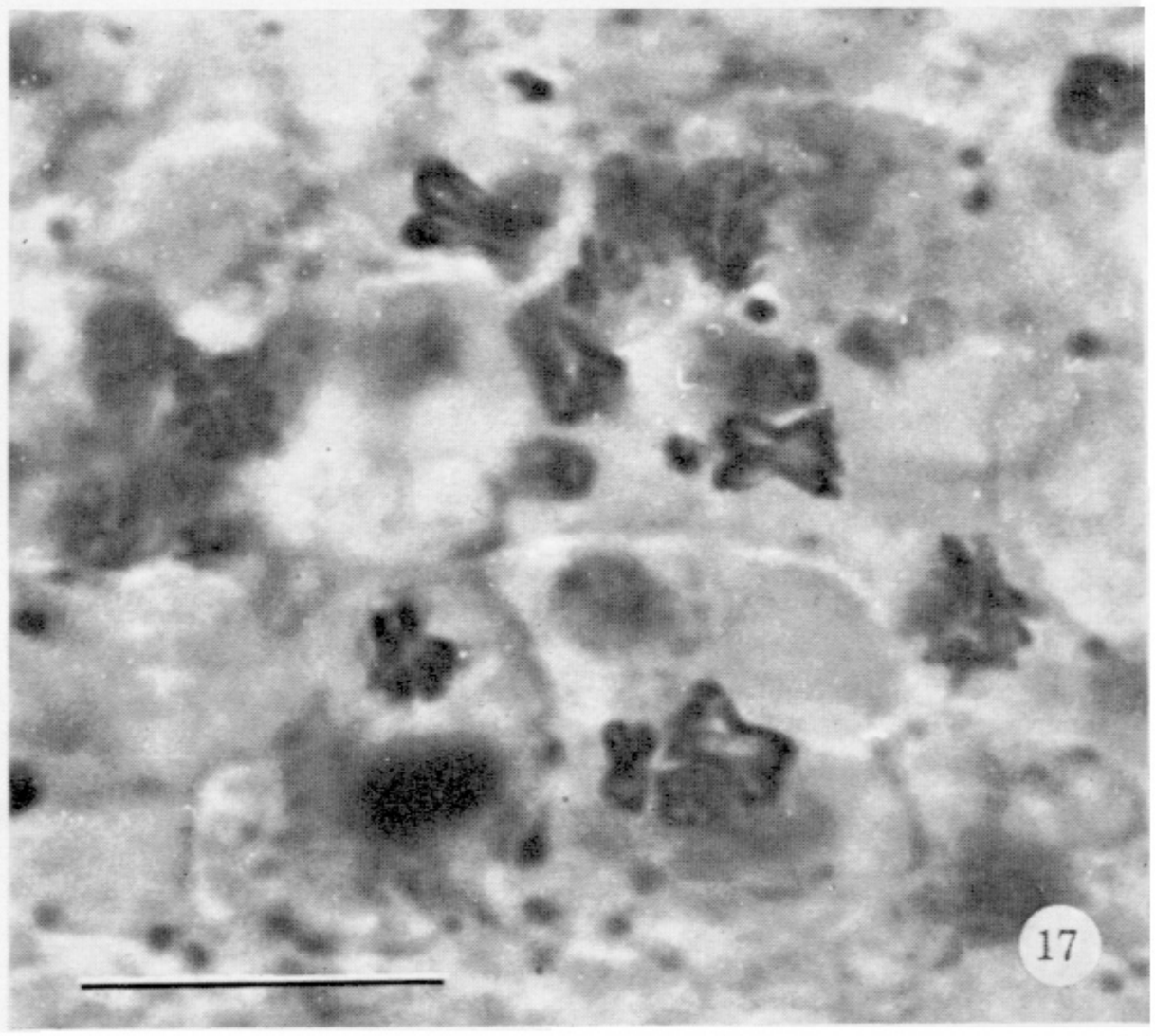

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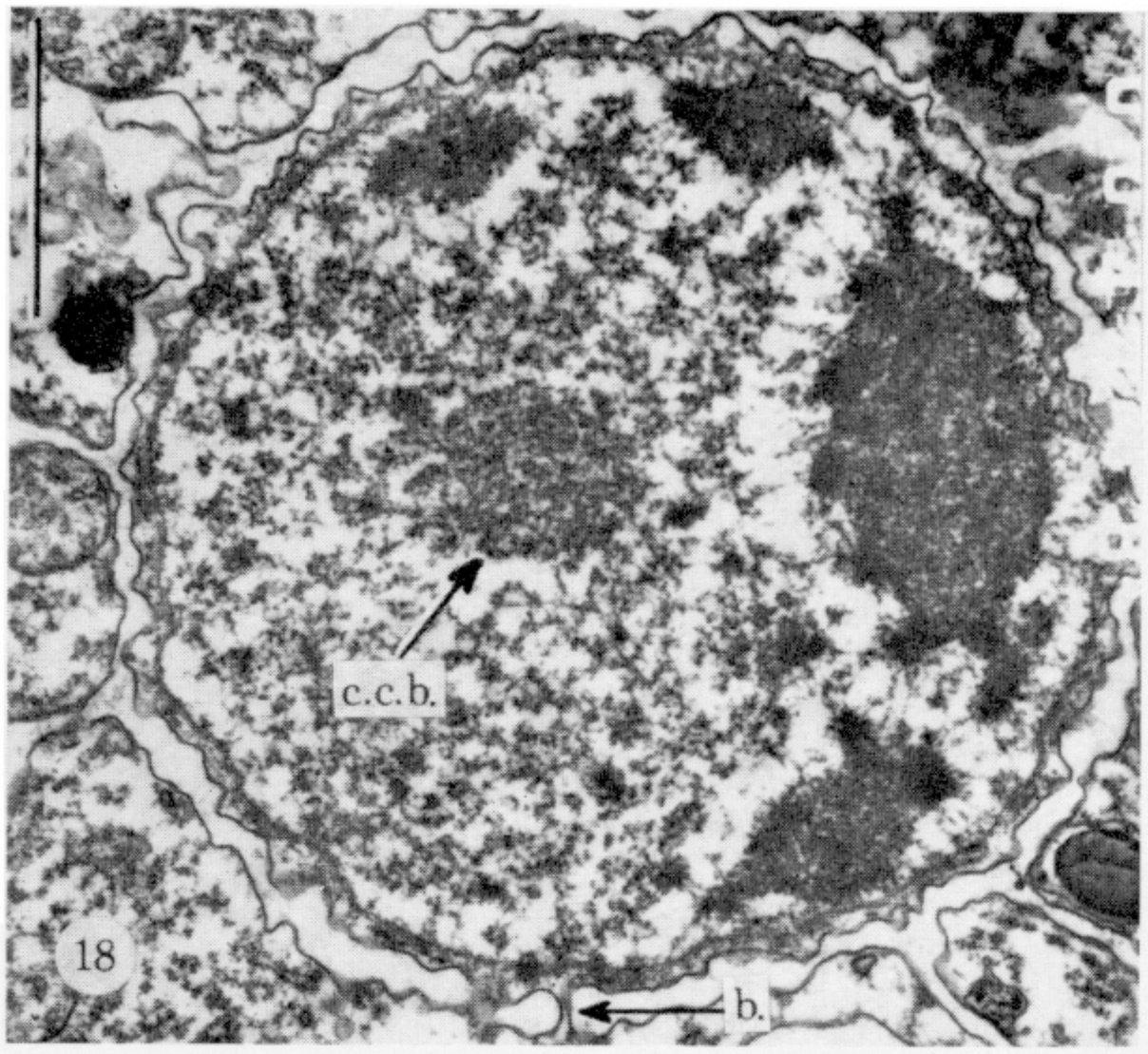

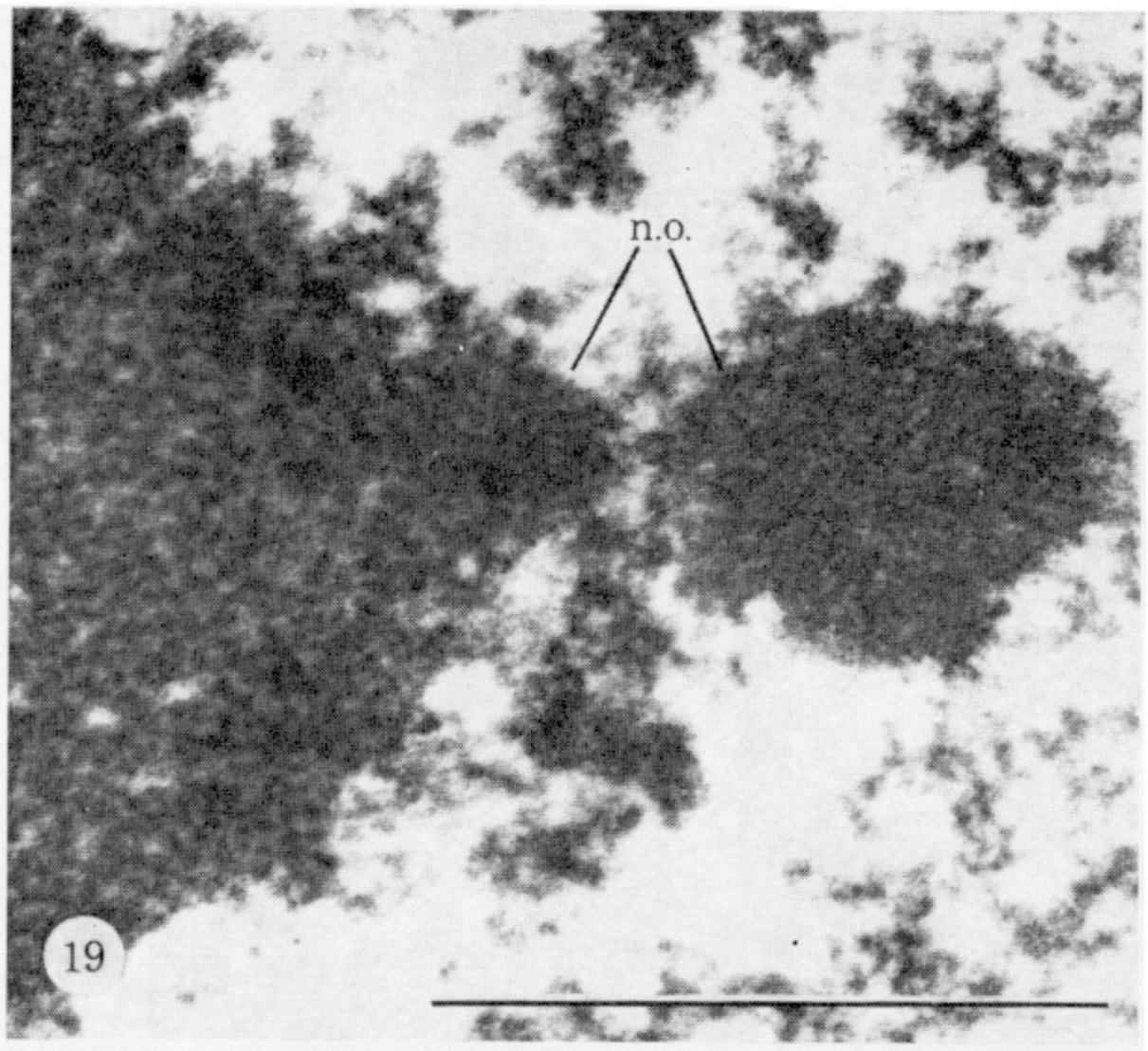

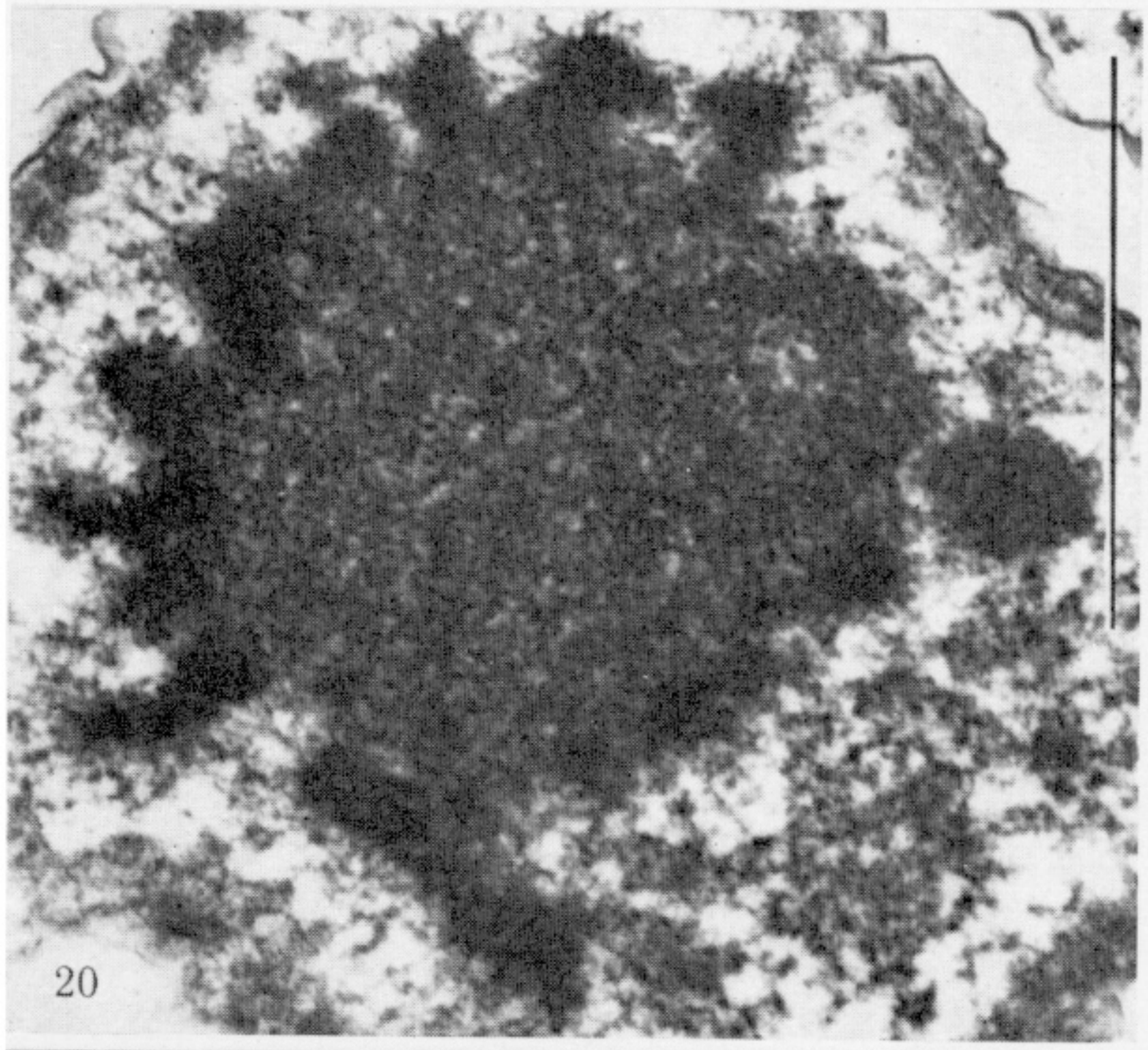

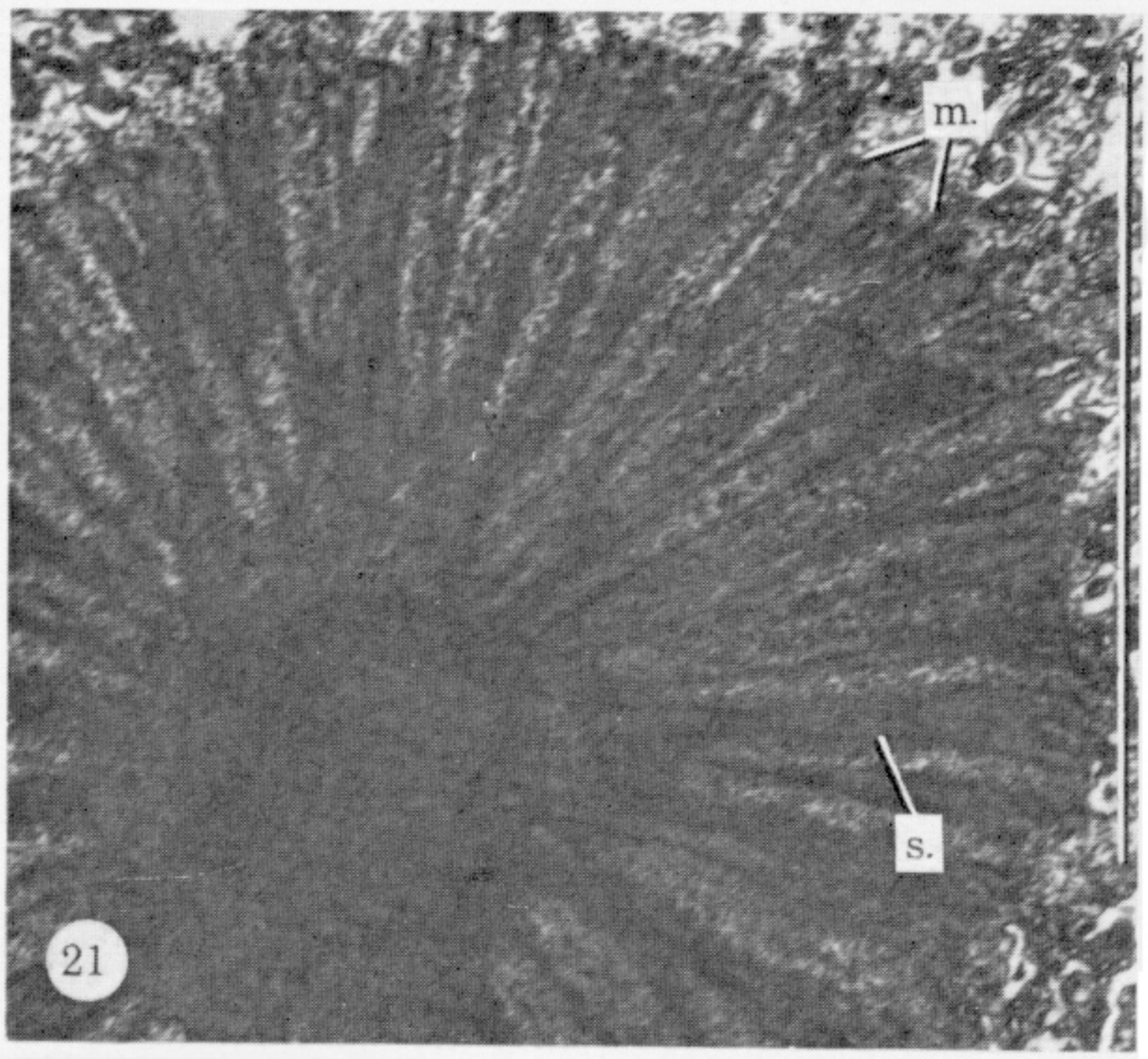

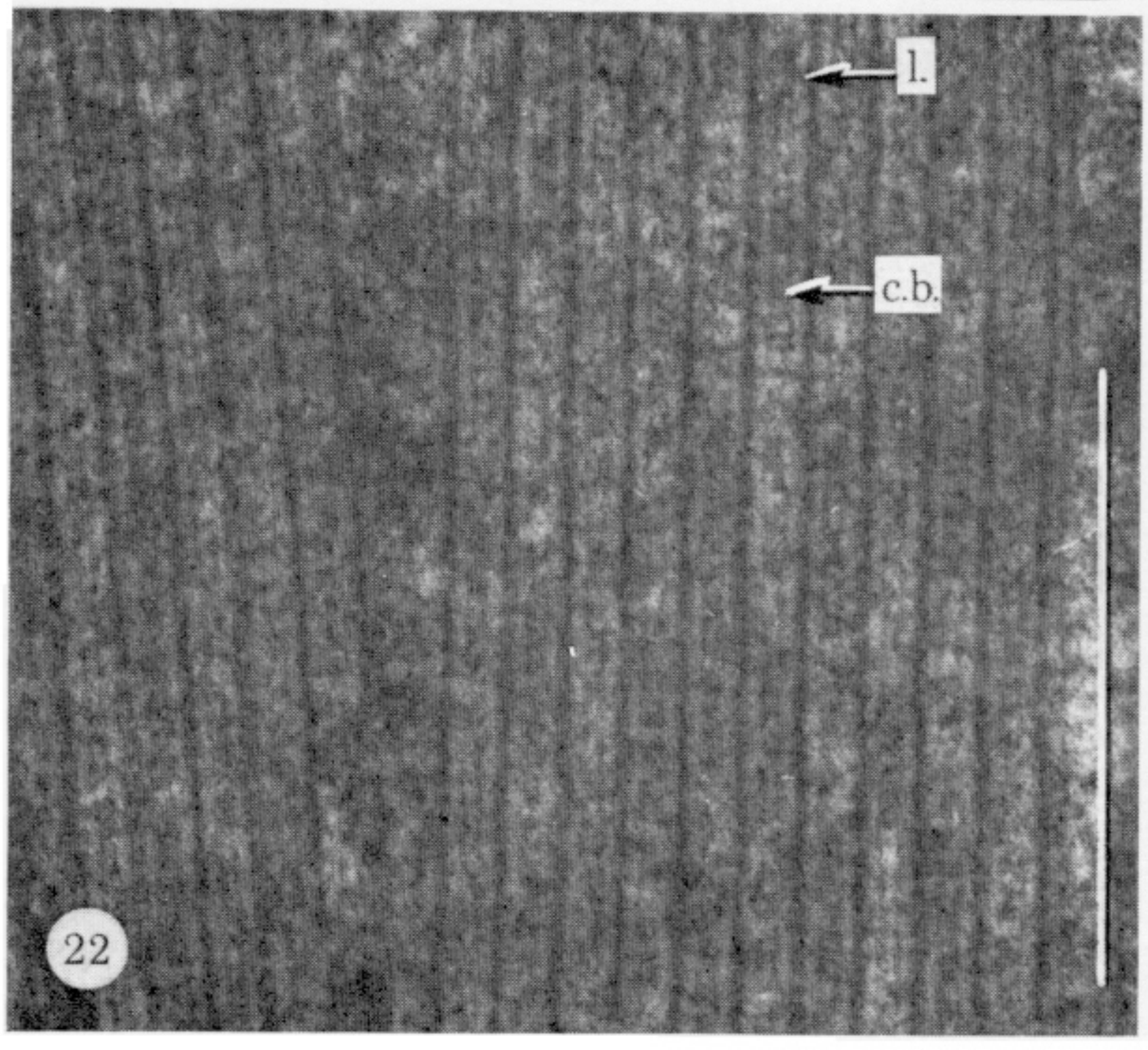

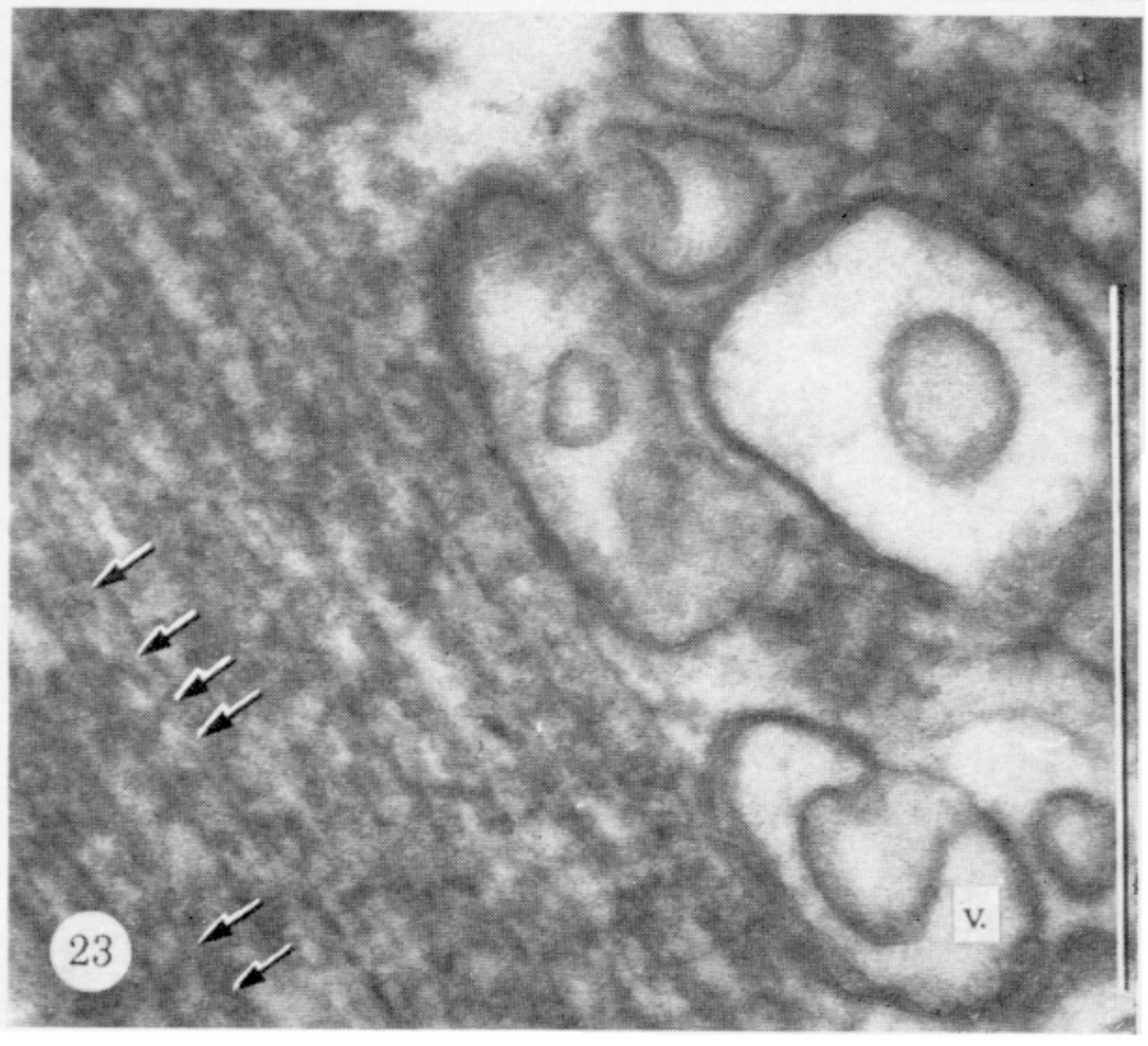

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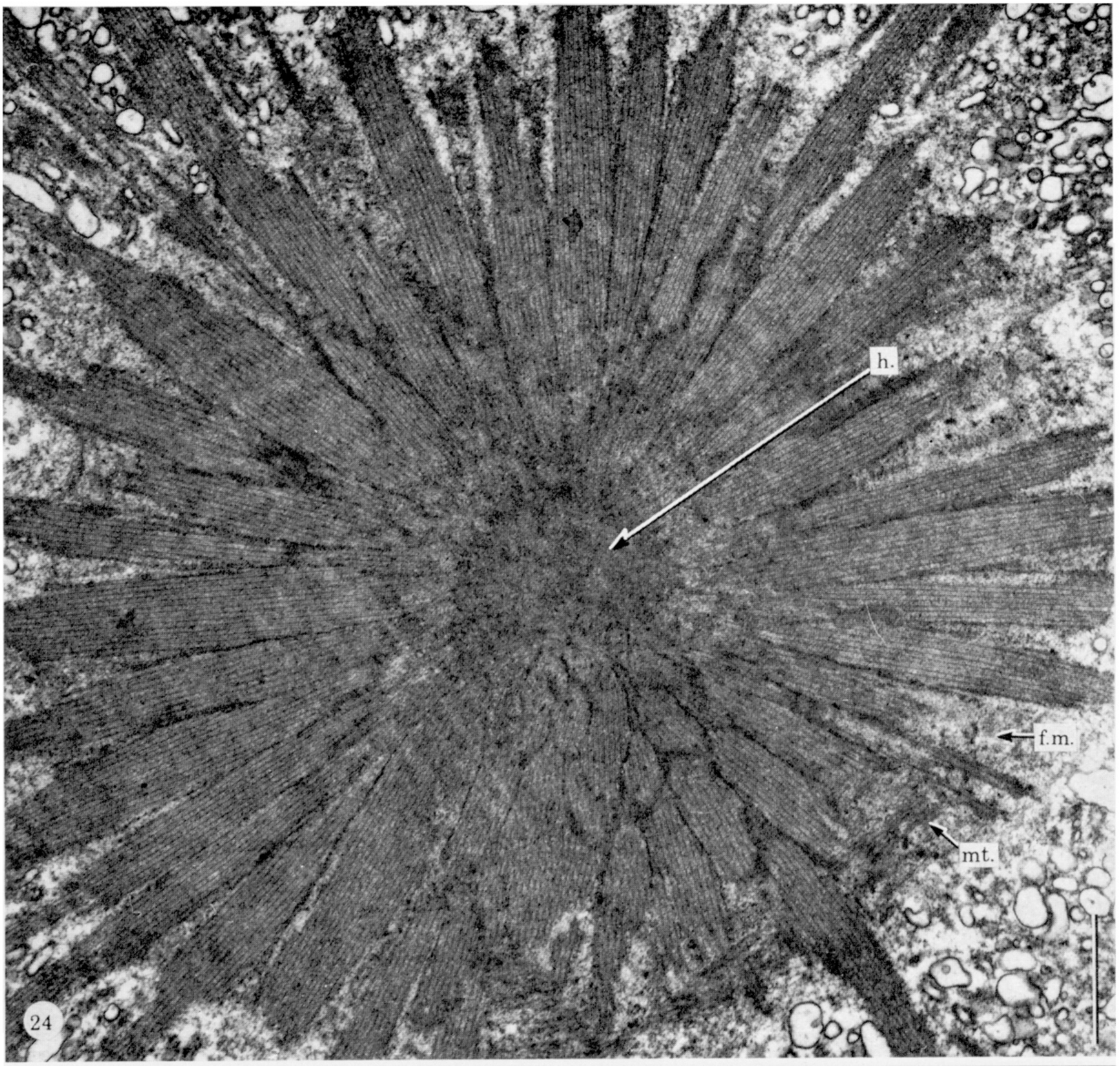

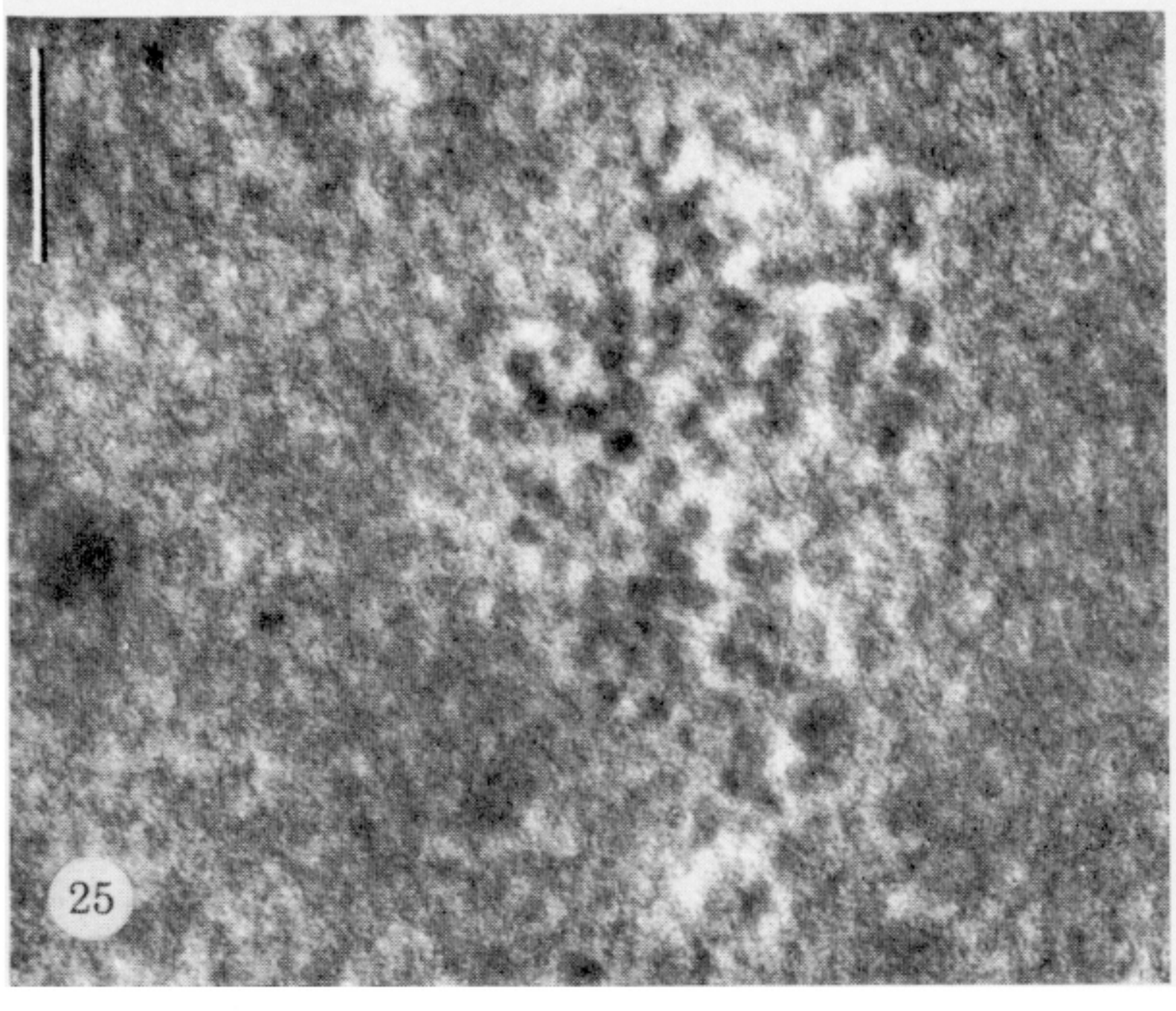

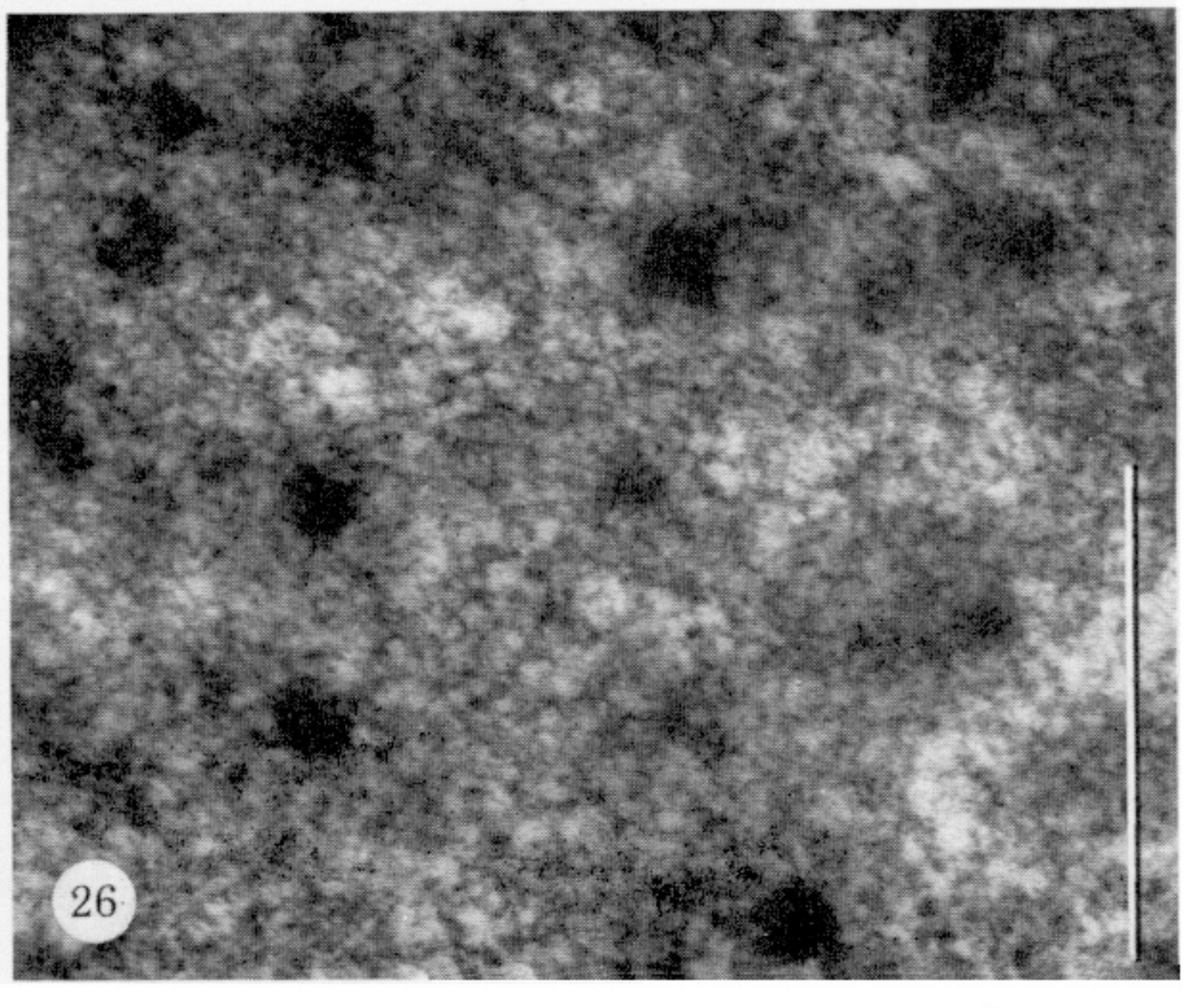

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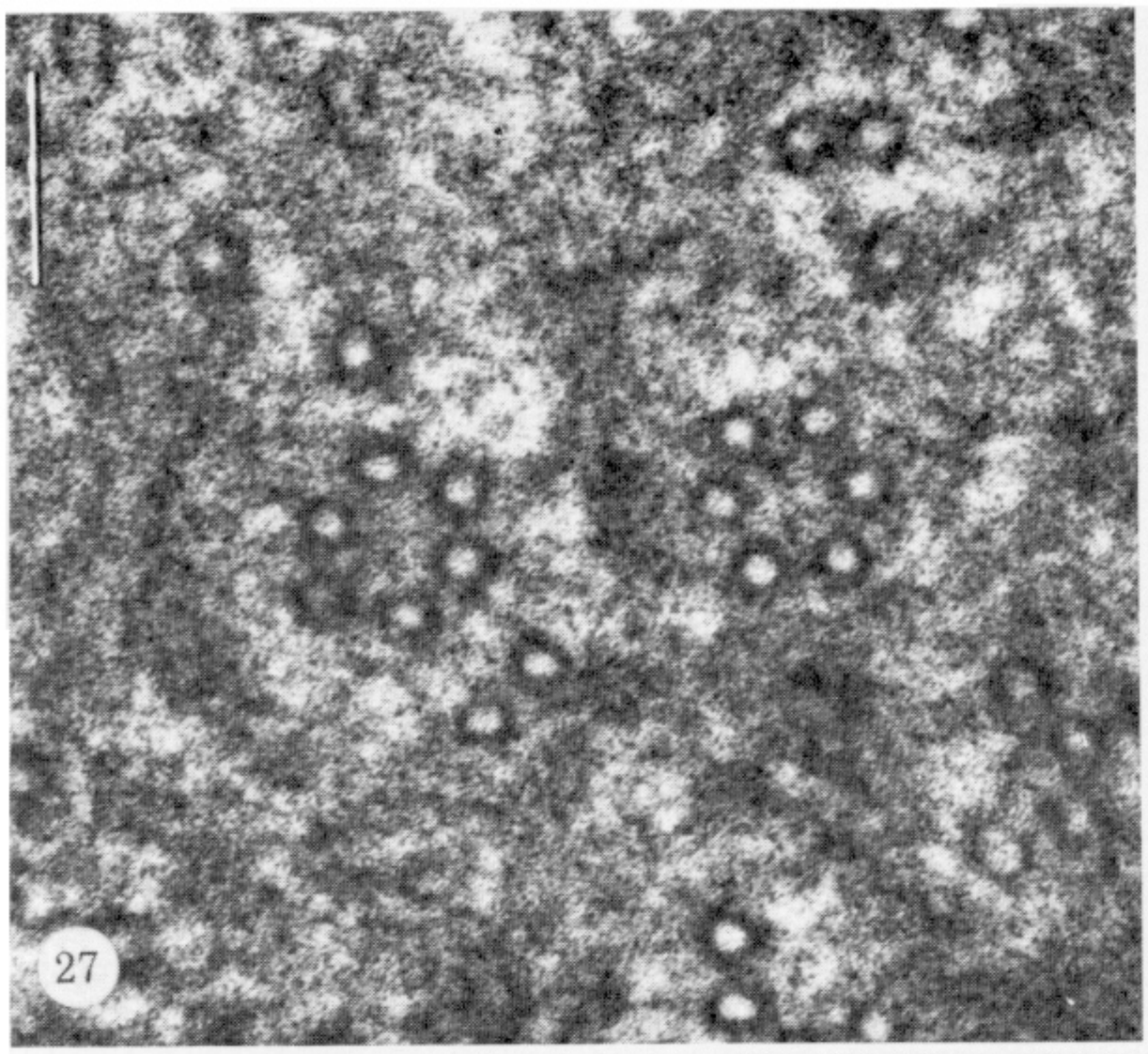

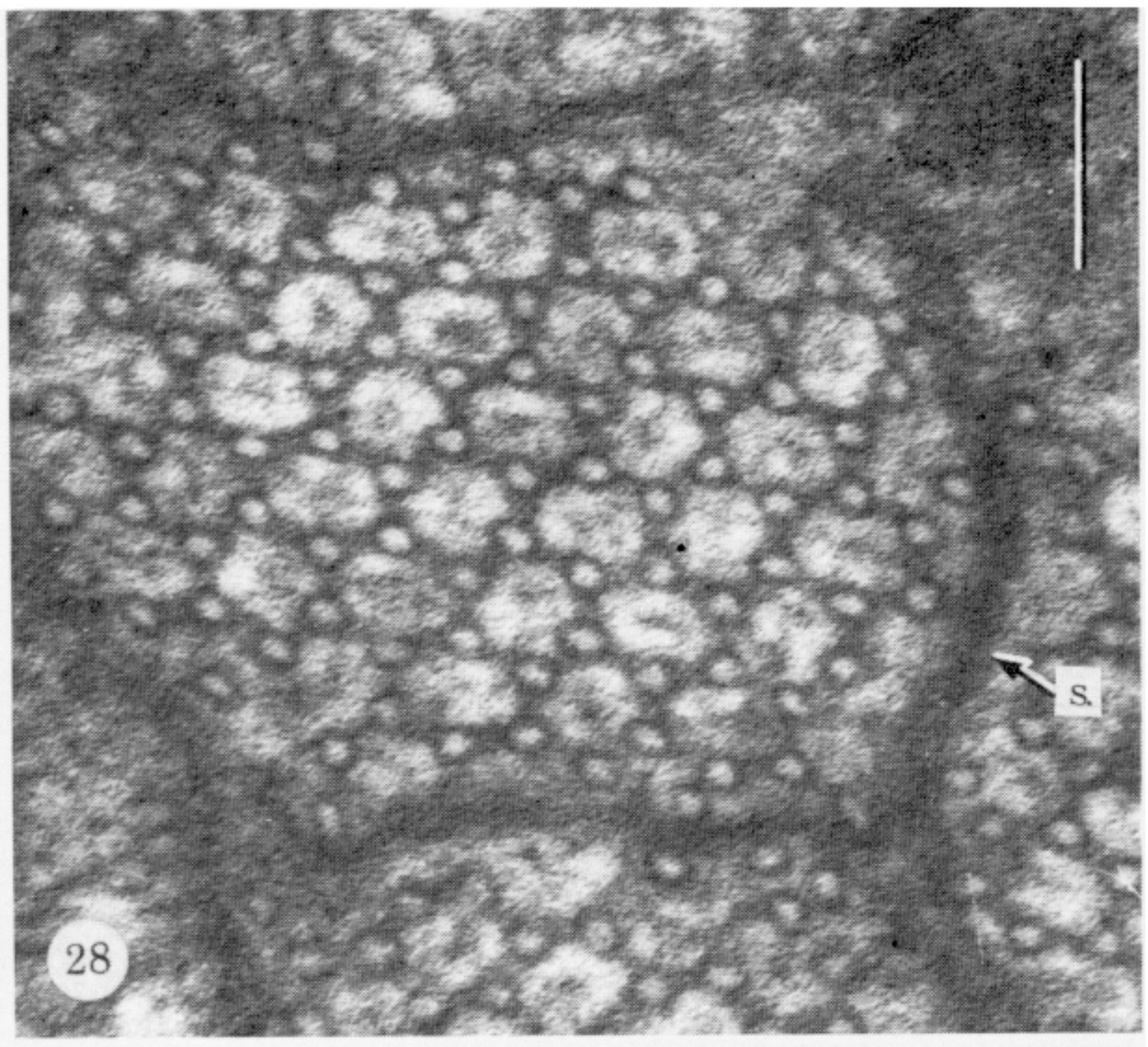

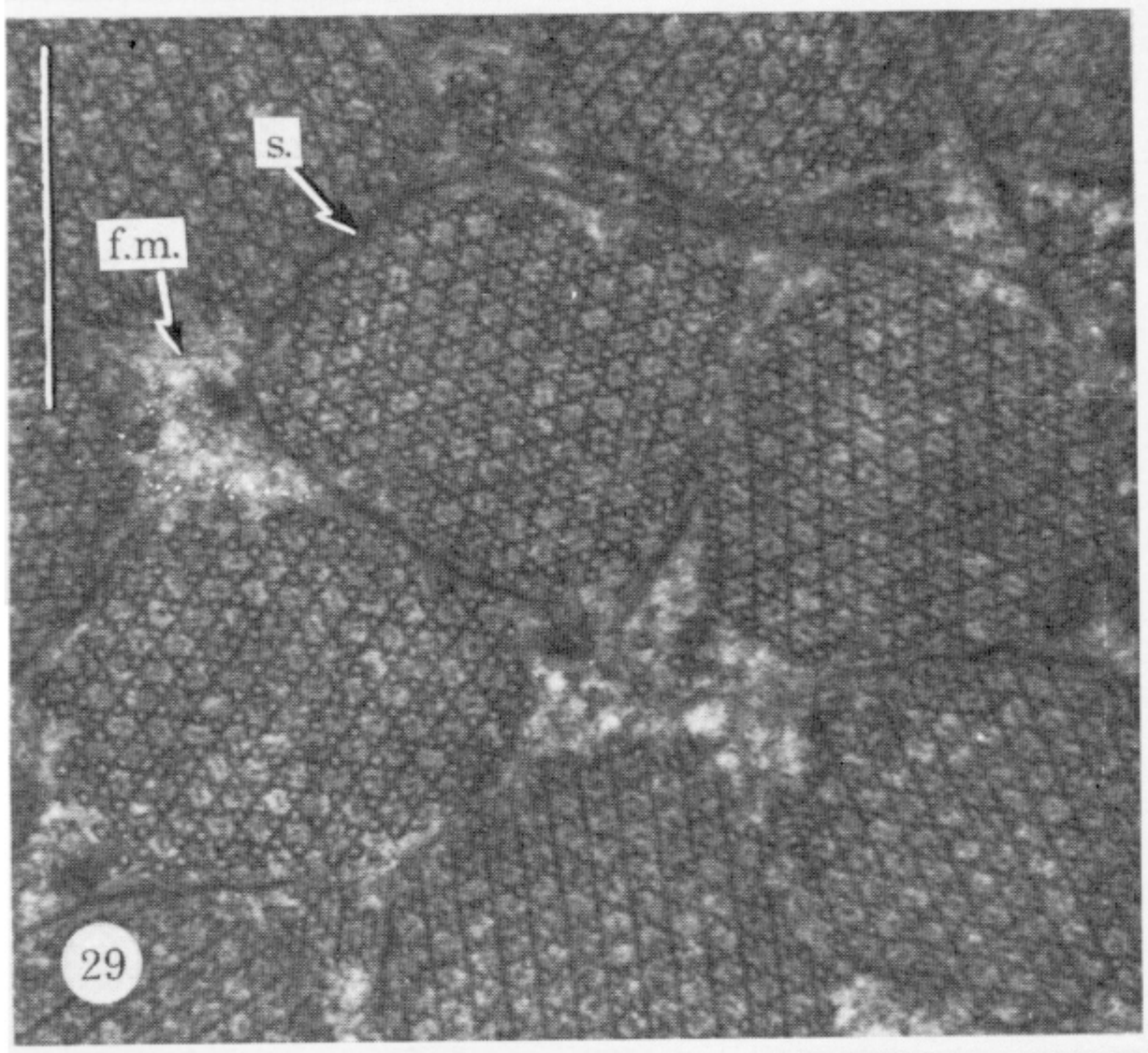

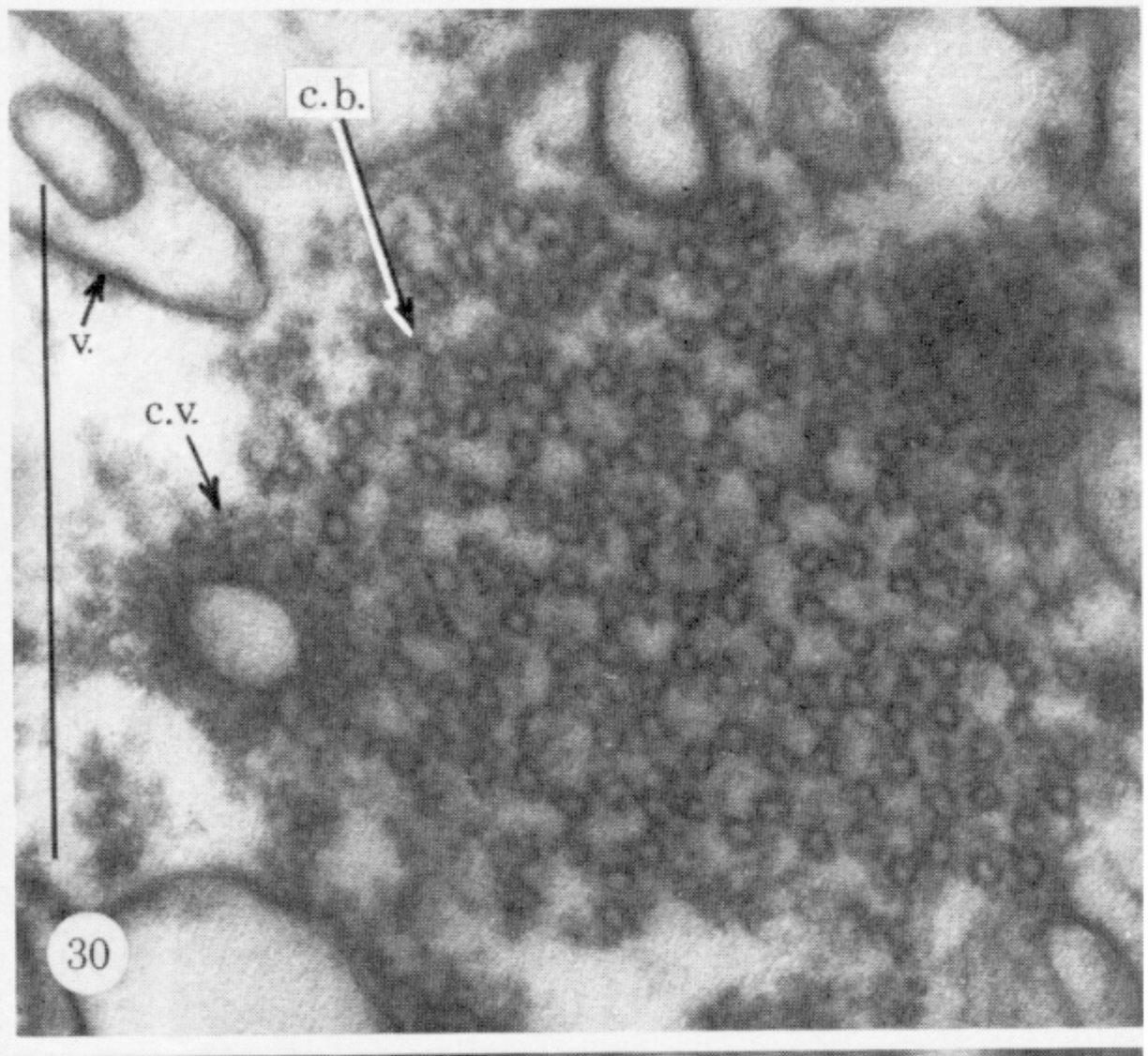

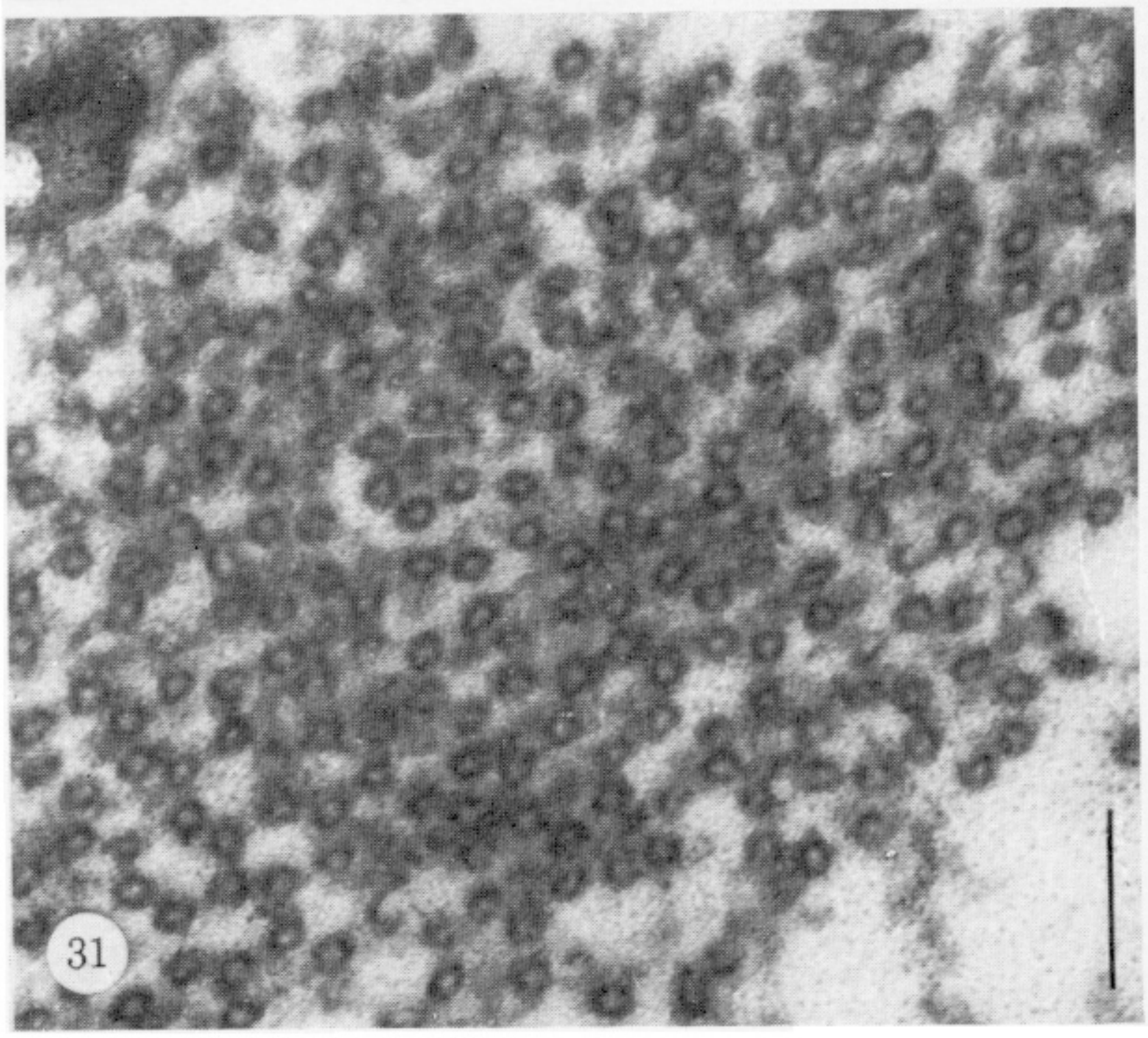

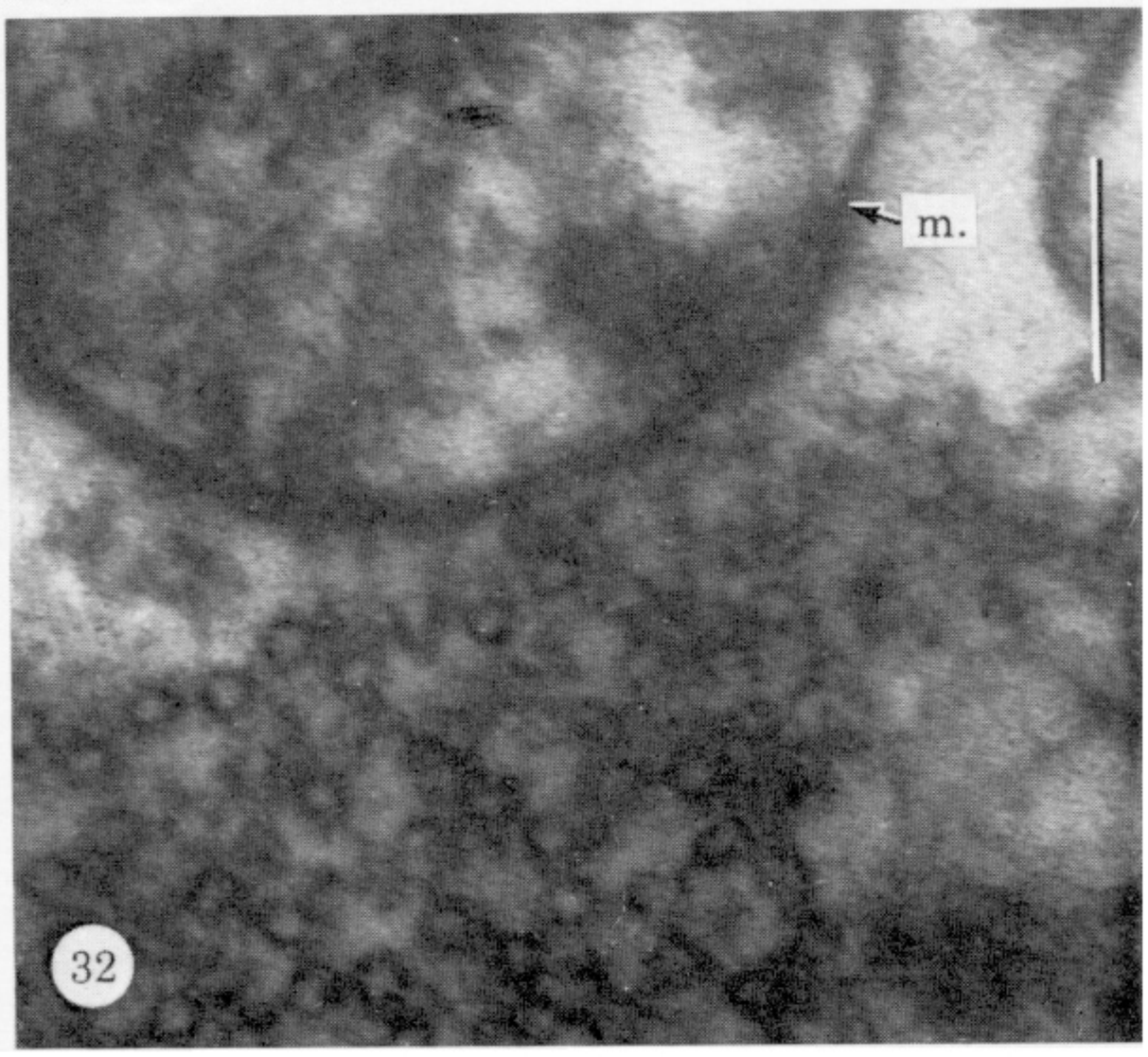

Figures 27–32. For description see opposite.