THE RHABDOMERE ORGANIZATION OF SOME NOCTURNAL PISAURID SPIDERS IN LIGHT AND DARKNESS

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The retinae of the posterior eyes of pisaurid spiders in the genus *Dolomedes* are described. They resemble those of Lycosidae, but the receptors are much larger, and proximal to the strips of tapetum upon which they rest the receptor axons are grossly dilated.

Each receptive segment contains two rhabdomeres, and pairs of rhabdomeres belonging to adjacent receptors are contiguous. Prolonged (6 h) illumination at physiological levels causes the rhabdomeres to diminish in volume by loss of membrane which is restored on return to darkness. When spiders are kept in darkness for 4–5 d, the rhabdomeres grow by the orderly addition of membrane to the microvilli until they completely fill the receptive segments, and such novel membrane is subsequently disassembled when the retina is illuminated. It is proposed that under normal conditions there is a balance maintained between the growth and destruction of rhabdomere membrane.

The paired rhabdomeres are flanked by the processes of supportive cells which exhibit much membrane amplification, and the supportive cell system extends below the tapetum completely to ensheath the swollen receptor axons, which are some 70–80 μm long. In dark-adapted retinae the supportive processes are shrunken; illumination causes them to swell, and the extracellular space between the interdigitations fills with electron-dense material derived from the breakdown of rhabdomere membrane. The material is passed basally and reintroduced into the receptor axons via an extensive system of endocytotic pleats. The tips of pleats often enclose pigment granules from the supporting system, and identical granules in various states of lysis are found within the axoplasm after exposure to light, thus implying that the pleats burst rather than merely transport material across their membranes. There is evidence that pleats may become detached. Exposure of retinae to infrared radiation also evokes breakdown of rhabdomere membrane, but the extracellular route is not employed.

The swollen axons are filled with whorls of rough endoplasmic reticulum, abundant Golgi bodies, and mitochondria. After long periods of darkness, all these systems are depleted, and the space they occupied becomes highly vacuolated. Light adaptation from dim light on a normal diurnal cycle evokes dilation of the cisternae of the endoplasmic reticulum, which pinch off smooth vesicles, and the Golgi bodies become highly active and produce coated vesicles in abundance. The relations between smooth vesicles and microvilli are ambiguous; precedents exist for supposing that smooth vesicles in the inter-rhabdomeral cytoplasm are pinocytotic and have been pinched off from the bases of the microvilli, but in *Dolomedes* there is some evidence to suggest that they may be identical with those manufactured by the endoplasmic reticulum and are also fusing with rhabdomere membrane.

Multivesicular and multilamellar bodies are the product of membrane fragments which have broken off from the rhabdomeres during light adaptation, and of coated vesicles produced by pinocytosis; they are transported within the receptors to the swollen axons where they undergo lysis.

It is proposed that in *Dolomedes* the rôle of the endoplasmic reticulum is to synthesize materials for the repair of rhabdomere membrane, and that the bulk of precursors to sustain this process is obtained by recycling the products of rhabdomere breakdown via the supportive cell system. The hypothesis is discussed in terms of current information about invertebrate retinae, and analogous processes which are well established for

those of vertebrates. *Dolomedes* do not move retinal pigment granules to modulate the shielding of their receptors, and it is likely that manipulation of the properties of photoreceptor membrane is the only strategy of adaptation available to them.

Introduction

Blest & Land (1977) have shown that in a nocturnal spider, *Dinopis*, the cuticular lens of each posterior eye has an *f*-number of only 0.58. Because the aperture cannot be stopped down, and there is no provision for the movement of protective screening pigment, the matter of how the retina deals with luminous fluxes which must daily extend over some 6–7 logarithmic units becomes of considerable interest. The receptive segments of light-adapted spiders, although some 20 μm in diameter and 55 μm deep, seemed, in observations with the light microscope, to be almost empty of materials with the histological reactions of rhabdomeres. It was suggested that *Dinopis* might employ an exaggerated version of the strategy implied by White & Lord (1975) for larval mosquitoes, and Behrens & Krebs (1976) for *Limulus*, both of which appear to partly demolish rhabdomeres in light and to reassemble them in darkness, and this hypothesis has now been confirmed (Blest, in the press). In *Dinopis*, the region of receptor axon proximal to the receptive segment is grossly swollen for a distance of some 150–250 μm, and it was supposed that material derived from the demolished rhabdomeres is stored there.

As a preliminary to the work on *Dinopis*, we have examined the retinal ultrastructure of some other nocturnal spiders in the Pisaurid genus *Dolomedes*. Although the retinae are quite differently organized, they share with *Dinopis* the possession of swollen receptor axons with comparable histological reactions. Here we describe the retina in the dark- and light-adapted states, and show that material is indeed removed from the rhabdomeres when the retina receives prolonged illumination within the normal physiological range of intensities and durations, and that it is returned to the receptors via the supportive cell system of the retina and recycled.

Materials and methods

New Zealand *Dolomedes* are large spiders whose systematics have not been recently revised. Of the three species used indiscriminately in this study, two may probably be assigned to *D. minor* Koch and *D. aquaticus* Goyen; they were obtained from creek-beds on Banks Peninsula and at Kaikoura (Canterbury, South Island). A third, very big species, at present undescribed, was obtained from the upper, forested reaches of the Kaituna Stream, Banks Peninsula. The spiders are all semi-aquatic, hiding beneath stones during the day, and emerging at dusk to rest with their first pair of legs touching the water surface. From this position they catch drifting insects trapped by the surface film. When alarmed, they pull themselves beneath the surface of the water, carrying a coating of air trapped by the hydrophobic hairs with which they are covered (Forster & Forster 1973).

Spiders were collected in the field by searching beneath stones. Those used in the preliminary experiments were merely kept in the shade after capture, and maintained in a dimly-lit room in vessels containing stones and an inch of water. They were fed on blowflies. For the later experiments, they were collected in plastic jars which were immediately placed in black plastic bags, and transferred to controlled régimes of illumination some 2–4 h later.

For light microscopy, spiders were rapidly dissected into Bouin's solution, a strip of cuticle

bearing the retina fixed for 24 h, the retinae detached from their lenses, and 10 μm wax-embedded sections cut and impregnated by a reduced silver method (Blest 1976).

For electron microscopy, light-adapted spiders were rapidly dissected under a primary fixative (2.5 % glutaraldehyde in 0.1 M cacodylate buffer with 0.14 M sucrose adjusted to pH 7.3) in the light. Dark-adapted spiders were placed under a dark red darkroom safelight, their legs and abdomens removed, a strip of cuticle bearing the retina excised and placed in fixative in the dark. In both cases the procedure was completed in 1.5–3.0 min, but weak electroretinograms could be recorded through the cuticle in response to similar red illumination; narrow-pass filters were not available. Retinae were freed from their lenses immediately after primary fixation for 1 h, washed in 0.1 M cacodylate buffer (pH 7.3, 30 min) and post-fixed in similarly buffered 1 % osmium tetroxide (30 min, room temperature). After washing briefly (2 min) in 10 % acetone, they were stained in 2 % aqueous uranyl acetate for 20–30 min, dehydrated through an acetone series, embedded in Spurr's resin and polymerized overnight at 60°C. Sections 50–70 nm thick (silver–pale gold interference colours) were cut on an LKB-Huxley or Ultratome III ultramicrotome, collected on 100- or 200-mesh copper grids coated with collodion, counterstained with lead citrate, and examined with a JEM 100B electron microscope.

TABLE 1. RÉGIMES OF LIGHT AND DARK ADAPTATION USED IN THE EXPERIMENTS

(Sequences read from left to right. 'Dim' light used for holding spiders before the experiment was measured in the same way as the intensities employed for adaptation, and gave spot photometer readings in the 2.2–62 cd/m² range. See text.)

	I	II	III	IV	V
1	dim light	fix	—	—	—
2	dim light	4 h 460 cd/m²	fix	—	—
3	dim light	4 h dark	2 h 460 cd/m²	2 ht dark	fix
4	dim light	560 cd/m²	fix	—	—
5	dim light	560 cd/m²	20 h dark	fix	—
6	dim light	1700 cd/m²	fix	—	—
7	dim light	1700 cd/m²	4 d dark	fix	—
8	dim light	6500 cd/m²	fix	—	—
9	dim light	6500 cd/m²	5 d dark	fix	—
10	dim light	5 d dark	1700 cd/m²	fix	—
11	dim light	6 h dark	fix	—	—
12	dim light	5 d dark	fix	—	—
13	dim light	5 d dark	6 h infrared	fix	—

With the exception of 2 and 3, all periods of light adaptation lasted for 6 h.
In the case of the experiment using infrared radiation alone, treatment 12 was repeated in parallel, and sequence 10 using an operating lamp at 3200 cd/m², as controls.

For purposes of light adaptation, spiders were individually restrained in clear plastic petri dishes. The sources of illumination were either 150, 250 or 275 W photofloods, or a Skylux Superminor portable operating lamp (Varanda Shadowless Lamp Co. Ltd, Tokyo), at 200–500 cm above the dishes, which were placed on a uniformly reflecting white surface. Light reflected off that surface varied from 560 cd/m² (275 W unfocused at 500 cm) to 6500 cd/m² (operating lamp at 300 cm) as measured by a Pentax spot photometer. A portable cool-air fan was used to maintain the animals at room temperature during adaptation.

These sources of illumination were not controlled for levels of infrared emission. The effects of infrared radiation in the absence of visible light were evaluated in a separate experiment. A 150 W photoflood source was used with an infrared filter with a cut-off point below 700 nm,

in conjunction with the cool-air fan to minimize secondary heating effects. Levels of infrared emission were monitored with a TIL 64 detector mounted in a similar plastic dish. Spiders thus exposed were kept in constant darkness for 5 d before treatment, and controls run in parallel killed spiders from darkness, and after 6 h exposure to mixed radiation from the operating lamp.

The treatments which the spiders received are described in table 1. Two spiders received each treatment; all posterior retinae from a given spider were found to be in the same state, and the retinal states from pairs of spiders receiving the same treatment were consistent. Careful orientation and trimming of the blocks and the radial disposition of the retinal elements allowed single sections to sample all levels of an individual retina.

The 'dim' light in which spiders were maintained gave readings between 2.2 and 62 cd/m^2.

Results

1. *Gross anatomy of the retina*

The retinae of the four posterior eyes are atypical in that they are trough-shaped instead of hemispherical, although they possess a grid-iron tapetum of the type common to Lycosidae and Pisauridae (Homann 1951, 1971). The shape of the retina is probably determined by optical adaptations to periodic immersion; here, it will merely be noted that the lenses, which are alike, have *F*-numbers in the range 0.8–1.0 when their front faces are in air. Those of the posterior median (p.m.) and posterior lateral (p.l.) eyes are identical in structure. The anterior eyes are not considered in this paper.

Figure 1 presents the gross arrangement of the retinal components. Beneath the lens and the glass cells which secreted it there is a thick layer of receptor somata, separated from each other by the thin lamellate processes of supportive cells. The somata give rise to receptive segments whose rhabdomeres are seated on strips of tapetum, and which are consequently ordered in lines along the lengths of the strips. Receptive segments composing a single line are contiguous, but each strip of tapetum bears two lines of receptors separated from each other and from the receptors of adjacent tapetal strips by the processes of supportive cells, which contain pigment granules. The pigment does not move in response to light and dark adaptation, and cannot be interposed between tapetum and receptive segment, so that the tapetum is not occlusible. Furthermore, the pigment granules do not extend distally beyond the outer boundaries of the receptive segments; thus, even if they were to some extent motile, they would be unable to screen the outer face of the retina. It follows that adaptation must rely upon processes occurring within the receptive segments themselves, and these fail to exhibit any gross photomechanical changes during light or dark adaptation.

As it traverses the grid of the tapetum, each receptor is compressed where it passes between adjacent strips, but, in silver-impregnated sections, fibrillar material filling the swollen axon can be seen to extend beyond the constrictions and into the receptive segments between the rhabdomeres.

The appearance of the receptors in longitudinal section resembles that of the homologous region of the *Dinopis* retina, but in *Dolomedes* there are fewer argyrophilic granules, and the swollen region is only some 70 μm long.

Although the overall arrangement of the retina is similar to that described for Lycosid spiders by Baccetti & Bedini (1964) and Melamed & Trujillo-Cenoz (1966), in *Dolomedes* each receptive segment is contiguous with its neighbours at the two faces composed of rhabdomeres,

whereas in Lycosids the segments are completely isolated by the processes of supportive cells. The latter are of two kinds: non-pigmented cells, whose nuclei lie distal to the receptive segments, extend narrow horizontal arms which radiate between many receptors and send numerous processes deep into the retina to the full extent of the swollen axons; and pigmented cells, whose

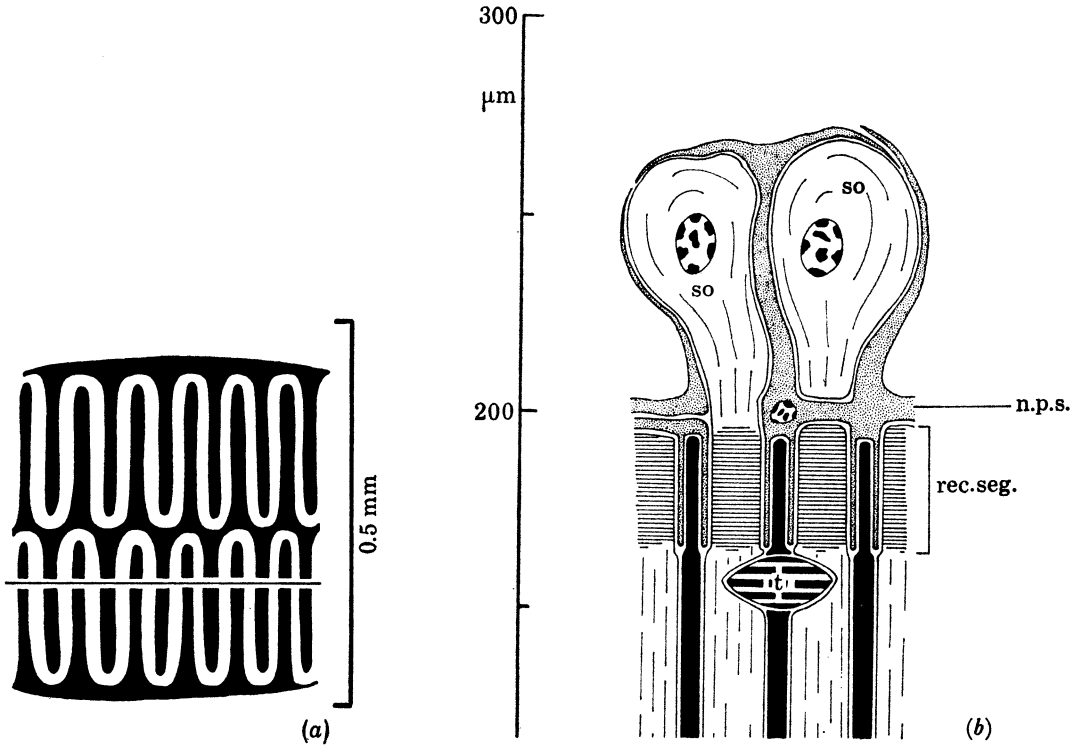


FIGURE 1. (*a*) Surface view of the central region of a posterior median retina, to show the disposition of the two wavy strips of tapetum (white) which underlie the rows of receptive segments. The line which intersects the lower strip gives the orientation of (*b*).

(*b*) Diagrammatic section perpendicular to the surface of the retina to show the arrangement of its components. The somata (so) of the receptors lie distally, and are ensheathed by sheets of processes derived from non-pigmented supportive cells (n.p.s.) whose nuclei lie just above the receptive segment layer. The rows of receptive segments (rec. seg.) are separated from each other by partitions composed of non-pigmented supportive cell processes and pigmented supportive cell processes (shown as solid black bars) whose nuclei lie basally between the axons of the receptors. Both types of supportive cells show much membrane amplification which is not represented in the diagram. Alternate supportive partitions are interrupted by strips of tapetum (t), of which one is shown. The receptor axons are swollen for a depth of some 150 μm so that their diameters correspond to those of the receptive segments. The scale is assumed to begin at 0 μm at the level at which the axons narrow abruptly as they leave the retina in bundles of the optic nerve. Full explanation in the text.

nuclei lie amongst the swollen axons, and extend processes distally which end at the outer margins of the receptive segments. Both kinds of supportive cell show much membrane amplification, and their processes interdigitate completely to sheath the receptors from the somata to the point at which the axons narrow to form tributaries of the optic nerve. Interdigitating membranes are bound by infrequent desmosomes. There are fewer supportive cells of either type than there are receptors enclosed by them.

Optic nerve fibres (figure 3, plate 1) are enveloped by sheathing cells which contain material with a banding pattern of the collagen type, but with a spacing of 12.5 nm (figure 4, plate 1). Insect collagens exhibit banding patterns which are usually in the 50–60 nm range, but may be as low as approximately 17 nm (Pipa & Woolever 1965; Harper, Seifter & Scharrer 1967).

2. *The ultrastructure of receptive segments*

(i) *Less then* 24 h *of dark adaptation*

For purposes of exposition, receptors from spiders which were kept in the light for varying periods, and then returned to the dark for less than 24 h are described first, and receptors at the end of periods of light adaptation and from spiders which were kept in the dark for 4–5 d are treated as departures from this intermediate state. It will be seen that descriptions of eyes as 'light-adapted' or 'dark-adapted' merely in terms of the régimes to which they have been exposed can be misleading. Retinae from spiders which have been in darkness for less than 24 h approximate in state to those from spiders maintained in a diurnal cycle with 'dim' daylight intensities and to those from spiders which have been light-adapted for 2 h.

A thin transverse section from light-adapted (2 h) receptive segments is shown at low magnification in figure, 2, plate 1. Between the rhabdomeres of each receptive segment there is a substantial volume of cytoplasm, containing vacuoles derived from, and in some cases continuous with, dilated fragments of endoplasmic reticulum, smooth and coated vesicles, and multivesicular and multilamellar bodies. The latter two structures are not regularly present, and are few in number. The two classes of vesicles, however, may be abundant. Fragments of smooth and rough endoplasmic reticulum are seen to be active, and there are free ribosomes and polyribosomes in the cytoplasm. In contrast to *Limulus* receptors (Lasansky 1967), the endoplasmic reticulum is nowhere regularly organized as subsurface cisternae (Rosenbluth 1962). Bodies with the staining reactions of glycogen also appear to be absent.

(ii) 6 h *of light adaptation*

After 6 h of light adaptation the rhabdomeres are to various degrees reduced. In extreme cases they may be short. The inter-rhabomeral cytoplasm contains larger, irregular vacuoles, but there are few vesicles of either kind, and fusion profiles are not observed. Multivesicular bodies are more prominent and many form clusters, and a variety of structures is seen which appear to represent transitions between fragments of membrane broken off from the microvilli and multivesicular or multilamellar bodies (figures 34 and 38). There are few ribosomes, and organized endoplasmic reticulum is scarcely in evidence. The receptive segment cytoplasm appears largely depleted of materials.

(iii) 5 d *of dark adaptation*

After 5 d of dark adaptation, the receptive segments are found to be filled with rhabdomeres which are for the most part orderly (figure 5). The microvilli have been greatly extended towards the central region of the receptive segment, and only a small volume of cytoplasm remains between them, largely depleted of organelles. There are many coated vesicles, and they are still seen to be attached to the tips of the microvilli. In addition, the rhabdomeres fit more tightly against the partitions of supportive cells, and this is achieved by a diminution in the volumes of the vertical ridges of non-pigmented supportive processes against which pairs of contiguous rhabdomeres are seated (figure 5, plate 2, and figures 20 and 21, plate 7).

In some receptive segments, the newly extended region of the microvilli is disorderly, and suggests that membrane has been added on at random. There are occasional membrane whorls of the kind which have been described as 'myelin figures' (e.g. Loew 1976; Behrens &

Krebs 1976) in similar contexts, but they are not frequent. Multivesicular bodies and multilamellar bodies are not in evidence.

(iv) 6 h *of light adaptation followed by* 4 d *of dark adaptation*

The rhabdomeres of receptors subjected to 6 h of light adaptation are reduced, sometimes dramatically, and never occupy more than about 40 % of the volume of the receptive segment. However, if such a period of light adaptation is followed by 4 d in the dark, it is found that the segments are filled with rhabdomeres: it is clear that reduction of rhabdomere membrane can be followed by its reconstitution and, since the experiments were conducted at levels of illumination matching those likely to be encountered by the spiders in their normal habitats, it must be assumed that such processes to some extent occur daily: they are definitely not pathological.

(v) 5 d *of dark adaptation followed by* 6 h *of light adaptation*

Spiders which have been light-adapted for 6 h at 1700 cd/m^2 after they have been allowed to fill their receptive segments with rhabdomere membrane during 5 d in the dark, prove to have demolished the novel membrane to much the same state as is found in spiders which have been light-adapted for a similar period at lower intensities, or have been stored under a normal indoor diurnal cycle (figure 6, plate 2). The addition of rhabdomere membrane in prolonged darkness is reversible.

Description of plate 1

Figure 2. Transverse section through lines of receptive segments from a spider maintained in room daylight on a normal diurnal cycle. Paired rhabdomeres belonging to adjacent receptive segments are separated by an extensive inter-rhabdomeral cytoplasm which is to various degrees vacuolated. Lines of receptors are divided from each other by wavy partitions of pigmented supportive cell processes. Vertical ridges of non-pigmented supportive cell processes are interposed between the pigmented partitions and the ends of the paired rhabdomeres, although they are not well resolved at this magnification. The latticed structures (bottom, centre and right), are tapetum, distorted by processing. See figures 15 and 16 in the text. (Magn. × 3280.)

Figure 3. Transverse section through the axons of the receptors where they have started to narrow as they enter a tributary of the optic nerve. They contain, at this basal level, numerous microtubules, and only a very sparse population of smooth vesicles and peripheral mitochondria. The sheathing cell lamellae which envelop them contain collagenous fibrils (figure 4). (Magn. × 14900.)

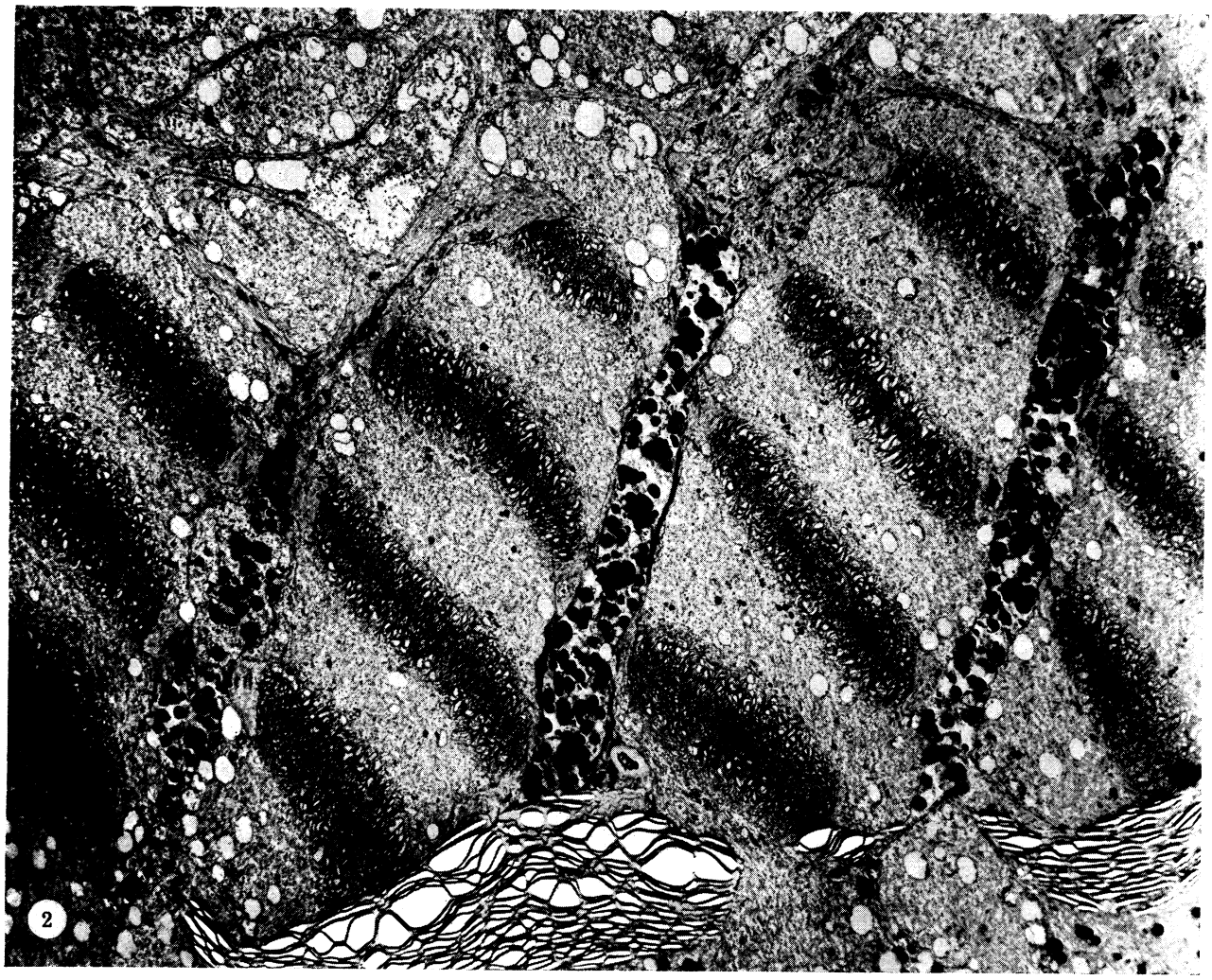
Figure 4. A sheathing cell fibril, showing a banding pattern of the collagen type with a periodicity of 12.5 nm. (Magn. × 79800.)

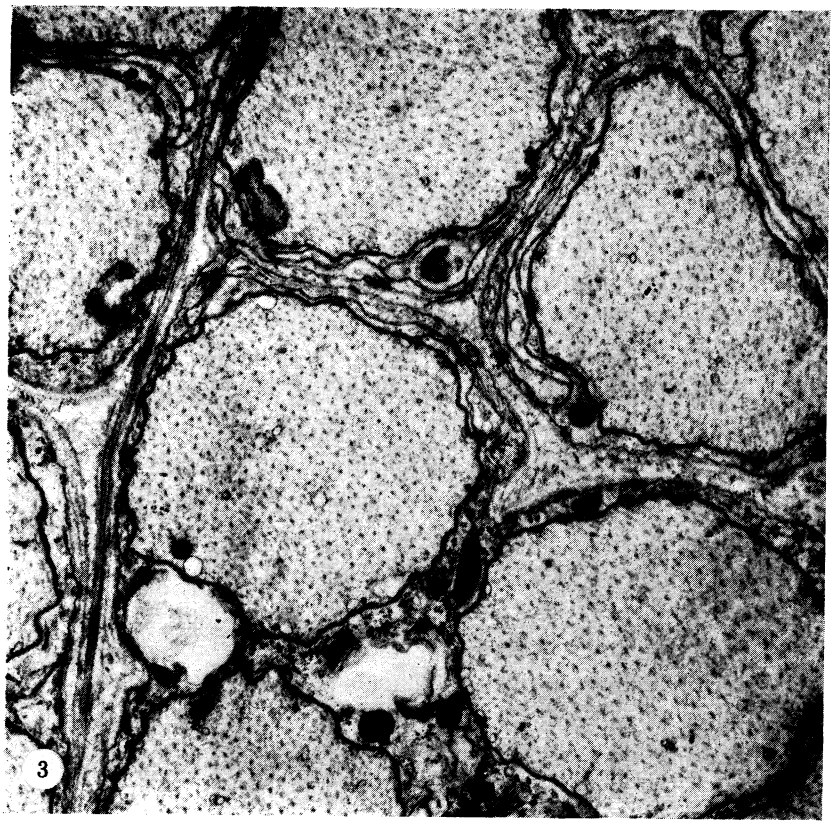
Description of plate 2

Figure 5. Transverse section of two adjacent paired rhabdomeres from a retina dark-adapted for 5 d. The receptive segments have filled with orderly rhadomere membrane, and the non-pigmented supportive ridges have flattened so that the lateral margins of the rhabdomeres are almost directly adjacent to the pigmented partitions. (Magn. × 7820.)

Figure 6. Transverse section through one pair of contiguous rhabdomeres from a spider dark-adapted for 5 d, and then light-adapted at physiological level (1700 cd/m^2) for 6 h. The rhabdomeres have been reduced from the state shown in figure 5 to dimensions similar to those found in spiders maintained in dim light. The non-pigmented supportive ridges have swollen, and now take up space previously occupied by rhabdomere microvilli, so that the transverse profiles of the rhabdomeres have shortened.

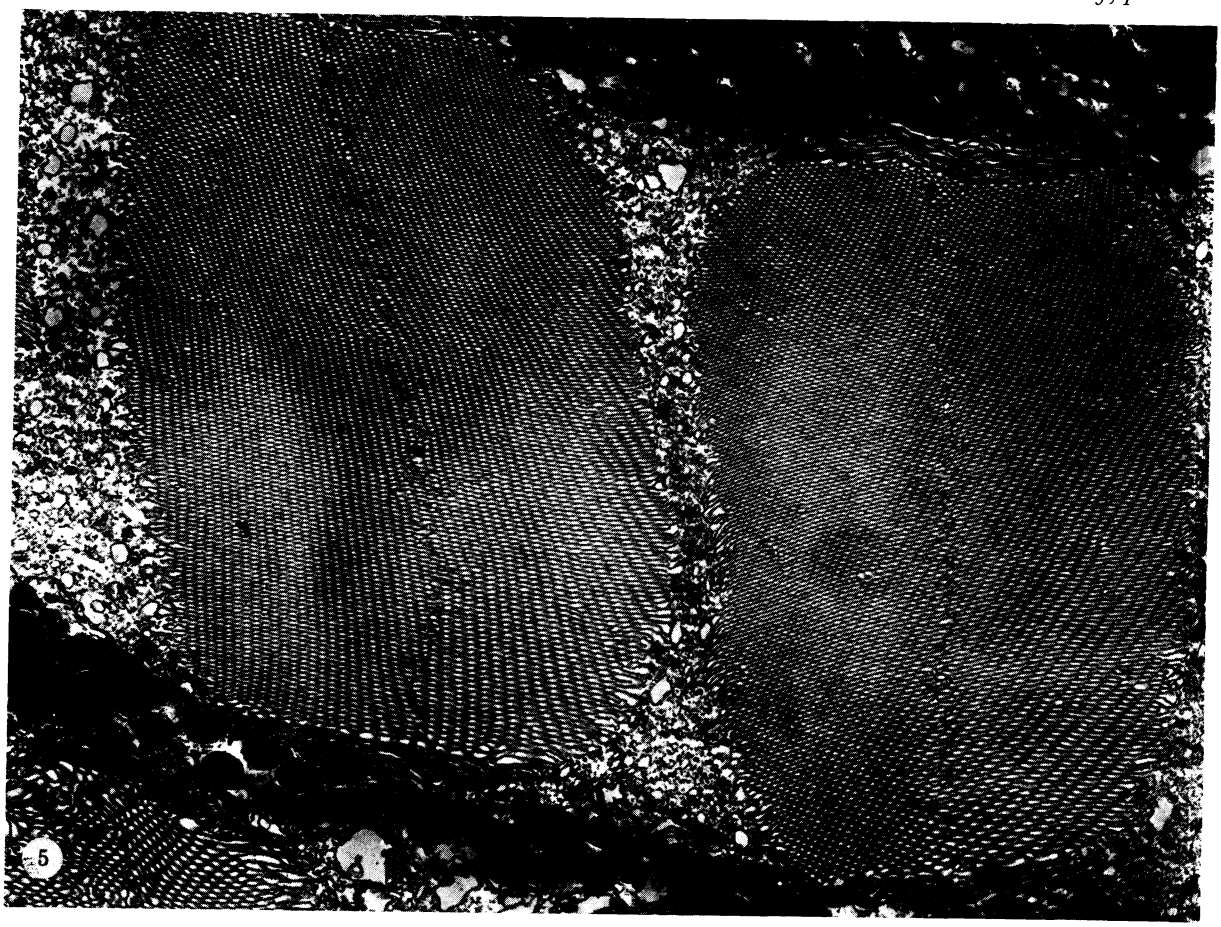
The area previously occupied by the rhabdomere membrane which has been lost can be seen as a faint 'halo', beyond which there are larger vacuoles and multivesicular bodies. Supportive cell partitions contain areas of electron-dense material, and the increased separation of the pigment granules indicates that the partitions, too have swollen. Despite the fact that the rhabdomeres are undergoing reduction, there are no arrays of smooth vesicles at their bases. See also figure 34 which shows part of the same section at higher magnification. (Magn. × 7820.)

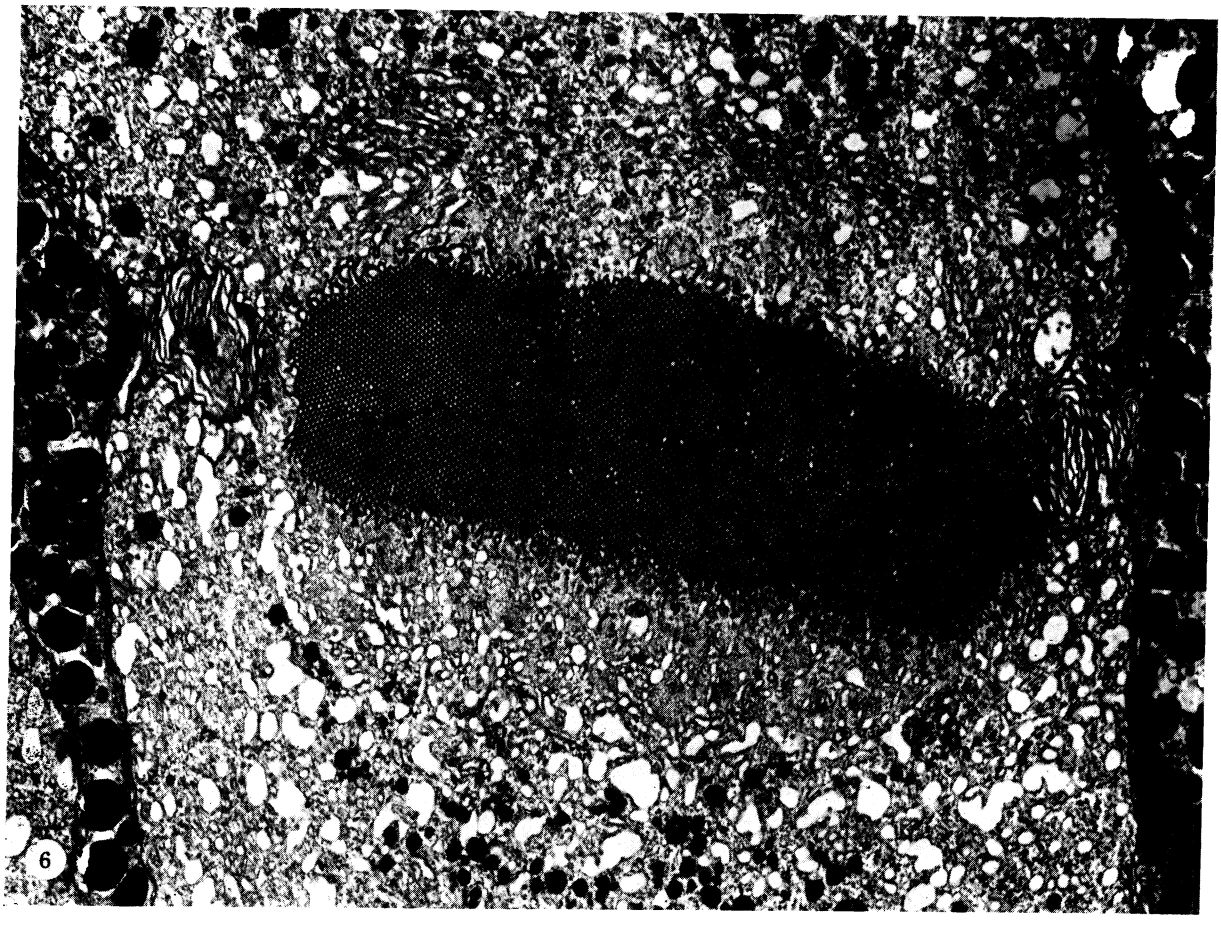




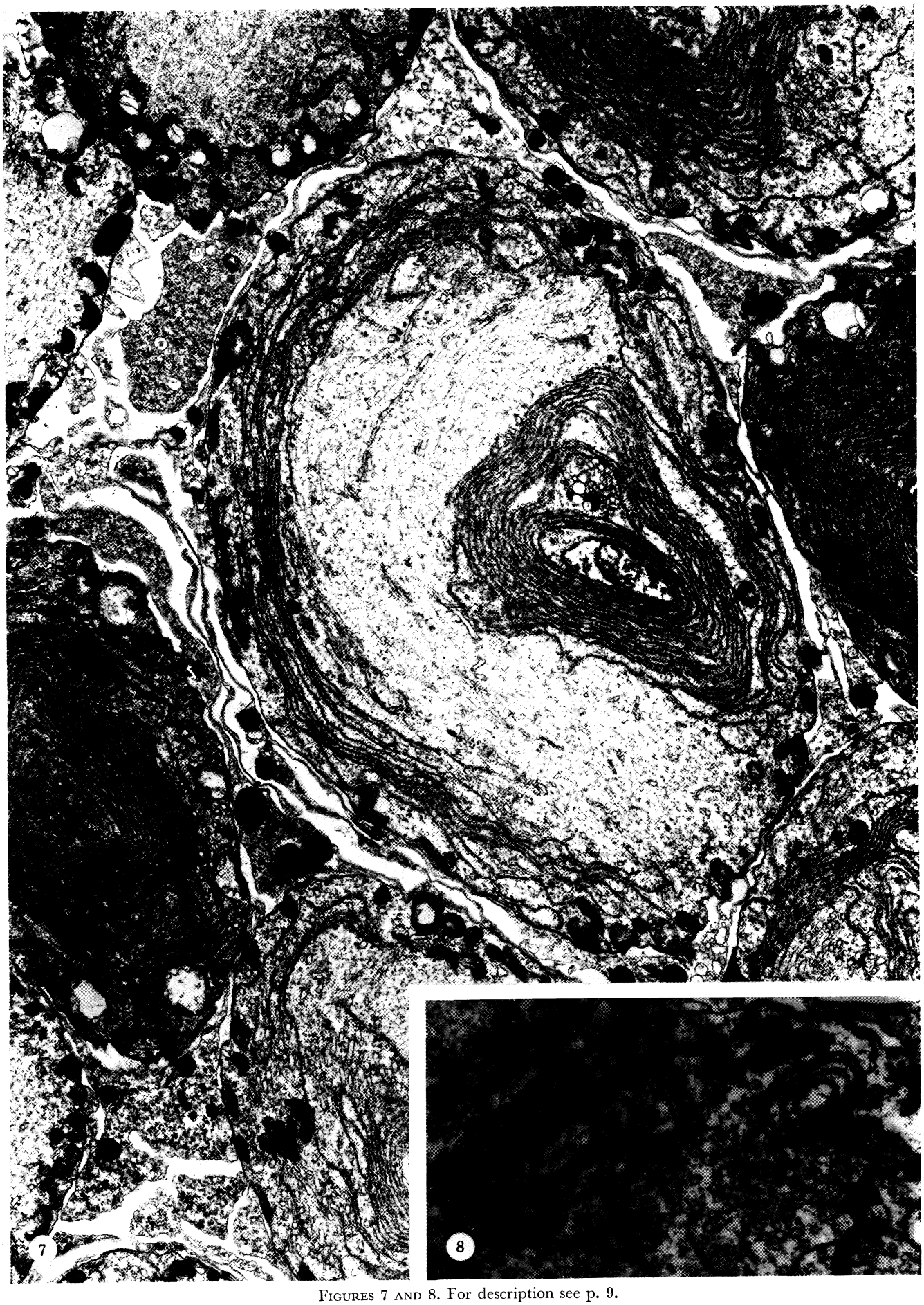
FIGURES 2–4. For description see opposite.

(*Facing p.* 8)

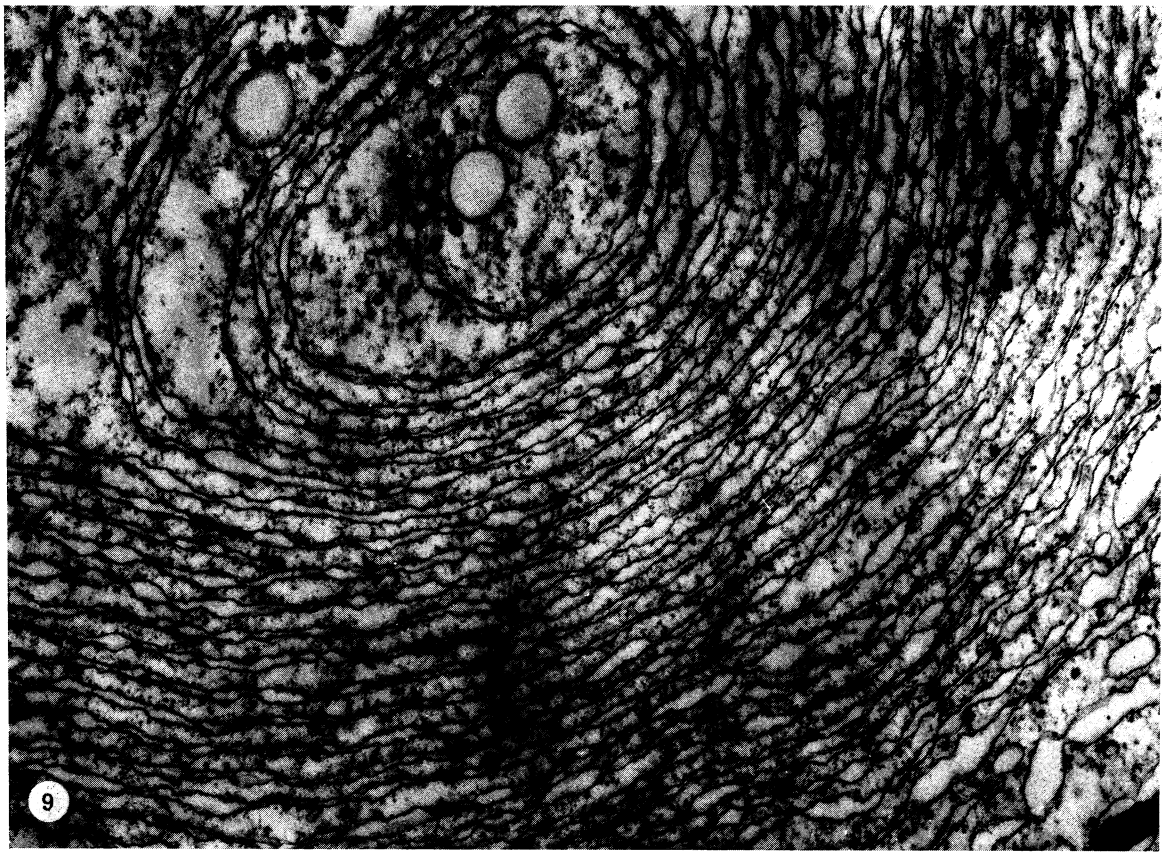


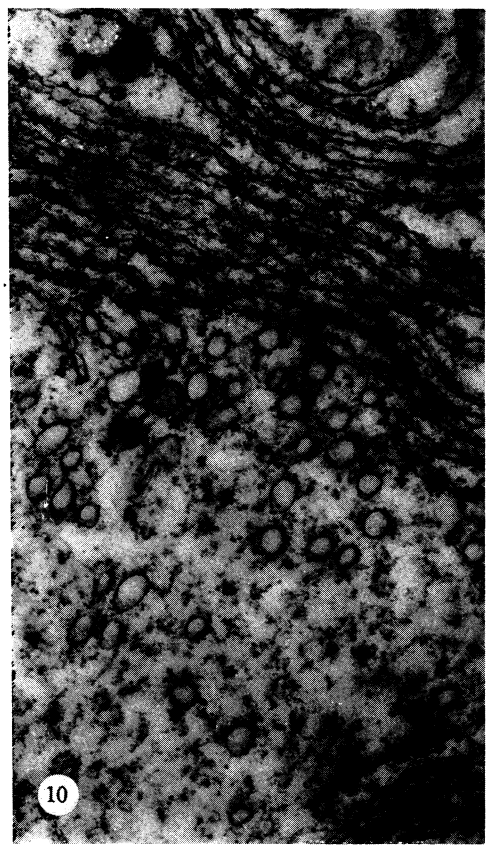


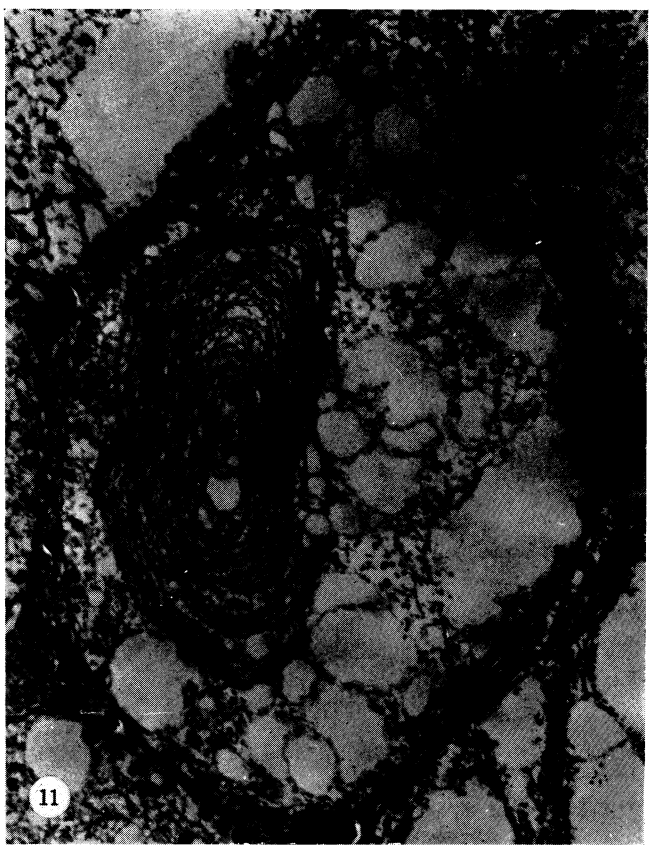
FIGURES 5 AND 6. For description see p. 8.



FIGURES 7 AND 8. For description see p. 9.







FIGURES 9–11. For description see opposite.

(vi) *Controls for the effects of infrared radiation*

An experiment in which infrared radiation alone was transmitted from a photoflood source showed that rhabdomere membrane is destroyed in the absence of visible light; the spiders were stored in the dark for 4 d before exposure, which lasted for 6 h. Output from the TIL64 monitor was 0.50 mA, whereas that from the operating lamp measured through the same filter at the appropriate working distance was only 0.01 mA. It is therefore unlikely that the reduction of rhabdomere membrane obtained with that source involves infrared radiation as its necessary component. This conclusion is supported by three lines of evidence: (*a*) the mechanism of reduction with infrared alone differs from that found in the presence of visible light (§3(iii) below); (*b*) Similar effects have been obtained in *Dinopis* in circumstances which preclude a significant infrared component (Blest, in the press); (*c*) Observations by White & Lord (1975) on rhabdomere reduction in mosquito larvae consequent upon illumination whose infrared component was filtered out closely resemble those reported here.

3. *Systems functionally associated with the receptive segments*

The somata and swollen axons of the receptors, and the supportive cell system can all be regarded as servicing the activities of the rhabdomeres. The following sections describe their ultrastructure and show that their behaviour does, indeed, correlate with the states of the receptive segments.

(i) *The swollen receptor axons*

Figure 7, plate 3, presents a transverse section through the swollen axons of an eye dark-adapted for 24 h from dim light. Conspicuous whorls of flat cisternae of rough endoplasmic reticulum occupy a major proportion of each sectioned profile, and longitudinal sections confirm that they consist of continuous sheets which occupy the greater part of the length of each axon, and may bulge up into the lower part of the cytoplasm between the rhabdomeres. Smooth,

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Description of plate 3

Figure 7. Transverse section through the region of swollen receptor axons below the tapetum in a spider dark-adapted from dim light for 20 h. Axons contain extensive whorls of rough endoplasmic reticulum with flattened cisternae, and mitochondria lie peripherally. Oblique neurofilaments can just be seen as faint striae in the axoplasm of the axon in the centre of the picture. The axoplasm contains numerous smooth vesicles derived from the endoplasmic reticulum. Supportive cell processes between the axons contain diffuse electron-dense material. (Magn. × 10 700.)

Figure 8. Part of a swollen axon sectioned basally just before narrowing to join the optic nerve, from the same preparation as figure 7. There are a few whorls of rough endoplasmic reticulum and a dense population of smooth vesicles. (Magn. × 15 100.)

Description of plate 4

Figures 9 and 10. Whorls of rough endoplasmic reticulum (e.r.) in the swollen axons typical of a retina light-adapted at 560 cd/m² for 2 h from dim light. Cisternae have dilated, and the population of ribosomes and polyribosomes adherent to them has diminished from the dark-adapted state shown in figure 7. Smooth vesicles are pinching off from the e.r. membranes. (Magns: figure 9, × 31 000; figure 10, × 23 100.)

Figure 11. Transverse section through the basal region of an axon from a retina dark-adapted for 5 d. The axoplasm is depleted and contains large vacuoles. The 'fuzzy', low-contrast appearance of the axon contents is typical of retinae in this state, and is not an artefact. (Magn. × 6800.)

tight membrane whorls of the type described as 'onion bodies' by Horridge & Barnard (1965) in the receptor cytoplasm of light adapted-locusts have not been found in the swollen axons or in any other region of the receptors. Some pinching off of the endoplasmic reticulum membranes is observed and gives rise to numerous smooth vesicles which can be seen to have accumulated throughout the axoplasm (figure 8, plate 3). There are also smaller numbers of coated vesicles; it will be shown below that these are derived from the Golgi apparatus, but the latter is not active in the dark-adapted retina, so that characteristic Golgi profiles are rarely seen. Abundant mitochondria lie as a peripheral cylinder in each axon, and there are many microtubles located basally. Neurofilaments, orientated obliquely with respect to the long axes of the axons, are found more distally beneath the tapetum in association with endoplasmic reticular whorls. There are a few multivesicular bodies.

The axons of retinae which have received 2 h of light adaptation begin to show a number of changes: the cisternae of the rough endoplasmic reticulum (figure 9, plate 4) expand, there is more pinching off of vesicles (figure 10), and there is a diminution in the number of ribosomes and polyribosomes attached to the dilated cisternae (figures 9 and 10). In addition, typical Golgi profiles are now seen, from which coated vesicles are in the process of derivation (figure 12, plate 5). Mitochondria still lie peripherally.

Light adaptation for 6 h extends these changes: the rough endoplasmic reticulum is now largely broken up and dispersed, and there is a diminution in the population of ribosomes and polyribosomes. The fragments of cisternae appear highly active. Numerous small, active Golgi profiles are surrounded by clouds of coated vesicles (figures 13 and 14, plate 5). The mitochondria have moved from their peripheral position to lie dispersed throughout the axoplasm, and they are both numerous and swollen. Neurofilaments are no longer seen. Although the small number of experiments which manipulated the infrared content of the illumination were primarily concerned with elucidating its effects on rhabdomere turnover, it was also noted that neurofilaments were preserved after treatment with weak infrared sources, and that strong infrared sources evoked greater movement and swelling of mitochondria than weak ones, as well as the disappearance of neurofilaments.

Description of plate 5

The Golgi apparatus, to demonstrate the various levels of activity described in the text.

Figure 12. Typical Golgi profile in a swollen axon from a retina light-adapted from dim light for 2 h. Coated vesicles are being pinched off from long, evenly apposed paired membranes. (Magn. × 112500.)

Figure 13. Active Golgi body: membranes are dilated and fragmented, and a moderate population of coated vesicles has accumulated. (Magn. × 67800.)

Figure 14. Highly active Golgi body: fragmented membranes are dispersed, and surrounded by a large population of coated vesicles. (Magn. × 59800.)

Description of plate 6

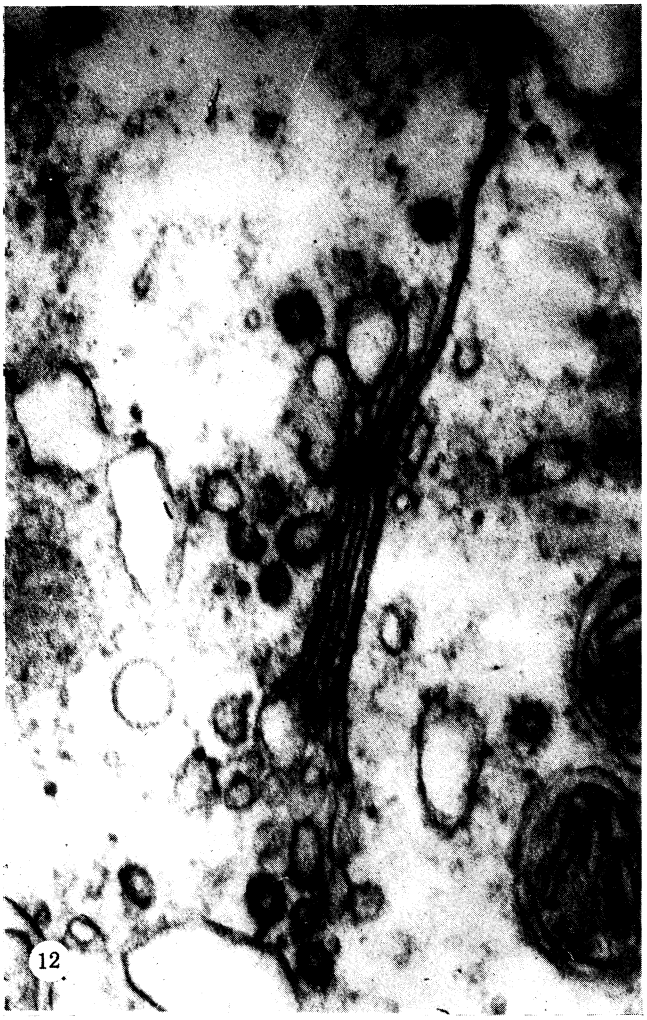
Figures 17–19. Supportive ridges and partitions of receptive segments in transverse section, to show the accumulation of electron-dense material during light adaptation.

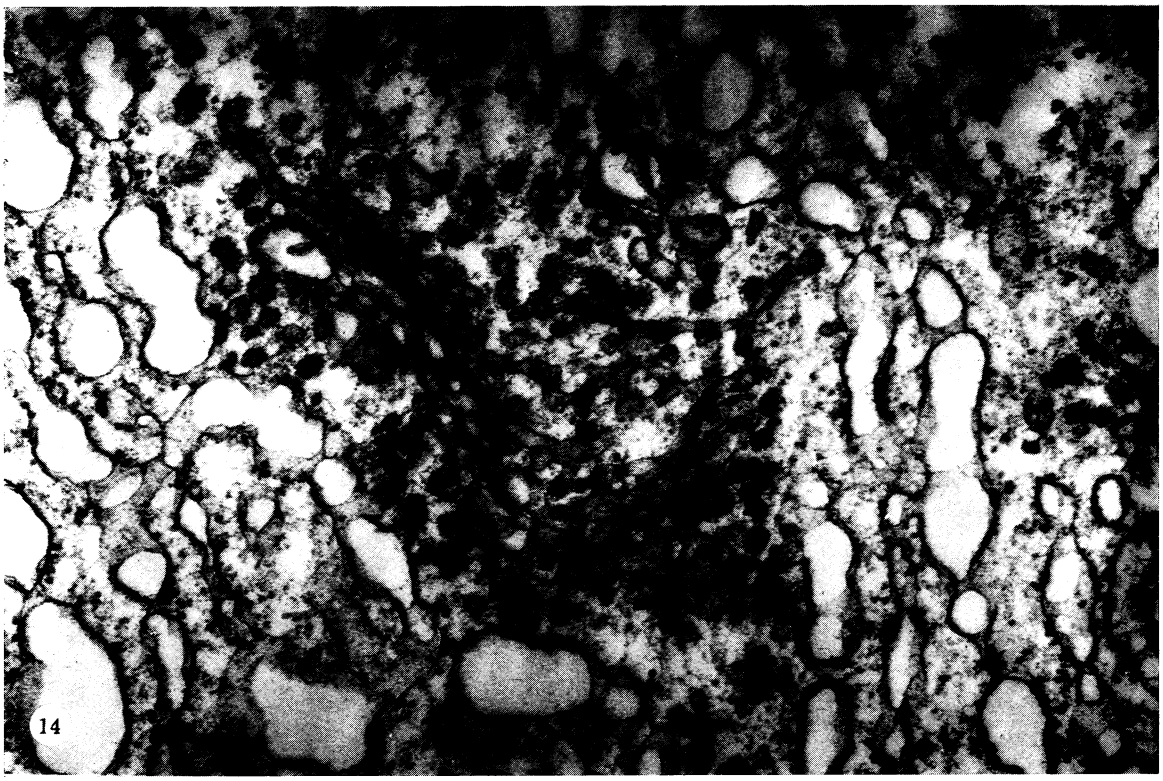
Figures 17 and 18. Diffuse areas of electron-dense material occupy the supportive ridges, and the membrane amplifications of the supportive cells are ill defined.

Figure 19. Large areas of electron-dense material lie in the pigmented partitions, and the processes of the pigmented supportive cells are swollen.

rh, rhadomeres; su, non-pigmented supportive ridge; e.d.m., electron-dense material.

The three preparations were from retinae light-adapted at 560 cd/m^2 for 6 h from dim light. (Magns: figure 17, × 50300; figure 18, × 30400; figure 19, × 23100.)





FIGURES 12–14. For description see opposite.

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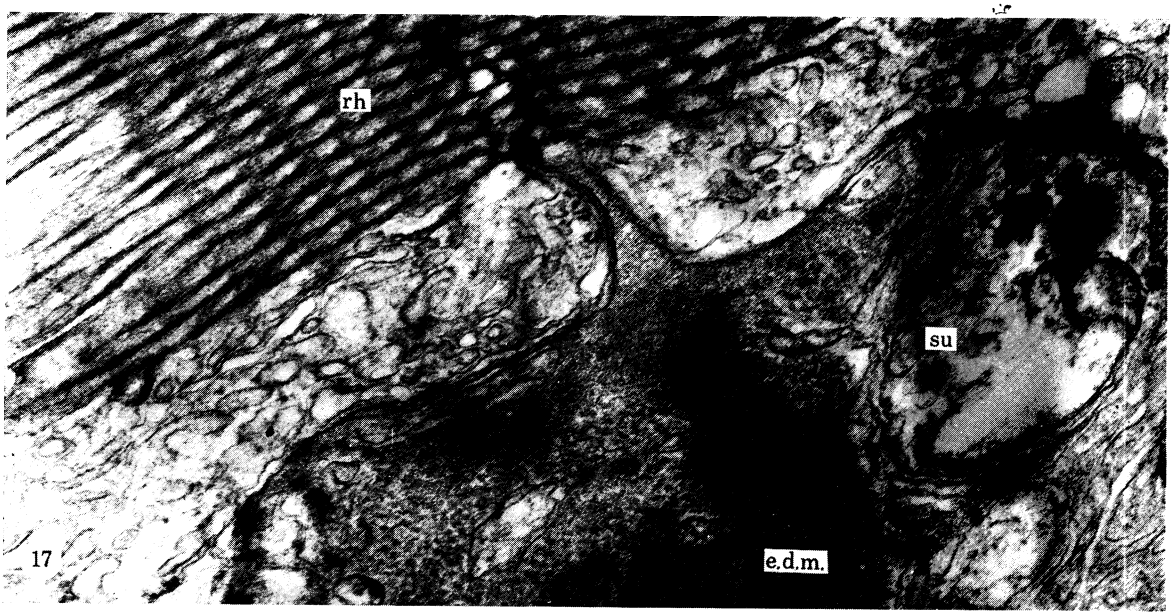
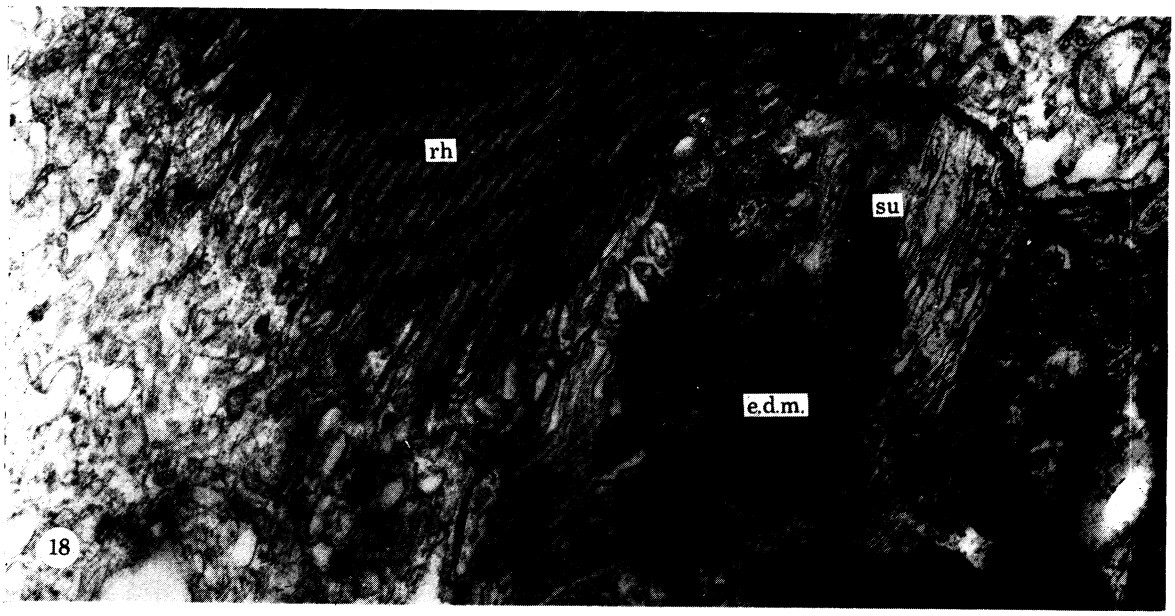
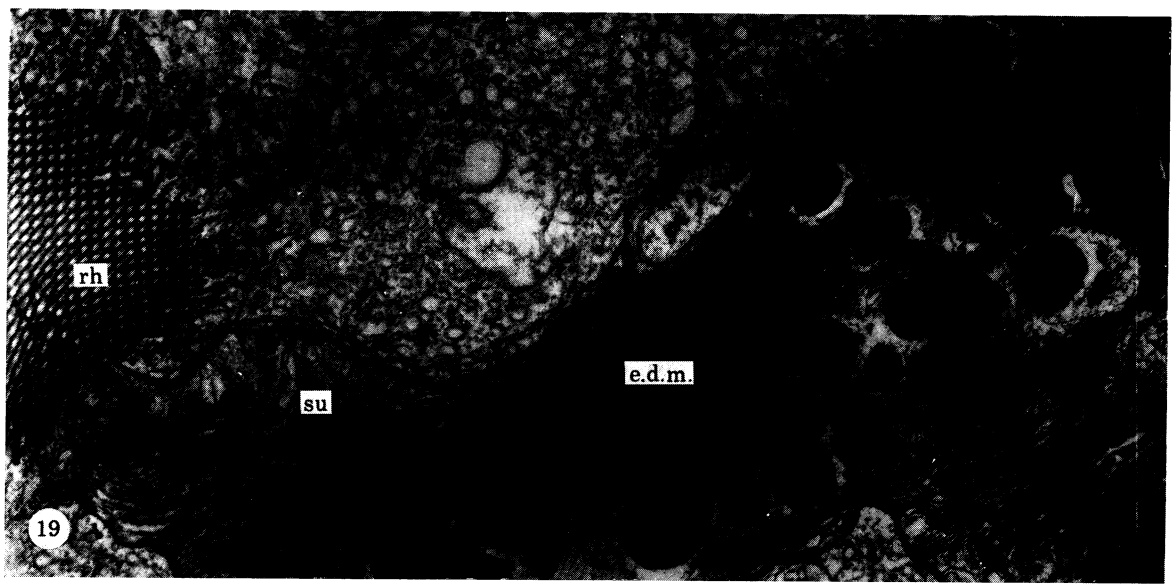
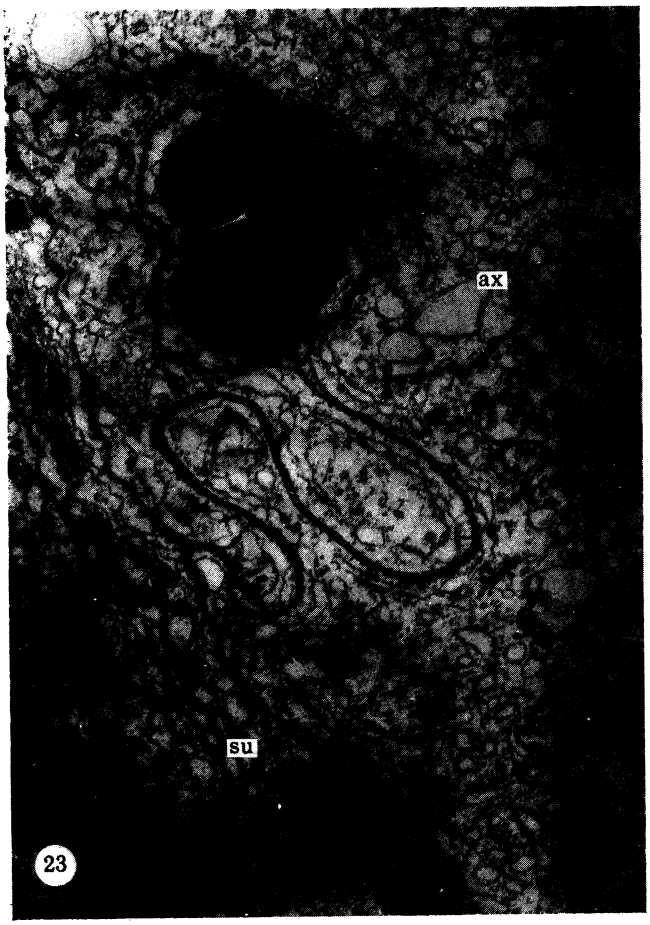


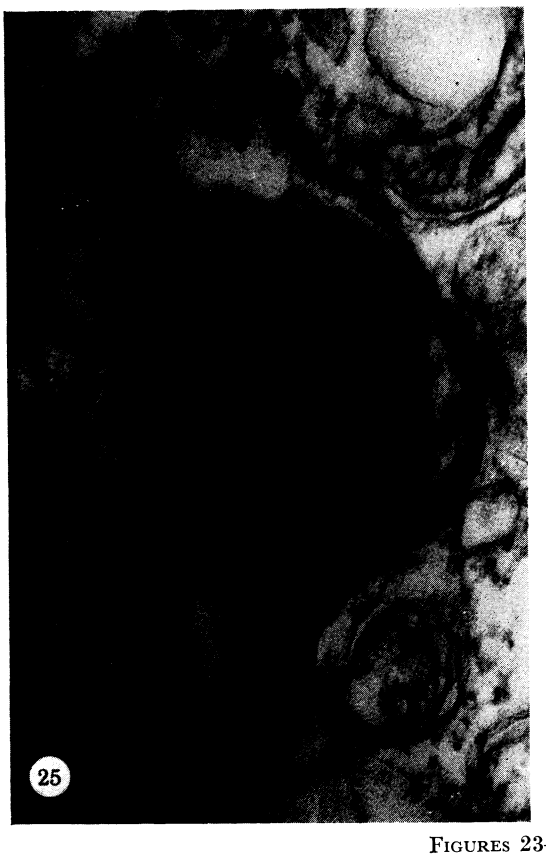


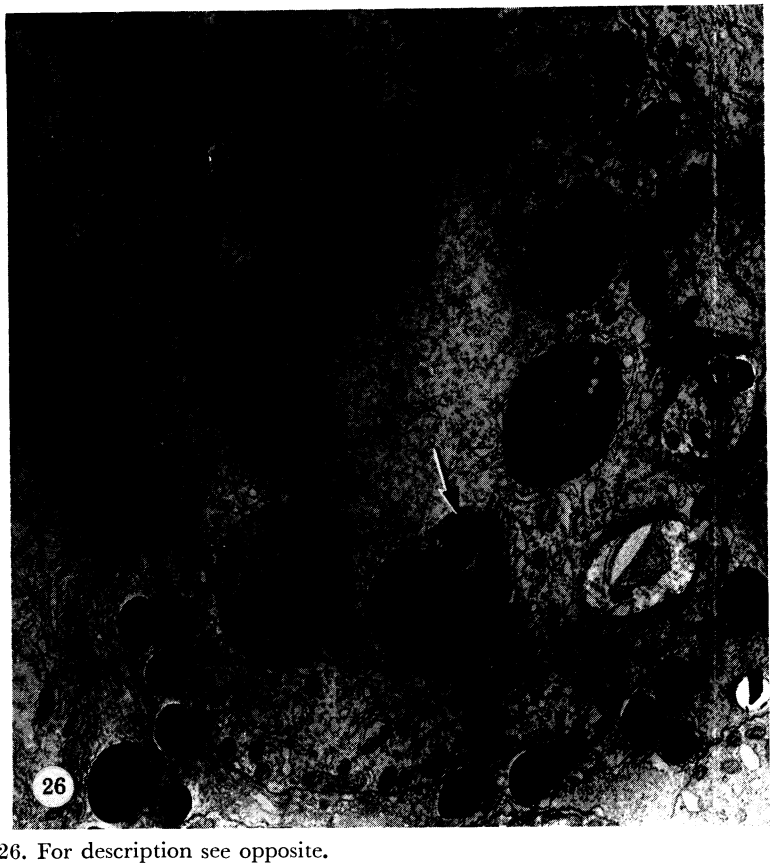


FIGURES 17–19. For description see p. 10.

FIGURES 20–22. For description see p. 11.







FIGURES 23–26. For description see opposite.

The axons of retinae which have been either dark adapted for 5 d from field conditions, or for 4 d after 6 h of light adaptation appear almost wholly depleted of materials. They contain a relatively modest amount of quiescent rough endoplasmic reticulum arranged basally in whorls (figure 11, plate 4), irregular, often very large vacuoles, small aggregates of coated vesicles, and mitochondria, once again peripheral in position, which appear somewhat shrunken. This depletion of the axon contents alone is enough to suggest that most of the activity seen in this region is concerned with the manufacture of materials required for the growth or maintenance of the rhabdomeres, and this interpretation will be further explored when we come to consider the behaviour of the various classes of vesicle. The location of the endoplasmic reticulum below the tapetum and remote from the receptive segments excludes the possibility that these massive changes linked to adaptation are primarily concerned with the modulation of the optical properties of the receptors or of local conductances within them, as has been plausibly suggested for other arthropods (Horridge & Barnard 1965; Lasansky 1967; Behrens & Krebs 1976).

Two further phenomena require mention: (*a*) the axoplasm contains scattered pigment granules in various stages of lysis in all states of retinal adaptation, and (*b*) there are numerous invaginations of the axolemma often containing components of the supportive cell system, arranged as longitudinal pleats. Both will be described together with the behaviour of the supportive cells.

Description of plate 7

Figure 20. Non-pigmented supportive cell membrane amplifications adjacent to the rhabdomeres after 6 h of light-adaptation at 1700 cd/m^2 from dim light. Both the processes and the extracellular space between them are dilated, and the latter contains electron-dense material which is most prominent next to the rhadomeres. (Magn. × 69500.)

Figure 21. Non-pigmented supportive cell amplifications after 4 d of dark-adaptation following light adaptation for 6 h at 1700 cd/m^2 from dim light. The reduction and flattening of the processes may be compared with the state of the supportive ridges of the preparation shown in figure 5, which was dark-adapted for 5 d from dim light. (Magn. × 69500.)

Figure 22. Section perpendicular to the retinal surface of supportive cell processes below the tapetum, after 6 h of light adaptation at 1700 cd/m^2 from dim light. Extracellular channels are filled with electron-dense material, a large area of which is conspicuous in the top right hand corner of the picture. (Magn. × 51700.)

Description of plate 8

Figure 23. An endocytotic pleat lying in the axoplasm of a swollen axon, from a retina light-adapted at 560 cd/m^2 for 6 h from dim light. Basally, the axolemmae of the pleat are closely apposed, and the dilated tip contains two pigment granules. ax, axoplasm; su, sheath of supportive cell processes. (Magn. × 30400.)

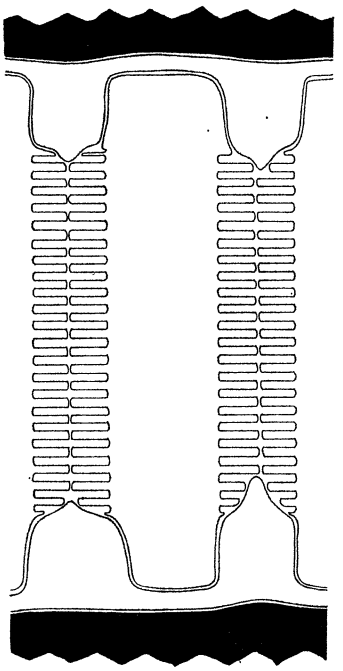
Figure 24. A crystal with a 9 nm periodicity, possibly protein, lying in one of the pigment bodies shown in figure 26 (arrowed). (Magn. × 133100.)

Figure 25. The tip of an endocytotic pleat containing a single pigment granule, from a retina light-adapted for 6 h at 1700 cd/m^2 following 5 d of dark adaptation. The pleat to which it belonged resembled the pleat shown in figure 28; it was W-shaped, and the three 'prongs' of the W were directed towards the centre of the axon, so that it could be interpreted as detached and lying freely in the axoplasm. The extreme tip of the pleat shows a profile which might be interpreted as either bursting or the start of lysis, although the possibility that it represents a region of obliquely sectioned axolemma cannot be excluded. (Magn. × 92100.)

Figure 26. Transverse section of the basal region of a swollen axon dark-adapted for 4 d following 6 h of light adaptation at 1700 cd/m^2 from dim light. Each of the four large bodies is composed of several lysing pigment granules. Intermediate stages have been seen between large bodies such as these, aggregates of pigment granules earlier in lysis, and single granules which are well preserved and presumably recently endocytosed. (Magn. × 11600.)

(ii) *The receptor somata*

The behaviour of the receptor somata is less clear-cut. They, too, contain rough and smooth endoplasmic reticulum, Golgi fragments and multivesicular bodies, but these organelles do not follow the state of the rhabdomeres very precisely. However, in the somata of light-adapted receptors the endoplasmic reticulum is always fragmented, whereas in those of dark-adapted receptors it is usually more organized, and may take the form of whorls of relatively flat cisternae similar to those found in the swollen axons of spiders which have received less than 24 h of dark adaptation. The soma cytoplasm may contain large peripheral vacuoles which are possibly derived from the system of irregular vacuoles found in the inter-rhabdomeral cytoplasm of light-adapted retinae.



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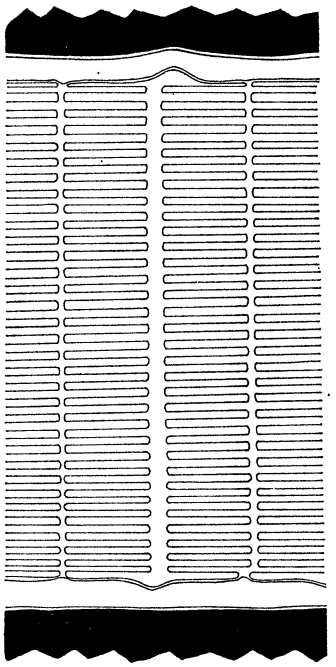

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FIGURES 15 AND 16. Diagrams of transverse sections of the receptive segments and supportive cells to show the nature of the changes in the dimensions of the rhabdomeres and the supportive cell ridges during the light-adapted (figure 15) and dark-adapted (figure 16) states. The spaces occupied by the processes of the non-pigmented (white) and pigmented (black) supportive cells have been drawn in outline, and no attempt has been made to represent their membrane amplifications, which are illustrated for the two states in figures 20 and 21. Full explanation in the text.

(iii) *The supportive cell system*

Pairs of rhabdomeres belonging to adjacent receptors are contiguous not only with each other, but with two perpendicular ridges of supportive cell processes (figures 15 and 16) composed of interdigitating membrane amplifications loosely bound by desmosomes. The topographical complexity of the system, and the fact that it is impossible to trace individual processes to their parent cells, means that desmosomal contacts may, in fact, be autosomes uniting different regions of the same cell. In receptors dark-adapted for 4–5 d the supportive ridges are flat and their processes quite closely apposed to each other (figures 5 and 21, plate 7). The effect of 2 h of light adaptation from dim light is to promote swelling of the ridges; the processes and the spaces between them both dilate, and the latter begin to become more notably electron-dense (figures

17 and 18, plate 6, and 20, plate 7). With longer periods of light adaptation, individual processes become ill defined, and the ridges acquire large areas of electron-dense material (figures 19, plate 6, and 20). At the same time, the ridges expand, so that they partially separate adjacent rhabdomeres, and where rhabdomere membranes are parted from each other in this way, microvilli are lost (figures 15 and 16). The paired rhabdomeres, therefore, as seen in transverse section, shorten in length as well as becoming thinner.

Electron-dense material, after 6 h of light adaptation, appears in all basal regions of the supportive system, including the processes sheathing the swollen axons (figure 22, plate 7). A comparison between retinae in different stages of rhabdomere reduction makes it clear that it appears first of all in the ridges, from which it migrates to the supportive cell partitions, and thence proximally to the regions below the tapetum, the densities observed becoming progressively less. At the level of the receptive segments, large masses are found among the pigmented processes (figures 6, 19), and similar masses of lesser density flank the swollen axons. Electron-dense material does not, however, invade the supportive cell interdigitations distally as far as the receptor somata.

Correlated with these changes, there are alterations in the states of the invaginated pleats of the axolemma at the level of the swollen axons (figures 23 and 25, plate 8). In retinae dark-adapted for 4–5 d the pleats are, for the most part, flat, with the paired membranes seen in sections tightly apposed except at the tips of the profiles, which often show vesicular formations (figure 23). The pleats of light-adapted retinae, on the other hand, are dilated, and they are invaded by electron-dense material and supportive cell processes (figure 25). Pigment granules may be carried into the pleats, and figures are seen which suggest that membrane containing them may be pinched off and come to lie in the axoplasm (figures 23 and 28, plate 10).

The simplest interpretation of these events is that the invaginations are, in effect, endocytotic pleats, and that material passed from demolished rhabdomeres outwards to extracellular space is transported to the region of the swollen axons and transferred to them. Pleats have not been seen bursting to release their contents, and such events would be expected to be of short duration; profiles of a kind which might correspond to bursting have been observed (figure 25), but they are ambiguous, and could be more plausibly interpreted as obliquely sectioned axolemma. Figures are also seen which suggest that pleats may break off and undergo lysis within the axoplasm (figure 28). Such configurations frequently appeared after 6 h of adaptation to the higher light intensities (e.g. 6500 cd/m^2), whereas pleats in retinae adapted for 6 h at 560 cd/m^2 were always intact and attached. The hypothesis that endocytosis is involved is also supported by two further observations: (*a*) in light-adapted retinae, diffuse electron-dense material is often seen lying peripherally in the axoplasm at depths corresponding to the usual levels of pleat penetration; and (*b*) pigment granules are found lying freely in the axoplasm in various stages of lysis (figures 24 and 26, plate 8, and figure 28), and the simplest explanation of their presence is that they have been carried inwards during the bursting of endocytotic invaginations.

The method by which material is transported by the supportive cell system has not been resolved; although Baccetti & Bedini (1964) and Melamed & Trujillo-Cenoz (1966) noted the presence of both endoplasmic reticulum and Golgi bodies in the cytoplasm of supportive cells, they are scarcely in evidence in *Dolomedes*, so that active transport of material seems unlikely.

Despite the small continuous loss of pigment granules from the supportive system which is implied, there is no indication that they are replaced.

An extracellular route for the disposal or recycling of degraded photoreceptor membrane in invertebrates has not hitherto been suggested, although analogous processes are well known in vertebrate retinae (see Discussion). We have shown that rhabdomere membrane synthesized in prolonged darkness is removed by a subsequent exposure either to visible light with a weak infrared component, or to infrared radiation alone. In the first case, irradiation is accompanied by bleaching of photopigment, whereas in the second, arthropod precedents imply that it is not. Does the deployment of the two routes of disposal differ according to whether or not photopigment is bleached?

Retinae exposed to infrared radiation alone (figure 27, plate 9) show that although multivesicular and multilamellar bodies are produced just as they are in response to visible light, (*a*) the extracellular space between the supportive cell processes does not become filled with electron-dense material, and (*b*) the swollen axons do not contain endocytotic pleats or pigment granules in the early stages of lysis. It would appear that the movement of material outwards from the receptive segments is associated with bleaching, and not with the thermal effects of infrared radiation; this finding strengthens the conclusion that the extracellular route is a normal physiological component of rhabdomere breakdown in *Dolomedes* and not an experimental artefact.

4. *Interactions between smooth and coated vesicles, multivesicular bodies, and rhabdomere membrane*

(i) *Smooth and coated vesicles*

Smooth vesicles are more numerous in the swollen axons than coated vesicles. In retinae adapted from dim light for 2 h, and in those which have received less than 24 h of dark adaptation, they are abundant; both their appearance, distribution throughout the swollen axons and

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Description of plate 9

Figure 27. Transverse section through two receptive segments from a retina exposed to infrared radiation alone (see text) after 5 d in the dark. The paired rhabdomeres are surrounded by 'haloes', showing that membrane has been lost during exposure (cf. figures 5 and 6). The supportive cell ridges have not swollen, the rhabdomeres show little shortening of their transverse profiles, and there is no electron-dense material in the extracellular space between supportive cell processes. (Magn. × 7250.)

Description of plate 10

Figure 28. An endocytotic pleat lying in a swollen axon. Both vesicular tips (v) are directed towards the centre of the axon, which suggests that this is a detached fragment. The arrowed profiles are suggestive of lysis rather than of obliquely sectioned membrane, although the latter interpretation cannot be excluded. Fragments have been seen in more advanced lysis which appear to be derived from detached pleats of the kind postulated here. From a retina light-adapted at 6500 cd/m^2 for 6 h from dim light. (Magn. × 92100.)

Figure 29. Two large coated vesicles attached to a dilated microvillar base. From a retina dark-adapted from dim light for 4 h, light-adapted at 460 cd/m^2, for 2 h ,and returned to the dark for a further 2 h. Vesicles of this type are thought to be of abnormal origin (see text). (Magn. × 215700.)

Figure 30. Two large coated vesicles lying free in the sub-rhabdomeral cytoplasm close to the field shown in figure 29. (Magn. × 215700.)

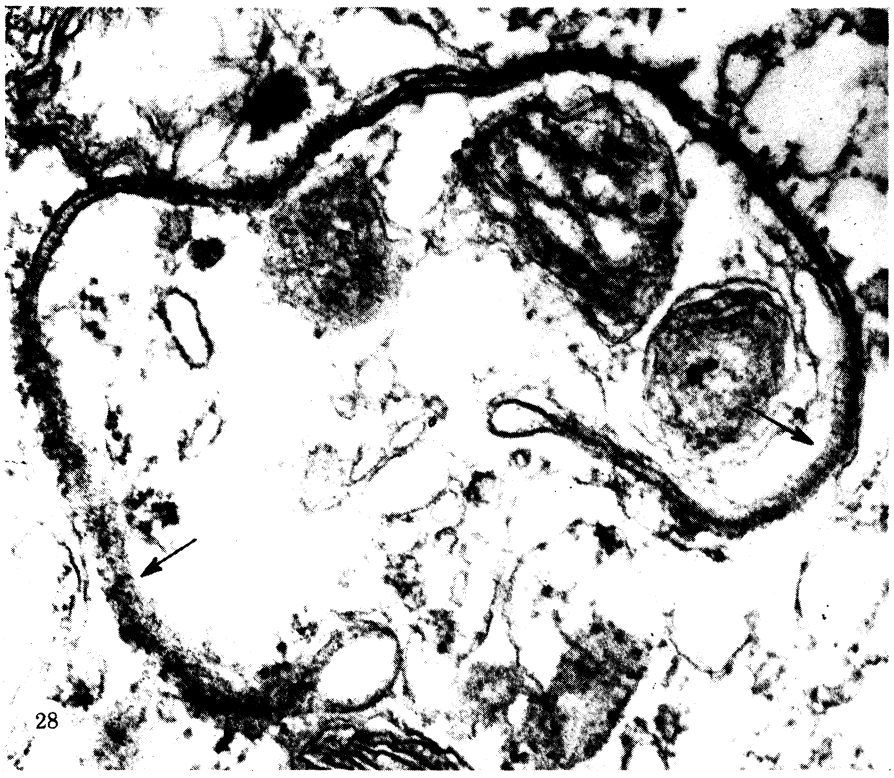
Figure 31. Clustered, dense vesicles lying in the axoplasm of a swollen axon. In *Dinopis*, identical clusters are produced from tubular endoplasmic reticulum (see text). The heavy staining is a typical feature. (Magn. × 24200.)

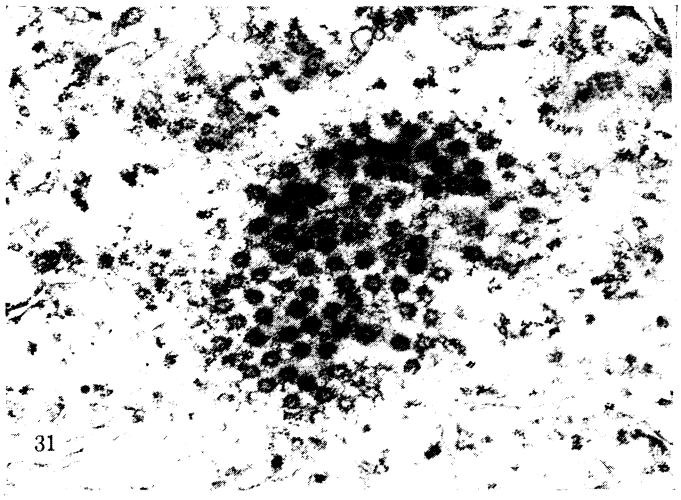
Figure 32. A detail of the cluster shown in figure 31 at higher magnification. (Magn. × 50000.)

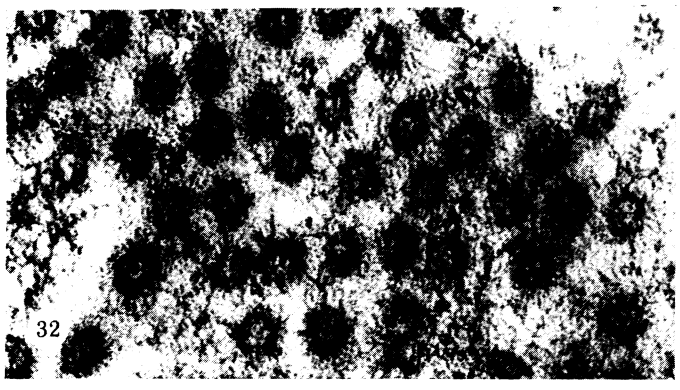
Figure 33. Coated vesicles disposed more or less linearly in relation to microvillar bases. (Magn. × 100000.)

FIGURE 27. For description see opposite.

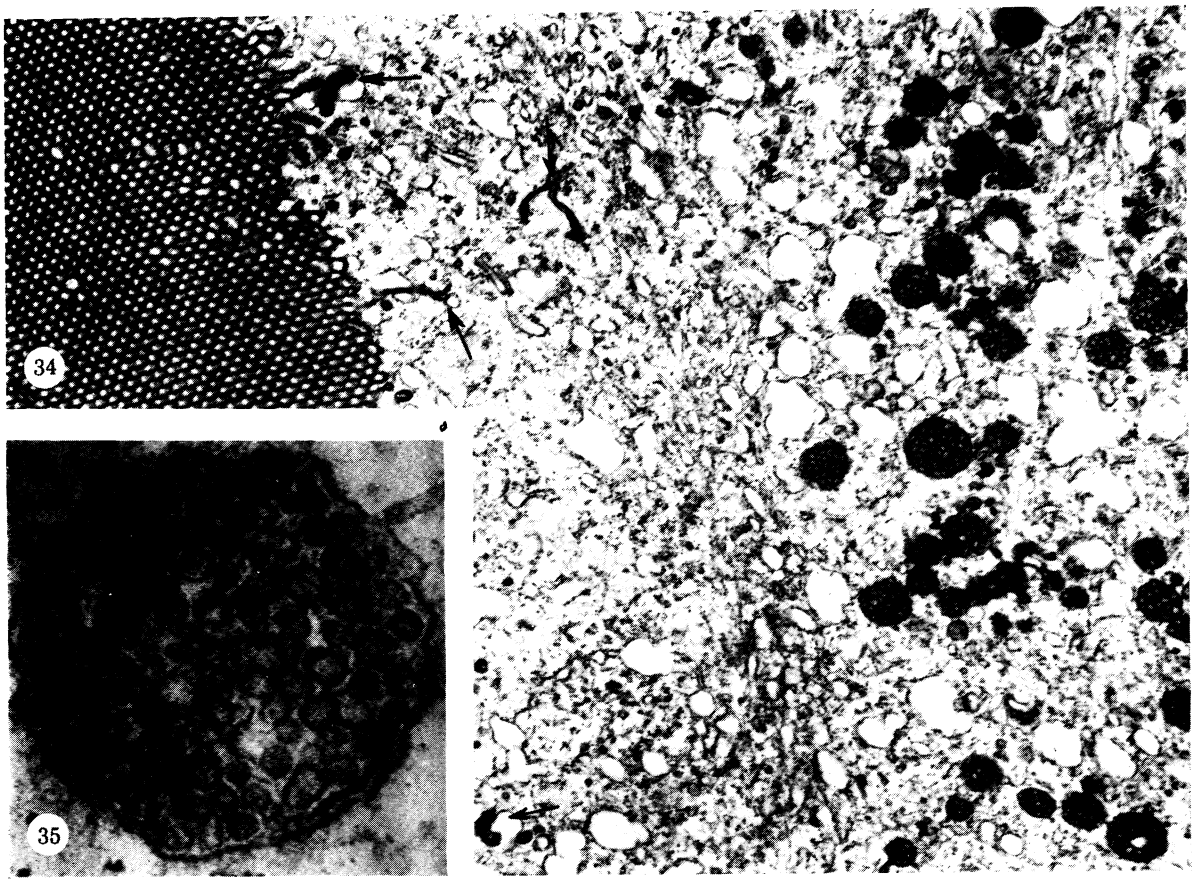
(*Facing p.* 14)

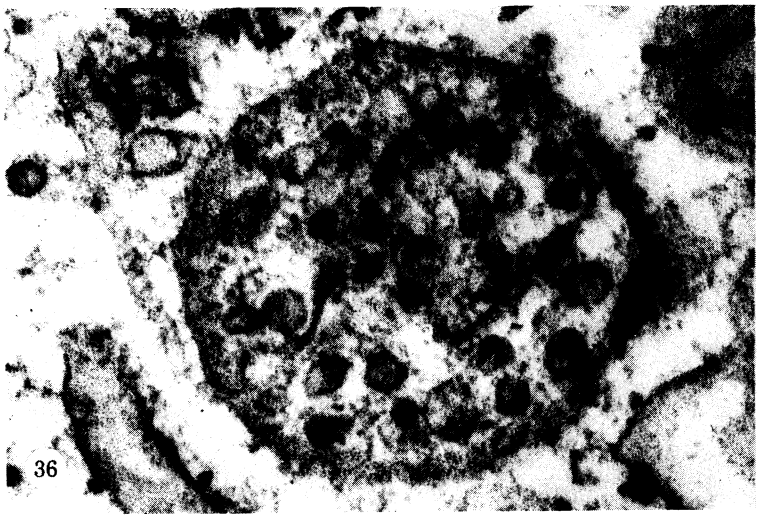


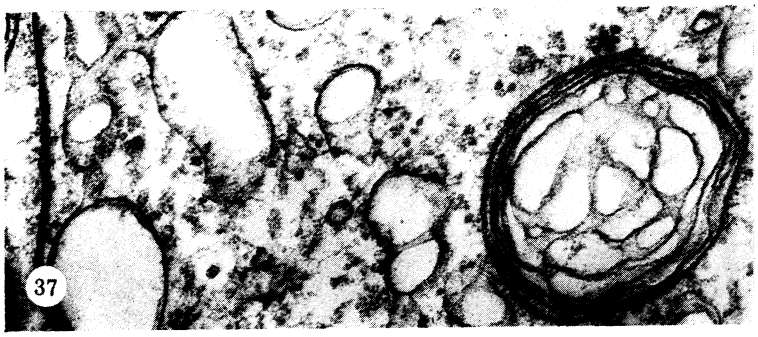


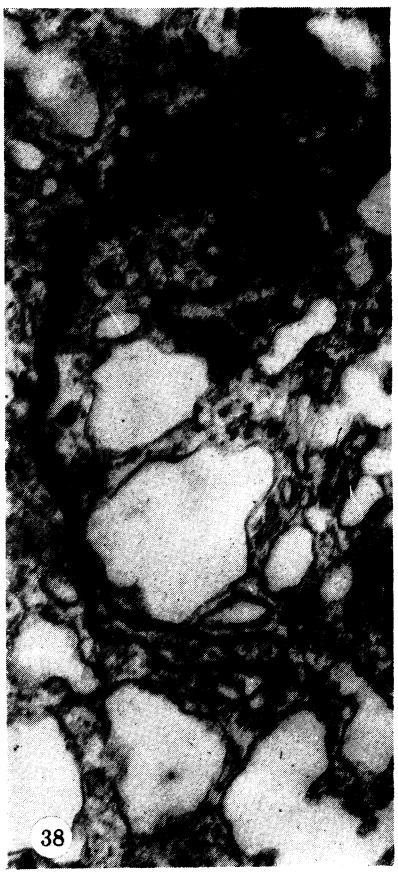


FIGURES 28–33. For description see p. 14.

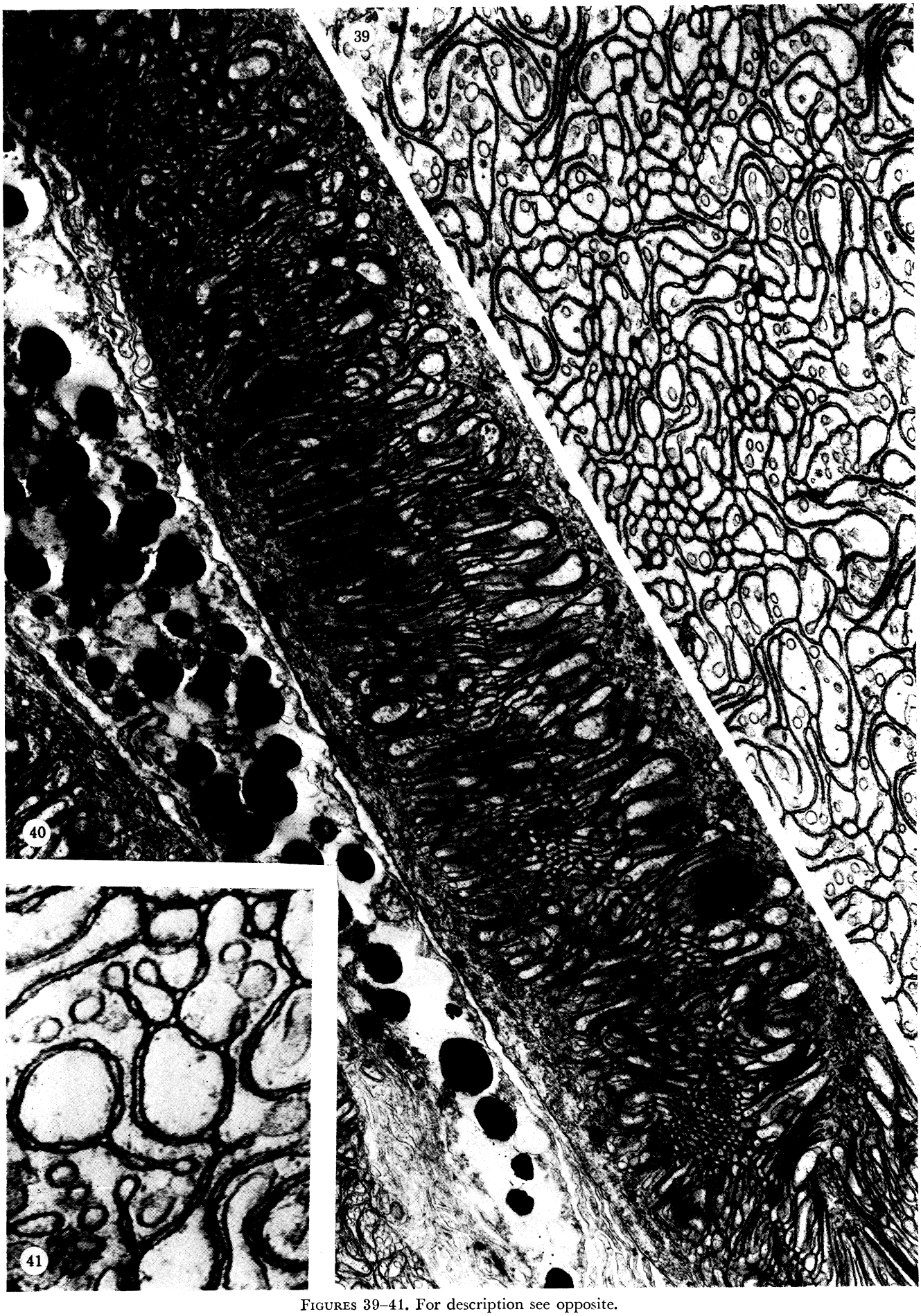








FIGURES 34–38. For description see p. 15.



FIGURES 39–41. For description see opposite.

the fact that similar vesicles can be seen to be pinched off in bulk from the copious rough endoplasmic reticulum imply that they may originate from that source. There are no synapses within the retina, and only single vesicles have been noted, infrequently, at the retinal end of optic nerve fibres, at points at least 1 mm distant from the first synapses of the visual pathway in the first optic glomeruli. Neither class of vesicle can be concerned with synaptic transmission.

The smaller population of coated vesicles in the swollen axons is derived from the Golgi apparatus, where they form as clusters. All states of the Golgi apparatus may be observed, from barely active formations whose distinct cisternae show elongated profiles pinching off at their tips, to highly active fragments lying at the centres of large assemblies of vesicles (figures 12–14). After prolonged dark adaptation, the Golgi apparatus is quiescent, and vesicles are sparse. Active production of coated vesicles may relate to the availability of material from the rhabdomeres which has been passed to the axons via the supportive cell system, for they are scarce in axons after prolonged dark adaptation (when all material is held in the rhabdomeres) and also in the axons of such dark-adapted retinae which have been subsequently light-adapted for 6 h, and in which the state of the supportive cell system suggests that recycled material has not yet been introduced into them.

In addition, a large cluster of electron-dense vesicles has been seen on one occasion in *Dolomedes* after 5 d of dark adaptation (figures 31 and 32, plate 10). In *Dinopis*, identical clusters are found in association with a highly active system of tubular endoplasmic reticulum during states of rapid membrane turnover (Blest, in preparation), and it is likely that their origin in *Dolomedes* is the same. If so, they are a potent source of confusion, for there may be no reliable way to distinguish between these structures, vesicles of the Golgi apparatus, and pinocytotic vesicles produced by rhabdomere breakdown, all of closely similar size and appearance.

Description of plate 11

Figure 34. Part of the receptive segment shown in figure 6 at higher magnification to show fragments of detached rhabdomere membrane (arrowed) and numerous multivesicular bodies in the inter-rhabdomeral cytoplasm. The microvilli remain orderly up to the margin of the rhadomere. (Magn. × 23 600.)

Figure 35. A multivesicular body from a swollen axon, still tightly assembled, and membrane-bound. (Magn. × 96 900.)

Figure 36. A multivesicular body undergoing lysis in a swollen axon. (Magn. × 133 100.)

Figure 37. A small multilamellar body (right) in a swollen axon. (Magn. × 67 800.)

Figure 38. A large fragment of detached photoreceptor membrane in the inter-rhabdomeral cytoplasm. At one end of the profile it is transitional to a multivesicular body, and is beading into numerous very small vesicles which appear to be coalescing to form larger, dense vesicles. The latter are larger than coated vesicles, and have different profiles. (Magn. × 51 700.)

Description of plate 12

Figures 39–41. Rhabdomeres from patches of receptive segments whose membranes are 'disordered'.

Figure 39. Disordered rhabdomere membrane in association with smooth and coated vesicles. (Magn. × 31 200.)

Figure 40. A longitudinal section (i.e. perpendicular to the retinal surface) through parts of two contiguous, disordered rhabdomeres, showing dilated microvillar bases, membrane whorls and incipient myelin figures. There are small regions of ordered microvilli. The inter-rhabdomeral cytoplasm contains numerous coated vesicles. (Magn. × 14 100.)

Figure 41. Part of the section shown in figure 39 at higher magnification, to display vesiculated membrane, and vesicles associated with it. (Magn. × 96 900.)

(ii) *Interactions between the microvilli and vesicles*

Observations of similar events to those described here in other arthropods have produced a fundamental difficulty of interpretation: are the relations between vesicles and the bases of microvilli to be interpreted as fusions of the one with the other, or are the vesicles being pinched off from the photoreceptor membrane? And, if fusions do occur, should they be considered to represent the carriage of materials for the maintenance or repair of membrane, or are they the product of lysosomes concerned with its destruction? Evidence from other groups will be considered in the Discussion.

(*a*) *Coated and smooth vesicles.* Profiles which might represent either fusion of vesicles with the bases of the microvilli or the pinching off of vesicles from them are frequently observed. Those which implicate coated vesicles are the least ambiguous (figure 33, plate 10): they are usually single events, in which one coated vesicle is seen to be attached to the base of a microvillus. Linear arrays of coated vesicles have sometimes been observed and the profiles of the microvilli to which they are attached often show some beading of a kind which suggests mass pinching off. Occasionally, and usually in the context of disordered membrane (see below), or of grossly dilated microvillar bases, profiles of much larger vesicles are observed (figures 29 and 30, plate 10). They are only seen in material whose dissection under fixative was accidentally prolonged, and their association with whorls of rhabdomere membrane makes it likely that they are of pathological origin. Although the interpretation of small coated vesicles associated with the microvilli is ambiguous in *Dolomedes*, recent studies of *Dinopis* make it clear that they are indeed pinocytotic in origin; White (1967, 1968) demonstrated that both coated and smooth vesicles in the sub-rhabdomeral cytoplasm of mosquito larvae incorporate ferritin from extracellular space. He also showed that such coated vesicles appear to lose their coats in the course of conversion to smooth vesicles, which are then assembled into multivesicular bodies. Eguchi & Waterman (1976) have provided more rigorous grounds, from freeze-fracture observations of crustacean retinae, for the belief that pinocytotic vesicles are assembled into multivesicular bodies, and that the latter are the main vehicles in their material for the carriage of degraded material away from the rhabdomeres.

It is not clear whether Golgi vesicles travel to the sub-rhabdomeral cytoplasm, although their abundant production implies that they play an important rôle. Coated vesicles are most abundant in the receptive segments during prolonged dark adaptation, but while such populations may be of pinocytotic origin, they could be derived either from the Golgi apparatus, or from the electron-dense clusters noted above.

The interpretation of the rôle of smooth vesicles is also uncertain from this material alone. In *Dinopis*, their production from endoplasmic reticulum and transport to the microvillar bases is unequivocal (Blest, in preparation). Itaya (1976) has proposed that in the shrimp, *Palaemonetes*, endoplasmic reticulum supplies materials for rhabdomere growth, and his hypothesis is consonant with our observations.

(*b*) *Distribution of vesicles in light and dark adaptation.* Some further evidence is provided by the distribution of smooth vesicles following different schedules of light- and dark-adaptation. As previously noted, they are abundant after both short periods of light and darkness. After long periods in light, however, they are few in number, or even absent altogether, despite the massive breakdown of membrane which results. Similarly, after prolonged darkness, they are almost wholly absent from the cytoplasm adjacent to the bases of the microvilli, and in this condition

both the swollen axons and the supportive system have been shown to be depleted of materials. An interesting observation concerns receptors which have been in darkness for 5 d, so that the receptive segments have filled with novel membrane, and then light-adapted for 6 h. It could be argued that if smooth vesicles represent the product of the breakdown of microvilli, the cytoplasm between the rhabdomeres must in this state contain an abundance of them; if they represent material added to the rhabdomeres, they should be infrequent or absent, for after prolonged dark adaptation the bulk of the resources of the receptors is incorporated in the rhabdomeres, the endoplasmic reticulum of the axons is scanty and depleted, and it can be seen that little material is available for recycling via the supportive cell system; it might be assumed that substrates for the synthesis of smooth vesicles are not yet present. When light adaptation follows prolonged darkness a considerable volume of rhabdomere membrane is lost, yet smooth vesicles are indeed absent from the inter-rhabdomeral cytoplasm (figure 6).

(iii) *Multivesicular bodies*

Although multivesicular bodies may be found in all states of adaptation, they are infrequent in retinae which have experienced long periods of dark adaptation, and most abundant in light-adapted eyes in which there are signs of membrane fragmentation within the receptive segments, where they may form clusters (figure 34, plate 11). Stages have been seen between membrane fragments detached from the bases of the microvilli (figure 38, plate 11), clusters of vesicles derived from them, and complete multivesicular bodies (figure 35). 'Tightly assembled' multivesicular bodies are found, also, in the somata and the swollen axons. In the latter site, configurations are seen suggestive of lysis (figure 36).

(iv) *Multilamellar bodies*

Multilamellar bodies are also found within the receptive segments, with a similar distribution to multivesicular bodies. Like them, they appear to be associated with the disposal of fragmented membrane, and, also, with the growth of disordered membrane whorls (see below). They too undergo lysis within the swollen axons (figure 37, plate 11). Eguchi & Waterman (1976) have demonstrated that a conversion of multivesicular bodies to multilamellar bodies is accompanied by a progressive degradation of the membranes of which they are composed.

In terms of the interpretation of the retinal system suggested here, both multivesicular and multilamellar bodies can be seen as mediating the recycling of photoreceptive membrane whose disassembly has caused fragmentation within the receptors. It must be emphasized that the bulks of multivesicular and multilamellar bodies observed are quite inadequate to account for the changes in rhabdomere volume, and these mechanisms of transport and conversion are probably of minor significance.

5. *'Disorderly' photoreceptor membrane*

A small proportion of receptors even from spiders which have been maintained in dim light possess disordered rhabdomeres (figures 39–41, plate 12). The two rhabdomeres of a given receptor are always in the same state, and receptors whose rhabdomeres are disordered may be found adjacent to those whose rhabdomeres are normal. More usually, receptors with disordered membrane are found in small patches. Although rhabdomeres which have been reconstituted in darkness often show broad fringes of disorderly membrane adjacent to the subrhabdomeral cytoplasm, attempts to provoke totally disordered restitution after prolonged light adaptation have not been successful.

Discussion

Light-dependent changes in rhabdomere volume

Alterations in rhabdomere volume dependent upon régimes of illumination have been examined in most detail by White & Lord (1975) for larval mosquitoes. The same effects observed in *Dolomedes* were found: rhabdomeres diminish in volume during periods of illumination, and are regenerated when the larvae are returned to darkness. The time courses of these events are, however, different. It is not meaningful to compare the rates at which rhabdomeres are reduced in the two cases because the eyes are of dissimilar construction, the experimental circumstances were not equivalent, and the present small samples were not followed throughout light-adaptation. It is clear, however, that in the larval mosquito, reduction of rhabdomere volume to equilibrium is followed, on return to darkness, by regeneration of membrane to completion within 5 h, whereas in *Dolomedes* regeneration is still proceeding 24 h after the cessation of light adaptation, and continues in the dark until the receptive segments are filled.

White & Lord (1975) have argued that the changes observed in mosquitoes, and probably in other arthropods as well must be regarded as part of the normal strategy of adaptation, and are not to be dismissed as pathological, in contradiction to some former opinions (e.g. Röhlich 1968), which held that rhabdomere reduction is an abnormal consequence of excessive illumination. Behrens & Krebs (1976) subscribe, cautiously, to the more recent view. In *Dolomedes* one of the two lower intensities of illumination employed during light adaptation matched those measured in the field in the same way for shaded banks at 15.00 h on a sunny spring afternoon (560 cd/m^2). New Zealand *Dolomedes* usually rest during the day beneath stones, but their habitats are subject to flash-flooding after heavy rain, and this is a frequent winter and spring event. It is likely that spiders flushed from their shelters are exposed to such levels of sustained illumination quite frequently, for New Zealand streams in spate may take a considerable time to subside. It is unlikely that their retinal physiology would not be adapted to deal with such emergencies, and, in any case, *Dolomedes* are sometimes seen resting or running on exposed sites during the day in direct sunlight (A. D. Blest, unpublished observations; Vida Stout and M. J. Winterbourne, personal communications). Female European *Dolomedes* often guard their egg-sacs fully exposed to daylight.

Previous work on the closely related Lycosidae (Baccetti & Bedini 1964; Melamed & Trujillo-Cenoz 1966) did not specify the light régimes under which the spiders were maintained, but these authors describe multivesicular bodies and vesiculation of the bases of microvilli which suggest that they were held in the light, and that similar consequences to those described here were in train when they were fixed for examination, albeit on a more modest scale. However, Eakin & Brandenburger's (1971) study of light-adapted salticid retinae seems to imply that such modifications of rhabdomere architecture are not found in that wholly diurnal family, for the bases of the microvilli are not equipped with associated vesicles, and the structure of the receptors, with the possible exception of those of layer 2 in the anterior median eyes, does not seem to include any machinery capable of sustaining such 'busy' processes. Findings on other arthropods emphasize that there is much variation between species in the stability of photoreceptor membranes (White & Lord 1975), but changes in rhabdomere volume have been observed in Crustacea (Röhlich 1968; Tuurala, Lehtinen & Nyholm 1966; Itaya 1976; Eguchi & Waterman 1976; Nemanic 1975), in *Limulus* (Behrens & Krebs 1976), and in cephalopod

molluscs (Young 1963), although the interpretation of the effects observed in ultrastructural terms is not always clear. In all these cases, dramatic changes in volume and in the transverse dimensions of microvilli probably follow régimes of exposure which do not correspond to those experienced by animals moving freely in their natural habitats, where they might be supposed to exercise some choice with respect to available illuminances, even though the intensities used were at physiological levels; it is likely that protracted periods of light adaptation and of recovery in the dark largely serve to reveal departures from a more usual state of balance between membrane loss and restitution (White & Lord 1975).

Loss and reconstitution of photoreceptor membrane

The unusual features of the spider retinae described here are the massive machinery contained in the swollen axons, and the supportive cell system for the return of material to them after its removal from the photoreceptor membrane.

Swollen axons are found in *Dinopis* (Blest & Land 1977); in adults of the other dinopid genus *Menneus* and the sparassid genera *Olios* and *Isopoda* (A. D. Blest, unpublished observations); and are illustrated by Homann (1951) for the retinae of an acanthoctenid. In *Dinopis* they take a more extreme form than those of *Dolomedes*. Pisauridae and Lycosidae are closely related, and the swollen axons of *Dolomedes* have probably been evolved from the only slightly dilated axons described for that family (Baccetti & Bedini 1964; Melamed & Trujillo-Cenoz 1966), where they also contain some endoplasmic reticulum. Neither the Italian nor the Venezuelan authors illustrated the endoplasmic reticulum which they noted to be present, but lack of space for its accommodation would clearly preclude its existence in the massive quantities which we have observed.

Similarly, the same two groups of workers described the membrane amplifications of the supportive cells, and noted that they contained electron-dense materials, but their failure to manipulate states of adaptation made it impossible for them to obtain a dynamic interpretation of their relations with the receptors. It seems likely, however, that similar processes of membrane loss and replenishment will prove to exist in lycosid spiders on a smaller scale.

Turnover of photoreceptor membrane is well known to occur in both vertebrate and some invertebrate retinae, and it is instructive to compare the processes postulated to take place in *Dolomedes* with those established in other groups. Vertebrate rods lose membrane by detaching packets of disks at their tips, and replacing them by the basal addition of membrane (Young 1969, 1971 *a*, *b*, *c*)). The cones of most species so far studied do not shed membrane at their tips, though those of some squirrels, and, possibly, man, shed membrane packets in a similar manner to rods (Anderson & Fisher 1976; Hogan, Wood & Steinberg 1974). The former authors discuss some problems of classification which the rod-like behaviour of nominal cones produces in this context. Membrane packets are phagocytosed by pigment epithelium after detachment, and phagosomes thus engaged present similar profiles to those of the myelin figures and multilamellar bodies seen in *Dolomedes* and other arthropods.

Young (1967) showed that the injection of [^{3}H]methionine into frogs and rats labelled a band of rod basal disk membrane which slowly migrated distally until it was incorporated into the pigment epithelium and disappeared. The rate of the process was not affected by whether the animals were kept in light or in darkness, although it has recently been shown that disk shedding is cyclical in both species; in rats it occurs at dawn and is under circadian control (LaVail 1976), while in frogs it is not, and is merely triggered by light (Basinger, Hoffman & Matthes 1976).

Incorporation of labelled amino acids into cones, however, is diffuse, both in species which do not shed membrane, and those that do (Anderson & Fisher 1976). It appears that vertebrate photoreceptors demonstrate two strategies of membrane repair: basal membrane addition (rods) and molecular insertion (cones), and, in so far as the major structural components are concerned, one mechanism of bulk loss (terminal membrane shedding).

Retinol, however, is transferred to the pigment epithelium from the receptors during light adaptation and restored to them on return to darkness; in the albino rat the first process reaches equilibrium in about 60 min, and the second nears completion after some 2 h (Dowling 1960).

Thus there are pertinent analogies between the interactions of pigmented epithelium and retina in vertebrates, and those between receptors and supportive cells in *Dolomedes*. In vertebrates, however, structural membrane protein is not, apparently, lost in bulk other than by distal membrane shedding.

Comparisons with invertebrate retinae are more difficult to make. In part, this is because of the widespread assumption that any demolition of membrane which may take place retains degraded product within the receptors, but the degree to which this is soundly based is not clear. Movement of material from receptors to supportive or shielding components might not be detectable by routine electron microscopy unless it were massive, and, given the assumption that multivesicular and multilamellar bodies are the primary vehicles for the carriage of degraded membrane away from the rhabdomeres, it is likely that the search for an extra-receptoral mechanism has been less than assiduous. Lasansky (1967) described an 'extracellular ground substance' in association with the retinal glia of *Limulus* which is remarkably similar to the electron dense material figured here, and the receptor membranes show similar invaginated pleats which are most marked in the case of the eccentric cells; external recycling of rhabdomere material was not suggested by either Lasansky or by Behrens & Krebs (1976), but does not seem to be excluded as a possibility by the published data. The former author suggested a trophic relation between glial cells and receptors. Materials for the repair of photoreceptor membrane have been assumed to be transported to their sites of incorporation as macromolecules, by analogy with vertebrate receptors.

Behrens & Krebs (1976) suggest that the coated vesicles which they observed in close association with the bases of microvilli, and in one instance, pinching off from them, are lysosomes mediating their disassembly (although in the same paper the authors also speculate that they may be fusing), but evidence as to whether the formation of pinocytotic vesicles may, in part, be modulated enzymically is so far lacking. Eguchi & Waterman (1976) have observed that acid phosphatase does not appear in a relation with pinocytotic vesicles until they form multivesicular bodies.

Eakin & Brandenburger (1976) interpret the paracrystalline assemblies of uniform spheres – the *Schaumstruktur* of Schwalbach, Lickfield & Hahn (1963) – found in the basal, perinuclear regions of snail photoreceptors as 'photic vesicles' which transport photopigment precursors to the photoreceptor microvilli, with which fusion has been seen, on one occasion, to take place. Photic vesicles were shown to arise from the Golgi apparatus (Eakin & Brandenburger 1967), and incorporation of [$^3H_2$] vitamin A into the latter was shown, in an autoradiographic study, to precede its appearance in the photic vesicles and the rhabdomeral microvilli (Brandenburger & Eakin 1970). This might suggest that in molluscs the systems responsible for membrane synthesis may be rather differently organized; however, Kataoka (1975) gives reasons for supposing that photic vesicles may, in fact, arise from endoplasmic reticulum. Whittle (1976)

argues for a primary rôle of endoplasmic reticulum in the synthesis of new membrane in invertebrate photoreceptors on comparative grounds.

Confirmation or disproof of the rôle of the endoplasmic reticulum which is proposed here must await further work, but it is worth noting that these very large and metabolically active spider receptors are peculiarly suitable for investigations which combine autoradiographic procedures and the use of inhibitors of protein synthesis with controlled régimes of illumination.

Disordered membrane

Loew (1976) has shown that the rhabdoms of *Nephrops norvegicus* respond to levels of illumination some three logarithmic units above those of their natural habitats by irreversible degeneration. The myelin figures and membrane whorls produced were similar to those described here, and have been seen in other Crustacea (Eguchi & Waterman 1967). Loew suggests that in those invertebrates whose rhabdoms are unstable, rhodopsins are major structural components which are needed to maintain membrane integrity, that bleaching causes configurational changes which, if they are not balanced by regeneration of rhodopsin, result in membrane breakdown, and that the relative thermal stabilities of opsin and rhodopsin (Hubbard 1959) may support this interpretation. So, too, may the freeze-fracture observations of Brandenburger, Reed & Eakin (1975), and Eakin & Brandenburger (1976), who demonstrated that particles whose dimensions (7.0–9.5 nm) were consonant with those of photopigment molecules, abundant on the cytoplasmic side of snail microvillar membranes, were much reduced in frequency in the light-adapted state; in this case, very long periods of adaptation were involved, and the authors stress that the effects were not found in short-term adaptation experiments. However, while such a model may account for the phenomena observed by Loew, and for the fragmented membrane seen in the inter-rhabdomeral cytoplasm of *Dolomedes* after long periods of light adaptation, the filling of whole receptive segments with disordered membrane (e.g. figure 40) seems more likely to relate to an inability to achieve ordered restitution after extreme degrees of disassembly.

Finally, it is of interest to note that the occurrence of small patches of receptors with completely disordered membrane was only seen in spiders from open, unshaded habitats, while they were absent from those taken from the beds of heavily shaded streams. M. F. Land has drawn our attention to the fact that the brightness of the sun's disk is some 5 logarithmic units above that of a piece of white card which it illumines, and suggests that spiders with disordered rhabdomeres may have rested in direct sunlight after flooding, and that such patches may preserve the retinal images of the sun. It does not seem possible to account for the presence of receptors with disordered membrane directly adjacent to normal receptors on the basis of an erratic penetration of fixative, because the effects are so sharply localized, and to invoke an unspecified pathological process seems, at this stage, to be unsatisfactory.

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References

Anderson, D. H. & Fisher, S. K. 1976 The photo-receptors of diurnal squirrels: disc shedding and protein renewal. *J. ultrastruct. Res.* **55**, 119–141.

Baccetti, B. & Bedini, C. 1964 Research on the structure and physiology of the eyes of a lycosid spider. I. Microscopic and ultramicroscopic structure. *Arch. ital., Biol.* **102**, 97–122.

Basinger, S., Hoffman, R. & Matthes, M. 1976 Photoreceptor shedding is initiated by light in the frog retina. *Science, N.Y.* **194**, 1074–1076.

Behrens, M. & Krebs, W. 1976 The effect of light-dark adaptation on the ultrastructure of *Limulus* lateral eye retinular cells. *J. comp. Physiol.* **107**, 77–96.

Blest, A. D. 1976 A new method for the reduced silver impregnation of arthropod central nervous systems. *Proc. R. Soc. Lond.* B **193**, 191–197.

Blest, A. D. 1978 The rapid synthesis and destruction of photoreceptor membrane by a dinopid spider: a daily cycle. *Proc. R. Soc. Lond.* B (in the press).

Blest, A. D. & Land, M. F. 1977 The physiological optics of *Dinopis subrufus* L. Koch: a fish lens in a spider. *Proc. R. Soc. Lond* B. **197**, 197–222.

Brandenburger, J. L. & Eakin, R. W. 1970 Pathway of incorporation of Vitamin A $^3H_2$ into photoreceptors of a snail, *Helix aspersa*. *Vision Res.* **10**, 639–653.

Brandenburger, J. L., Reed, C. T. & Eakin, R. M. 1975 Freeze-fracture study of photoreceptors of dark and light-adapted snails. *Am. Zool.* **15**, 782.

Dowling, J. E. 1960 Chemistry of visual adaptation in the rat. *Nature, Lond.* **188**, 114–118.

Eakin, R. W. & Brandenburger, J. L. 1967 Differentiation in the eye of a pulmonate snail *Helix aspersa*. *J. ultrastruct. Res.* **18**, 391–421.

Eakin, R. W. & Brandenburger, J. L. 1971 Fine structure of the eyes of jumping spiders. *J. ultrastruct. Res.* **37**, 618–663.

Eakin, R. W. & Brandenburger, J. L. 1976 Understanding a snail's eye at a snail's pace. *Am. Zool.* **15**, 851–863.

Eguchi, E. & Waterman, T. H. 1967 Changes in retinal fine structure induced in the crab *Libinia* by light and dark adaptation. *Z. Zellforschung* **79**, 209–229.

Eguchi, E. & Waterman, T. H. 1976 Freeze-etch and histochemical evidence for cycling in crayfish photoreceptor membranes. *Cell. Tiss. Res.* **169**, 419–434.

Forster, R. R. & Forster, L. M. 1973 *New Zealand spiders: an introduction*. Auckland: Collins.

Harper, E., Seifter, S. & Scharrer, B. 1967 Electron microscopic and biochemical characterisation of collagen in blattarian insects. *J. Cell Biol.* **33**, 385–394.

Hogan, M. J., Wood, I. & Steinberg, R. H. 1974 Phagocytosis by pigment epithelium of human retinal cones. *Nature, Lond.* **252**, 305–307.

Homann, H. 1951 Die Nebenaugen der Araneen. *Zool. Jb.* (*Anat.*) **71**, 56–144.

Homann, H. 1971 Die Augen der Araneen. *Z. Morphol. Tiere* **69**, 201–272.

Horridge, G. A. & Barnard, P. B. T. 1965 Movement of palisade in locust retinula cells when illuminated. *Q. Jl micr. Sci.* **106**, 131–135.

Hubbard, R. 1959 The thermal stability of rhodopsin and opsin. *J. gen. Physiol.* **42**, 259–280.

Itaya, S. K. 1976 Rhabdom changes in the shrimp, *Palaemonetes*. *Cell Tiss. Res.* **169**, 419–434.

Kataoka, S. 1975 Fine structure of the retina of a slug, *Limax flavus* L. *Vision Res.* **15**, 681–686.

Lasansky, A. 1967 Cell junctions in ommatidia of *Limulus*. *J. Cell Biol.* **33**, 365–383.

LaVail, M. M. 1976 Rod outer segment disc shedding in rat retina: relationship to cyclic lighting. *Science, N.Y.* **194**, 1071–1074.

Loew, E. R. 1976 Light, and photoreceptor degeneration in the Norway lobster, *Nephrops norvegicus* (L). *Proc. R. Soc. Lond.* B **193**, 31–44.

Melamed, J. & Trujillo-Cenoz, O. 1966 On the fine structure of the visual system of *Lycosa* (Araneae, Lycosidae). I. Retina and optic nerve. *Z. Zellforsch. Anat.* **74**, 12–31.

Nemanic, P. 1975 Fine structure of the compound eye of *Porcellio scaber* in light and dark adaptation. *Cell Tiss. Res.* **7**, 453–468.

Pipa, R. L. & Woolever, P. S. 1965 Insect neurometamorphosis. II. The fine structure of perineural connective tissue, adipohaemocytes and the shortening ventral cord of a moth, *Galleria mellonella* (L). *Z. Zellforsch.* **68**, 80–101.

Röhlich, P. 1968 Fine structural changes induced in photoreceptors by light and prolonged darkness. In *Symposium on neurobiology of invertebrates* (ed. J. Salanki), pp. 95–109. New York: Plenum Press.

Rosenbluth, J. 1962 Subsurface cisternae and their relationship to the neuronal plasma membrane. *J. Cell Biol.* **13**, 405–421.

Schwalbach, G., Lickfeld, K. G. & Hahn, M. 1963 Der mikromorphologische Aufbau des Linsenauges der Weinbergschnecke (*Helix pomatia* L.). *Protoplasma* **56**, 242–273.

Tuurala, O., Lehtinen, A. & Nyholm, N. 1966 Zu den photomechanischen Erscheinungen im Augen einer Asselart *Oniscus asellus* L. *Ann. Acad. Sci. Fenn.* A (*IV Biol.*) **99**, 1–8.

White, R. H. 1967 The effect of light deprivation upon the ultrastructure of the larval mosquito eye. II. The rhabdom. *J. exp. Zool.* **166**, 405–425.

White, R. H. 1968 The effect of light and light deprivation upon the ultrastructure of the larval mosquito eye. III. Multivesicular bodies and protein uptake. *J. exp. Zool.* **169**, 261–278.

White, R. H. & Lord, E. 1975 Diminution and enlargement of the mosquito rhabdom in light and darkness. *J. gen. Physiol.* **65**, 583–598.

Whittle, A. C. 1976 Reticular specialisations in photoreceptors: a review. *Zool. Scripta* **5**, 191–206.

Young, J. Z. 1963 Light and dark adaptation in the eyes of some cephalopods. *Proc. zool. Soc. Lond.* **140**, 255–272.

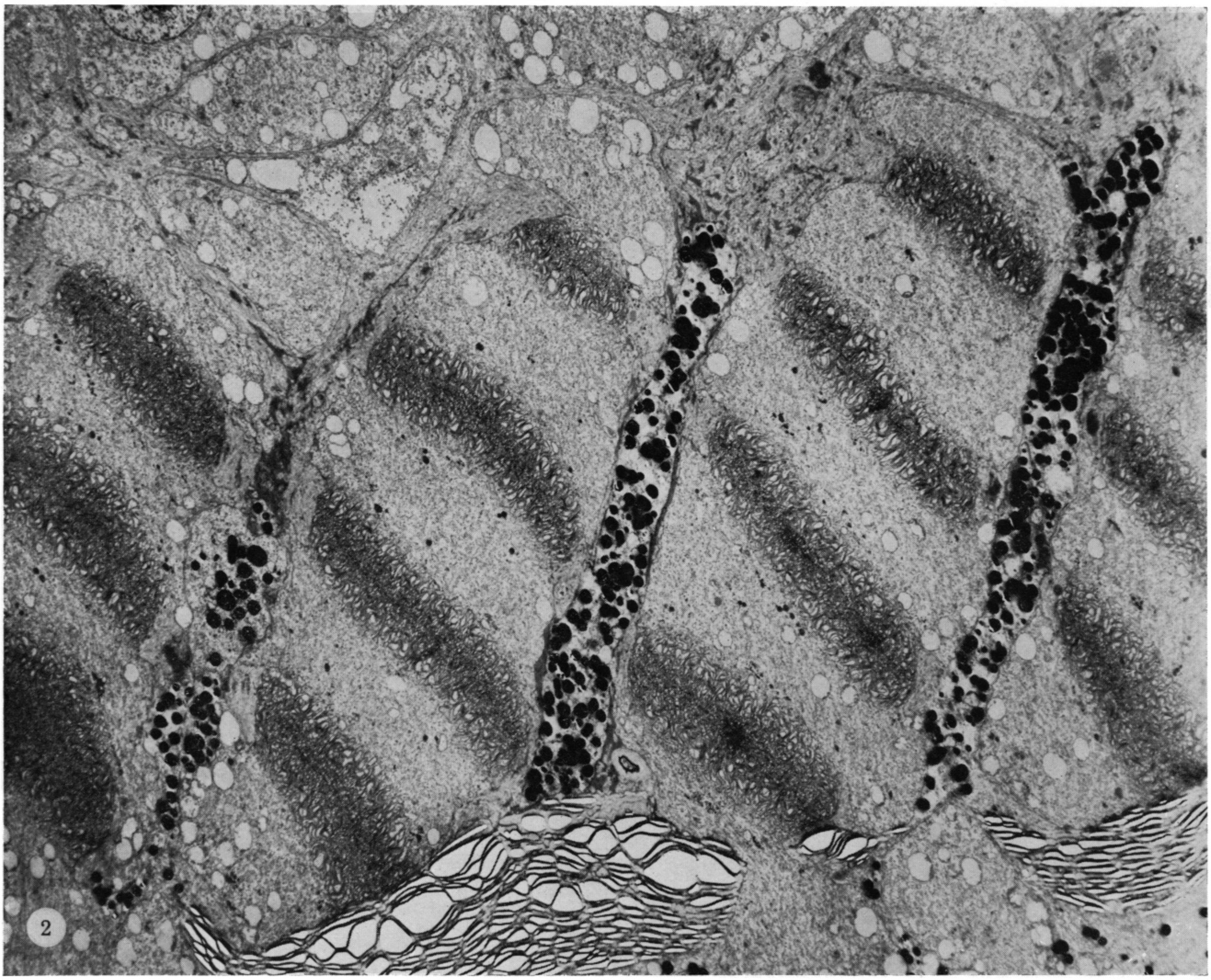
Young, R. W. 1967 The renewal of photoreceptor cell outer segments. *J. Cell Biol.* **33**, 61–72.

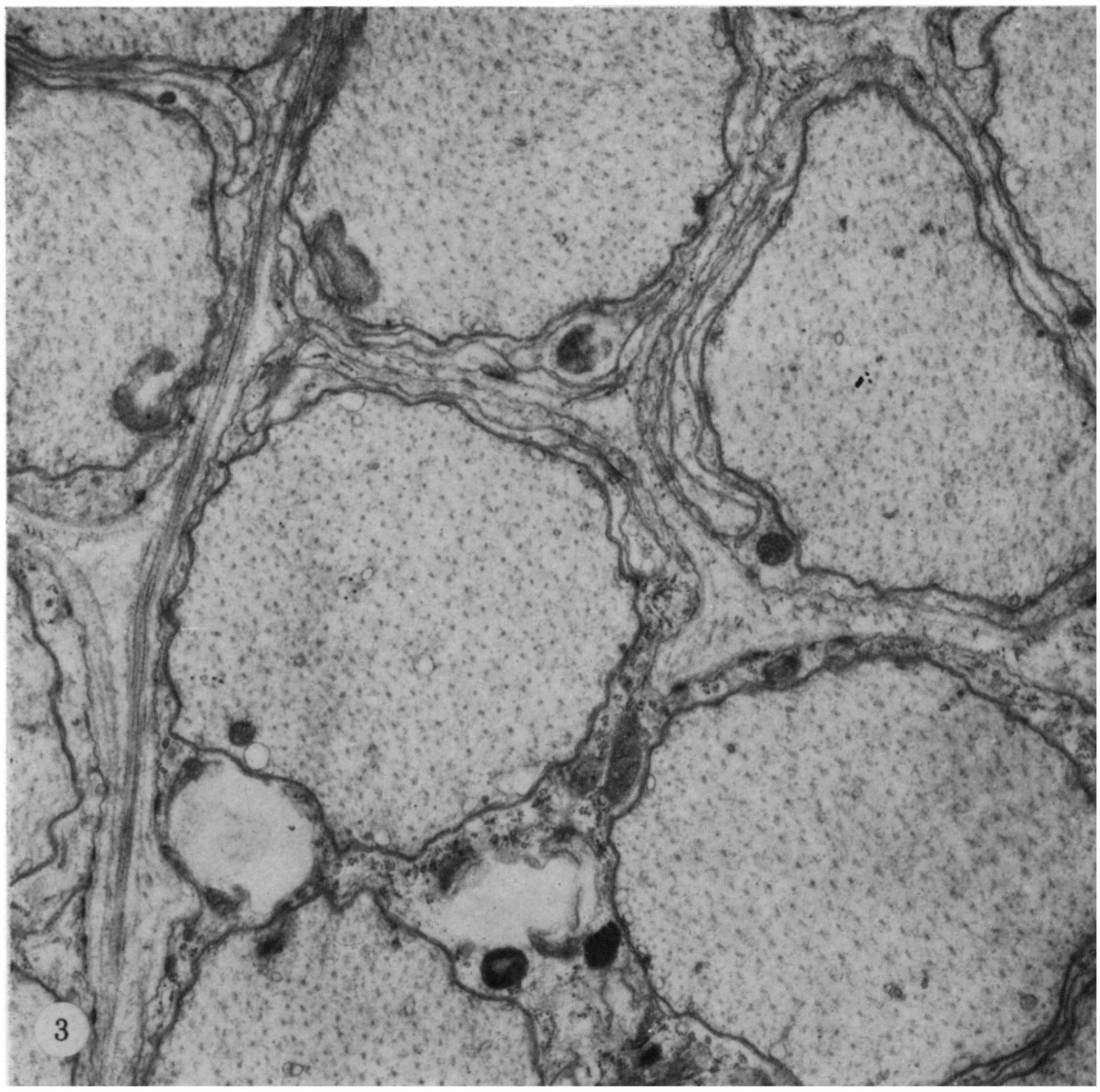
Young, R. W. 1969 A difference between rods and cones in the renewal of outer segment protein. *Invest. Ophthalmol.* **8**, 222–231.

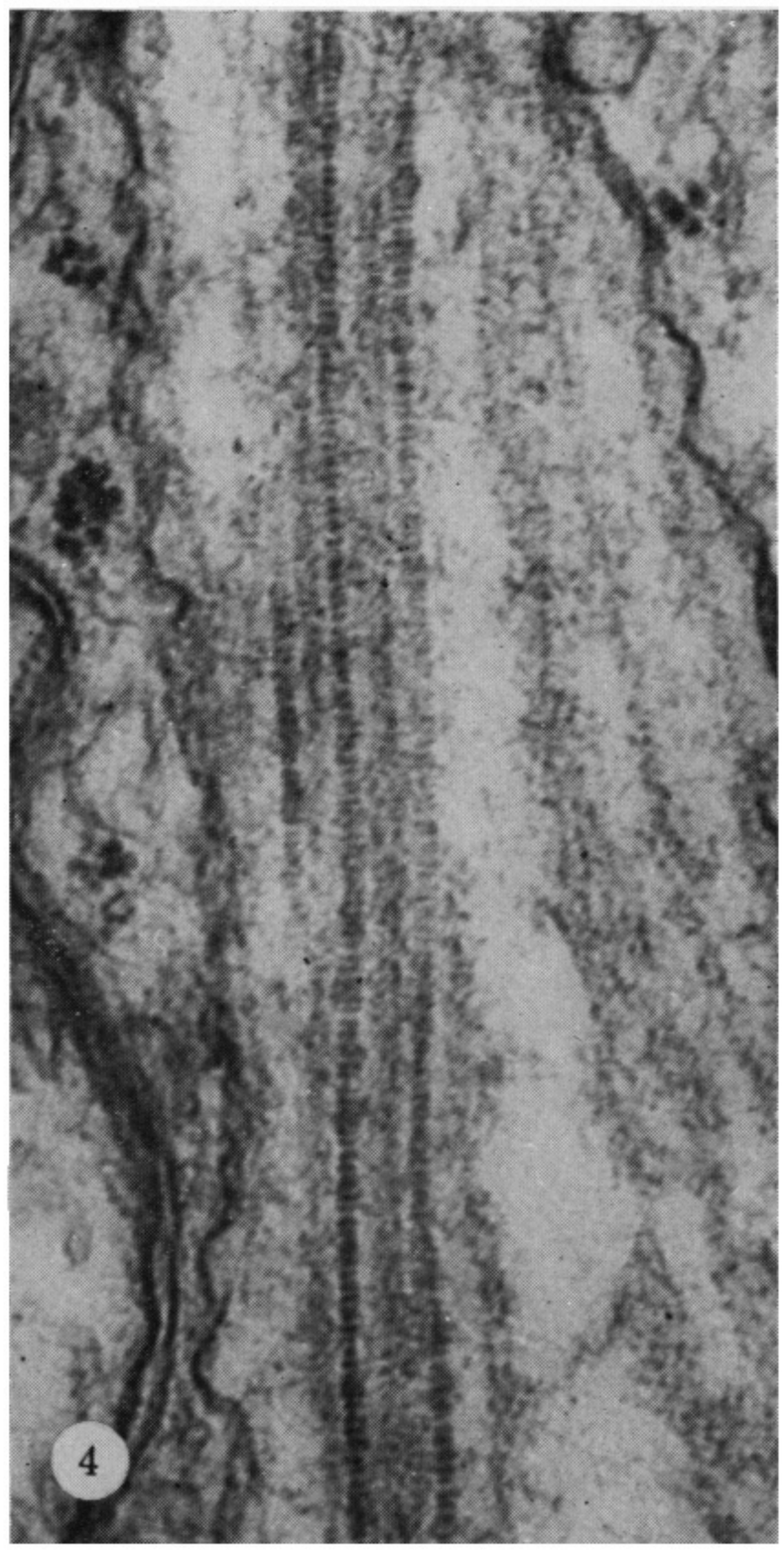
Young, R. W. 1971*a* An hypothesis to account for a basic difference between rods and cones. *Vision Res.* **11**, 1–5.

Young, R. W. 1971*b* The renewal of rod and cone outer segments in the rhesus monkey. *J. Cell Biol.* **49**, 303–318.

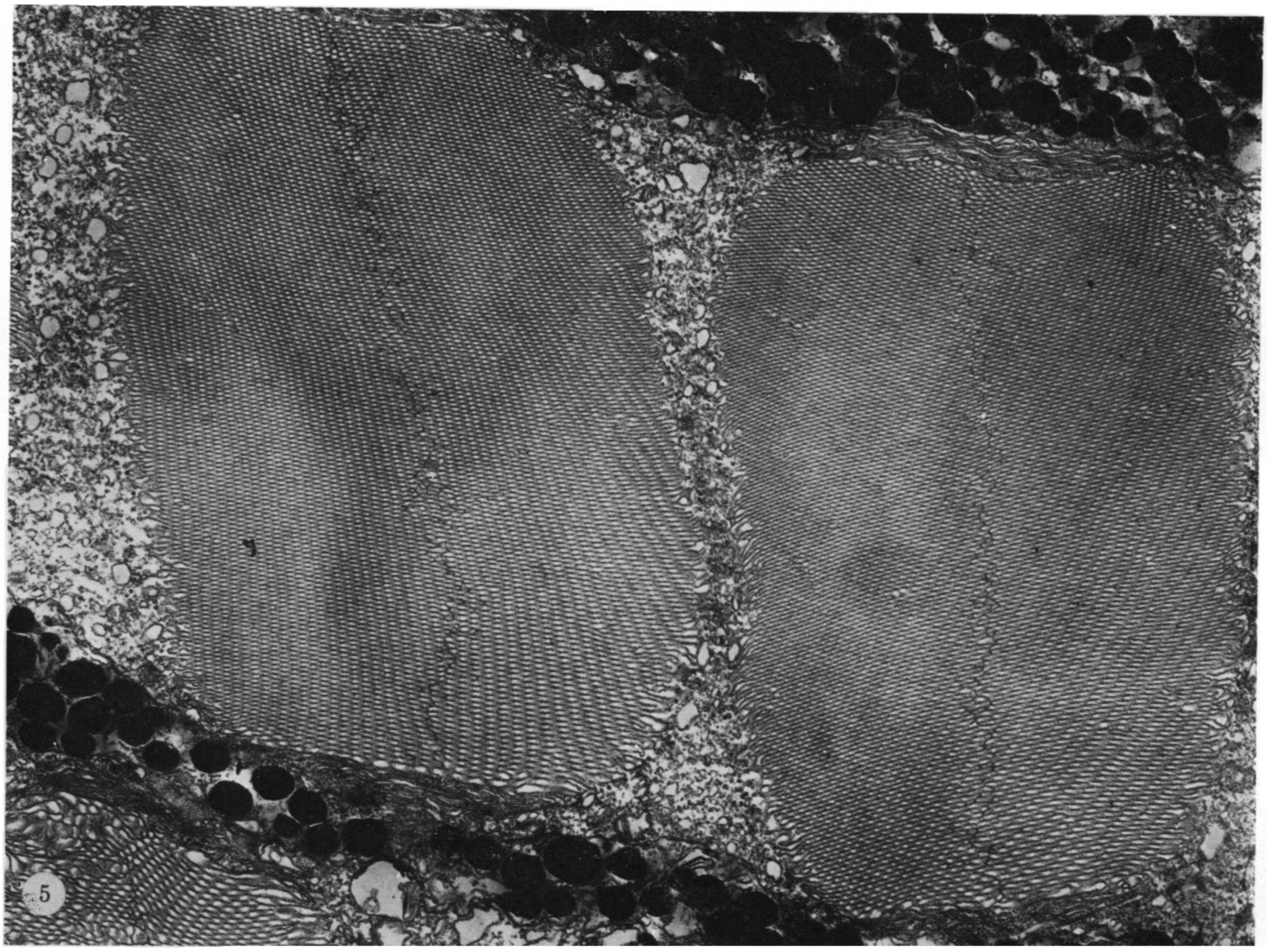
Young, R. W. 1971*c* Shedding of discs from rod outer segments in the rhesus monkey. *J. ultrastruct. Res.* **34**, 190–203.

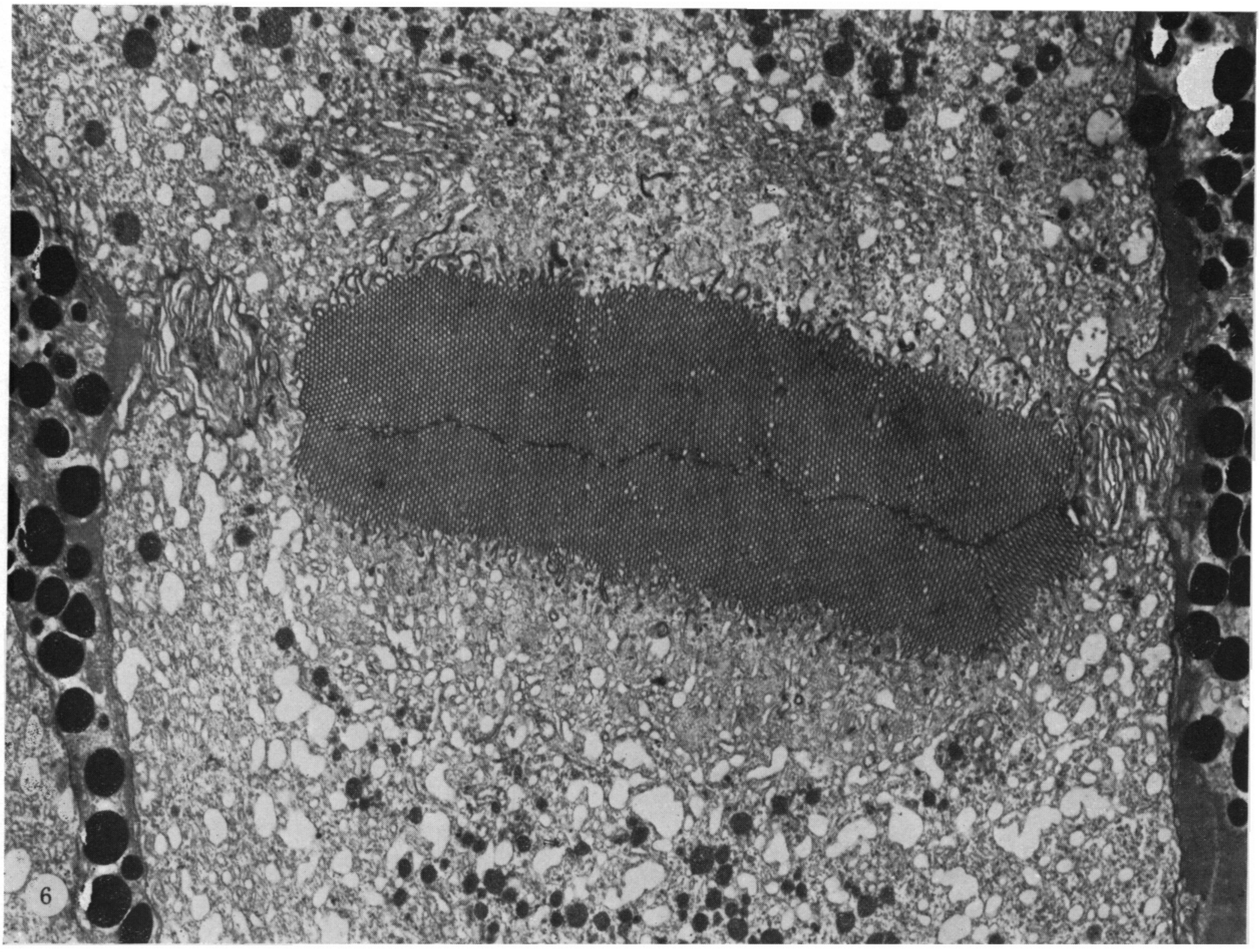




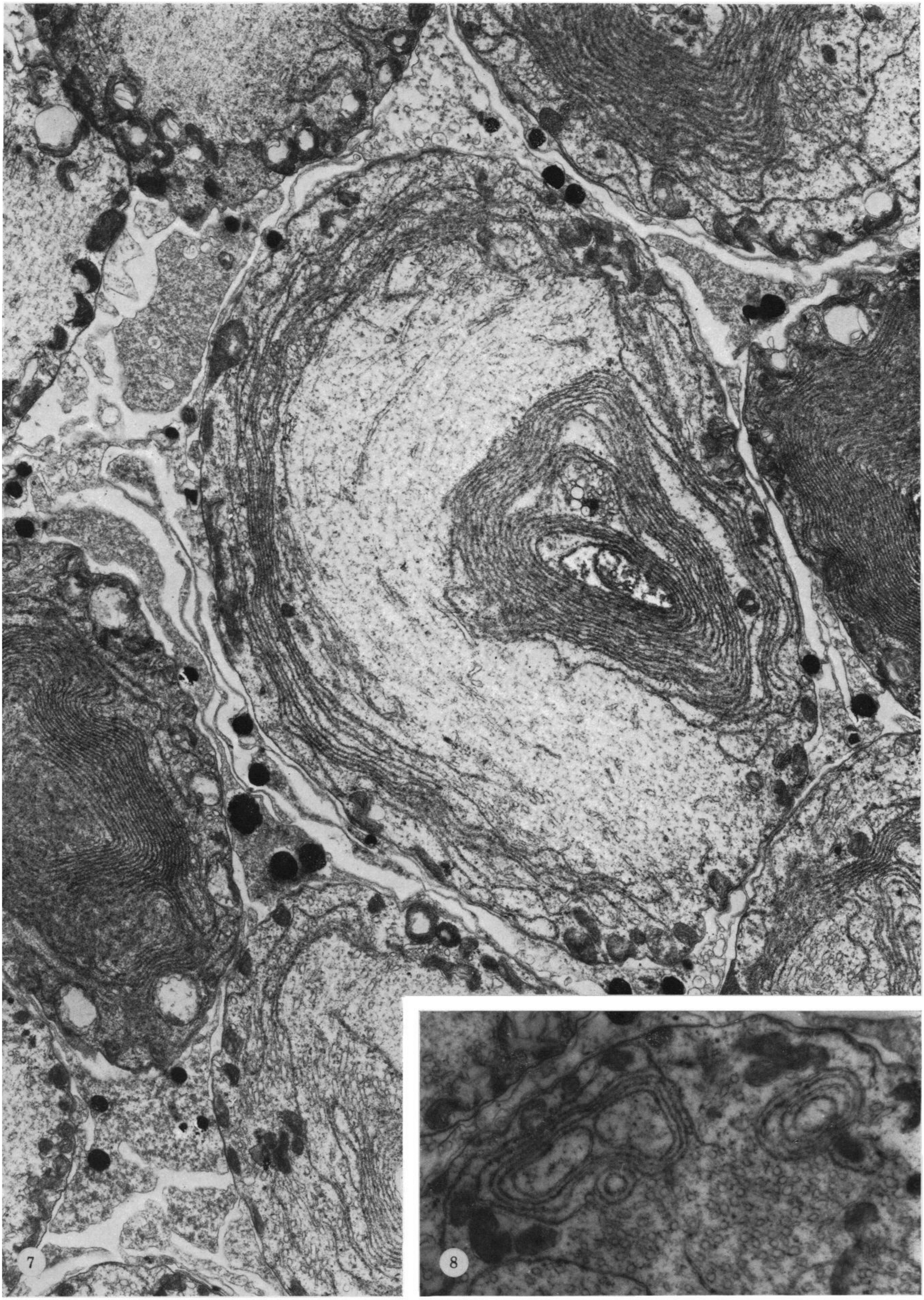


FIGURES 2–4. For description see opposite.





FIGURES 5 AND 6. For description see p. 8.



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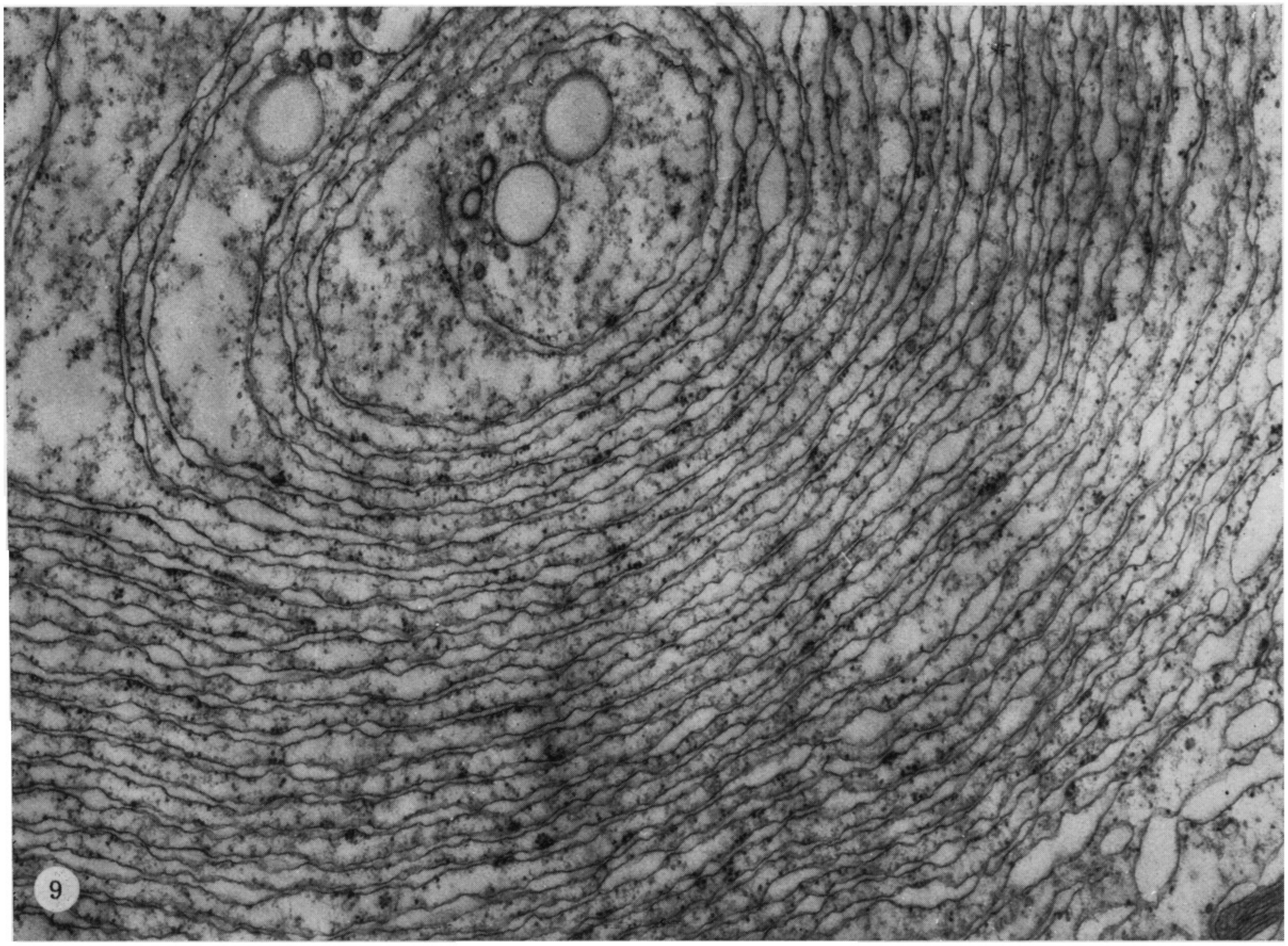
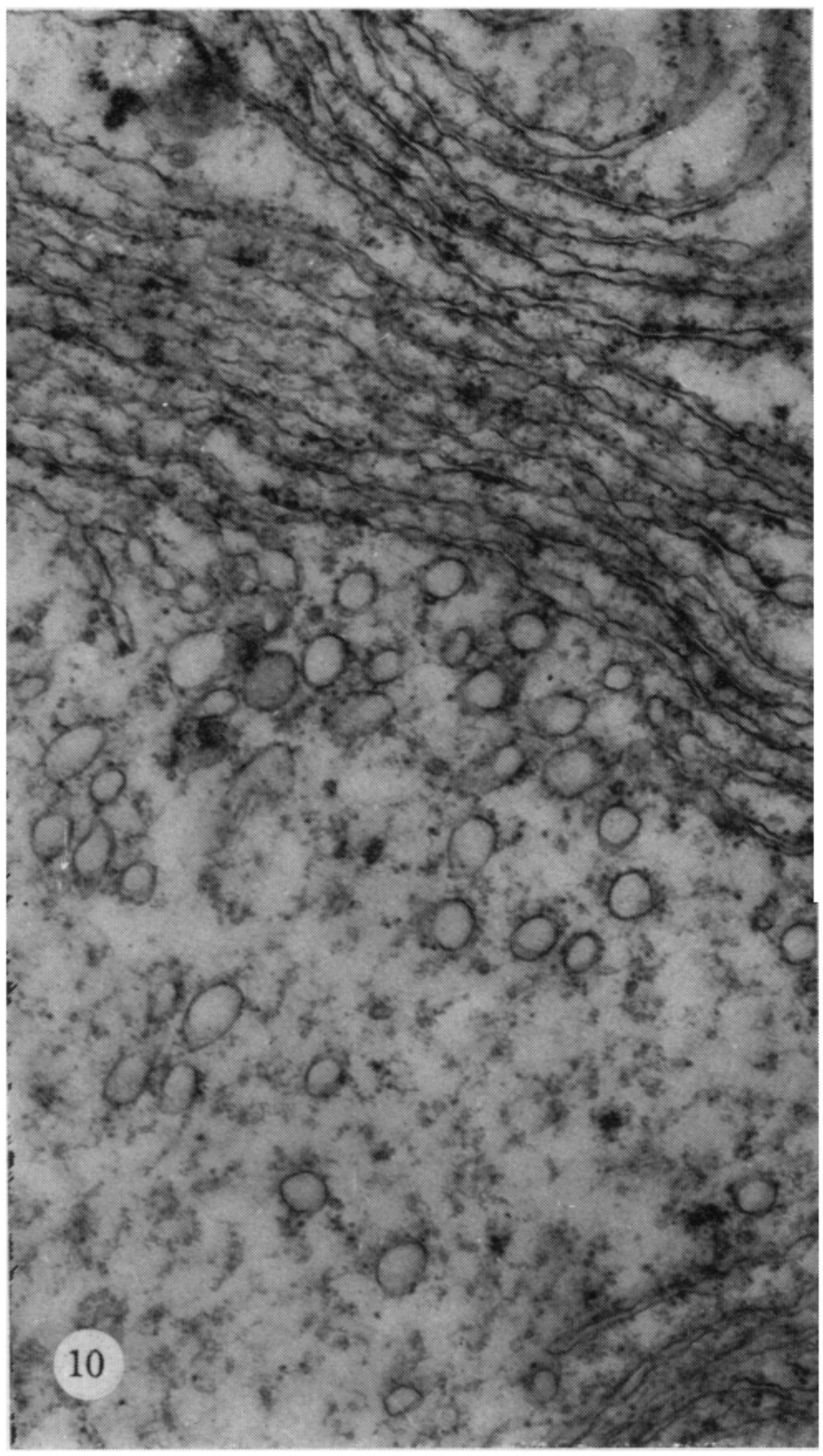
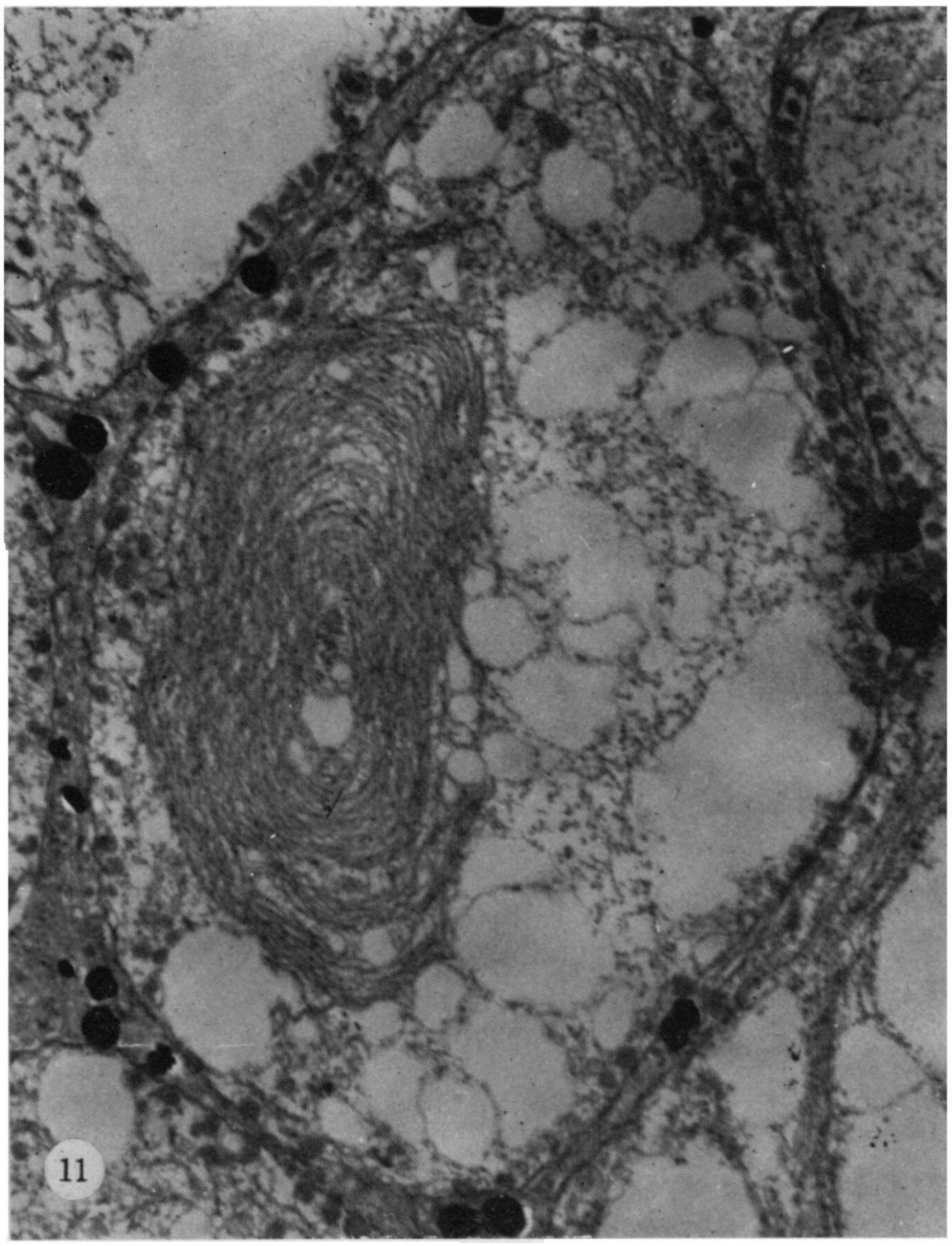
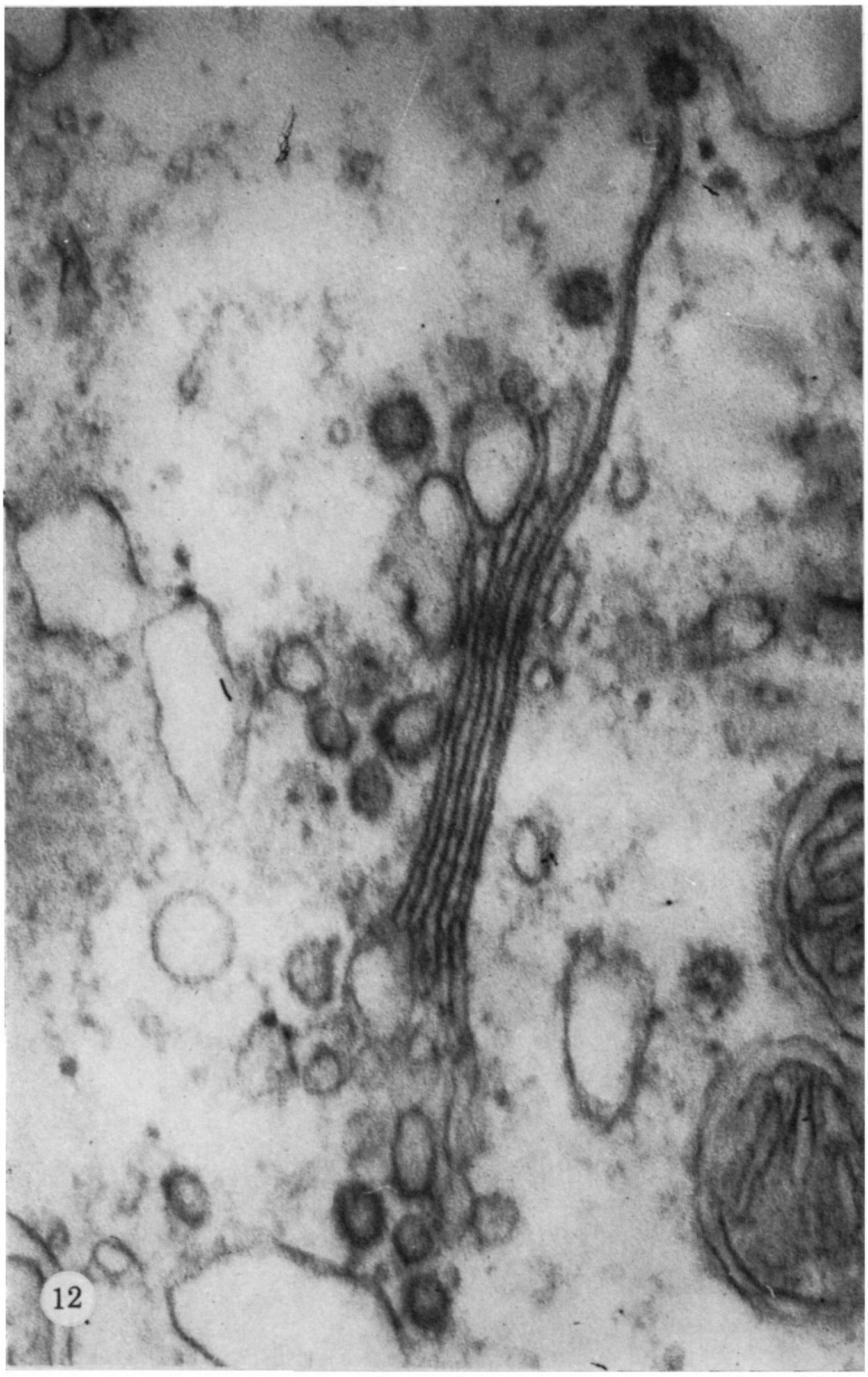
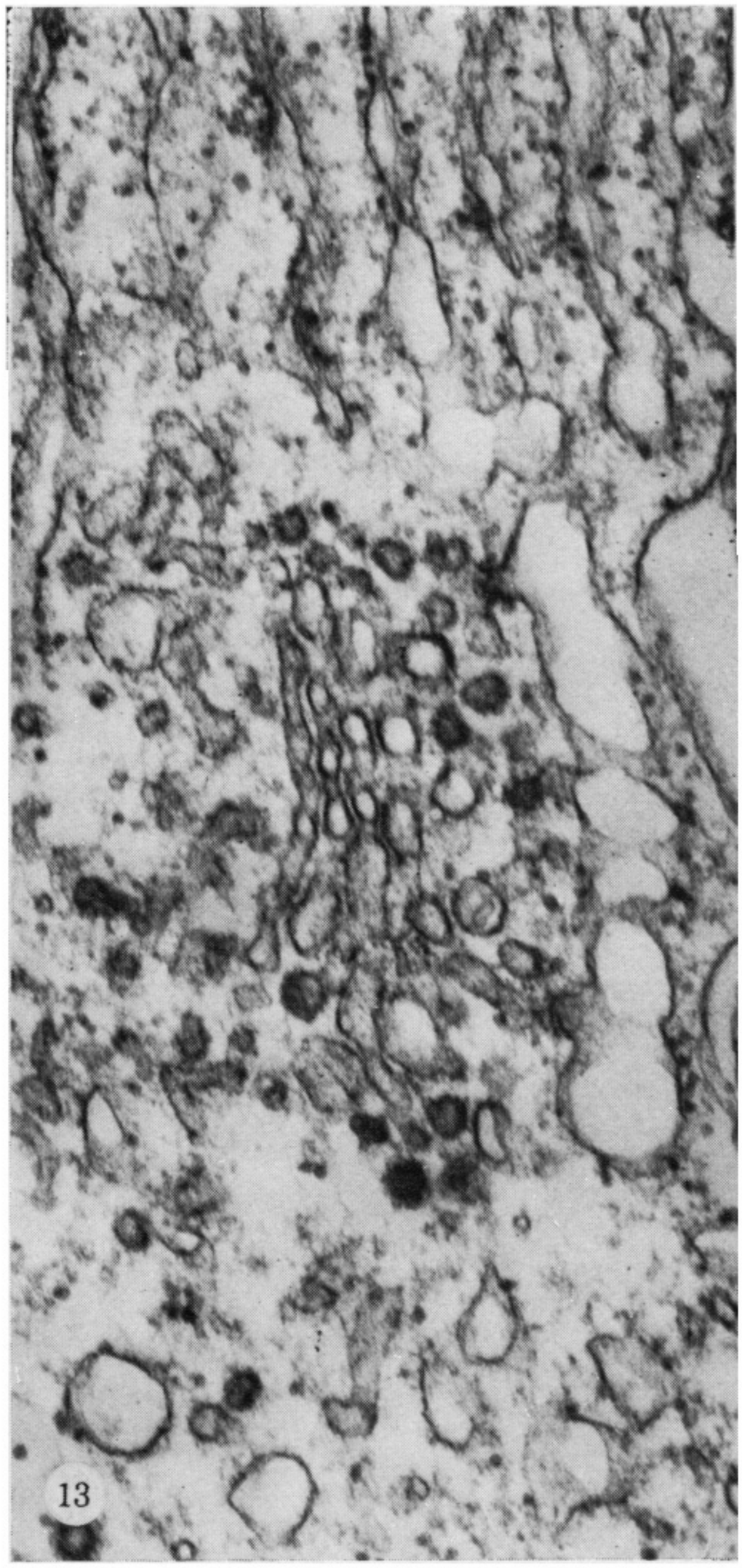


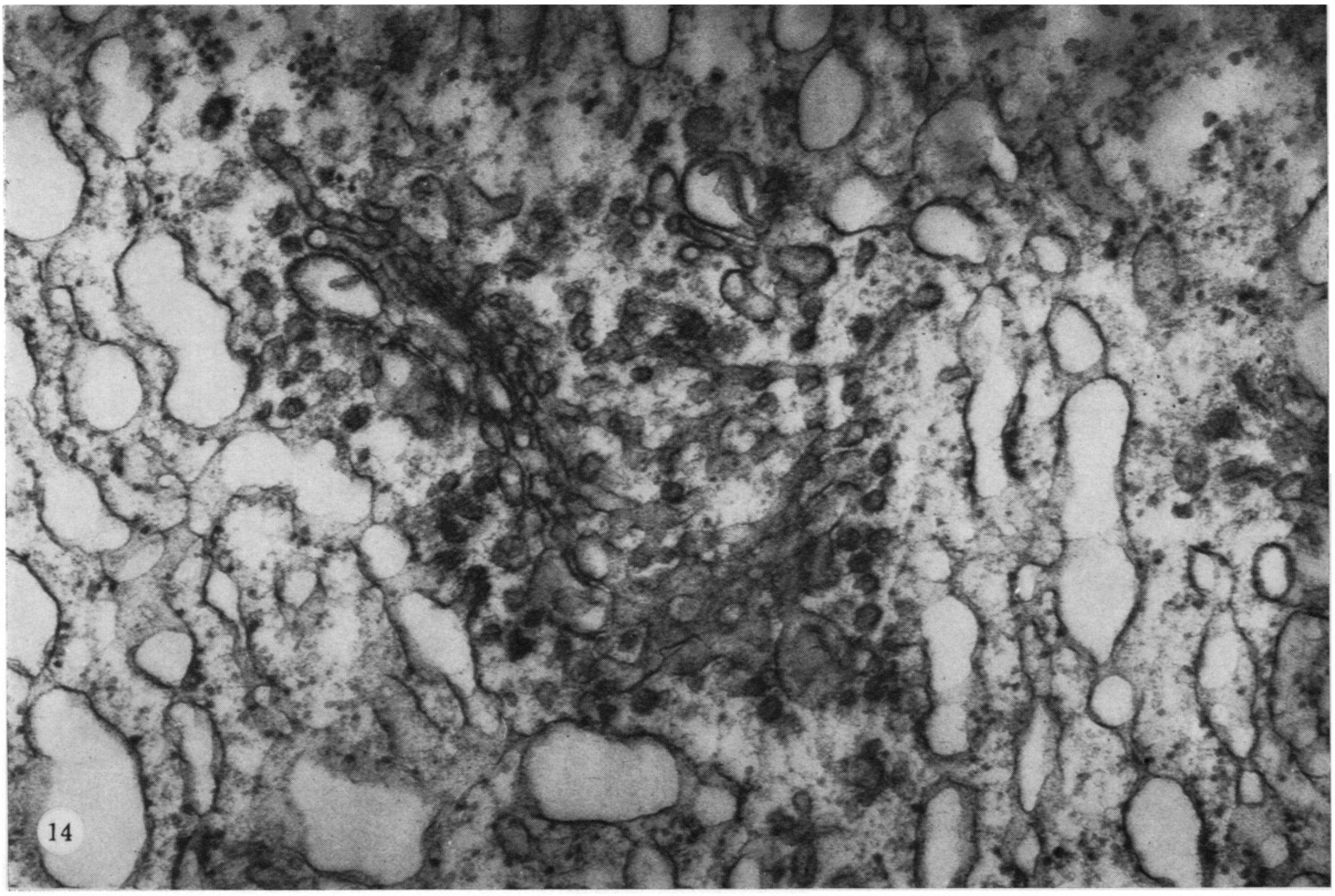




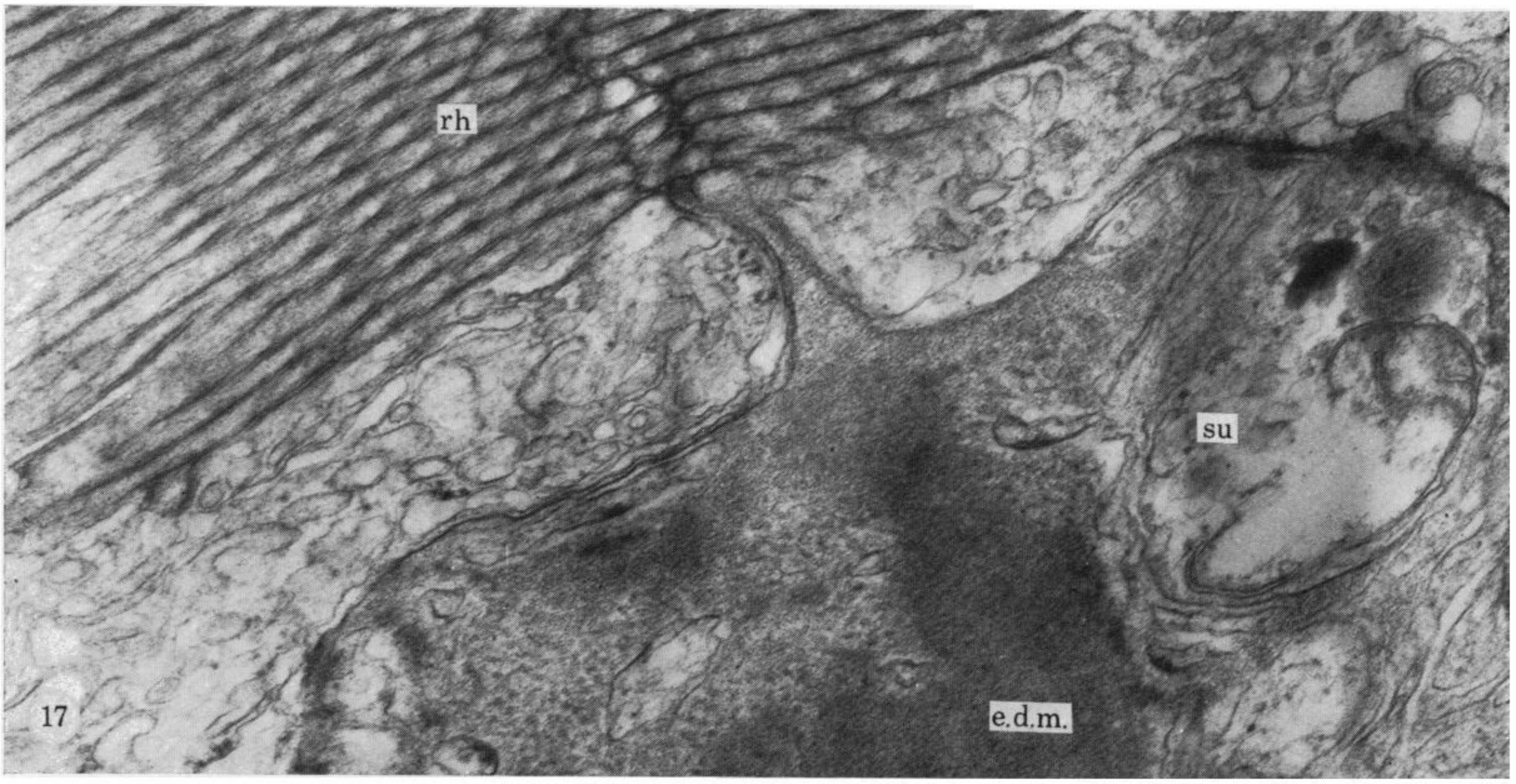
FIGURES 9–11. For description see opposite.

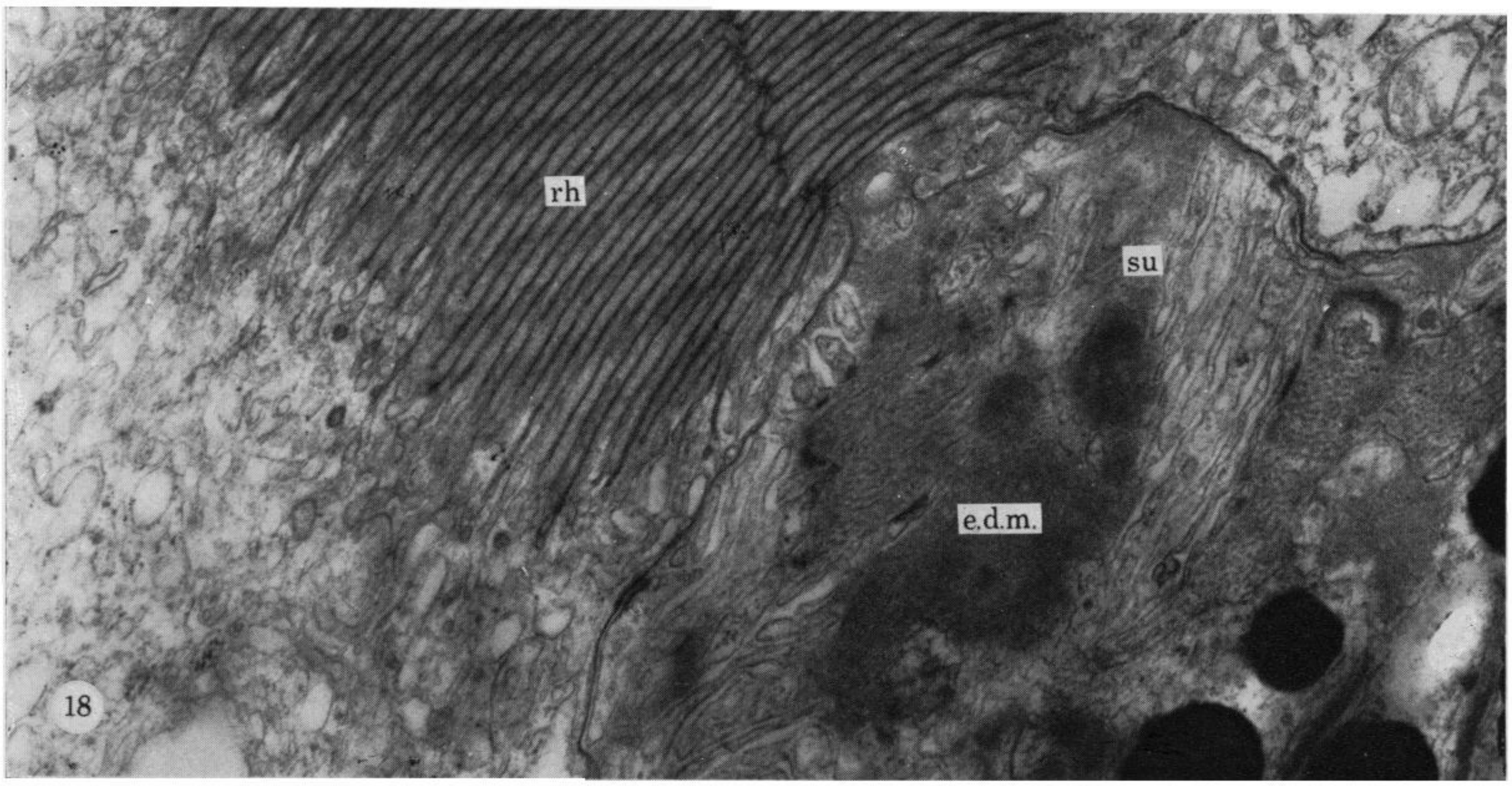


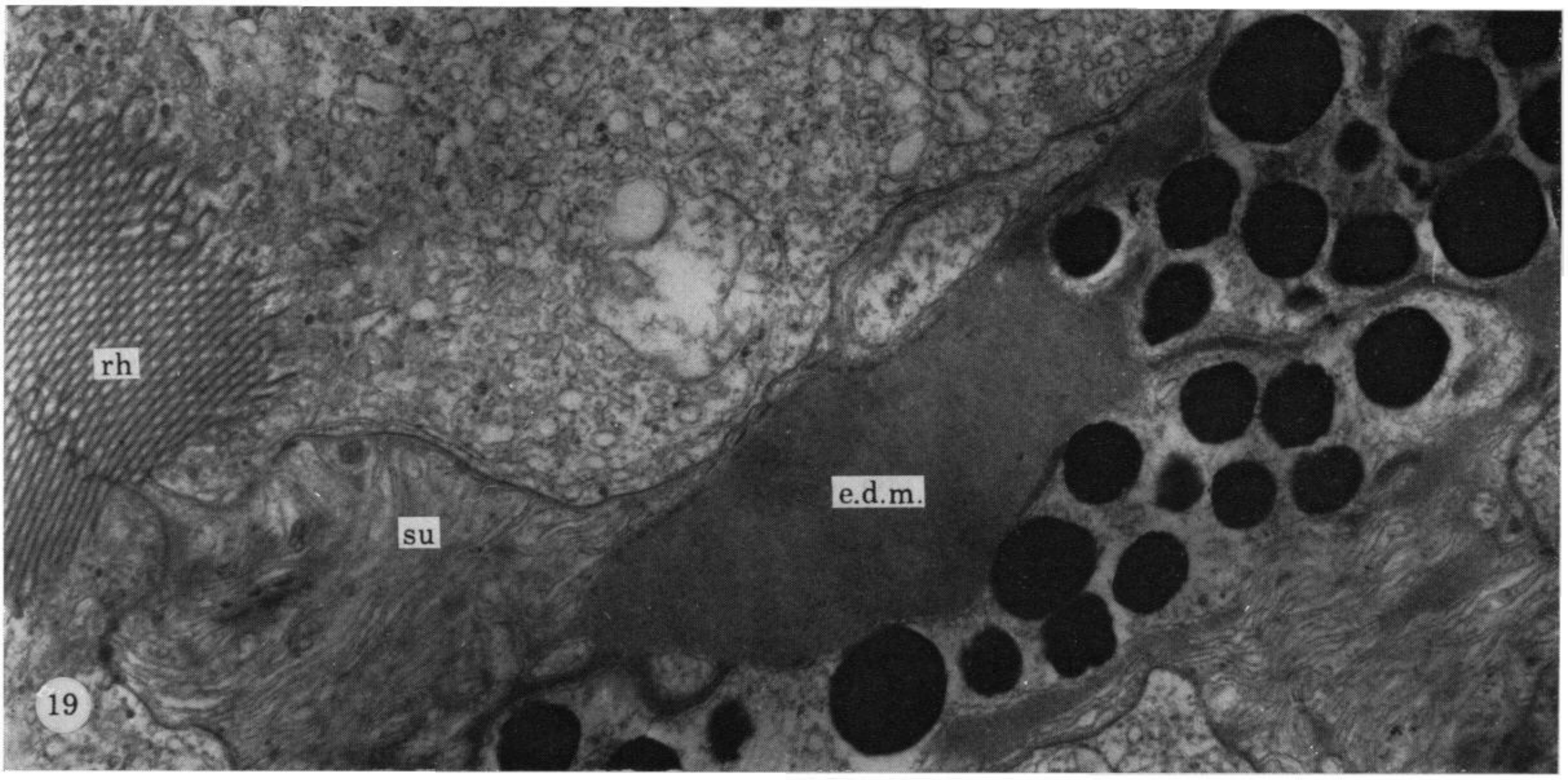




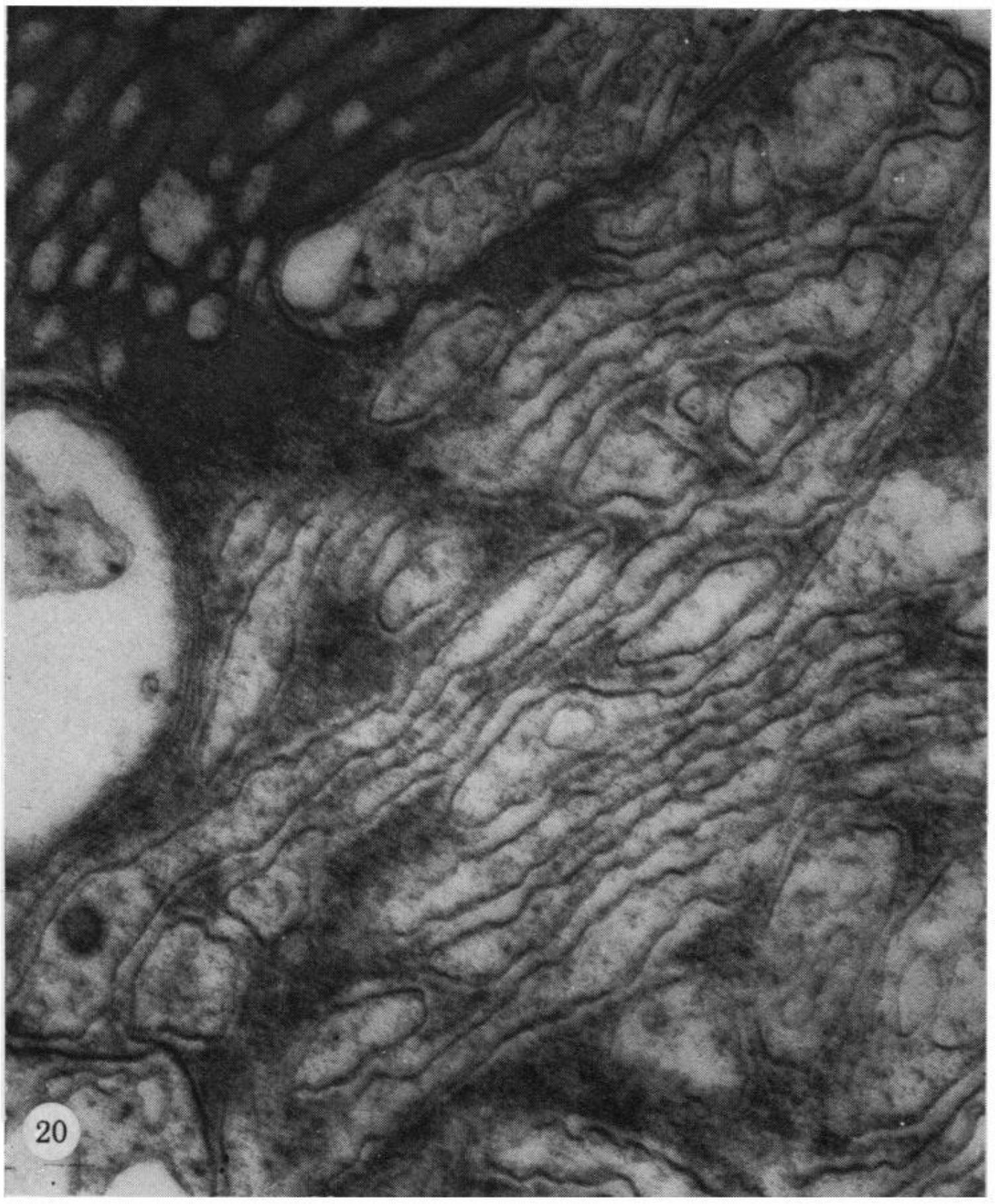
FIGURES 12–14. For description see opposite.



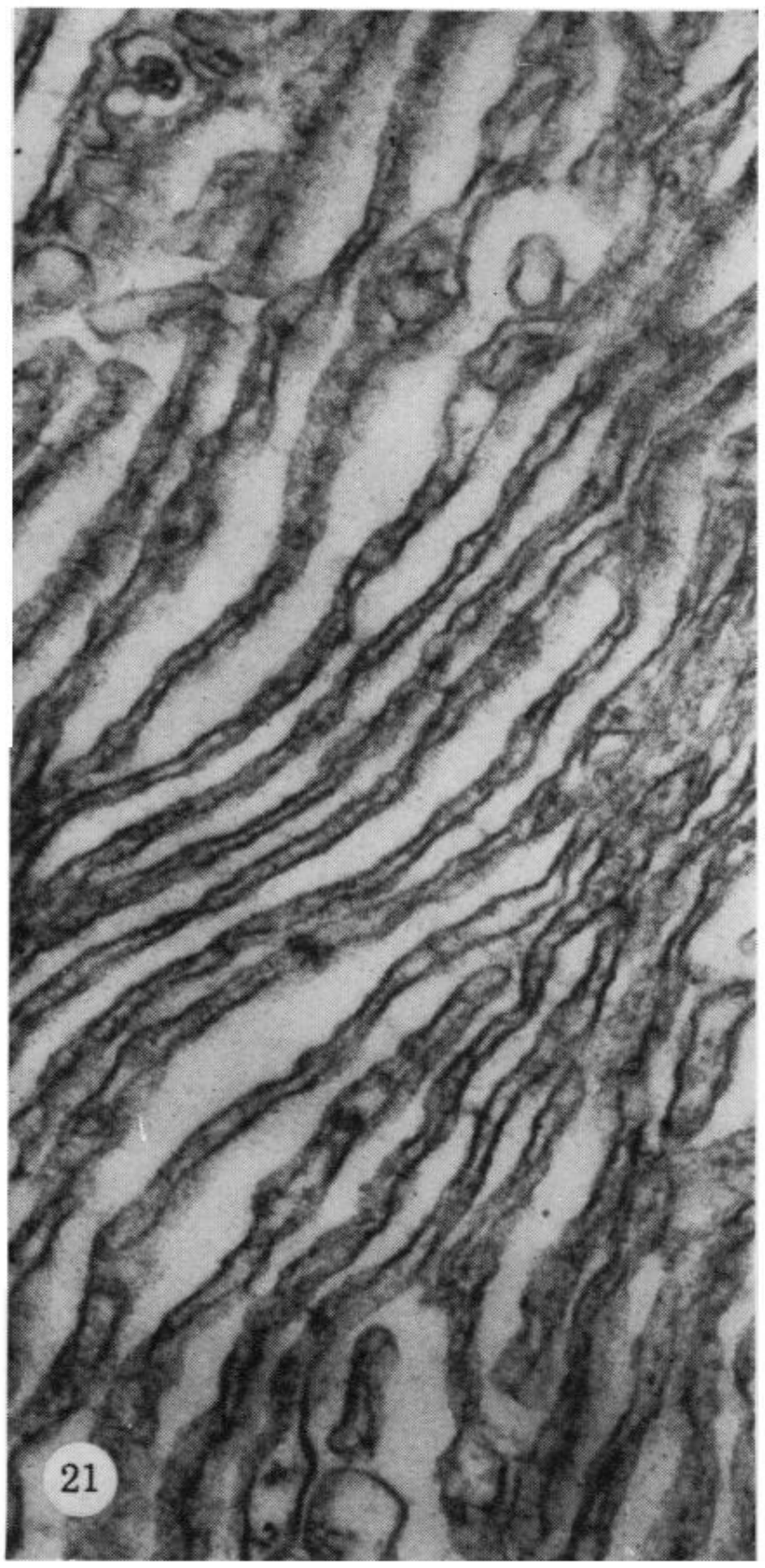




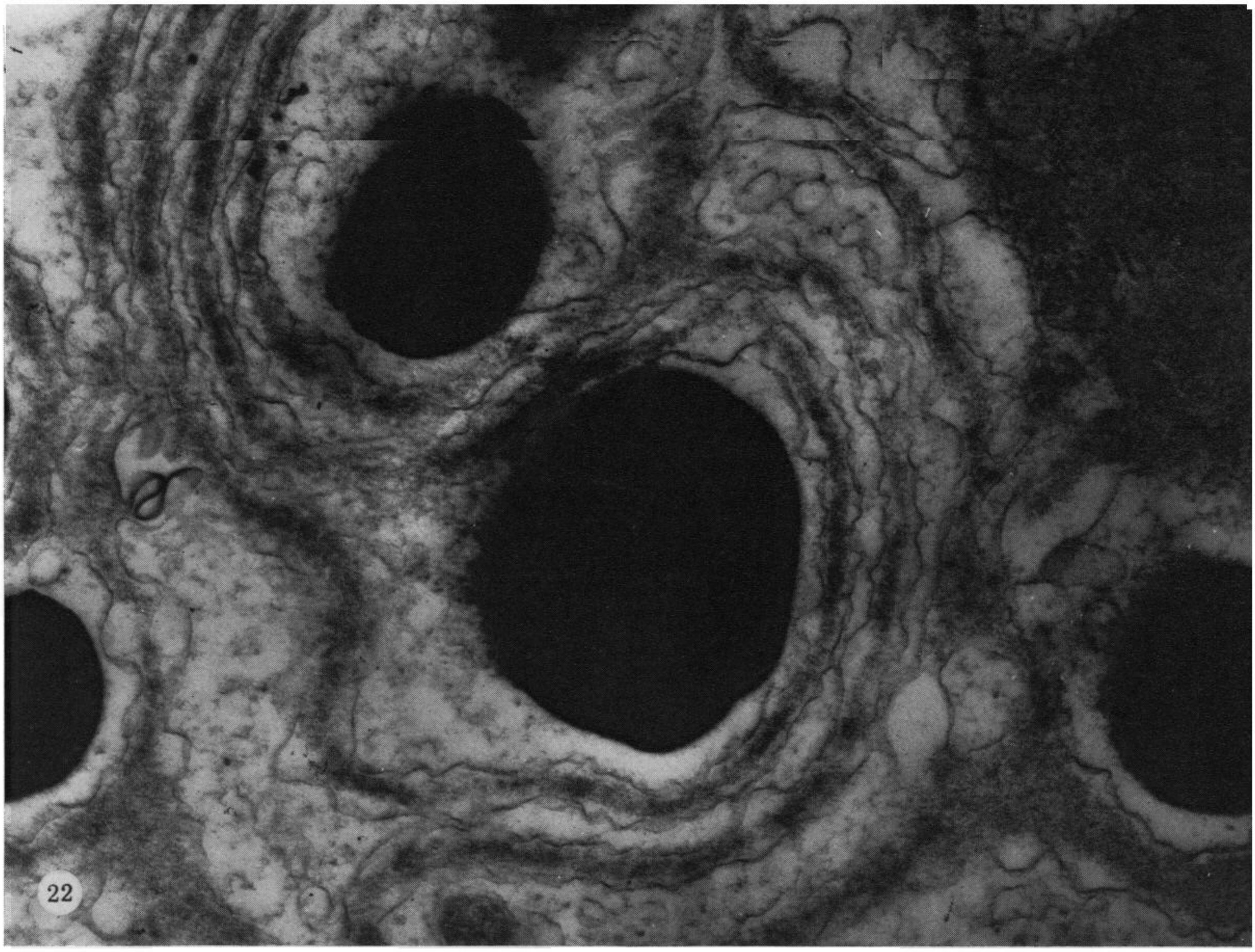
FIGURES 17–19. For description see p. 10.



20

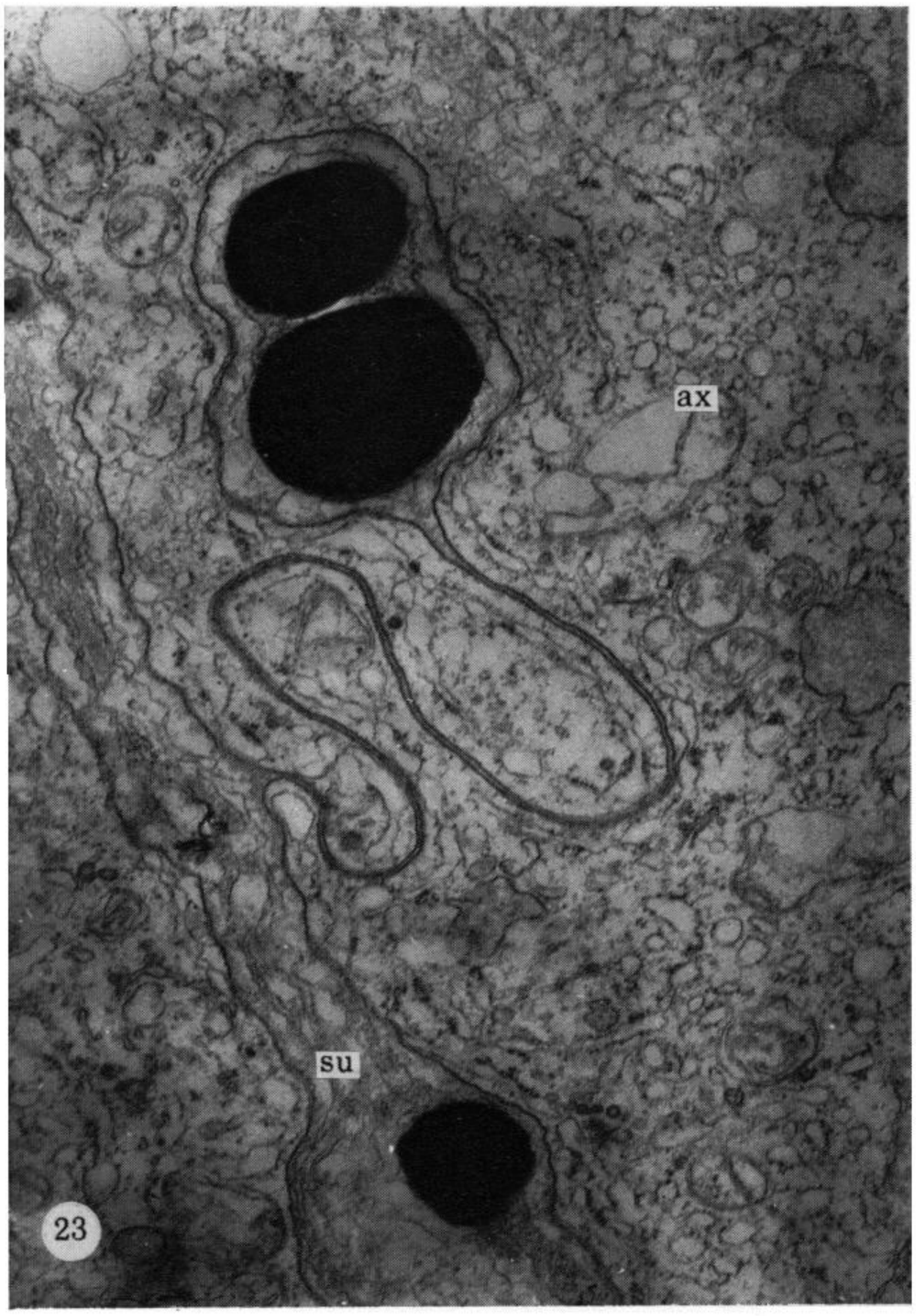


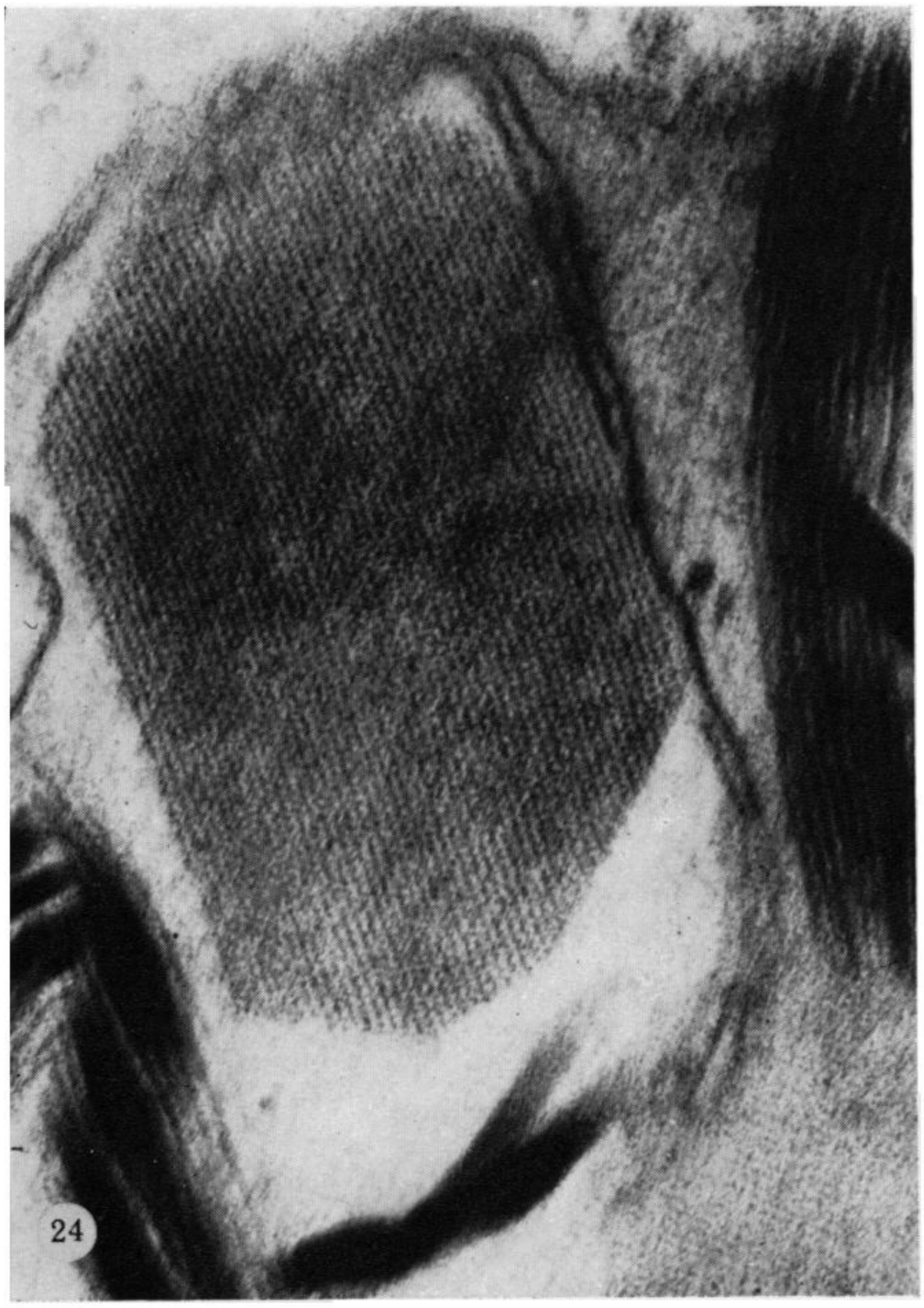
21

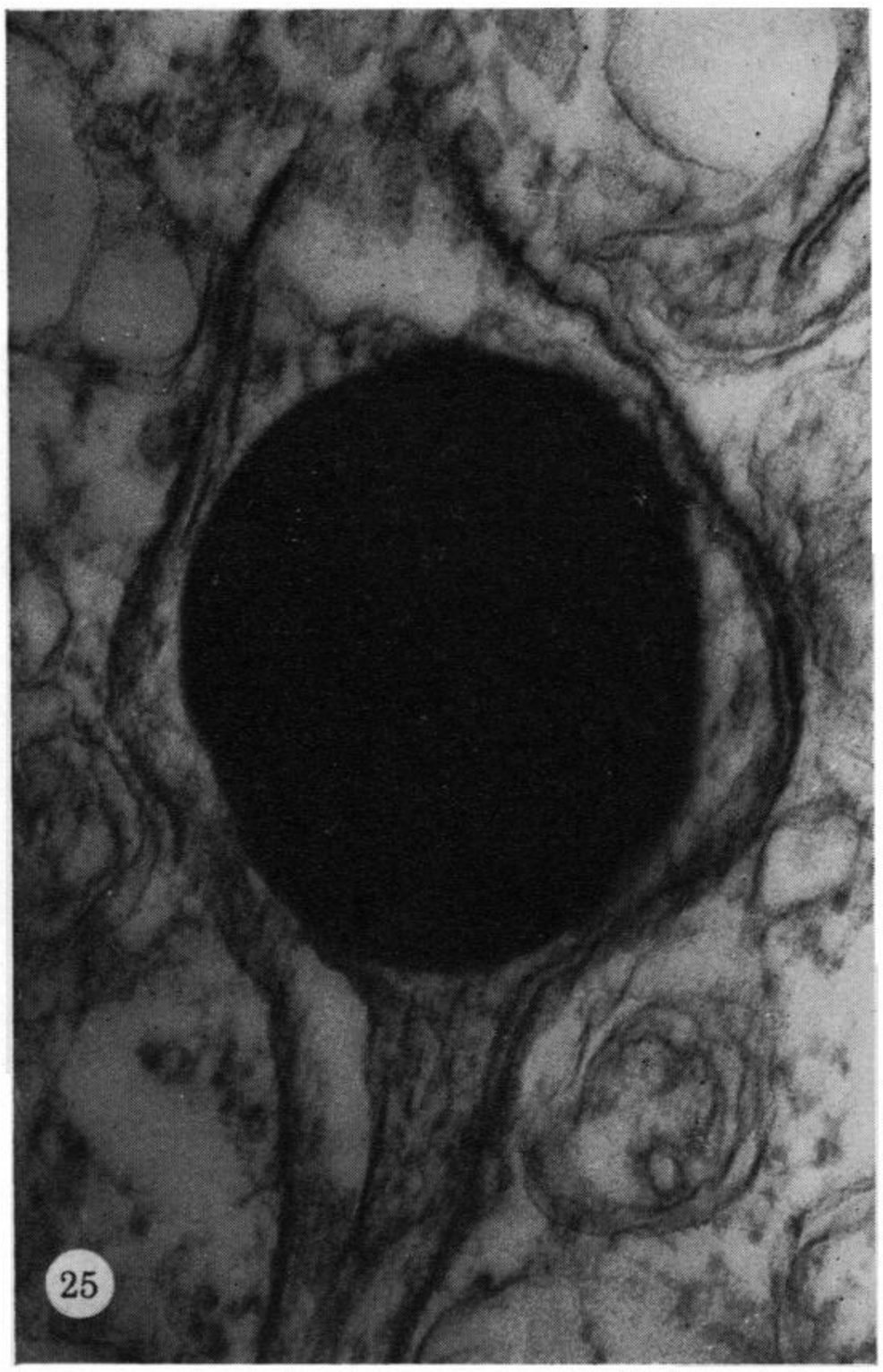


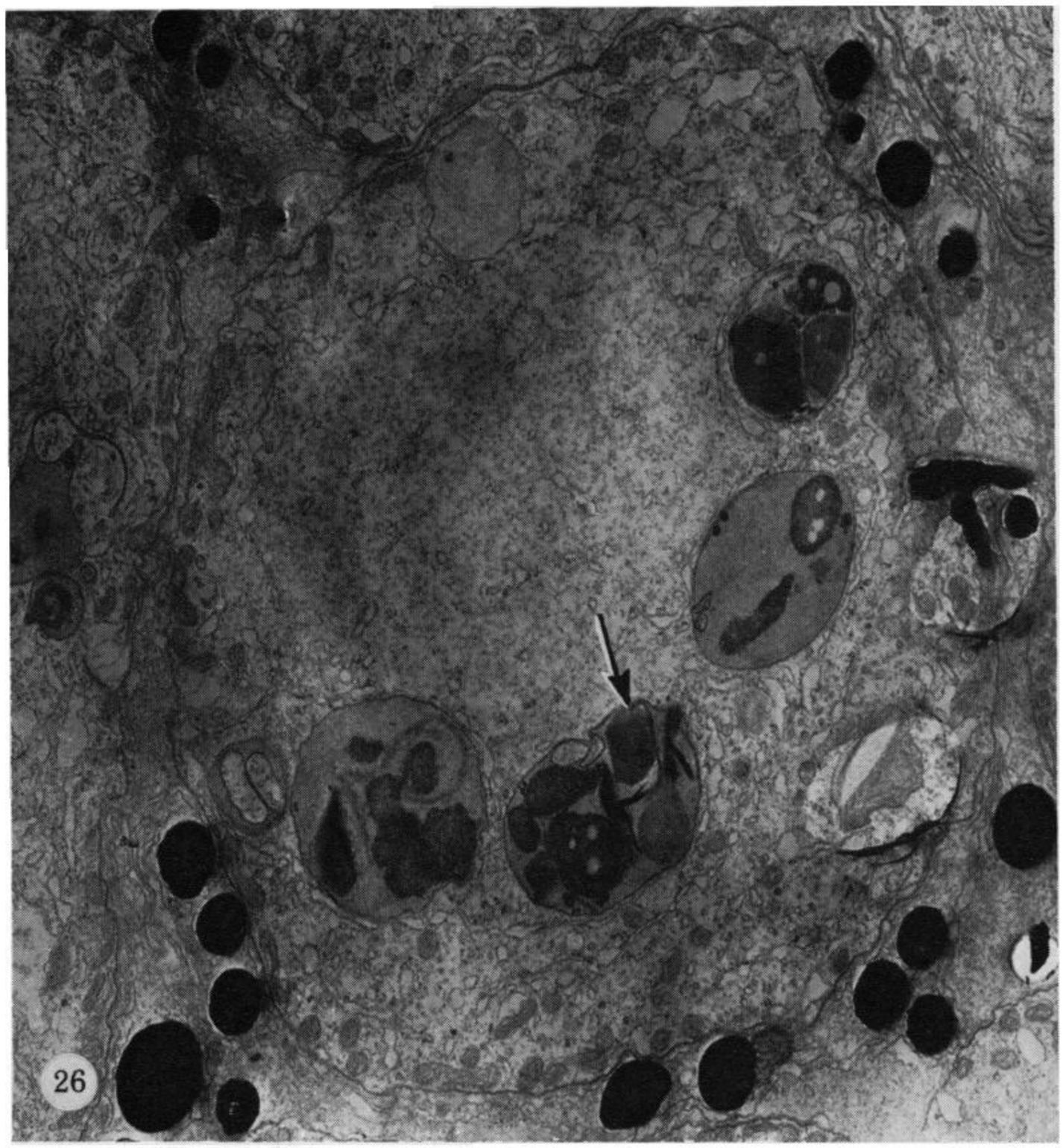
22

FIGURES 20–22. For description see p. 11.









FIGURES 23–26. For description see opposite.

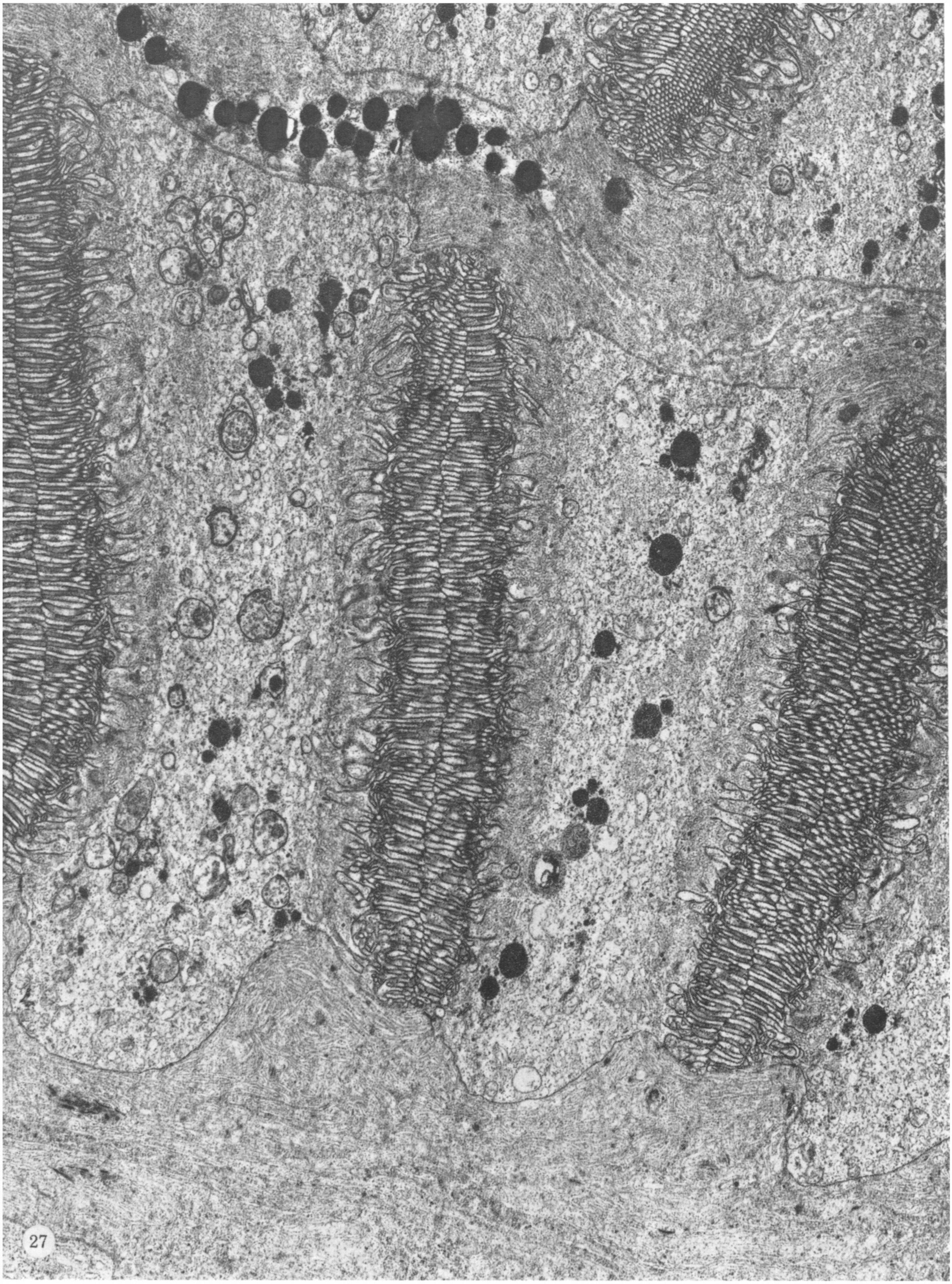
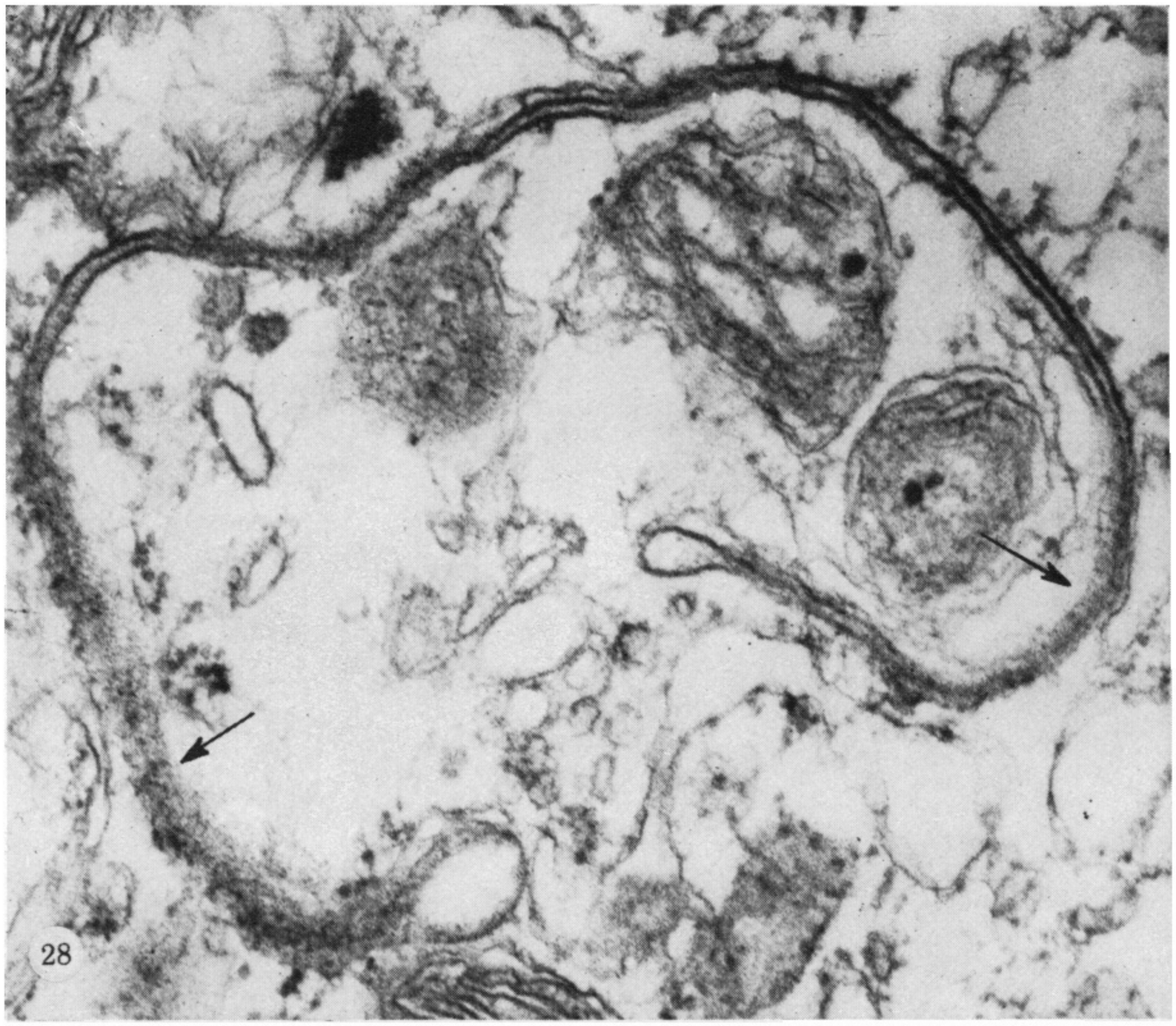
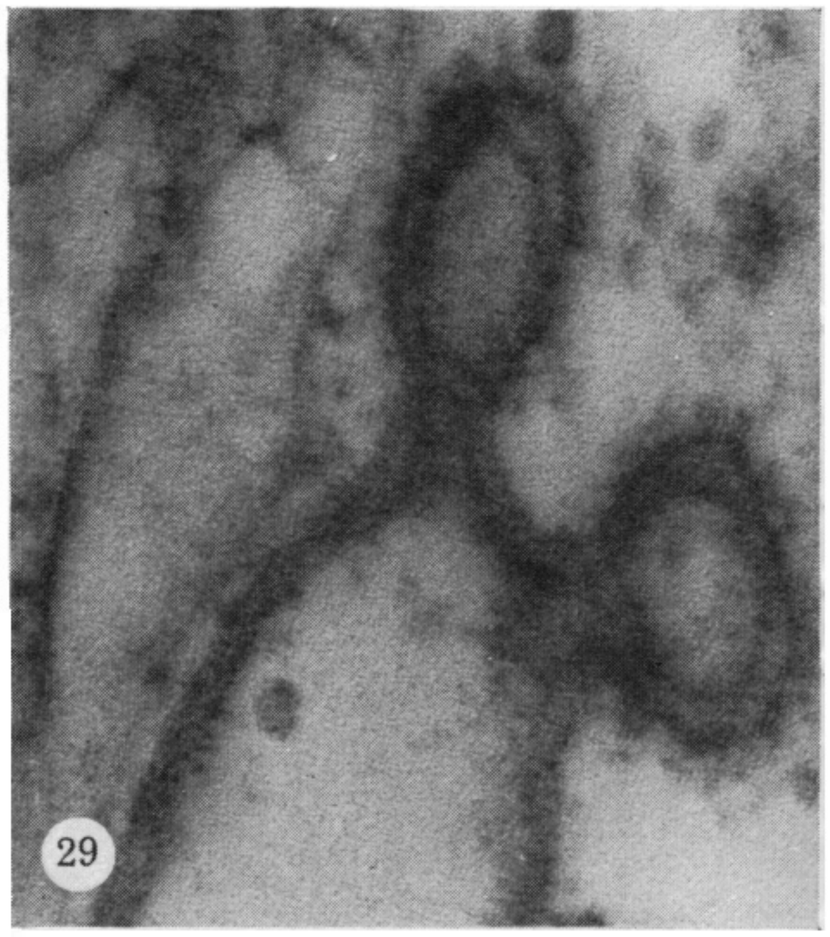


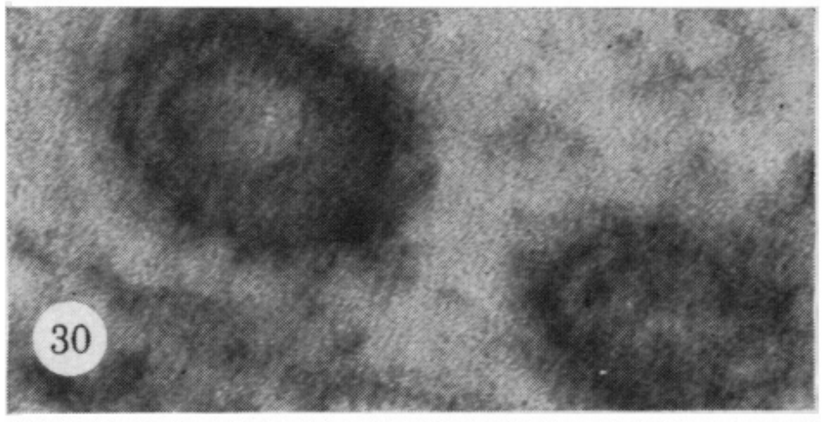
FIGURE 27. For description see opposite.



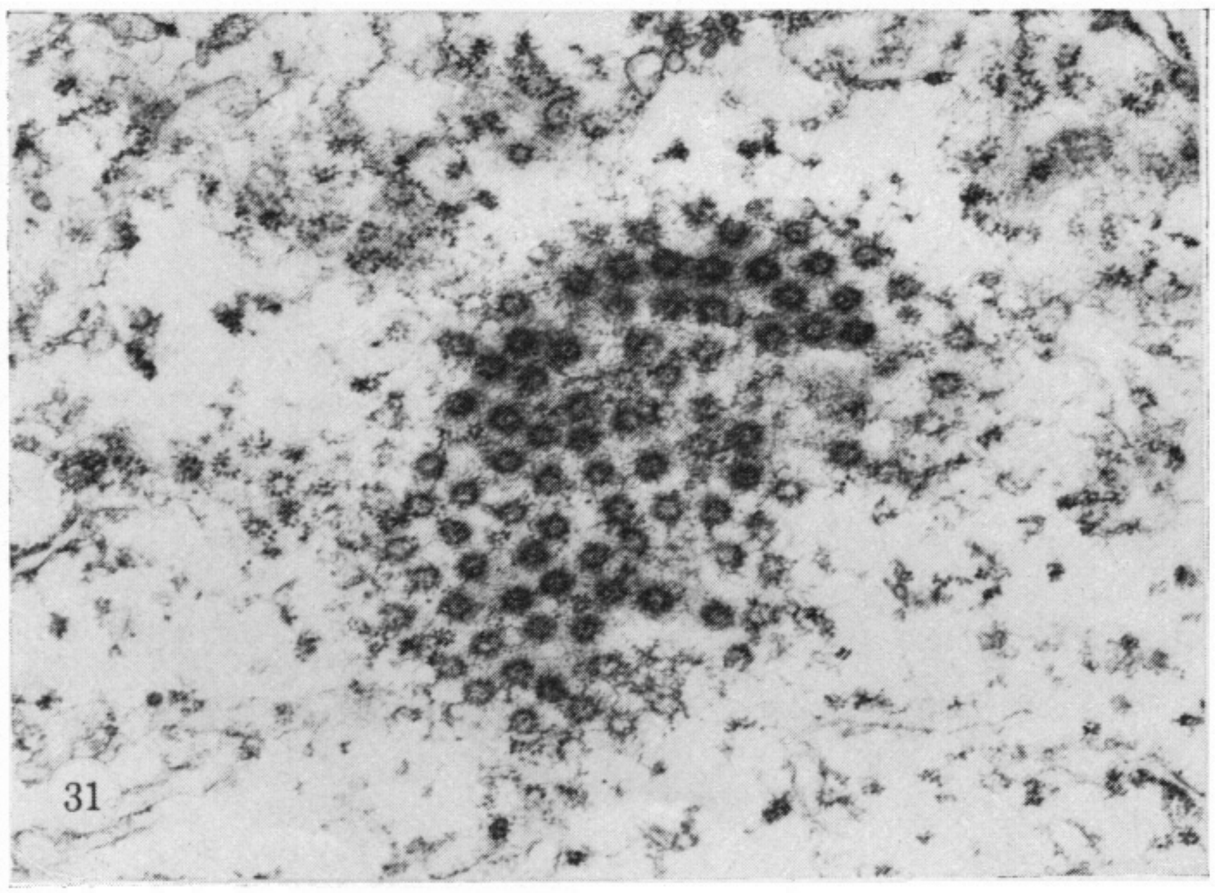
28



29



30



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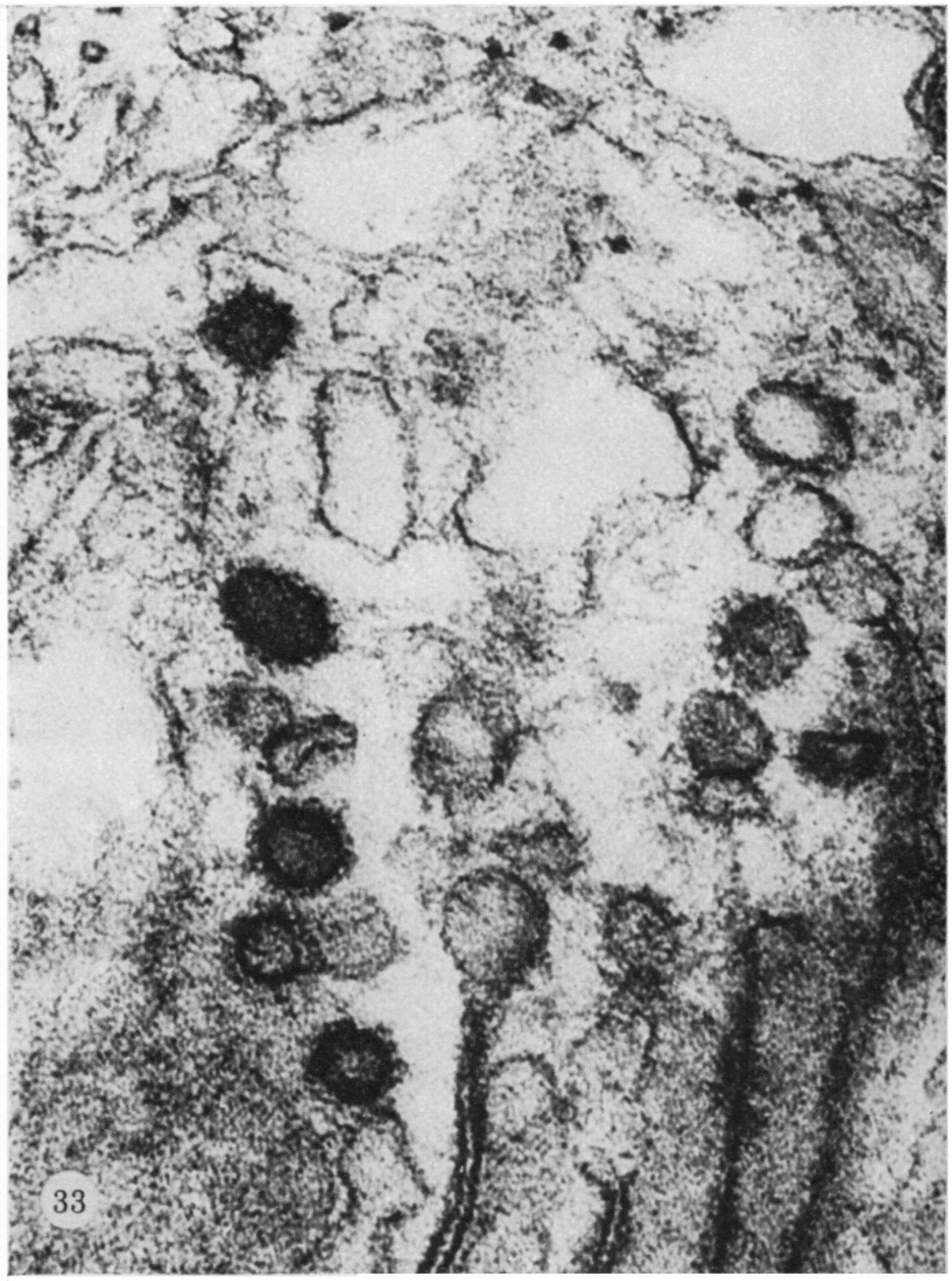
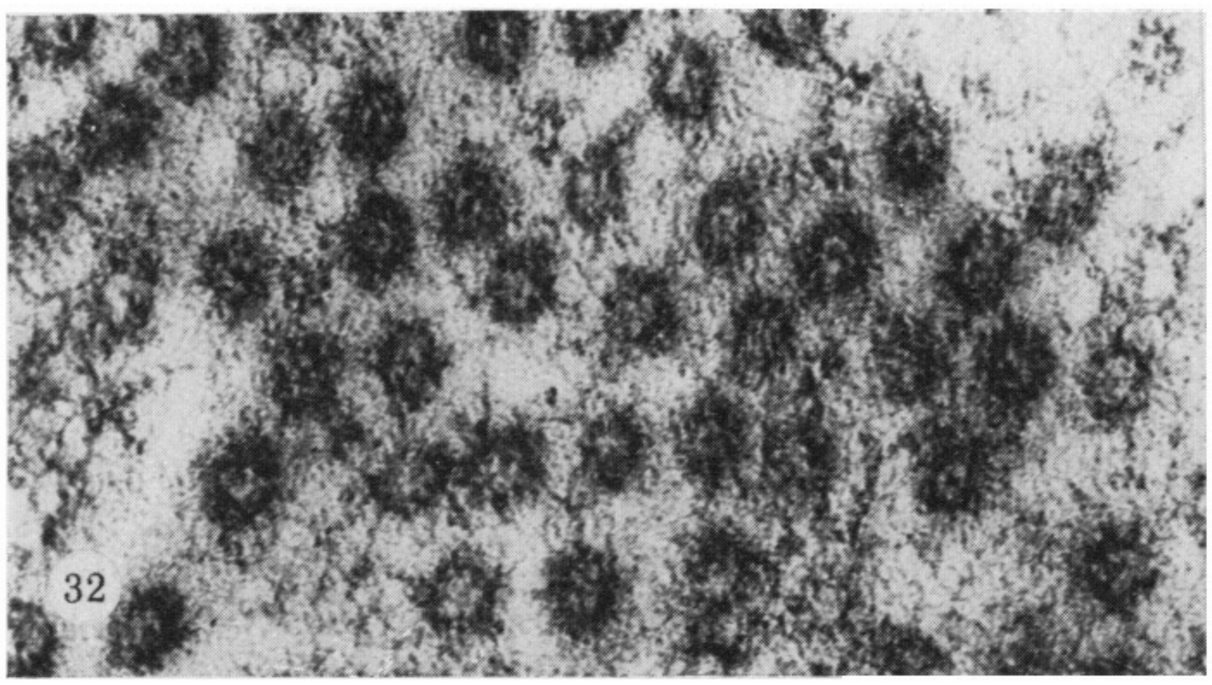


33



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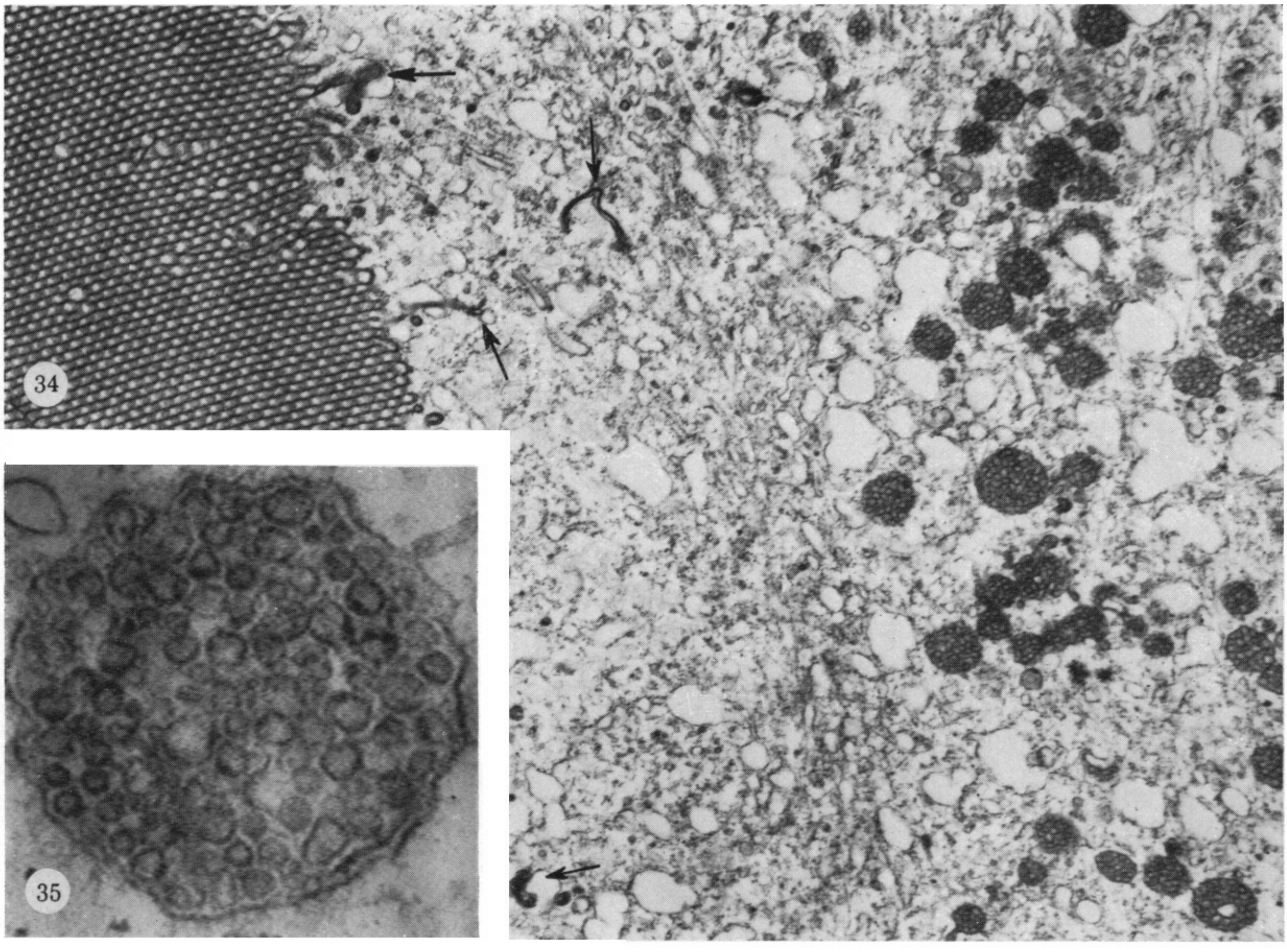
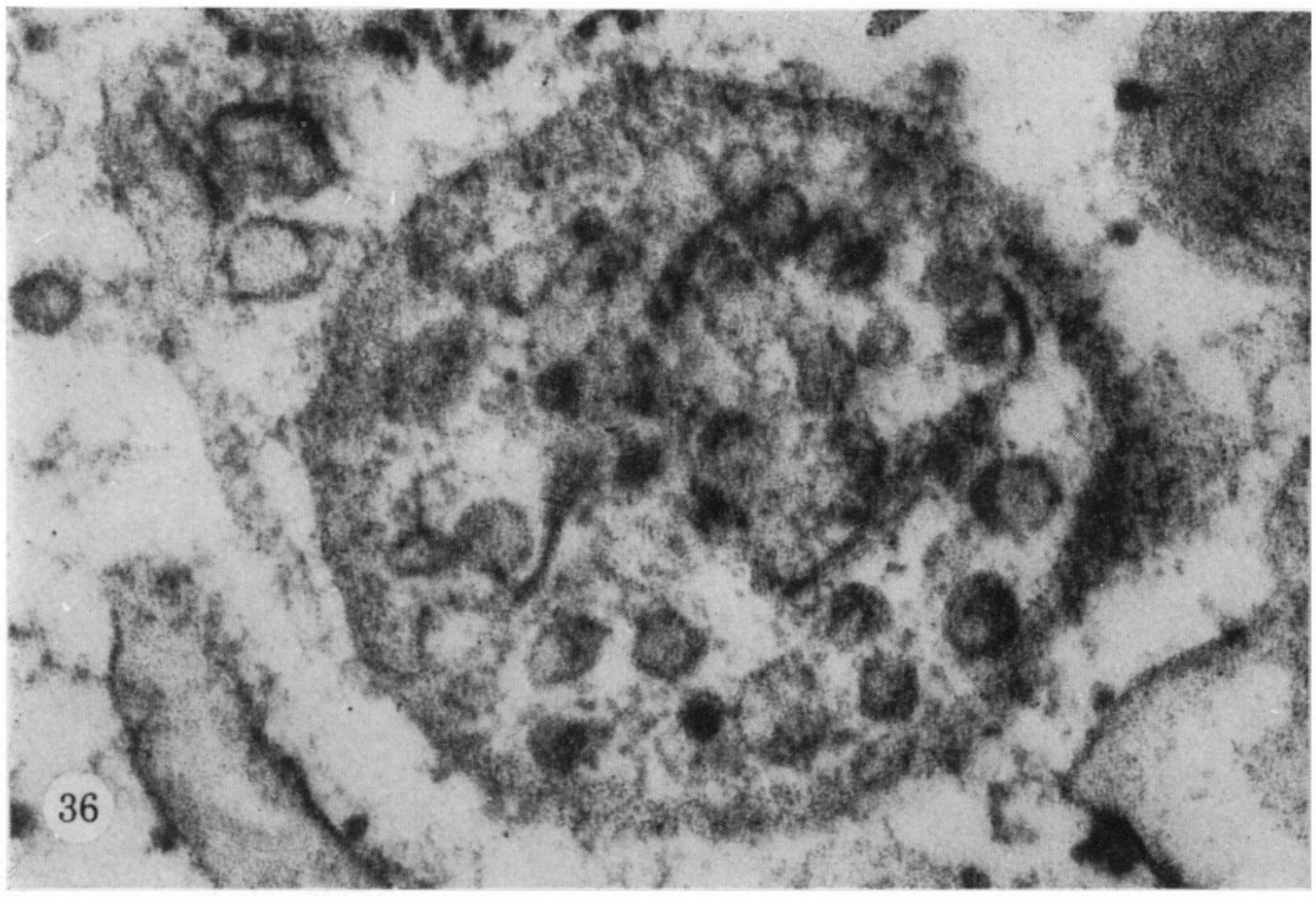
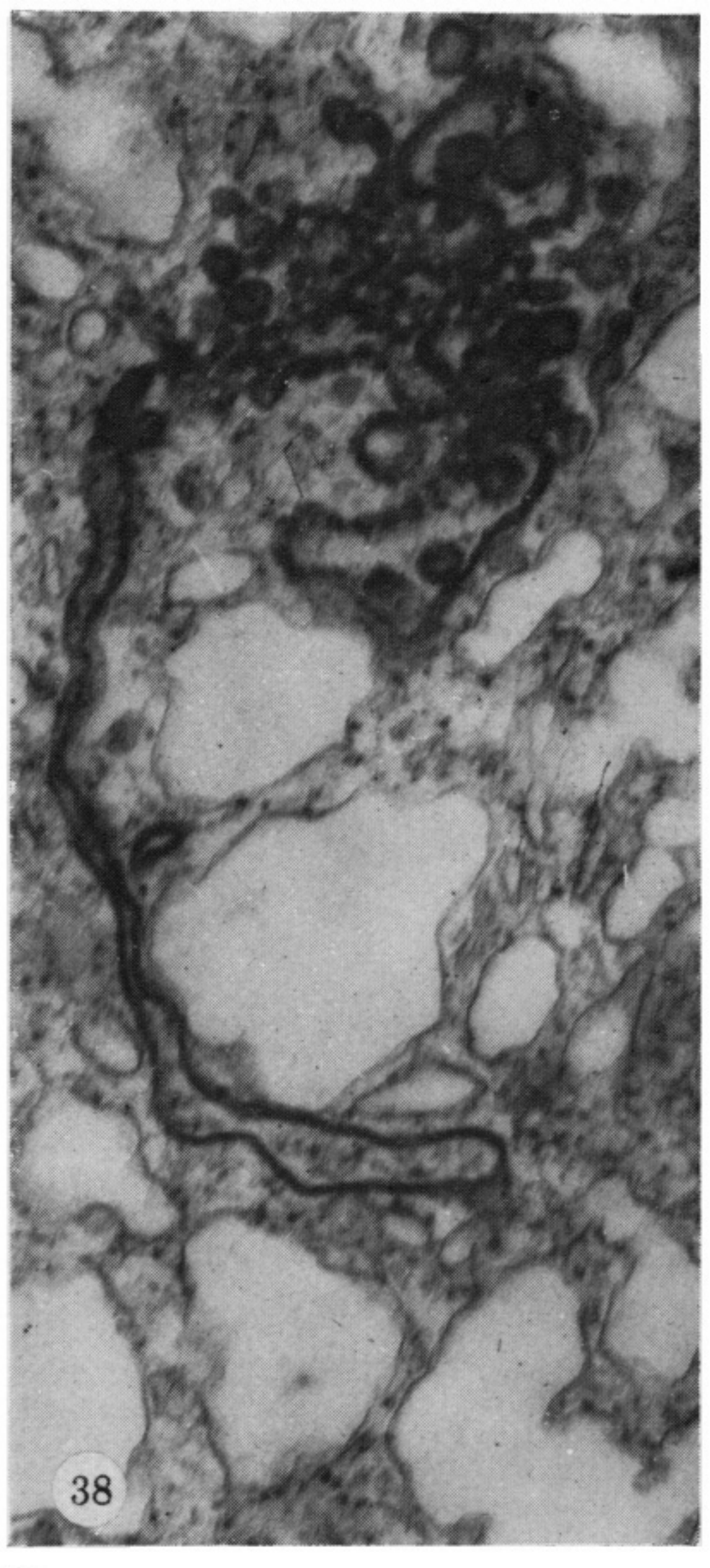
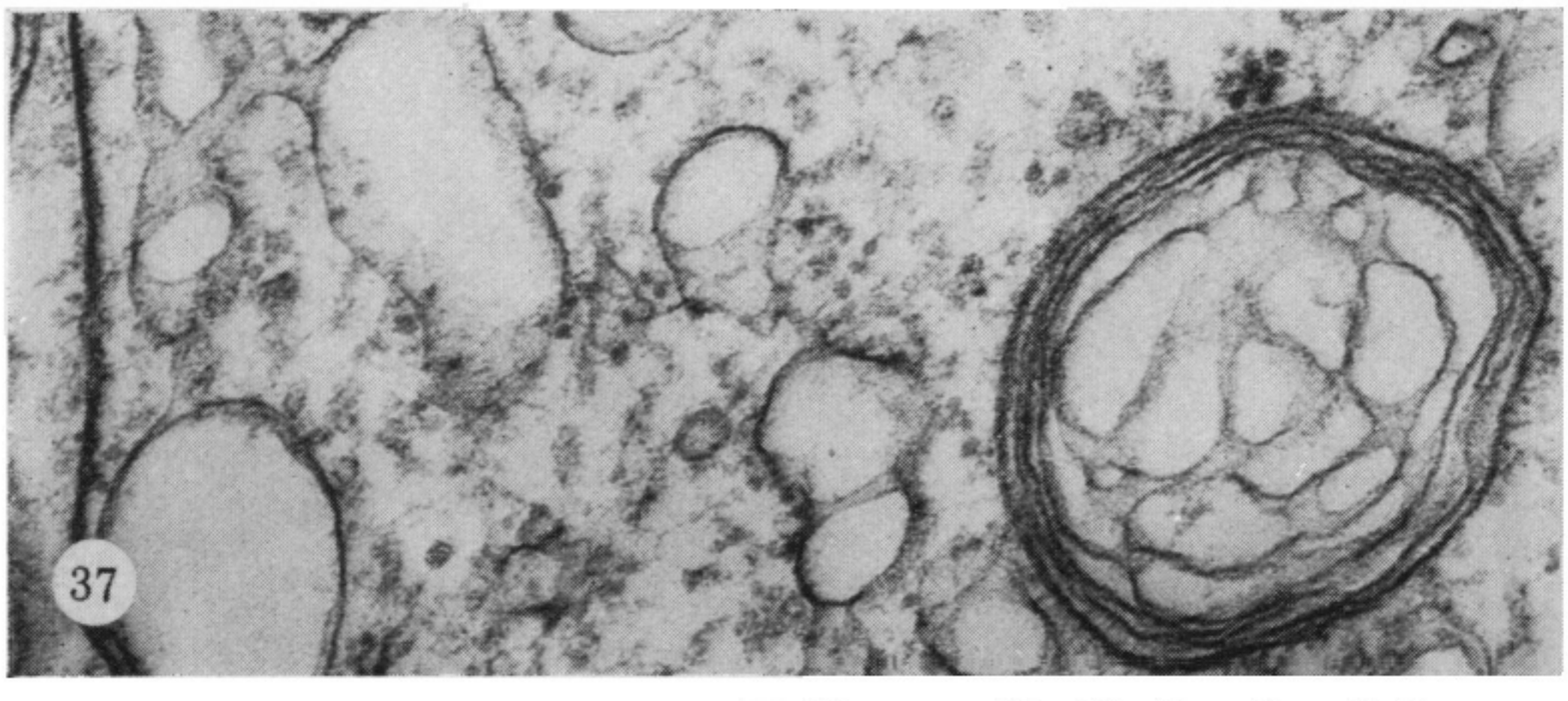
FIGURES 28–33. For description see p. 14.

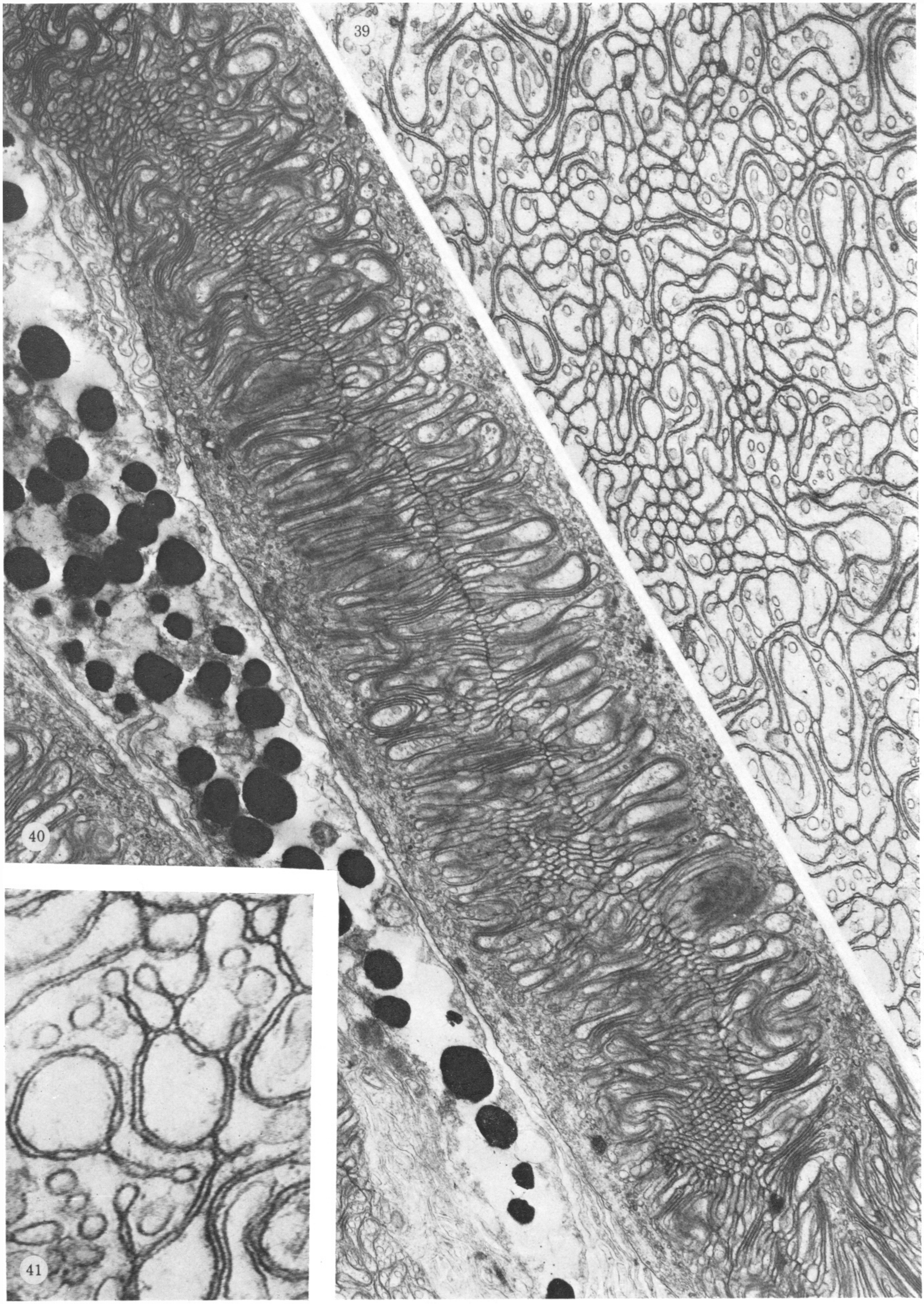








FIGURES 34–38. For description see p. 15.



FIGURES 39–41. For description see opposite.