FINE STRUCTURE AND DEVELOPMENT OF THE ZYGOSPORE OF *RHIZOPUS SEXUALIS* (SMITH) CALLEN

By LILIAN E. HAWKER and A. BECKETT
Department of Botany, University of Bristol

(*Communicated by H. E. Hinton, F.R.S.—Received* 13 *April* 1971)

[Plates 5 to 15]

CONTENTS

Vol. 263. B. 848. (Price £1.75; U.S. $4.55) 9 [Published 18 November 1971

The development of the zygospore of *Rhizopus sexualis* (Smith) Callen was followed by light microscopy, transmission electron microscopy and scanning electron microscopy. Details of all stages, including dissolution of the fusion wall, delimitation of the gametangia by septa, and formation of the complex wall of the zygospore are described and illustrated. Changes in number, form and distribution of the organelles and the behaviour of the nuclei are described. The probable function of the cytoplasmic organelles and the possible mechanisms controlling development are discussed.

I. Introduction

Species of *Rhizopus*, *Mucor* and allied genera are among the commonest and best known fungi, but many details of their structure and development remain obscure. The present report attempts to give as complete an account as present techniques permit of the structural changes taking place during the initiation and maturation of the zygospores of *Rhizopus sexualis* (Smith) Callen, a homothallic species (Callen 1940) which forms both multispored sporangia and zygospores in culture under appropriate conditions. Zygospores form freely on malt agar over a range of temperature from 10 to 20 °C (Hawker, Hepden & Perkins 1957; Hepden & Hawker 1961; Hawker & Hepden 1962; Hawker, Abbott & Gooday 1968).

The fine structure of aerial vegetative hyphae, taken from the margins of young colonies of species of *Rhizopus*, including *R. sexualis*, was described by Hawker & Abbott (1963*a*). L. E. Hawker & P. McV. Abbott (unpublished data) also made a preliminary study of stages in development of sporangia of *R. sexualis* and *R. stolonifer* (syn. *nigricans*). Since the results obtained agreed closely with those of Bracker (1966, 1968) for the ultrastructure of developing sporangia of *Gilbertella persicaria* (Eddy) Hesseltine, no further work on sporangial structure was undertaken by the present writers. Ultrastructural changes during the germination of the sporangiospores of several species of *Rhizopus* have been reported by Hawker & Abbott (1963*b*), Ekundayo (1966) and Buckley, Sommer & Matsumoto (1968).

II. Methods

R. sexualis was grown on malt agar plates or on disks or strips of twice-boiled Cellophane laid on the surface of such a plate, at a temperature of 20 °C. After 2 days zygospores at all stages were present.

Zygospores were examined with the light microscope, the transmission electron microscope and the scanning electron microscope.

(*a*) *Light microscopy*

A Zeiss photomicroscope was used to study fixed, stained material and, with the aid of phase-contrast lenses, living material also.

Methods of fixation and staining were as described by Hawker *et al.* (1968).

(*b*) *Electron microscopy*

(i) *Transmission electron microscopy*

Whole colonies growing on Cellophane disks or directly on agar were immersed in either 2% aqueous potassium permanganate or 4% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2. Fixation in $KMnO_4$ was either for 45 min at room temperature with the first 15 min under vacuum, or for 1 to 2 h at 4 °C. Glutaraldehyde fixation was for 3 h, at 4 °C. Following fixation mycelial colonies containing zygospores were scraped from the Petri dishes and transferred

to centrifuge tubes for washing in either distilled water ($KMnO_4$-fixed material) or phosphate buffer (glutaraldehyde-fixed material). Specimens fixed in aldehyde were post-fixed overnight at 4 °C with 2 % osmium tetroxide in 0.1 M phosphate buffer at pH 7.2. They were then washed in buffer and rinsed in distilled water prior to dehydration in a graded ethanol series. All specimens were soaked for 1 to 2 h in 2 % uranyl acetate at the 70 % ethanol stage. Finally they were soaked in propylene oxide and embedded in Epon.

Blocks were trimmed to aline the zygospore and the specimen was photographed with the light microscope to record the stage of development. Sections were cut on an LKB ultramicrotome with either glass or diamond knives, stained with lead citrate (Reynolds 1963) and examined with an AEI EM 6B electron microscope.

(ii) *Scanning electron microscopy*

Whole zygospores were fixed briefly by immersion in 4 % glutaraldehyde as described above, washed in distilled water and mounted in a drop of water on to stubs which had been coated with either Haupt's adhesive or double-sided Cellotape. The stubs were plunged into liquid nitrogen and then rapidly placed in a freeze drying apparatus modified after Jensen (1962). Frozen dried material was coated with gold palladium while rotated at *ca.* 100 rev/min. Zygospores were also fragmented by gently grinding while immersed in liquid nitrogen. A suspension of these spore fragments was mounted and prepared for microscopy as described for whole spores above. Specimens were examined in a Cambridge Instrument Company Stereoscan electron microscope.

III. Definition of stages in zygospore development

Development of the zygospore involves a series of events taking place in an orderly sequence, but overlapping so that two or more processes may proceed simultaneously. Hawker *et al.* (1957) defined seven stages of development. Observations with the electron microscope have revealed that these stages are less clearly defined than was then thought and accordingly, while the main divisions suggested by Hawker *et al.* have been retained, some of these have been subdivided as follows.

A: *early progametangial stage.* Progametangia just recognizable, slightly swollen, apices just in contact and fusion wall forming.

B: *progametangial stage.* Progametangia enlarged, carrot-shaped, contents denser than in supporting hyphae, dissolution of fusion wall beginning, but wall not breached (figures 1 and 2, plate 5).

C: *early gametangial stage.* Gametangia distinguishable under light microscope by their denser contents, septal rings growing inwards but not readily visible in whole spores by light microscope, fusion wall still not breached (figures 3 and 9, plates 5 and 6).

D: *gametangial stage.* Gametangia clearly delimited by cross-walls, fusion wall still not breached but considerably eroded.

E_1: *early gametangial fusion stage.* Fusion wall just breached, secondary thickening of septa and lateral walls of gametangia just beginning (figure 4, plate 5).

E_2: *fusion stage.* Fusion wall reduced to a shallow ring, ornamentation of the lateral walls developing but not complete, suspensors fully inflated (figures 5 and 10, plates 5 and 6).

F: *young zygospore stage.* Spore barrel-shaped, equatorial groove marking position of original

fusion wall still visible but filling out, brown pigmented wart initials clearly visible (figures 6, 11 and 12, plates 5 and 6).

G_1: *enlarged zygospore stage.* Spore enlarged, more or less spherical, black pigmented warts fully developed (figures 7 and 13, plates 5 and 6).

G_2: *mature zygospore stage.* Tertiary inner wall formed (figure 8, plate 5).

Six or more examples of each stage were sectioned and examined by electron microscopy.

IV. General morphohology of cytoplasmic organelles of *R. sexualis*

(*a*) *Nuclei*

The nuclei are of similar appearance in both vegetative hyphae (Hawker & Abbott 1963*a*) and reproductive structures (figures 16 to 19, plate 7). The interphase nucleus in the developing zygospore possesses a nuclear envelope which is perforated by pores, none of which has been seen to be plugged. Nuclei, particularly in actively growing structures, are often interconnected by endoplasmic reticulum, or groups of two or more nuclei may be surrounded by at least one more or less continuous membrane. In glutaraldehyde/osmium-fixed material these membranes are seen to be rough, i.e. studded with ribosomes (figure 23, plate 8).

In permanganate-fixed material the nucleoplasm is homogenous. An electron-dense nucleolar region is seen in glutaraldehyde/osmium-fixed material.

(*b*) *Mitochondria*

The mitochondria resemble those described for other Zygomycetes and for higher fungi (figures 16, 19, 20, 23, 24 to 27, plates 7 and 8, 9). The cristae, which appear as parallel flat

Key to lettering on plates

AV	alined vesicles	M	mitochondrion
CI	crystalline inclusion	MVB	multivesicular body
CR	cisternal ring	N	nucleus
ER	endoplasmic reticulum	OPW	outer layer of primary wall
EWZ	end wall of zygospore	OWZ	outer wall of zygospore
FW	fusion wall	Pl	plasmodesmata
G	glycogen	S	suspensor
GS	gametangial septum	SW	suspensor wall
GV	glycogen vacuole	V	vesicle (V(i)α etc. as listed on p. 76)
IPW	inner layer of primary wall	Va	vacuole
IWZ	inner wall of zygospore	W	wart
L	lipid droplet	Zp	zygophore
Lo	lomasome	Z	zygospore
LW	lateral wall		

Description of plate 5

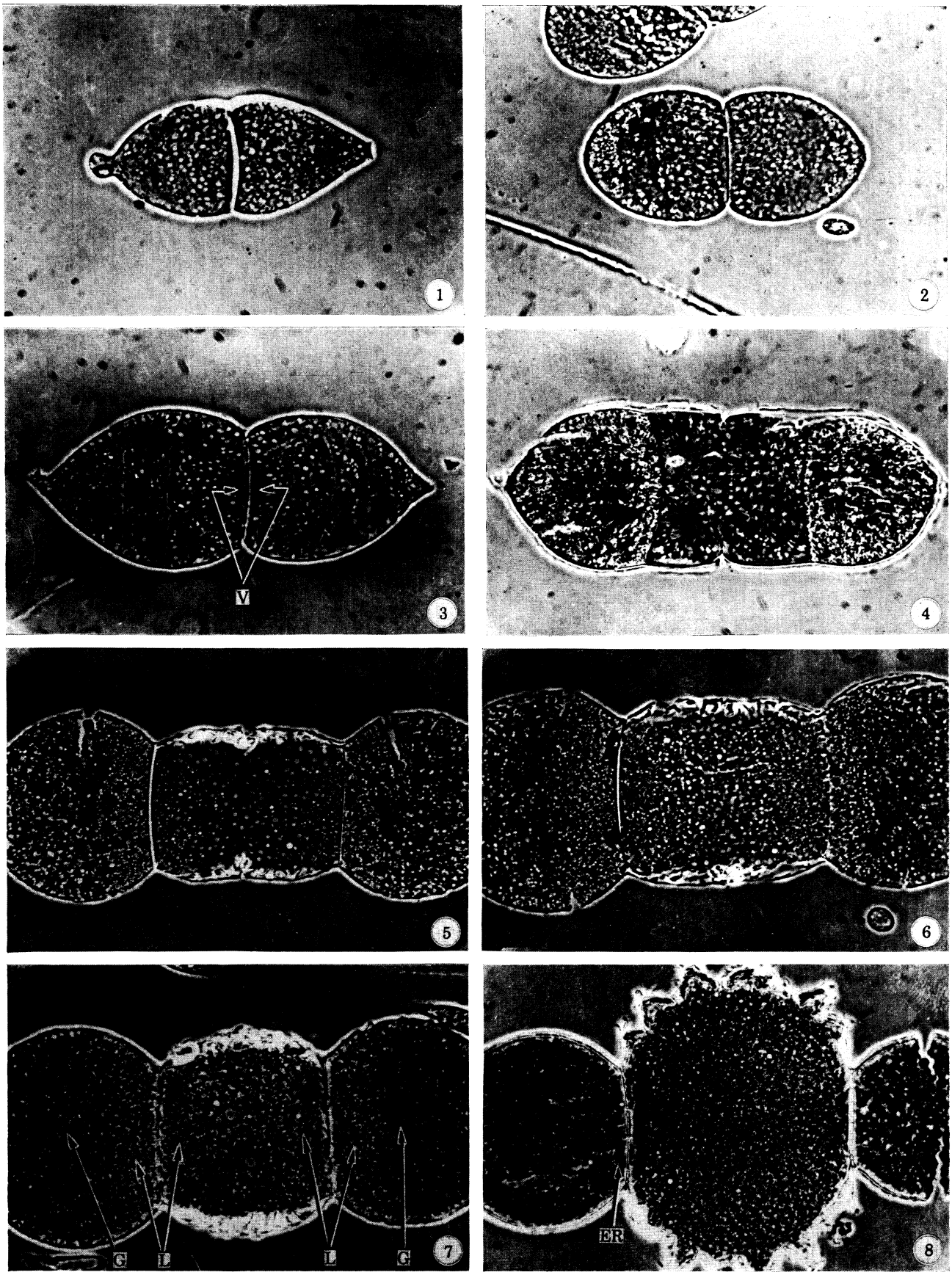
Figures 1 to 8. Light micrographs of longitudinal sections of Epon embedded material (× 500).

1, 2. Progametangia separated by intact fusion wall. The accumulation of vesicles along both sides of the fusion wall can be seen as a clear grey zone (figure 2) and the zonation of mitochondria, nuclei, lipids and glycogen is also evident.

3. Delimitation of gametangia by cross-septa.

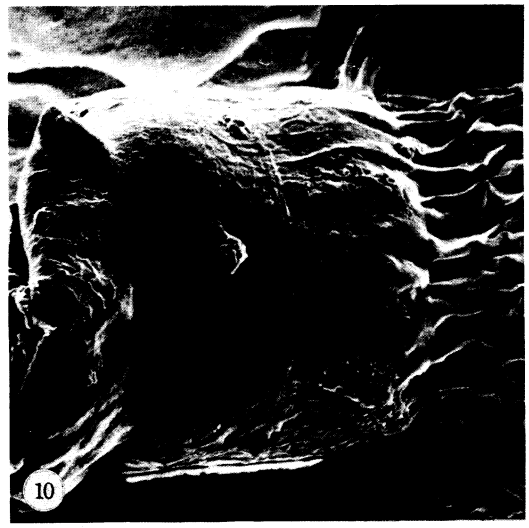
4. Break down of the fusion wall and mingling of contents in zygospore initial.

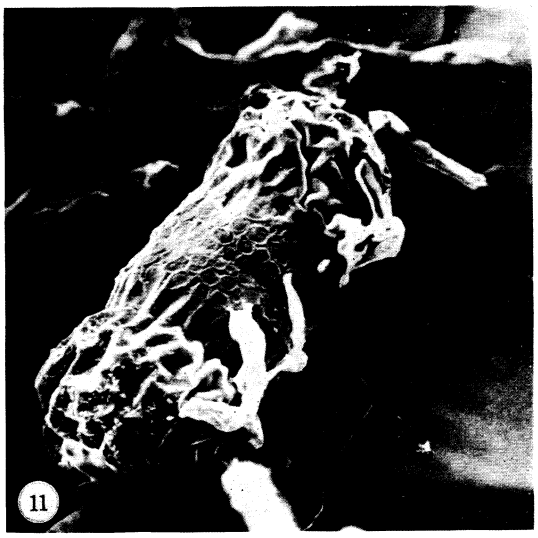
5 to 8. Stages in development of suspensors and zygospore. Note ornamentation of zygospore wall, change in shape of suspensors and zygospore, accumulation of lipid and glycogen and the association of E.R. masses with the zygospore end walls.

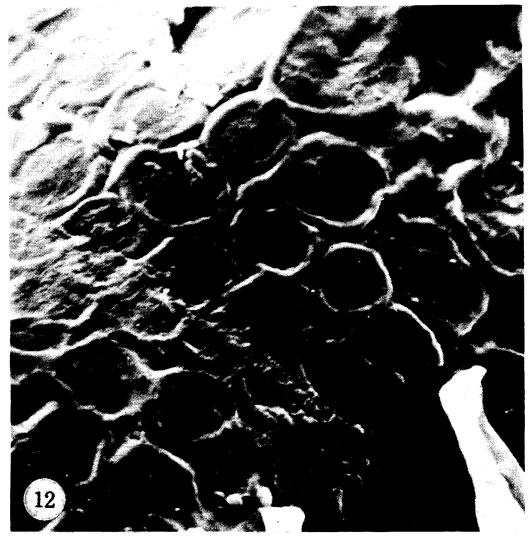


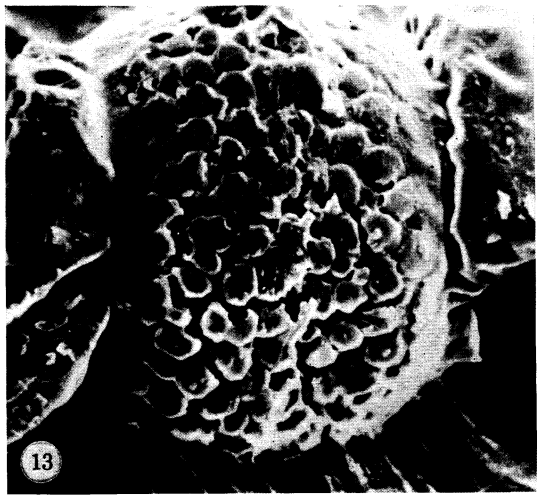
FIGURES 1 to 8. For legends see facing page

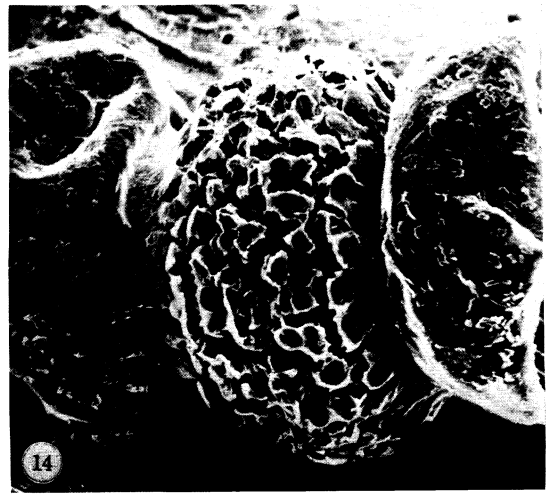
(Facing p. 74)











FIGURES 9 to 15. For legends see facing page

plates in both permanganate-fixed material and in material fixed with glutaraldehyde and osmium, vary in number with different levels of developmental activity but are usually numerous and may fill the mitochondrion (figures 20, 23 and 24, plates 8 and 9). Serial sections show that the shape of the mitochondria varies from spheroid to ovoid to cup-shaped. This variation is generally correlated with the stage of development of the zygospore, so that in the young zygospore most mitochondria are spheroid to ovoid, while in maturing zygospores mitochondria are often highly irregular in shape, forming rings and cup-like structures (figures 41 to 44 and 48, plates 12 to 14). Where cytoplasmic streaming is active, mitochondria become elongated in the direction of the current. The diameters of these organelles are usually within the range 0.3 to 2.0 μm. Mitochondria in the suspensors of old zygospores often become enclosed within a vesicle and break down to form an irregular mass of membrane (figures 54, 55, plate 15).

(*c*) *Endoplasmic reticulum* (*E.R.*)

In vegetative hyphae (Hawker & Abbott 1963*a*) and in the mature zygospores, endoplasmic reticulum is sparse and of a lamellate type. During the development of the zygospore localized aggregations of lamellate and/or tubular E.R. occur at certain stages of development. These aggregations are described and their probable functions are discussed in the account of development given below (pp. 87, 95).

(*d*) *Ribosomes*

The general distribution of R.N.A. was shown by Korson's stain (Hawker *et al.* 1968). Numerous ribosomes (diam. *ca.* 20 nm) are seen in glutaraldehyde/osmium-fixed specimens (figures 20, 23, plate 8). The majority are free in the endoplasm, but rough E.R. has been seen associated with nuclei in glutaraldehyde-fixed material (figure 23, plate 8).

(*e*) *Microbodies*

Bracker (1967) drew attention to the occurrence in fungal cells of vesicles which resemble the plant microbody isolated by Mollenhauer, Morré & Kelly (1966). These microbodies bounded by a single membrane are usually less than 1 μm in diameter and are frequently associated with E.R. cisternae. They often contain amorphous or crystalline inclusions and may be involved in enzyme compartmentalization or protein storage.

Such bodies are rare in the vegetative mycelium (see X-bodies, Hawker & Abbott 1963*a*), but during the development of the zygospore several types of microbodies can be distinguished which vary in morphology according to the fixative used. These may be listed as follows:

Description of plate 6

Figures 9 to 14. Stereoscan electron micrographs showing stages in development of the zygospore and ornamentation of the lateral wall.

9. Early stage in zygospore formation – probably equivalent to that shown in figure 3, plate 5. Ornamentation of the lateral wall is not yet evident from the outside surface (× 470).

10. Early stage of lateral wall ornamentation. Disks of wall material are apparent on each side of the groove which marks the point where the fusion wall joins the lateral wall (× 1000).

11, 12. A slightly later stage than that in figure 10. Each disk of wall material represents what will be one wart (× 470 and × 2400 respectively).

13, 14. Stages in zygospore development showing the cracking and tearing of the outer primary wall as the warts enlarge and protrude towards the outside surface (× 700 and × 500 respectively).

15. Light micrograph of a section through part of a 14-day-old zygospore wall showing several constituent layers and the remains of the torn primary wall on the tip of the wart (× 1120).

(i) *Seen in $KMnO_4$-fixed material*

(α) Small membrane-bound, electron dense, spherical vesicles, regularly associated with E.R. membranes and measuring *ca.* 70 nm in diameter (figures 21, 22, plate 8).

(β) Membrane-bound vesicles ranging from 0.2 to 0.7 μm in diameter with electron dense contents (figures 16 to 19, 21, 22, 24 to 30, 34 to 37, 41 to 44 and 53 to 57, plates 7 to 13 and 15).

(γ) Vesicles with a diameter ranging from 0.1 to 0.7 μm with electron-transparent lumina (figure 19, plate 7; figures 21, 22, plate 8).

(ii) *Seen in glutaraldehyde/osmium tetroxide-fixed material*

(α) Small vesicles with electron-dense centres (figure 20, plate 8), which possibly correspond to the α type vesicles of $KMnO_4$-fixed material.

(β) Vesicles measuring 0.1 to 0.4 μm in diameter with fibrous or sparsely granular contents (figures 20, 23, plate 8).

(γ) Vesicles similar to the last but devoid of electron-dense contents. These vesicles may represent a developmental phase of type (ii) β (figure 20, plate 8).

(Δ) Electron-dense vesicles measuring 0.3 to 0.6 μm in diameter frequently seen with a ring-like morphology (figures 20, 23, plate 8). This characteristic form may arise as a result of a deep invagination into the microbody or by an engulfing by the microbody of a smaller vesicle or cisternum.

In addition to the microbodies described above, numerous vesicles and vesicular structures may be seen at various stages of zygospore formation. Multivesicular bodies have occasionally been seen in the region of the dissolving fusion wall (figures 39, 40, plate 11). Cisternal rings are present in large numbers during the later stages of zygospore maturation and are usually located in the peripheral regions of the zygospore inside the developing melanized lateral walls (figures 35, 38, 41 to 44, plate 11 to 13) and in the suspensors. These cisternae are distinguished from the E.R. by their regular dilated profiles, the electron-transparent nature of the lumen between the cisternal membranes, and the absence of any apparent membraneous connexion with E.R. Lomasomes (Girbardt 1958, 1961; Moore & McAlear 1961) are frequently seen at the sites of deposition of wall material (figures 36 and 37, plate 11).

(*f*) *Food materials*

(i) *Glycogen*, which appears mainly as α-particles, seen as aggregations of relatively electron dense granules (figures 24 to 27, 34, 35, 38, 41 to 44 and 52 to 55, plates 9, 12 to 13 and 15), is present at all stages and is distributed irregularly throughout the cytoplasm. The presence and distribution of glycogen was confirmed by histochemical staining (Hawker *et al.* 1968).

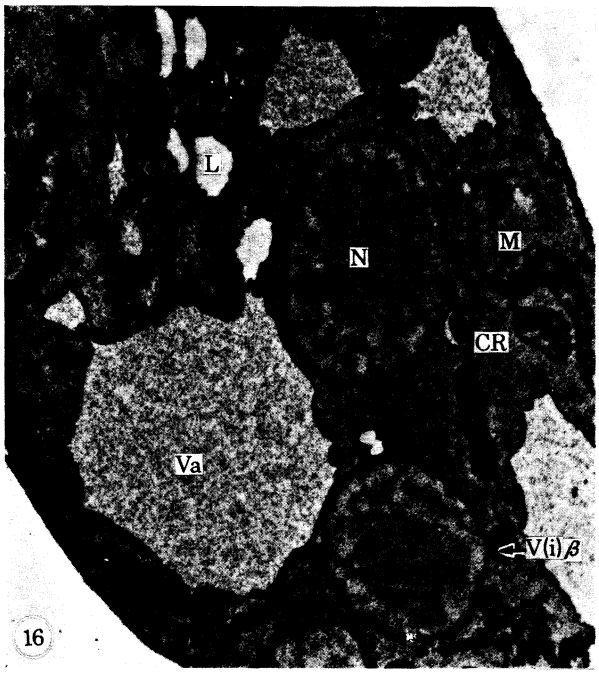
Description of plate 7

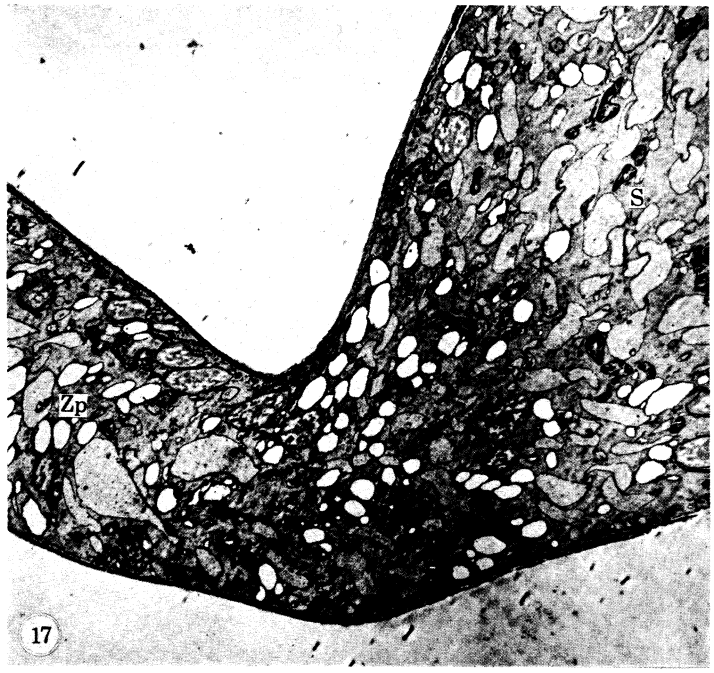
Figure 16. Longtitudinal section through part of a zygophore. $KMnO_4$ fixation (× 10750).

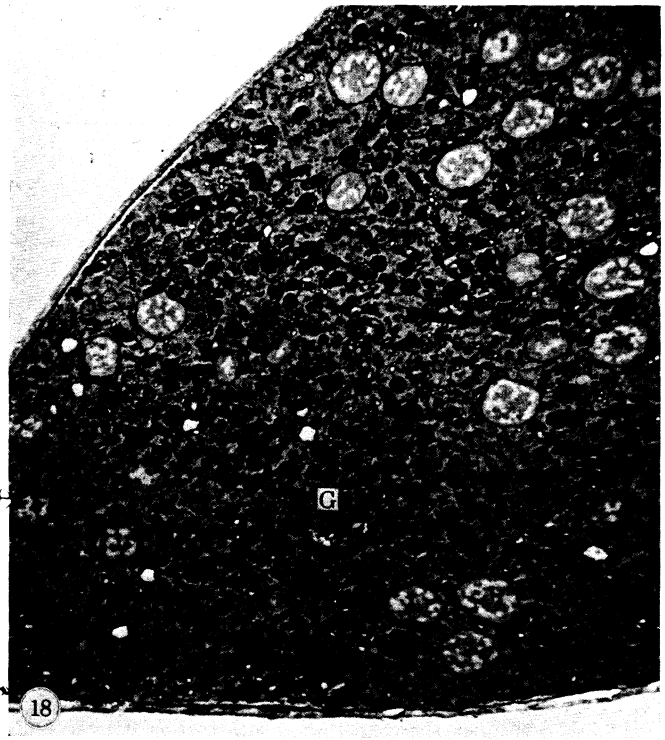
Figure 17. Longitudinal section through the hind region of a suspensor and part of the parent zygophore. $KMnO_4$ fixation (× 2550).

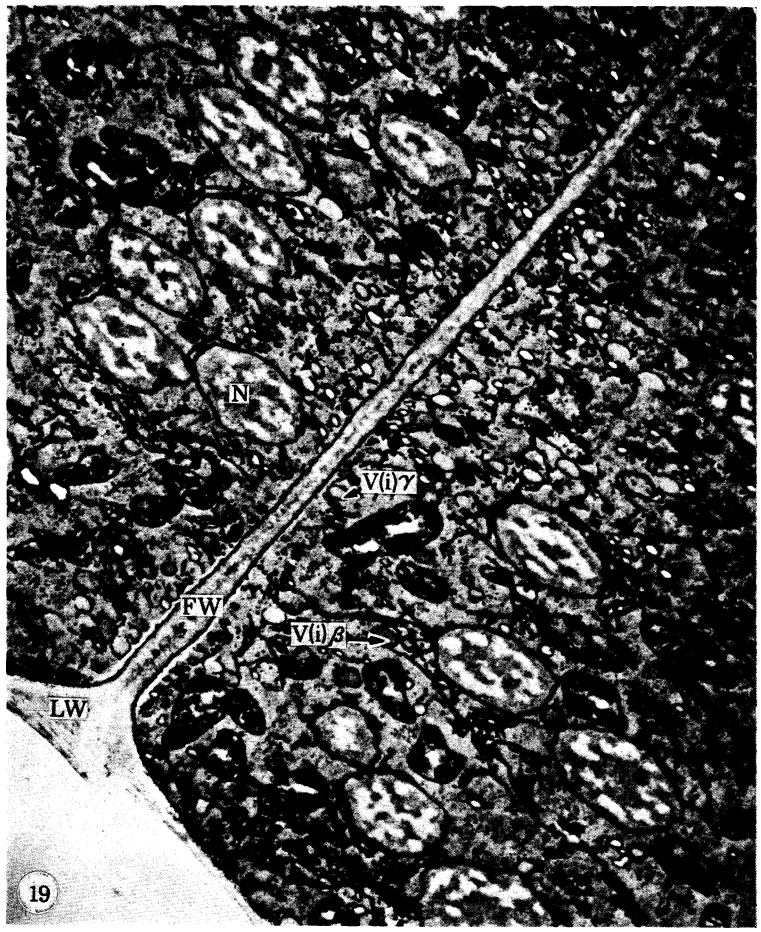
Figure 18. Longitudinal section through the hind region of a progametangium showing the accumulation and coalescence of glycogen vacuoles (G). $KMnO_4$ fixation (× 3300).

Figure 19. Longitudinal section through parts of the apical region of two fused progametangia showing the zonation of organelles and accumulation of vesicles (microbodies) of various types on each side of the fusion wall. $KMnO_4$ fixation (× 6600).



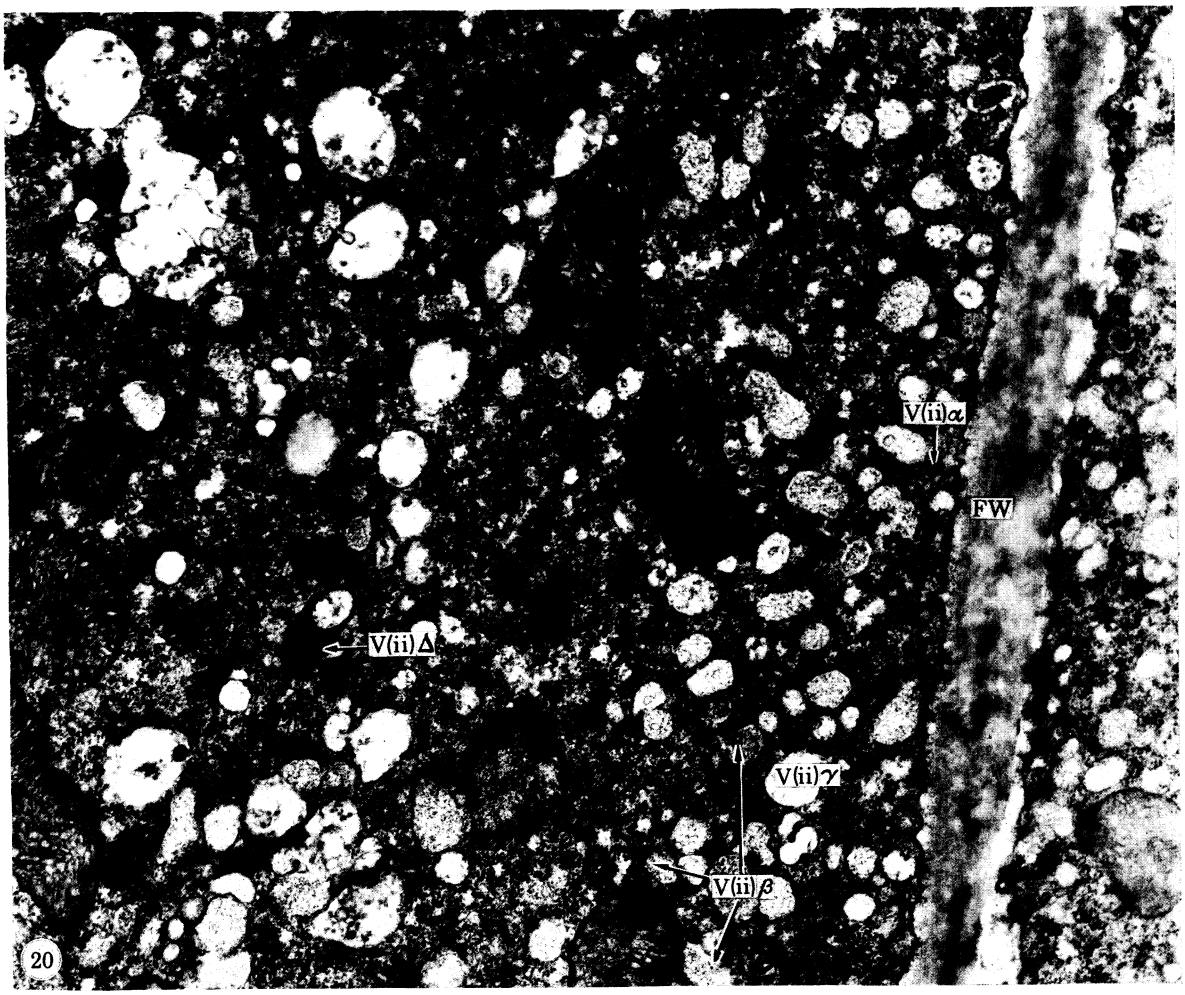


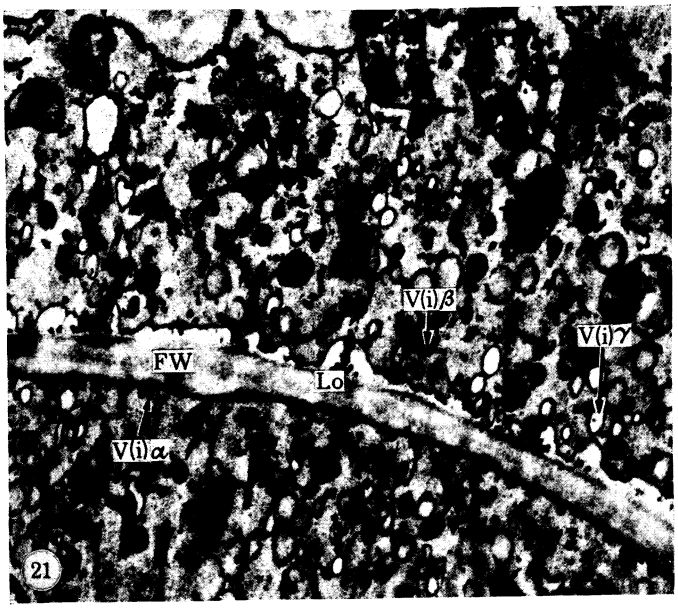


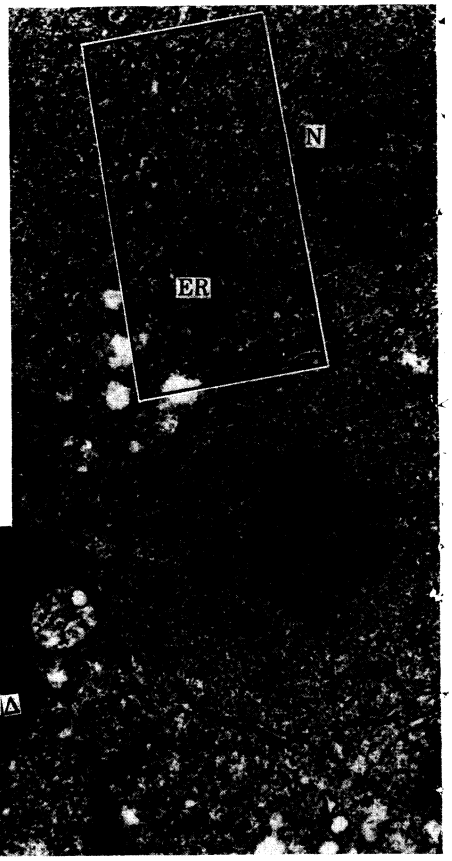


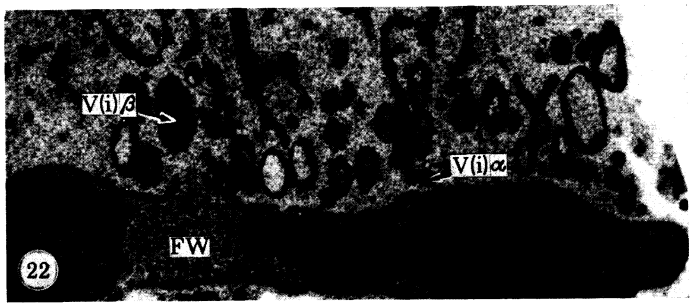
FIGURES 16 to 19. For legends see facing page

(*Facing p.* 76)









FIGURES 20 to 23. For legends see facing page

In the progametangia and later in the suspensors, glycogen may also be enclosed within vacuoles. Fusion between the vacuoles occurs as the zygospore develops so that in mature stages large irregular membrane-bound glycogen masses can be seen occupying much of the space within the suspensors (compare figures 1 to 8, plate 5, figure 18, plate 7, figures 52, 53, plate 15). In the mature zygospore there are few such vacuoles (figure 57, plate 15).

(ii) *Lipid droplets.* The distribution of lipid droplets is readily seen in sectioned material with the light microscope where they appear as spherical-ovoid transparent globules (figures 1 to 8, plate 5). When fixed with $KMnO_4$ and viewed in sections with the electron microscope, lipid droplets are more variable in outline but have electron transparent centres surrounded by a narrow dense line. This dense boundary layer sometimes resembles a membrane but does not possess the unit-membrane structure (figures 16, 17, 24 to 26, 29, 30, 34 to 36, 40 to 44, 48, 49 and 52 to 57, plates 7, 9 to 11, 13 to 15).

V. Origin of the zygospore initial

(*a*) *The zygophore and conjugation*

Young colonies of *R. sexualis*, growing under favourable conditions of nutrition, temperature, aeration and H-ion concentration, produce numerous aerial hyphae growing upwards from the surface mycelium behind the advancing margin of the colony. These aerial hyphae are coarser than the marginal hyphae but, as pointed out by Hawker *et al.* (1968), resemble them in the distribution of organelles (Hawker & Abbott 1963*a*) and in their response to histochemical stains.

Studies of the ultrastructure of the apices of vegetative hyphae of a number of fungi (McClure, Park & Robinson 1967; Girbardt 1969; Grove, Bracker & Morré 1970) reveal a zoned arrangement of organelles. The extreme tip contains large numbers of small vesicles but larger organelles are lacking. Just behind the tip is a zone containing fewer vesicles but numerous mitochondria; further from the tip nuclei also are present. *Phycomyces blakesleeanus* shows a similar apical zonation of organelles in the young sporangiophore (Peat & Banbury 1967). The vegetative hyphal tip of *R. sexualis* has not been examined in such detail but observations made with the light and electron microscopes indicate a similar arrangement.

The wall of the zygophore is very thin over the extreme apex, but further from the tip it is slightly thicker and consists of a thin, relatively electron dense outer layer and a thicker less electron dense inner one.

According to the conditions under which they are growing, and particularly to the temperature, the aerial hyphae of *R. sexualis* give rise to sporangia or to zygophores or remain purely vegetative (Hawker *et al.* 1968). Callen (1940) has fully described the morphology of the

---

Description of plate 8

Figure 20. Longitudinal section through the apical regions of two fused progametangia showing the concentration of vesicles, mitochondria and ribosome. Glutaraldehyde/osmium tetroxide fixation (× 19500).

Figures 21, 22. Longitudinal sections showing two stages in the dissolution of the fusion wall. $KMnO_4$ fixation (× 13500 and × 32000 respectively).

Figure 23. Longitudinal section through part of a progametangium showing rough E.R. associated with nuclei and two microbodies of type V(ii) △. Glutaraldehyde/osmium tetroxide fixation (× 38000). The area within the rectangle is enlarged as figure 23*a* and shows the rough E.R. associated with nuclear envelope (× 63500).

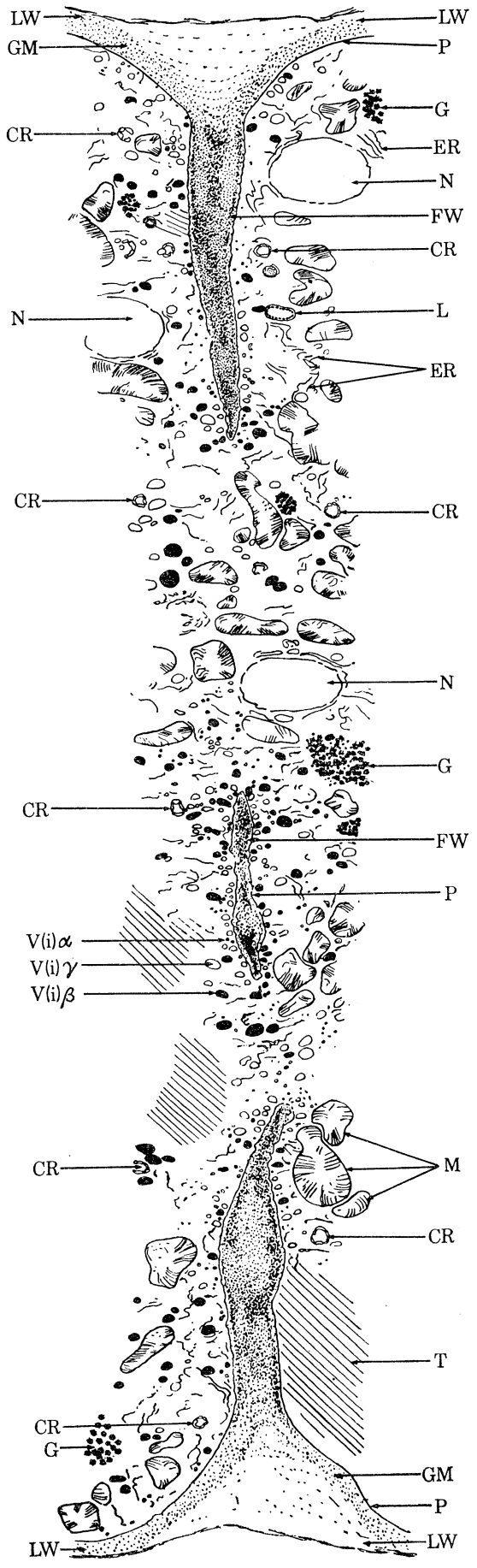


FIGURE 58. The distribution of cytoplasmic vesicles and other organelles in relation to dissolution of the fusion wall. Drawn to scale from a series of overlapping electron micrographs of permanganate-fixed material. *Key to lettering and shading*:

FW remains of fusion wall (density of stippling corresponds to electron density and presumably to stage of dissolution)
LW lateral walls of gametangia
GM gelatinous material filling groove between the two gametangia
P plasmalemma
N nucleus
M mitochondrion (cristae, often very crowded, not drawn accurately, but position and distribution comparable to that shown in the electron micrographs in all examples)
CR cisternal ring
ER membranous endoplasmic reticulum
V cytoplasmic vesicles (those of type V(i)α, p. 76, and V(i)β with electron-dense contents shown solid black, those of type V(i)γ with relatively electron-transparent contents shown in outline only)
L single lipid body present
G coarsely stippled areas indicate patches of glycogen
T shaded areas represent parts of section too badly torn for accurate reconstruction

zygophores which usually arise as stout branches from the aerial hyphae. The tips of the latter may themselves function as zygophores. If conditions continue to be suitable the zygophores conjugate in pairs and enlarge to form the progametangia. Treatment with Korson's stain indicates that the R.N.A./D.N.A. ratio which is high in vegetative hyphal tips is reduced in those about to conjugate. This relatively low ratio persists in the developing progametangia. Hawker *et al.* (1968) show that zygospore initiation takes place only after an increase in the relative concentration of D.N.A. in the zygophore.

(*b*) *Further development of the progametangia*

After apical contact is made between two zygophore branches (stage A, p. 73) these enlarge rapidly and alter in shape to form the progametangia, the apical walls of which become addressed to one another and fuse together to form the fusion wall (figures 1 to 3 and 19, plates 5 and 7).

At stage A the young progametangia have a structure essentially similar to that of the parent zygophores. As they enlarge they become first carrot-shaped and then turnip-shaped (stage B), the greatest diameter being just behind the plane of contact between them. The flattened apical walls of the paired progametangia are in contact and fused together over a circular area of diameter almost as great as that of the adjacent zone of maximum inflation, leaving a shallow equatorial groove, partially filled with amorphous material (figures 4, 9, and 19, plates 5 to 7). The fusion wall rapidly becomes thickened, its appearance indicating that this is the result not of the deposition of new wall material but rather of gelatinization and subsequent swelling. At the same time the lateral walls become thickened but are not gelatinous.

As the progametangia enlarge and as new material flows in from the zygophores the numbers and distribution of organelles alter. (Figures 18 and 19, plate 7) shows the distribution of organelles at stage B, i.e. when the progametangia have reached almost their final size and shape prior to delimitation of the gametangia. Hawker & Gooday (1969) described this distribution and their description has been confirmed and amplified by recent more detailed studies. The regions immediately next to the fusion wall contain numerous microbodies and some mitochondria. Behind these thin apical zones are broader ones containing large numbers of both nuclei and mitochondria. Ribosomes are present in the endoplasm throughout the progametangia but are particularly numerous in the zones adjacent to the fusion wall (figure 20, plate 8), a distribution which corresponds to that of R.N.A. as indicated by Korson's stain (Hawker *et al.* 1968). In the region farther away from the fusion wall nuclei and mitochondria are most numerous in the peripheral layers but are fewer in the central core which is packed with food reserves, largely glycogen, lipid droplets and storage vacuoles containing polysaccharide material (figure 18, plate 7). Glycogen extends into the layers adjacent to the fusion wall, but lipid droplets and storage vacuoles are absent from these layers. This distribution of organelles and organic food materials is confirmed by histochemical staining.

VI. The dissolution of the fusion wall

Soon after its formation the fusion wall, which is originally a flat plate of approximately uniform thickness, becomes progressively broken down by a process of dissolution which begins at the centre of the wall and spreads outwards (Hawker & Gooday 1969). The plasmalemmas on either side of the fusion wall become wrinkled and pockets of electron-transparent material

develop between the plasmalemmas and the fusion wall, the outline of which is no longer sharply defined (figures 20, 21, plate 8). Within these pockets, membrane-bound vesicles with electron-dense contents are frequently present, as a result of which the edge of the wall appears to be studded with numerous lomasome-like structures (figures 21, plate 8). Numerous microbodies (of types (i) α and (i) β, p. 76) are present in the zone of cytoplasm on either side of the fusion wall. The close association of these vesicles with the plasmalemma (figure 21, plate 8) is significant. When the fusion wall has reached an advanced stage in dissolution the plasmalemma breaks down at numerous places and wall contents are apparently released into the cytoplasm of the gametangia (figure 22, plate 8). The distribution of the vesicles on either side of the wall (figures 2, 3, 20 to 22, plates 5, 8; figure 58), suggests that they are concerned with the passage of materials across the plasmalemma but the direction of such movement cannot be determined from electron micrographs alone.

At the time of completion of the gametantial septa, the fusion wall has already been considerably reduced in thickness, particularly at the centre where the first rupturing later occurs. The timing of this process in relation to gametangial septum formation varies a little but the fusion wall is still unbreached when the septum has been completed. At this stage nuclei and other organelles are densely crowded in zones adjacent to the fusion wall (figure 19, plate 7). Histochemical staining (Hawker *et al.* 1968) shows a concentration of both R.N.A. and D.N.A. adjacent to the dissolving wall.

Fusion of the gametangia takes place soon after their delimitation. Breakdown of the fusion wall begins at or near the centre and one or more irregular-shaped holes soon develop (figure 58) The remaining sections of the wall become irregular in thickness (figure 22, plate 8). Soon a peripheral fringe is all that remains of the fusion wall (figure 4, plate 5). This persists for some time but finally, in normal zygospores, disappears entirely.

As the fusion wall breaks down, the number of vesicles (particularly of type (i) β), in the adjacent cytoplasm increases. The nature of the contents of the various types of vesicles present was not determined.

VII. The delimitation of the gametangia

Formation of the septa cutting off the apical portions of the progametangia to form the gametangia (stage C) may begin slightly earlier in one progametangium than in the other. The earliest stages of septum formation cannot readily be followed by light microscopy. A slight localized decrease in density of the cell contents is the first indication that cross-walls are

---

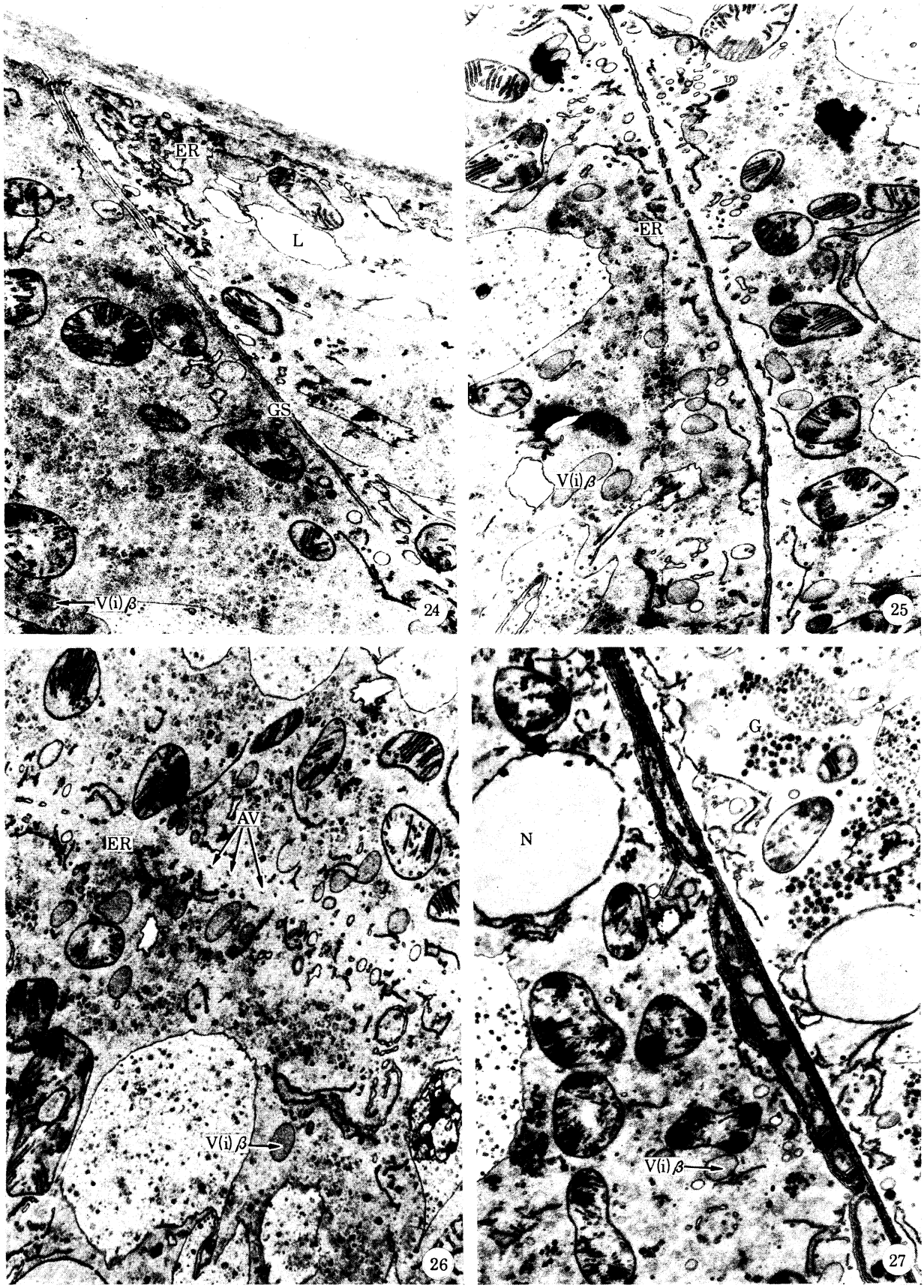
Description of plate 9

Figure 24. Longitudinal section through part of a progametangium at an early stage in septum formation. The septum projects backwards at an acute angle. The future gametangium is to the left and suspensor to the right. $KMnO_4$ fixation (× 15000).

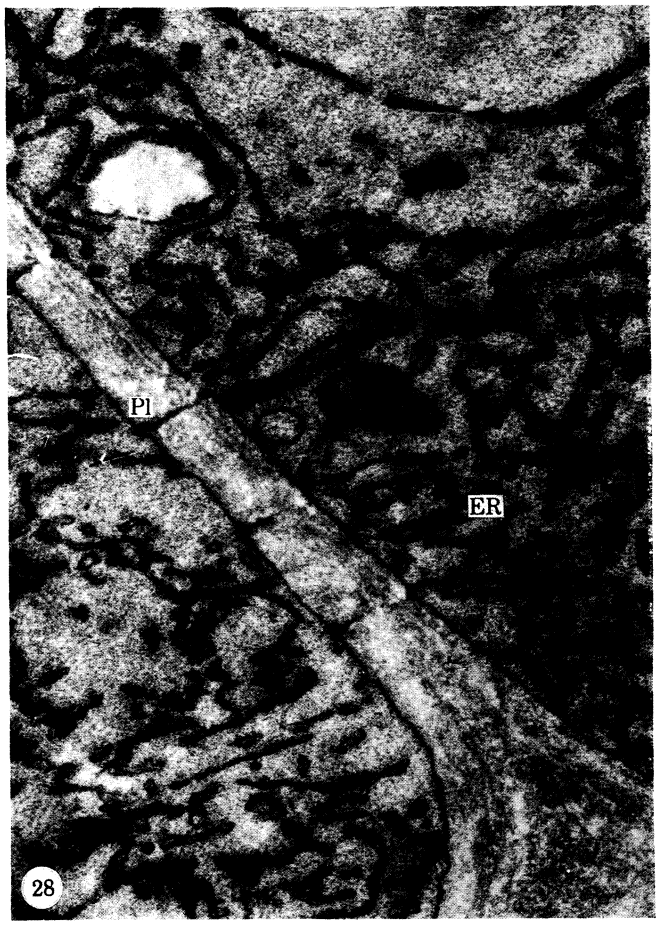
Figure 25. Longitudinal section showing the fusion of coalesced vesicles to form the septum. E.R. occurs on each side of the developing septal plate. (Micrograph by Mrs M. A. Gooday.) $KMnO_4$ fixation (× 15000).

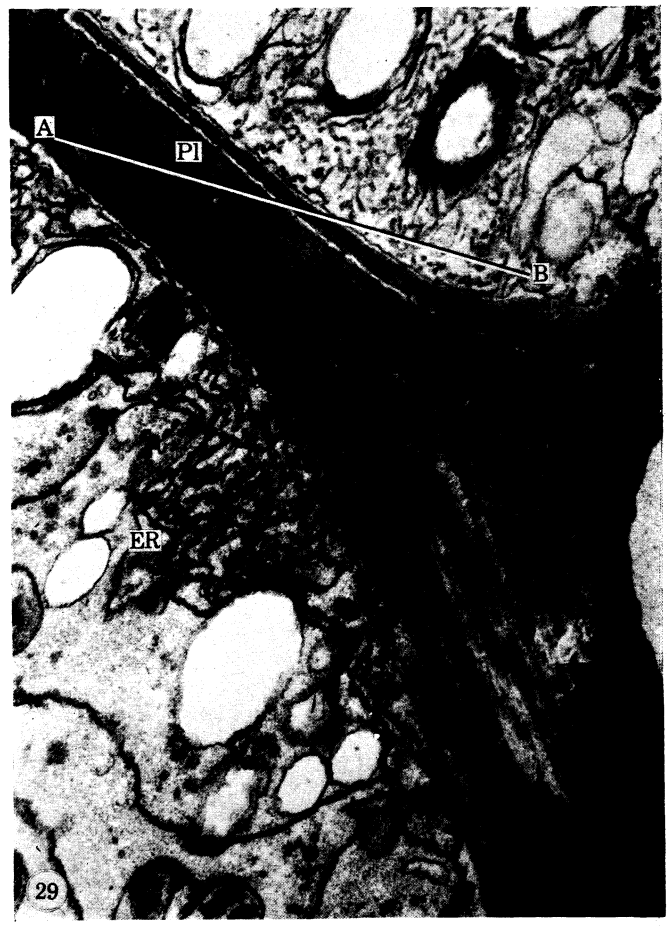
Figure 26. Tangential longitudinal section through part of the gametangial septum. Aligned vesicles are surrounded by E.R. and microbodies. (Micrograph by Mrs M. A. Gooday.) $KMnO_4$ fixation (× 15000).

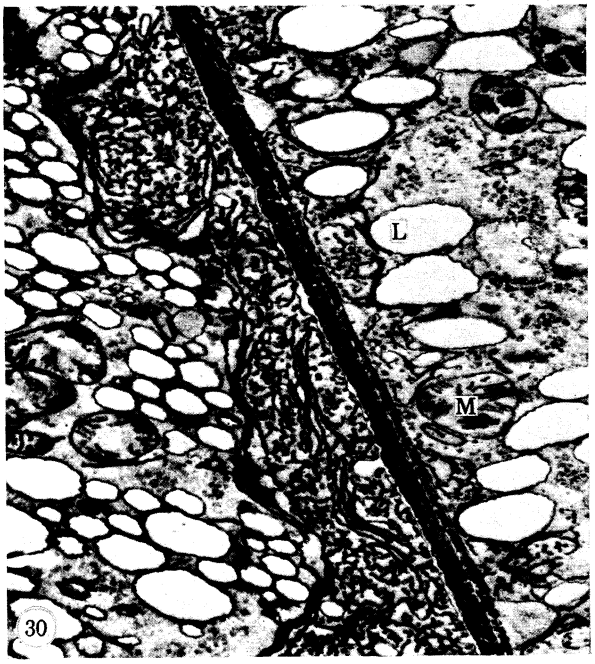
Figure 27. Longitudinal section through the partially thickened cross-wall showing abnormal development resulting in regions of double wall being laid down. (Micrograph by Mrs M. A. Gooday.) $KMnO_4$ fixation (× 15000).

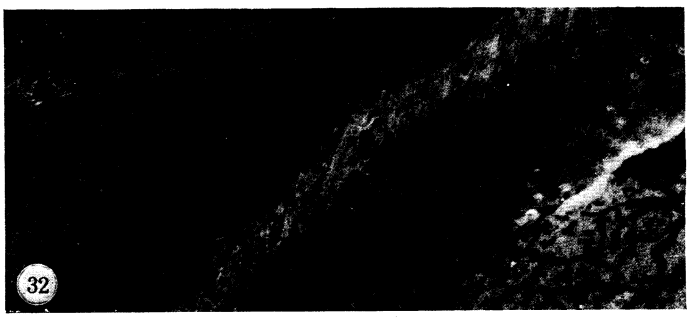


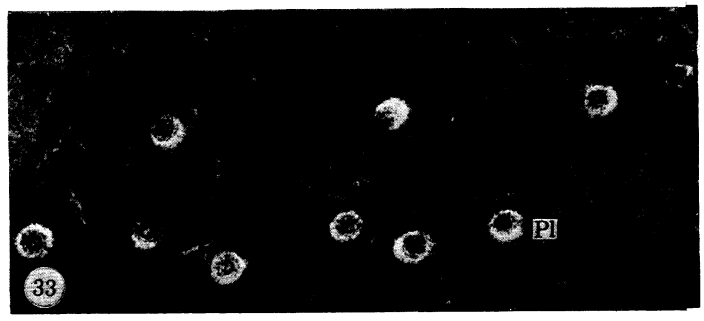
FIGURES 24 to 27. For legends see facing page











FIGURES 28 to 33. For legends see facing page

beginning to develop inwards from the progametangial wall. In such a specimen the electron microscope reveals that a very thin septum is already partially developed, extending about one-third of the way across the cell. At this early stage the partial septum is at an acute angle with the progametangial wall directed away from the apex or gametangial initial (figure 24, plate 9).

Electron micrographs show the presence of microbodies of types (i) α and (i) β described previously (p. 76) on each side of the septum and also of cisternal rings together with sections of E.R. (figures 24 to 26, plate 9).

The advancing edge of the incomplete septum is very thin and undulating, consisting of an inner electron-transparent plate between two unit membranes. Radial sections show that the plane of the septum is continued inwards for a short distance by alined but disconnected vesicles, circular or elongated in section, of structure comparable to that of the newly formed septum (figure 26, plate 9). Tangential sections (figure 25, plate 9) passing near to the advancing inner edge of the septum show a larger number of these alined vesicles, as might be expected. Near the advancing edge of the septum these vesicles tend to coalesce and often overlap in a manner resembling roof tiles (Hawker & Gooday 1967). Fusion of these overlapping vesicles (figure 25, plate 9), results in the centripetal growth of the septum. This method of wall formation causes the undulating appearance of the young septum as seen in section. These undulations finally disappear as the wall begins to thicken and as the central gap finally closes.

The probable part played by cytoplasmic streaming in the initiation of septum formation and in the formation of the wall from alined vesicles is discussed later (p. 90).

As an individual vesicle (or a coalesced line of these) becomes incorporated in the septum, the unit membrane surrounding it fuses with the plasmalemma enveloping the incomplete septum. The resulting plasmalemmas of the gametangium and of the cut-off parts of the progametangium (the suspensor cell) are therefore continous with and similar to the original plasmalemma of the progametangium.

Before the septum is complete, thickening of this new wall begins next to the original peripheral wall of the progametangium and progresses inwards towards the centre of the closing septal plate. The septum, as seen in section, is thus a narrow-based wedge (figure 24, plate 9). New wall material is similar in appearance to that comprising the original progametangial wall and is laid down between the two new plasmalemmas. The wall material, however, is not continous across the septum but is laid down immediately next to each plasmalemma leaving

---

Description of plate 10

Figures 28, 29. Longitudinal sections showing stages in the development of the cross-wall between the zygospore and suspensor. Plasmodesmata traverse the wall and masses of E.R. can be seen on both sides. Asymmetrical thickening of the wall leads to a greater deposition of dense material on the zygospore side (to the left of the figures). $KMnO_4$ fixation ($\times$ 15000 and $\times$ 10750 respectively). Figure 28 stained with lead citrate only. (Micrograph by Mrs M. A. Gooday).

Figures 30, 31. Longitudinal sections through parts of the cross-wall showing the spatial relationships between the wall, E.R., lipids and mitochondria. In both figures the zygospore is to the left. $KMnO_4$ fixation ($\times$ 10750 and $\times$ 30000 respectively).

Figure 32. Oblique transverse section passing through the cross-wall in the plane A–B shown in figure 29. Variations in the fibrous texture of the wall are visible and numerous plasmodesmata can be seen. $KMnO_4$ fixation ($\times$ 17000).

Figure 33. Transverse sections through part of the cross-wall showing plasmodesmata. $KMnO_4$ fixation ($\times$ 68000).

a thin electron-transparent layer between two layers of secondary wall. This central layer corresponds to the lumina of the vesicles from which the primary septum has developed. At intervals along the septum fine tubules can be seen connecting the cytoplasts of gametangium and suspensor cell and similar to the plasmodesmata of plant cells (Hawker, Bracker & Gooday 1966; Hawker & Gooday 1967) (figures 28 to 33, plate 10).

As the septum thickens the endoplasmic reticulum which, was originally sparse and scattered, becomes aggregated into complex masses along both sides of the wall (figures 28 to 31, plate 10); beginning in the angle between this wall and the lateral one and developing first on the suspensor side. Parallel studies with light and electron microscopes show that the septum then thickens rapidly by the deposition of new wall material presumably at the plasmalemma wall interface on each side of the septum. This material, however, is laid down more rapidly and deposition continues longer on the gametangial side of the septum than on the suspensor side (figures 29 to 31, plate 10). Thus the secondary wall surrounding the gametangium is very thick and eventually forms part of the zygospore wall, while that next to the suspensor remains relatively thin. Both layers, however, remain firmly stuck together by the still visible electron transparent layer. The plasmodesmata are still very conspicuous and can be seen to be continuous with the tubular masses of endoplasmic reticulum on either side of the wall (figures 30, 31, plate 10).

During the delimitation of the gametangia their primary lateral walls become thickened to an extent comparable to that seen in the suspensors. The outer region becomes more electron dense than the thicker inner part and becomes torn and irregular at the surface as the cell expands.

VIII. Formation of the complex zygospore wall

The wall of the mature zygospore is a complex structure consisting of several distinct layers. Although many details of its development and final form have long been known (Vuillemin 1904; Dangeard 1906; Ling-Young 1930) most text-books of mycology give an incomplete or actually incorrect account. Various authors have used the terms exospore, perispore, mesospore and endospore to denote layers of the complex wall, but since a particular term may have been used in several different senses, the present writers consider it advisable not to use them and to refer instead to primary, secondary, tertiary and quaternary wall material according to the order of its deposition.

Description of plate 11

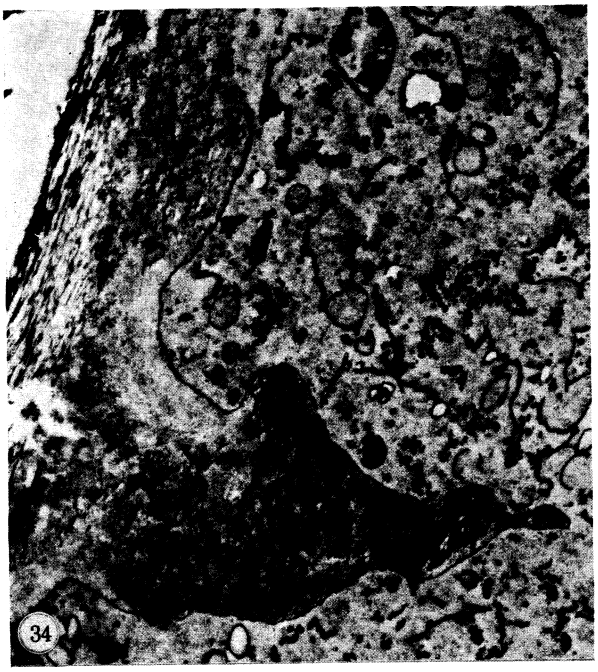
Figures 34, 35. Longitudinal sections showing the two opposite ends of the broken down fusion wall and their association with early stages in lateral wall ornamentation. Note the presence of numerous ring cisternae (figure 35, cf. figure 38) $KMnO_4$ fixation (× 10750 and × 6000 respectively).

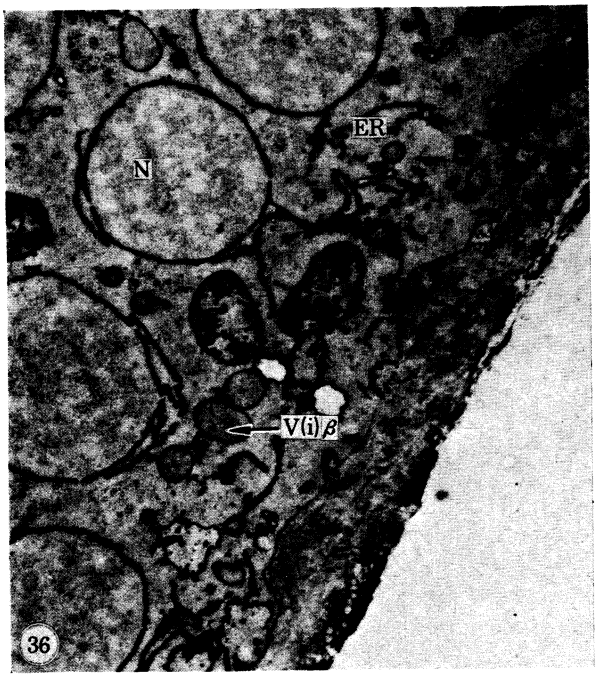
Figure 36. Longitudinal section through part of the zygospore lateral wall showing secondary wall deposition and associated nuclei, E.R. mitochondria, microbodies and ring cisternae. $KMnO_4$ fixation (× 10750).

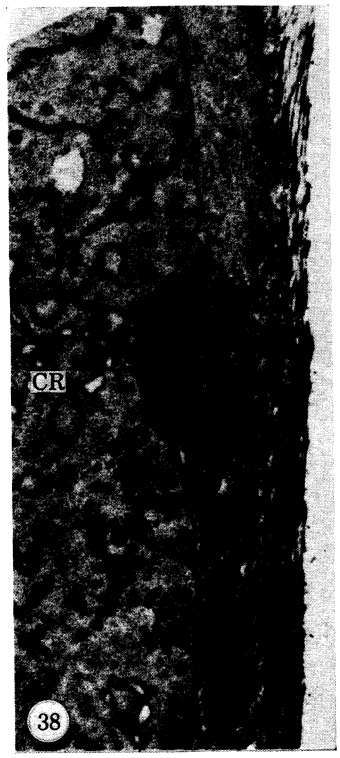
Figure 37. Longitudinal section through part of the lateral wall at a very early stage in secondary wall deposition. Lomasome-like bodies occur at the plasmalemma – cell wall interface. $KMnO_4$ fixation (× 18000).

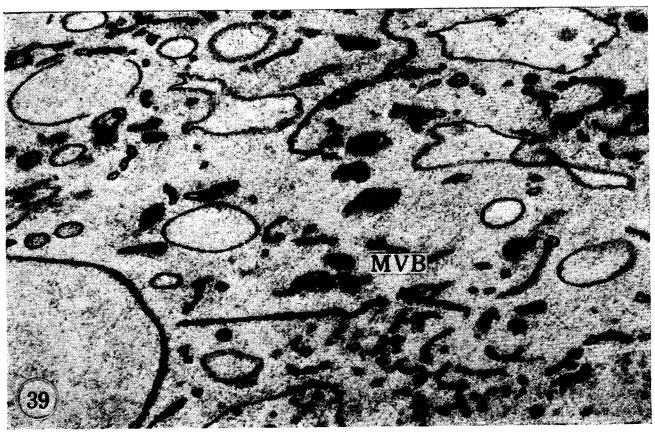
Figure 38. As for figure 37 but showing a later stage. $KMnO_4$ fixation (× 17000).

Figures 39, 40. Transverse sections through part of the zygospore initial in the region near the fusion wall. Multivesicular bodies occur, apparently by the enclosure of small vesicles within membrane packets. $KMnO_4$ fixation (× 30000 and × 45000 respectively).









FIGURES 34 to 40. For legends see facing page

(*Facing p.* 82)

(*a*) *The end walls*

As described above (p. 81) secondary thickening of the gametangial septa, which become the end walls of the zygospore, begins immediately after the septal plates are completed and proceeds more rapidly on the gametangial sides of the septa than on the sides adjoining the suspensors (figures 29 to 31 and 44, plates 10 and 13). This process continues until the end walls of the spore have reached a considerable thickness (*ca.* 1·7 μm) and at the same time dark pigment, presumably melanin, develops in the wall. Communication through the wall is maintained, at least until the spore is of full size, by the plasmodesmata already referred to (p. 82). When mature spores are treated with liquid nitrogen the suspensor cells are often torn off, revealing the end walls of the spore (figure 51, plate 14).

(*b*) *The ornamented lateral wall*

Meanwhile the lateral wall of the zygospore develops its characteristic ornamentation which, under the light microscope, is seen as black truncated roughly conical warts. The wart initials become visible soon after the completion of the gametangial septa, before the fusion wall separating the two gametangia has completely dissolved (figures 34 to 38, plate 11), and while a shallow equatorial groove indicating the original plane of fusion is still present. The form of the developing warts is best shown by the stereoscan electron microscope (figures 9 to 14, plate 6). The warts develop rapidly and become hard, black and opaque (figure 15, plate 6), rendering observation of the contents of the maturing zygospore increasingly difficult.

The earlier workers with the light microscope (Vuillemin 1904; Dangeard 1906; Ling-Young 1930) thought that the warts arose *de novo* and formed a new wall distinct from the original gametangial one. Hawker & Gooday (1968) using electron microscopy came to a similar conclusion, but none of their preparations showed the very early stages of wart initiation described below. It is now clear, that the warts are actually initiated by the deposition of secondary material at particular spots on the inner surface of the original primary wall and at first not clearly demarcated from it.

The beginning of wart development in the gametangial wall is shown by electron microscopy to occur at stage E_1 (p. 73) and to begin in the regions adjacent to the disintegrating fusion wall. This is readily seen both with the transmission electron micrscope in sectioned material (figures 34, 35, plate 11), and with stereoscan microscopy of whole spores (figures 10 to 12, plate 6). The earliest stages of this secondary deposition of wall material are recognized by the localized appearance of lomasome-like structures on the inner side of the lateral wall (figure 37, plate 11). Further deposition leads to the formation of discrete, regularly spaced blocks of wall material which project inwards towards the centre of the zygospore (figures 36, 38, plate 11). The lomasome-like bodies frequently occur along the margins of these projecting blocks and the adjacent cytoplasm contains numerous microbodies of types (i) α and (i) β together with cisternal rings and E.R. (figures 38, 41 to 44, plates 11 to 13). This secondary wall material is of similar appearance to that being laid down on the zygospore side of the gametangial septa and differs from that of the original lateral walls in its greater electron density and stratified appearance, features which become accentuated during later stages of development (figures 41 to 44, 48, 49, plates 12 to 14; figure 59)

Once laid down the wart initials develop rapidly, first becoming shaped like inverted saucers (figure 41, plate 12) and finally resembling irregularly-shaped inverted flower pots (figures 42

to 44, 49, 50, plates 12 to 14). The sections of wall between them become thickened but electron transparent, suggesting gelatinization and subsequent swelling rather than a deposition of new material (figures 43, 44, 48, 49, plates 12 to 14). During the ornamentation of the lateral walls, numerous microbodies of type (i) β (p. 76) occur around the periphery of the zygospore and in addition cisternal rings occur abundantly between and to the inside of the developing warts

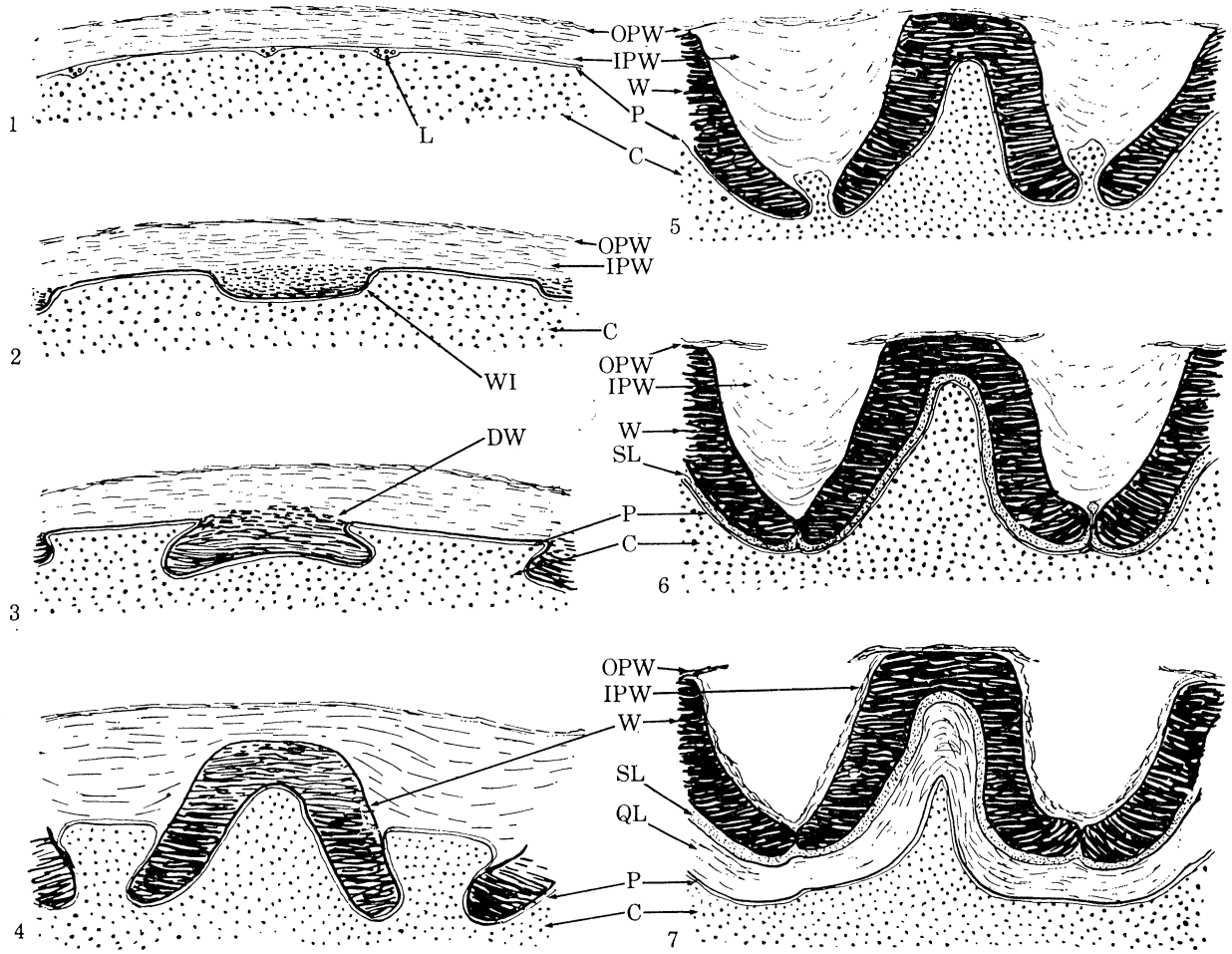


FIGURE 59. Development of lateral wall of zygospore (diagrammatic). *Key to lettering:*

OPW	outer layer of primary wall	SL	'smoothing' (tertiary) layer
IPW	inner layer of primary wall	QL	quaternary layer(s)
WI	wart initial	P	plasmalemma
DW	developing wart	C	cytoplasm
W	fully developed wart	L	lomasome-like body

1. Primary wall before inflation of zygospore, showing thin electron-dense outer layer, thicker less electron-dense inner one, and scattered lomasome-like bodies.

2. Blocks of secondary material (wart initials) developing locally on inner surface of primary wall.

3. Wart initials growing by deposition of secondary material at the rims to give saucer shaped pigmented masses.

4. Warts becoming flower-pot shaped by further growth at rims, inner layer of primary wall becoming gelatinous and swollen. Note pockets of cytoplasm between warts.

5. Rims of warts nearly touching, inner layer of primary wall showing stress lines, pockets of cytoplasm between warts much reduced.

6. Edges of warts touching, warts lined with tertiary smoothing layer, outer layer of primary wall torn.

7. Thick stratified impermeable layer of quaternary material laid down inside smoothing layer, inner gelatinous layer of primary wall has collapsed as a horny skin enveloping the warts.

(figures 34 to 38, 41 to 44, 49, plates 12 to 14). These cisternal rings give rise to characteristic vesicles, with electron-transparent contents, which appear to be incorporated into the gelatinizing primary wall between the warts (figures 41 to 44, plates 12, 13). However, in later stages of development (e.g. figure 43, 44, plates 12, 13), the wart material is quite distinct from the primary wall and frequently during sectioning warts fall out leaving clear spaces, the shape of inverted flower pots within the original wall.

The plasmalemma closely lines the saucer-like wart initials and balloons outwards between them. The cytoplasm fills the hollows beneath the saucers and extends into the 'balloons' of plasmalemma between them (figure 48, plate 14). Stereoscan pictures show that the outer layer of the original wall is stretched at the wart initials but is wrinkled between them, almost certainly as a result of shrinkage of the spore during fixation and coating (figure 12, plate 6).

Wart initiation is soon followed by the disappearance of the equatorial groove marking the position of the recently dissolved fusion wall. As the spore enlarges and becomes first barrel-shaped and finally approximately spherical the warts develop further by the deposition of wall material at the rims of the 'saucers' leading to the final inverted flower-pot shape. At the same time the bases approach one another until they are more or less in contact. Stereoscan microscopy of the inside of the warts at this stage shows adjacent rims to be separated by an irregular groove (figure 45, plate 14). It is not certain whether the cytoplasm which ballooned out between the rims is retracted or if some may be nipped off by the bases of adjacent warts as these approach each other. Partly disintegrated cytoplasm and organelles have not been seen in this position and hence it seems more likely that the living cytoplasm is retracted into the spore. Meanwhile the outer layer of the primary wall between the wart apices becomes torn, but remnants of it remain attached to the warts for some time (figures 14, 15 and 49, plates 6, 14). In transmission electron micrographs of thin sections amorphous material is seen between the warts and appears to be remains of the original inner part of the primary wall, although the possibility that it includes some cytoplasm cut off by the closing of the gaps between the warts is not excluded (figures 48, 49, plate 14).

(*c*) *Formation of an inner impermeable wall*

Shortly after the closure of the gaps between the warts a thin layer of tertiary material is laid down. Stereoscan microscopy of broken pieces of wall shows that this 'smoothing' layer obscures the lines of junction of the rims of the warts but otherwise follows their contours (figures 46 and 48, plate 14). In section the smoothing layer is seen to consist of more uniform and less electron-dense material than that comprising the warts.

Up to this stage the zygospore wall is permeable to fixatives and the contents of the spore can be readily studied. Further layers of wall material are rapidly laid down beneath the smoothing layer (figure 59), and the wall becomes impermeable to fixatives and stains. Stereoscan viewing of fragmented mature spores shows that cleavage occurs at the junction of the tertiary smoothing layer and the later formed or quaternary one(s). Where this innermost wall is fractured it presents a stratified appearance and may be formed of several closely adpressed layers. The whole of this inner wall follows the shape of the smoothing layer which acts as a mould. In stereoscan pictures the separated inner wall shows spikes corresponding to the interior of the warts (figures 47, 50, 51, plate 14). No satisfactory ultra-thin sections of this wall have been made.

IX. Changes in cell contents during zygospore development

(*a*) *Distribution and behaviour of nuclei*

The distribution and numbers of nuclei change as the spore develops.

As the progametangia enlarge the nuclei are mostly in the peripheral zone and along both sides of the fusion wall (p. 79; figure 19, plate 7). After delimitation of the gametangia the majority of the gametangial nuclei congregate near the fusion wall (p. 80). After breakdown of the latter and as the young spore enlarges, most of the nuclei again take up a peripheral position, often immediately below the developing warts (p. 83; figures 36, 41, 42, 44 and 49, plates 11 to 14).

Table 1. Counts of nuclei in progametangia, gametangia and young zygospores

stage of development of zygospore	estimated numbers of nuclei†
1. Progametangia (stage B of p. 73)	167
2. Gametangia not completely delimited (stage C)	138 (total for both gametangia)
3. Gametangia completely delimited (stage D)	306 (total for both gametangia)
4. Fusion wall breaking down (stage E_1)	165
5. Slightly later stage in break down (stage E_2)	145
6. Young spore enlarging and wall ornamentation beginning to develop (stage F)	274

† The figures given are each from only one zygospore initial but for all stages more than one specimen was examined and estimates made were of the same order as those given in the table. The figures set out in the table were in every stage estimated from the clearest set of photographs.

Observation of nuclear behaviour in living spores is difficult, particularly in the later stages, owing to the rapid darkening and thickening of the zygospore wall. Electron micrographs show paired nuclei and others in the process of either division or fusion but nuclear behaviour cannot be deduced from these.

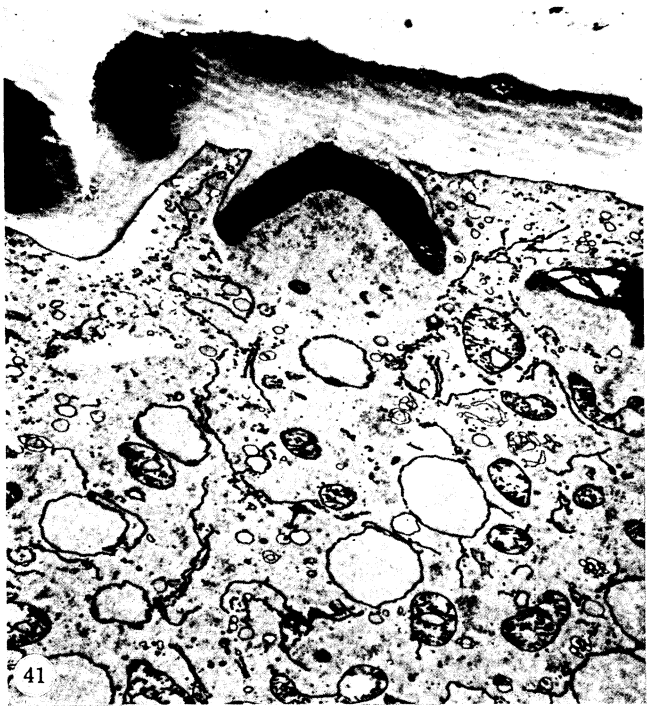
Zygospores of *R. sexualis* at different stages of development were stained with Feulgen's stain and serial photographs were taken at different depths of focus. The number of nuclei present was estimated from these photographs. The results are set out in table 1.

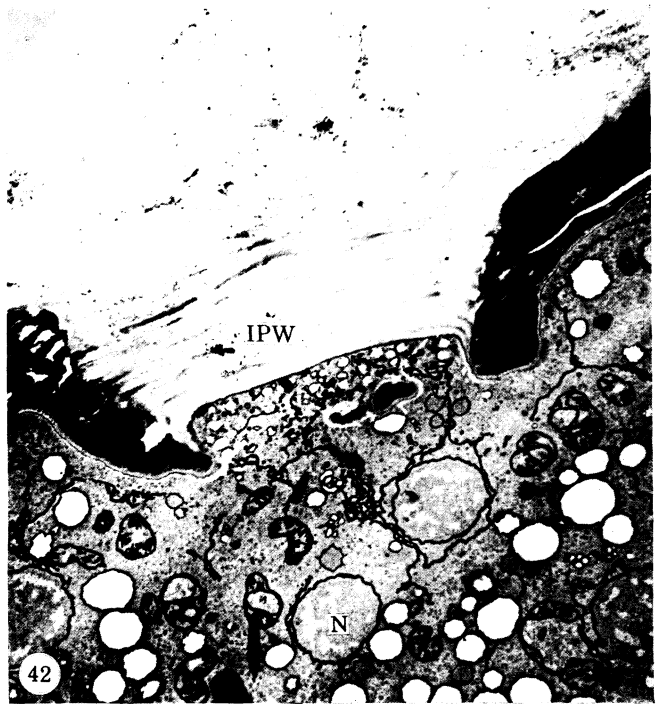
The figure of 167 nuclei from a pair of conjugating progametangia is the total number observed, including both the parts destined to be the gametangia and those expected to form part of the suspensor cells. It was not possible at this stage to determine the exact limits of the future gametangia. Hence it is not surprising that the number counted in incompletely delimited gametangia is smaller than that in the parent progametangia. Until the septa cutting off the gametangia are complete, nuclei may still pass into the latter from the suspensors. The increase in numbers in the gametangia after completion of the septa, but before the fusion wall is breached, reflects divisions of the gametangial nuclei. This was actually seen to take place in living material and was suggested also by the preponderance of pairs of nuclei in electron micrographs. Nuclei congregate at the earliest point of breakdown of the fusion wall. The

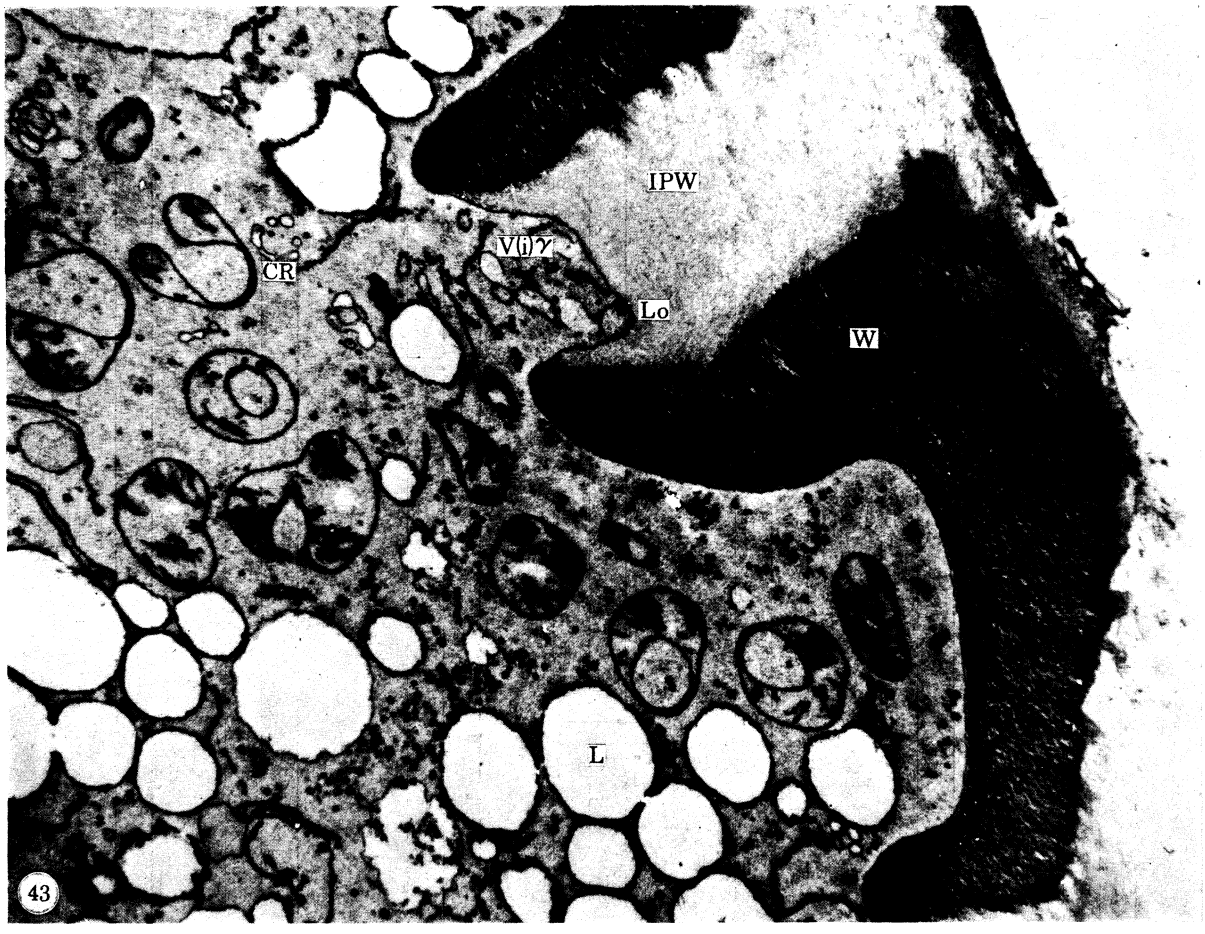
---

Description of plate 12

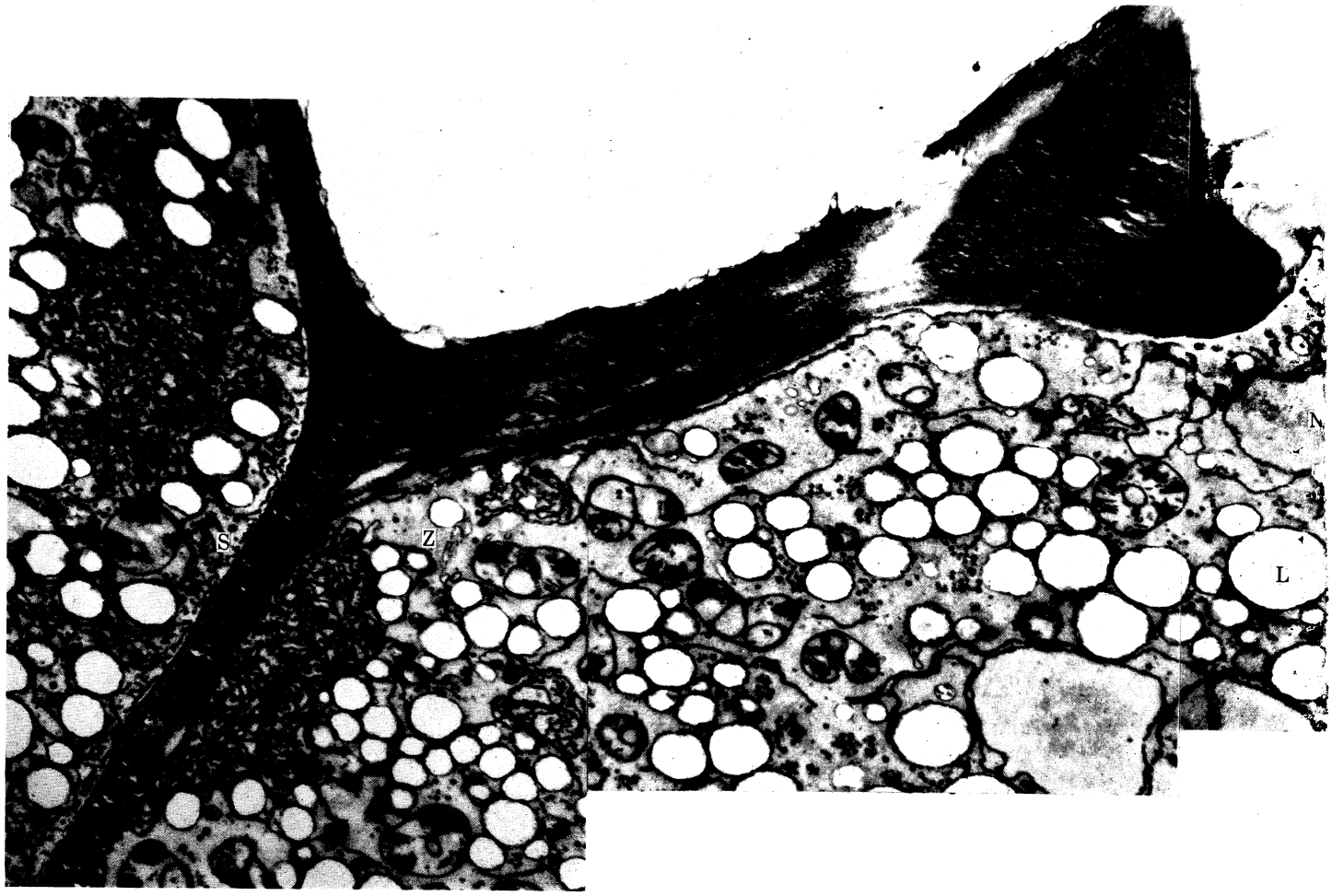
Figures 41 to 43. Longitudinal sections through parts of the zygospore lateral wall showing stages in the deposition and development of the wart-like ornamentations. Note the stress marks in the gelatinizing inner layer of the primary wall. Numerous ring cisternae occur immediately beneath the wall. $KMnO_4$ fixation (×4800, ×4800 and ×15000 respectively). (Figure 43, micrograph by Mrs M. A. Gooday.)







FIGURES 41 to 43. For legends see facing page


S
Z
L

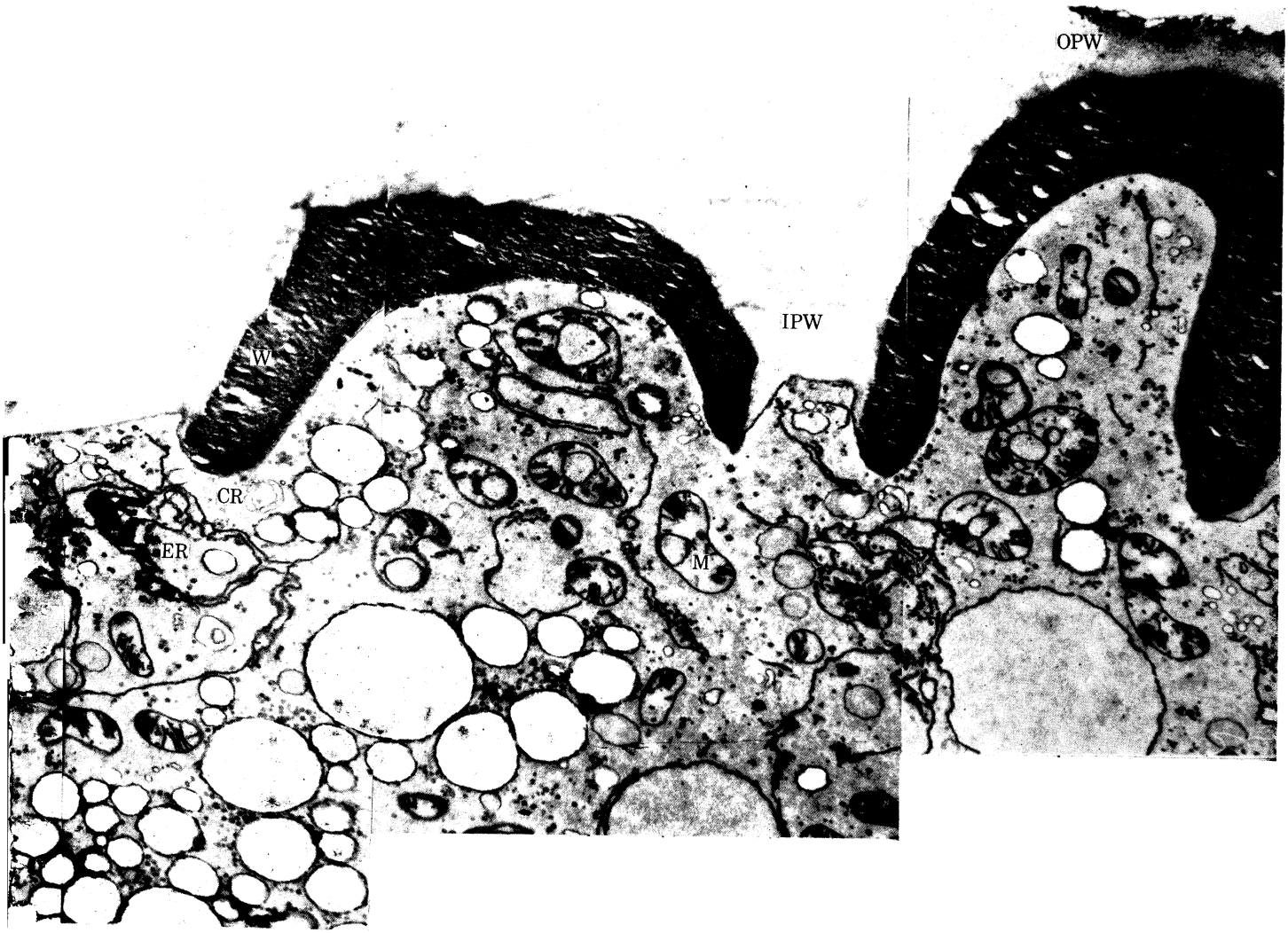
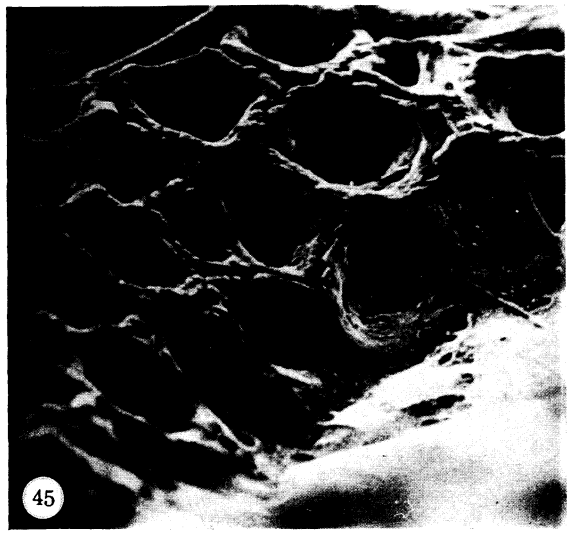
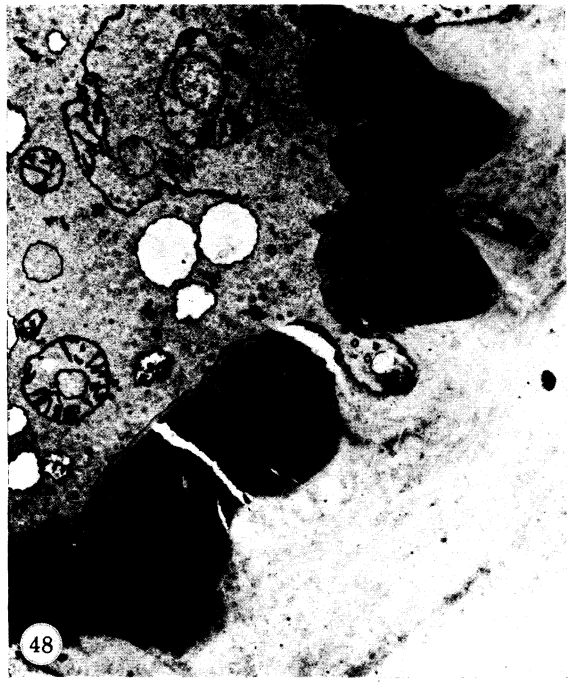
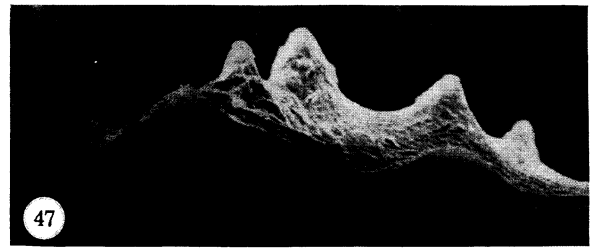
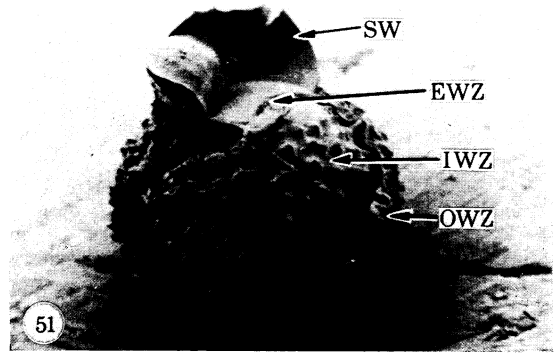


FIGURE 44. Photomontage of a longitudinal section through part of the zygospore wall showing the formation of the wart-like ornamentation by deposition of secondary wall material within the gelatinizing inner layers of the primary wall. Note the uniform deposition of secondary material on the zygospore side of the end wall. $KMnO_4$ fixation (×4285). (Micrographs by Mrs M. A. Gooday.)









FIGURES 45 to 51. For legends see facing page

approximate halving of the number immediately wall break down occurs, together with an increase in size of the nuclei, suggests that numerous fusions rapidly take place involving most if not all the nuclei. After these presumed fusions paired nuclei of normal size are seen again and the figures in the table are further evidence of an early division of the fusion nuclei. There is no evidence as to whether or not this division is meiotic.

(*b*) *Mitochondria*

The distribution of mitochondria at various stages in spore development has been stated in the appropriate places (pp. 77 to 85). As the spore enlarges they increase in number and become invaginated and irregularly lobed (figures 41 to 44 and 48, plates 10 to 14). In the suspensors mitochondria break down within membrane packets (see below).

(*c*) *Endoplasmic reticulum* (*E.R.*)

E.R. is closely associated with nuclei throughout the development of the zygospore (figures 18, 19, 23 25, 27, 35, 36, 41 to 44 and 49 , plates 7 to 9, 11 to 13). In the progametangia during dissolution of the fusion wall, ribosomes are present on the surfaces of this E.R. (figure 23, plate 8). Sheets of E.R. membrane also occur on either side of the alined vesicles during formation of the gametangial septum (figures 25, 26, plate 9). Once the septum is complete and secondary wall development begins, large masses of E.R. accumulate along the edges of the septum (figures 24, 28 to 31, 44, plates 9 to 10 and 13). In the maturing zygospore a complex membrane system is formed adjacent to the end walls within which numerous lipid globules are interspersed (figure 56, plate 15). E.R. is less abundant in the suspensors but in later stages of development is seen surrounding the mitochondria which subsequently degenerate into an irregular membrane mass (figures 54, 55, plate 15). Such a degeneration of mitochondria within membrane packets was observed by Bracker (1966) in sporangia of *Gilbertella persicaria*.

(*d*) *Accumulation of reserve food material*

Polysaccharide reserves build up in the suspensors and the compartmentalization of these reserves within vacuoles can readily be seen beginning in the central region of the suspensors farthest from the septum (figure 18, plates 7, see also figures 1 to 8, plate 5). In the zygospore glycogen increases in amount but remains scattered throughout the cytoplasm while the major volume of the spore becomes occupied by large numbers of lipid globules (figure 57, plate 15).

Description of plate 14

Figures 45, 46. Stereoscan electron microscope views of the inside of the wart-like ornamentations of the zygospore wall. Note how the rims grow together (figure 45) and are later covered by a further 'smoothing' layer of tertiary wall material (figure 46) (× 1700 and × 2000 respectively). Figure 45, micrograph by Mrs B. Thomas.

Figure 47. Stereoscan electron micrograph of the fractured edge of the composite, inner (quaternary) layers of wall material which form the impermeable covering immediately outside the protoplast (× 2000).

Figure 48. Tangential longitudinal section of part of the zygospore wall showing the formation of the 'smoothing' layer beneath the warts, the 'ballooning' of cytoplasm between the warts and the gelatinizing wall. $KMnO_4$ fixation (× 10750).

Figure 49. Transverse section through part of the zygospore wall showing the torn outer layer of primary wall parts of which adhere to the tips of the warts. $KMnO_4$ fixation (× 2250).

Figures 50, 51. Stereoscan electron micrographs of broken zygospores showing the relationship of the inner, impermeable layers to the outer 'warty' layers. Figure 51. Micrograph by Mrs B. Thomas (× 950 and × 370 respectively).

Larger vacuoles also occur in smaller numbers and histochemical staining suggests that these contain polysaccharide material. These results confirm those of Hawker *et al.* (1968).

X. Formation of abnormal azygospores or twin zygospores

Synchronization of development in the two conjugating progametangia, or later in the gametangia, is frequently not exact. As already pointed out erosion of the fusion wall may begin earlier on one side than the other and the gametangial septum in one progametangium may be initiated and complete its development earlier than in the other. Usually these slight differences are insufficient to prevent normal development of the spore. Occasionally, however, the differences are considerable. If development of one progametangium is much faster than that of its partner, the first may be fully developed before the other is delimited. The fully formed gametangium then proceeds to form an azygospore and the tardy one aborts.

If breaching of the fusion wall is delayed too long after delimitation of the gametangium the ornamentations normally confined to the lateral walls continue along both sides of the fusion wall and twin spores develop. It is not clear whether these are azygospores or whether limited breaching of the fusion wall takes place allowing intermingling of gametangial nuclei and other contents.

The incidence of such abnormalities increases with the length of time of an isolate in artificial culture.

XI. Discussion

(*a*) *Possible mechanisms regulating zygospore development*

The development of the zygospore includes a number of complex processes which are described in detail above (pp. 77 to 85). These take place in a more or less uniform sequence, but there is considerable overlap so that contrasted processes (e.g. wall degradation and wall building) may be taking place simultaneously in adjacent regions of the same cell. There must, therefore, be a complex pattern of control mechanisms which trigger off and regulate these varied developmental sequences. The present study of ultrastructural changes between initiation and maturation of the zygospore suggests some possible clues to the nature of some parts of the regulating systems.

(i) *Fusion of the zygospore tips and enlargement of the progametangium*

The ultrastructure of the tip of a young zygophore is similar in appearance to that of a vegetative hypha. It differs chemically, however, in having a higher D.N.A./R.N.A. ratio. Hawker *et al.* (1968) showed that environmental conditions, such as low temperature, may reverse this

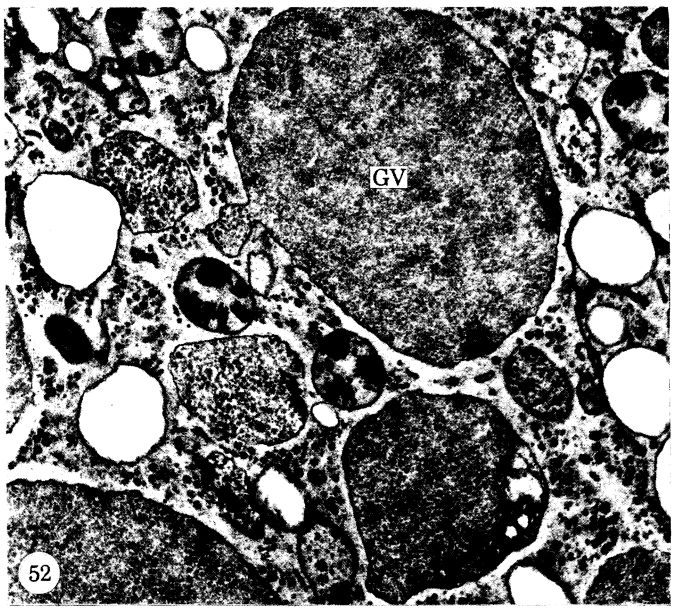
---

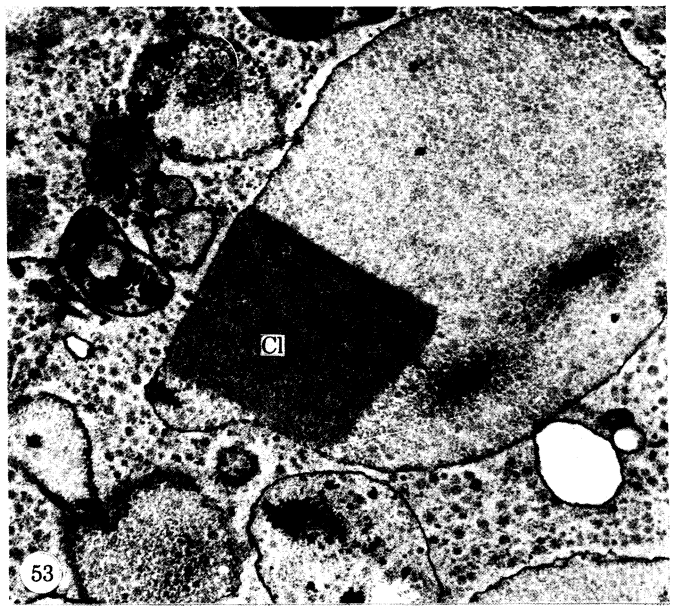
Description of plate 15

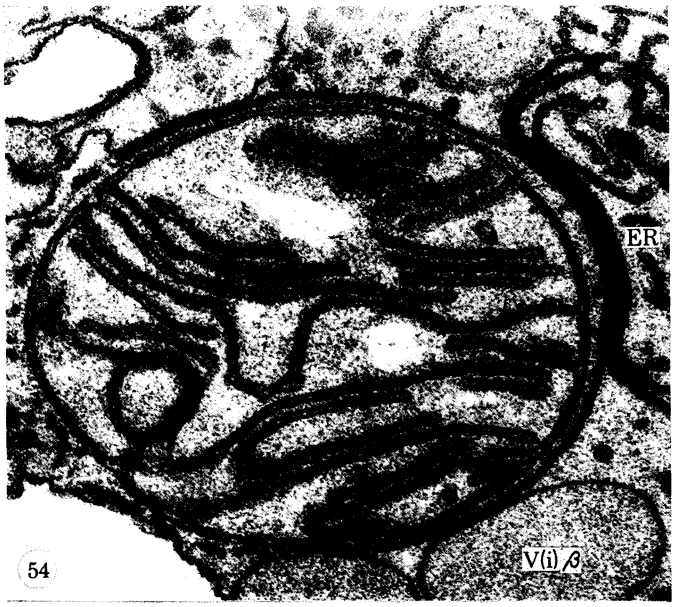
Figures 52, 53. Longitudinal sections through parts of the suspensor showing the accumulation of glycogen within membrane bound 'packets'. Crystalline inclusions also occur in some vacuoles (figure 53). $KMnO_4$ fixation (× 10750 and × 15000 respectively).

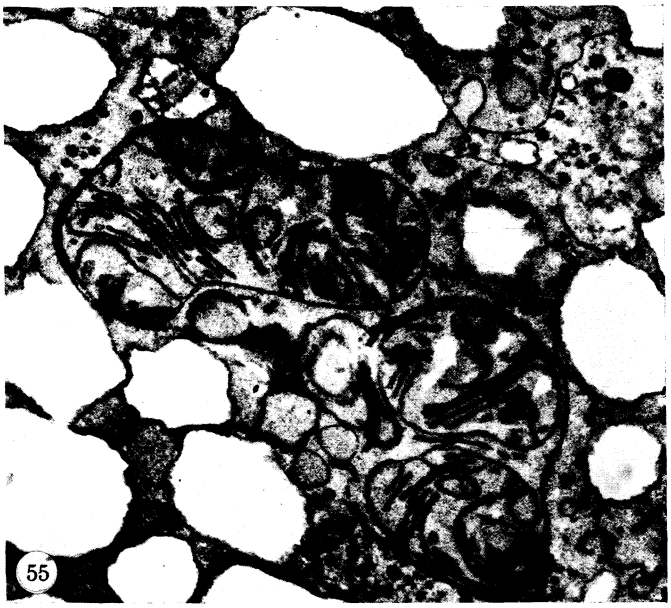
Figures 54, 55. Longitudinal sections through parts of the suspensor showing the enclosure and break down of mitochondria within E.R. membranes. $KMnO_4$ fixation (× 60000 and × 22500 respectively).

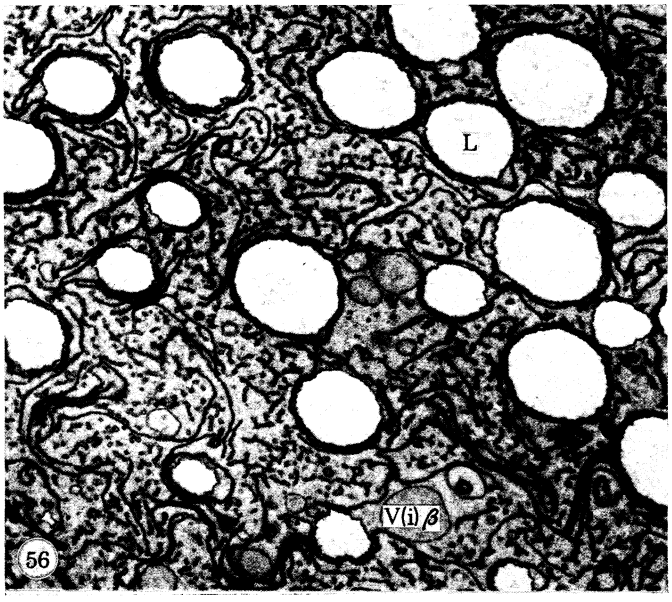
Figures 56, 57. Transverse sections through parts of the nearly mature zygospore. Figure 56 shows the E.R. masses interspersed with lipid globules in the region adjacent to the end wall. Figure 57 shows the accumulation of lipids and cytoplasmic glycogen in the centre of the zygospore. $KMnO_4$ fixation (× 17000 and × 4500 respectively).

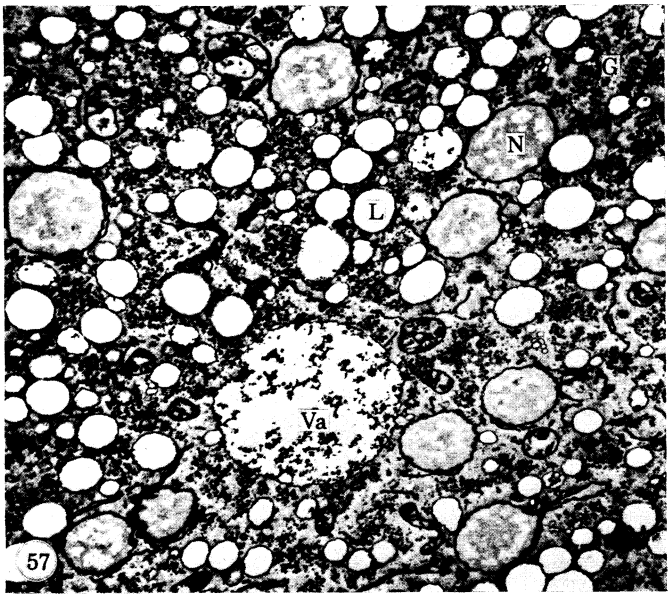












FIGURES 52 to 57. For legends see facing page

ratio and that the zygophore initial may then revert to the vegetative condition or may develop into a sporangiophore.

The mechanism by which two suitable zygophore tips make contact with each other has not been studied in *R. sexualis*, but it is assumed that volatile chemical substances are concerned, as in *Phycomyces blakesleeanus* (Banbury 1955) or *Mucor mucedo* (Gooday 1967). Volatile substances have been shown to be active in zygospore initiation and early stages of development (Hepden

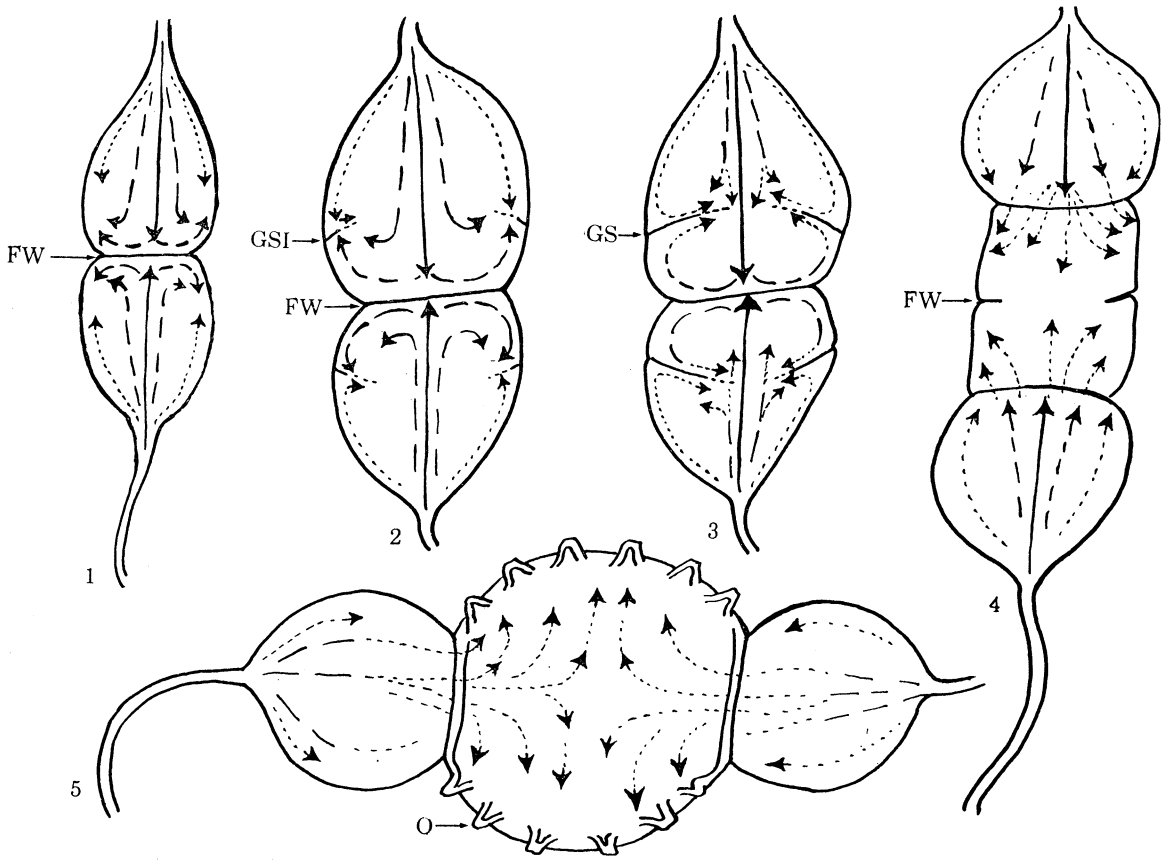


FIGURE 60. Probable patterns of cytoplasmic streaming in developing zygospore. (Diagrammatic, not drawn to exact scale.) *Key to lettering*:

FW, fusion wall;
GSI, gametangial septum initial;
GS, gametangial septum;
O, ornamentation (i.e. warts).

Relative strength of currents represented by unbroken line (strong current), broken line (less strong) and dotted line (weak).

1. Progametangia, showing deflexion of strong central current by fusion wall.
2. Deposition of wall material initiating septum formation at point where currents meet.
3. Possible streaming mechanism causing alinement of E.R. and vesicles formed from it, resulting in coalescence of latter to form ingrowing edge of septum.
4. Currents flowing into suspensors checked by completion of septa, leading to inflation of suspensors. Weak currents entering young zygospore via plasmodesmata.
5. Suspensors fully inflated, resistance of suspensor wall causing water to be pumped through plasmodesmata leading to inflation of zygospore.

& Hawker 1961; Hawker & Hepden 1962) but it is not known whether these are identical with those inducing zygotropic movements.

The rapid flattening of the apical walls of the conjugating zygophore branches and the enlargement and alteration in shape of the gametangia may be brought about by pressure from

cytoplasm streaming towards the fusion wall (figure 60). The lateral walls on either side of this wall (i.e. in the extension zone of the original hyphae) are still extensible and since elongation is checked by fusion of the zygophore tips, lateral inflation results. As the progametangial wall ages and solidifies from the base, the subsequent change in shape of the expanding progametangium from carrot-shape to turnip-shape would be expected. The apical walls of hyphae (Hunsley & Burnett 1970) have been shown to consist of a thin layer of microfibrils and it is not surprising that when two such tips are pressed together their walls become completely fused, showing no trace of their original separate identities. The increase in thickness of the wall after fusion has the appearance of gelatinization rather than of the deposition of new fibrillar material, although matrical material may infiltrate into the wall across the plasmalemma.

The zonation of organelles in the progametangium (p. 79, plate 7) is similar to that in a vegetative hyphal tip. This zonation is maintained by pressure from the rear in association with the rapid development of storage vacuoles in the basal part of the progametangium. The numbers of organelles are, however, greater than in the hyphal tip, presumably as a result of division of those originally present, or of reinforcements carried from the rear by the observed cytoplasmic streaming or a combination of these factors.

(ii) *Dissolution of the fusion wall*

In a study of the dissolution of the fusion wall of *Phycomyces*, Sassen (1962) concludes that the whole process is a chemical one and that no trace of microfibrillar residue remains. Observation of the similar process in *Rhizopus* indicates that here also the fusion wall is entirely dissolved leaving no detectable solid residue. The vesicles which, as described on p. 76 and illustrated in plate 8 and figure 58, are present in large numbers in the neighbourhood of the dissolving wall, almost certainly function as the means of transport of hydrolytic enzymes to the wall and of degraded wall material from it.

The occurrence of aggregations of vesicles between the wall and the plasmalemma, resulting in lomasome-like structures (figure 21, plate 8), is also a striking feature of the break down process as already described (p. 79). Whether these vesicles are enzyme packets, the deposition and rupture of which at the wall surface could initiate dissolution, or whether they contain degraded wall substances ready for transport through the plasmalemma and away from the wall is not known. Their relation to the vesicles within the cytoplasm is also unknown.

(iii) *The initiation and formation of the gametangial septa*

The initiation of the gametangial septa as inward growing rings and their continued inward growth by the coalescence of pre-alined vesicles may be plausibly explained as the result of the direction of the flow of the cytoplasm (figure 60, (1), (2) and (3)). Local differences or gradations in the elasticity of the lateral wall may also play a part.

Observations of the pattern of cytoplasmic streaming in the living progametangium are difficult since the flow is readily stopped by slight disturbance of the culture. Nevertheless, on a number of occasions a flow pattern has been observed which suggests a possible mechanism of septum siting and alinement (figure 60).

In the progametangium the flow of cytoplasm is most rapid along the central axis and least so at the periphery. As the central stream impinges on the fusion wall it spreads outwards, slows down and flows backwards inside the lateral wall to meet the inflowing peripheral currents. The backward flowing current may well carry with it vesicles containing degraded material

from the fusion wall. This material would be deposited where this current meets and checks the inflowing one. Such a deposition of wall material at a particular distance from the fusion wall could initiate the septum as a ring-like structure. The formation of such a ring would in turn deflect the converging streams of cytoplasm inwards and could bring about the observed alinement of E.R. and of the special vesicles which by coalescence complete the septum. The observation (p. 81) that on its formation the septal ring is deflected strongly backwards so that in section it is seen to form an acute angle with the suspensor wall (figure 24, plate 9) could be explained if the flow of cytoplasm back from the fusion wall area is stronger than that coming towards it from the parent hypha.

The final closure of the septum may be made possible by the progressive neutralization of the central stream of cytoplasm by the currents flowing inwards along both surfaces of the septal plate (figure 60 (3)).

Irregularities in the currents along the surfaces of the septum or perhaps their deflexion by a large organelle, such as a mitochondrion, may be responsible for the not infrequent double or treble septum formation, where either a small bubble of cytoplasm is enclosed in similar walls (figure 27, plate 9) or the septum may be double for a large part of its area. No such double septa have been detected in mature spores. It is not known whether these abnormalities are resolved in later developments or whether they lead to abortion of the zygospore.

The method of septum formation by the coalescence of previously alined vesicles is reminiscent of that described for higher plants (Bajer 1968; Newcomb 1969 and contained references). Septum formation in the plant cell, however, proceeds from the centre outwards, in contrast to the inward growing gametangial septum of *Rhizopus*, and is associated with nuclear division. In the plant cell alinement of the vesicles is thought to involve the phragmoplast microtubules. A spindle of course plays no part in the formation of the *Rhizopus* septa. Microtubules of a different origin could be present but the fixatives used in the present study would not be expected to preserve them. The fact that they have not been demonstrated does not therefore preclude the possibility of their presence. It may be that alinement of the vesicles is solely due to cytoplasmic streaming, in which case resemblance to the plant cell is illusory. Such an alinement of vesicles has not been seen during the formation of septa in vegetative hyphae of higher fungi (Littlefield & Bracker 1971),

Once the gametangial septa are complete they must form a considerable barrier to the passage of water into the gametangia since only the fine plasmodesmata permit communication between suspensors and gametangia. This offers an explanation of the inflation of the suspensors prior to that of the zygospore itself. It may be suggested that when the limits of extensibility of the lateral walls of the suspensor are reached or, more probably when this extensibility is reduced by secondary thickening of these walls, the turgor pressure of the dilated suspensor forces water through the plasmodesmata into the zygospore which in turn inflates. This inflation of the suspensors and later of the zygospore would result in the observed flattening of the septal plates, and a usually only temporary bulging of these into the spore (figures 4 to 8, plate 5).

In some other genera of the Mucorales, e.g. *Mucor*, the suspensors do not inflate to become spherical, as in *Rhizopus*, and it must be assumed that the suspensor walls in the former are either less extensible from the start than are those of *Rhizopus* or become solidified by secondary thickening at an earlier stage.

(iv) *The problem of simultaneous degradation and deposition of cell-wall material*

Before dissolution of the fusion wall has proceeded far and while this wall still forms a continuous barrier between the two progametangia, the opposite process of wall building commences nearby, leading to the delimitation of the gametangia by transverse septa. The fusion wall is not breached until after a thin but complete septum has been formed across the progametangium cutting off the gametangium from the suspensor cell. Considerable secondary thickening of these septa and of the lateral walls of the gametangia takes place before all traces of the fusion wall have disappeared. Thus in the same cell and in close juxtaposition, the fusion wall is being degraded simultaneously with the deposition of wall material nearby. It is probable that material removed from the fusion wall is actually used in the formation of at least the primary septal walls as already suggested (p. 91). The secondary material laid down later is of different appearance from the fusion wall. Material from the latter would necessarily undergo considerable changes before it could be reused in this way. The same morphological types of vesicle are associated with dissolution and the deposition of both primary and secondary wall material. For a brief period before the septum closes all three processes take place simultaneously in different parts of the gametangium.

If the balance between wall precursors and wall polymers decides whether degradation or construction takes place, small local differences in physical factors, e.g. levels of oxygen or carbon dioxide, might determine the direction of change. If, however, the chemical nature of the material being deposited (i.e. the secondary wall material) is different from that of the fusion wall the two processes might proceed under identical conditions and the same transport vesicles might carry both types of material.

(v) *The development of the ornamented lateral wall of the zygospore*

One of the most difficult problems is the interpretation of the mechanism(s) resulting in the formation of the ornamented lateral wall of the zygospore (p. 83). Some tentative suggestions may, however, be made.

Observations of the changes in shape of the gametangia (and later of the zygospore) and suspensor cells show that the latter attain their full size and inflated shape before any considerable expansion of the young zygospore takes place (p. 73) and before ornamentation of the lateral wall of the zygospore is initiated. Thus there is a change in the site of lateral wall extension from the suspensor to the fused gametangium and at no time are the suspensor cells and the zygospore expanding equally rapidly. Such an arrangement would prevent more than minimal stretching of the septal plates. Deposition of secondary wall material, subsequent to delimitation of the gametangia and cessation of suspensor wall expansion, is thus taking place simultaneously over relatively stable areas (i.e. the septa now forming the end walls of the zygospore) and over rather rapidly expanding areas (i.e. the lateral walls of the gametangia and later of the zygospore). As reported on p. 83 the layers of secondary material laid down on the inner surfaces of the gametangial septa are continuous and uniform in thickness. These layers continue for a short distance along the adjacent lateral wall (plate 13). Over the greater part of the lateral wall, however, the secondary material, although similar in appearance to that lining the end walls, is laid down in separate blocks spaced more or less regularly over the inner surface of the primary lateral wall. It seems possible that this arrangement is brought about by the continouus expansion of the curved surface on which the material is being deposited. It is significant that block formation is visible first in the equatorial region of the spore where primary wall

expansion is greatest. Narrow zones of continuous secondary material occur along the zygospore lateral wall from its junctions with the septa and extend as finger-like projections for some distance along the lateral wall (figure 16, plate 7).

A small rubber balloon was used as a model. After partial inflation it was coated with a

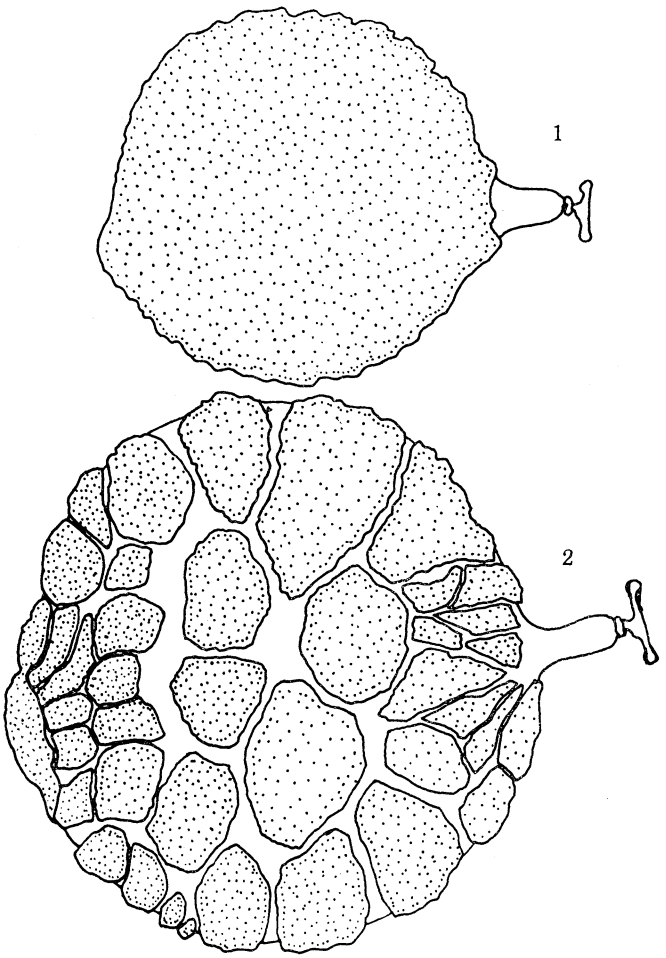


FIGURE 61. Diagrammatic sketch showing the pattern of cracking that occurs when a partially inflated balloon, coated with a layer of inelastic 'Polyfilla' (1), is further inflated (2).

proprietary cellulose-based plaster ('Polyfilla') and when this covering had dried was further inflated. The cracking of the plaster resulted in a configuration remarkably similar to that of the secondary material of the zygospore wall (figure 61). The cracks radiated out from the apex and base of the balloon giving finger-like blocks of plaster similar to the blocks of secondary wall material fringing the end walls of the zygospore. The plaster on the expanding equatorial part of the balloon broke up into irregularly hexagonal blocks reminiscent of the wart initials of the spore wall. It would be possible to construct a more exact model, but the simple one employed is sufficient to support the hypothesis that the arrangement of the warts is the result of deposition of non-extensible secondary material on an expanding primary wall.

Once the blocks of secondary wall material are laid down it is not surprising that small organelles tend to collect in the bays between them as the spore expands, just as flotsam collects in small indentations of a shore or stream bank. Among these are 'transport vesicles' similar

to those previously associated with wall degradation and construction. Additional wall material thus tends to accumulate at the edges of the blocks which grow to form the characteristic 'flower-pot' warts. These show stratification consistent with such a mode of growth. The direction of growth must be regulated by the position of the available new material. This in turn is controlled by the sluggish movements in the cytoplasm set up by continued entry of water and dissolved materials through the end walls (figure 60 (4) and (5)) and the changes due to the accumulation of lipids and other materials in the central part of the spore.

As the 'flower pots' grow (figure 59) the stresses on the primary lateral wall increase (figure 49, plate 14) leading to the tearing of the thin outer fibrous and non-gelatinous layer. Once the rims have met and adhered together further expansion of the lateral wall is prevented and further deposition of wall material results in the continuous 'smoothing layer' described on p. 85 and shown in figure 59 and figures 46 and 48, plate 14. At the same time the remains of the primary wall are cut off from the supply of nutrients and collapse on to the flower pots as the thin gelatinous layer shown in figure 15, plate 6. In stereoscan pictures (figure 51, plate 14), of mature spores this gelatinous outer layer is not visible, except for torn fragments adhering to the wart apices, and has presumably dried to form a thin horny covering.

(vi) *Quaternary wall formation*

From the behaviour of spores when broken by treatment with liquid nitrogen it is obvious that after the deposition of the thin smoothing layer, or tertiary wall, further wall layers differ in structure since the wall shears at this level (figures 47, 50, 51, plate 14). Moreover, attempts to fix and section older spores show that not only is the spore now impermeable to the fixatives used, but that the texture of this innermost or quaternary wall differs from that of the outer wall (comprising primary, secondary and tertiary material) so that the layers tear apart.

After the coalescence of the flower-pot rims immediately prior to tertiary wall formation, the mitochondria exhibit changes characteristic of a reduction in available oxygen (figure 48, plate 14). It seems likely, therefore, that partial anaerobiosis develops during the deposition of the smoothing layer, until a threshold level is reached leading to differences in structure and composition of the subsequently formed wall layers.

Finally one may conclude that wall deposition ceases altogether, either because supplies of wall material precursors become exhausted or as a result of a degree of anaerobiosis sufficient to inactivate the operative enzymes.

(*b*) *Nuclear fusions and divisions*

The behaviour of the nuclei during the formation and maturation of the zygospores of the Mucorales is still uncertain in all but a few species. The rapid development of black pigment in the zygospore wall of most species makes it difficult to follow internal events in living material, while the hardening of the wall and its final impermeable nature (p. 85) render fixation and sectioning extremely difficult.

In some species with transparent walls the events can be readily followed, e.g. *Endogone lactiflua* (Hawker 1954), where it can be seen that one nucleus from each gametangium migrates into the zygosporic bud where karyogamy occurs. The remaining nuclei abort. Fusion is followed by rapid and repeated nuclear division. Cutter (1942*a*, *b*) concluded, from examination of a number of species representing several genera, that the stages at which karyogamy and meiosis take place are not the same in all species and that the numbers of pairs of nuclei which

fuse also differ. He distinguished four groups. He did not study *R. sexualis* but considered that in the morphologically similar but heterothallic species *R. stolonifer* (syn *R. nigricans*), while some gametangial nuclei aborted, a number of pairs fused to give fusion nuclei which remained undivided during maturation of the spore. He suggested that meiosis occurred during germination of the zygospore. In some species of *Mucor* and *Zygorhynchus* he concluded that all the gametangial nuclei fused in pairs and that meiosis took place during spore maturation. Cutter's evidence is not entirely conclusive and his work has never been independently confirmed.

In the present study, although there is evidence of nuclear fusions and divisions taking place at particular stages in zygospore formation in *R. sexualis*, details of fusion and meiosis have not been obtained. The data presented in table 1 strongly suggest that most of the gametangial nuclei fuse in pairs immediately after the breaching of the fusion wall and that these fusions are quickly followed by division of the fusion nuclei. If this is a reduction division *R. sexualis* would fall into Cutter's first group, in which he places *Mucor* and *Zygorhynchus*, and would differ from *R. stolonifer* where, according to Cutter, meiosis is delayed until spore germination takes place. Although a few zygospores have been induced to go through the earliest stages of germination (Hepden 1959) none has so far produced a germ sporangium. Even if one could be obtained it would be unlikely to assist in the interpretation of a homothallic species.

No indication of abortion of nuclei, as postulated by Cutter in *R. stolonifer* and reported by Hawker (1954) in *Endogone*, was seen in *R. sexualis*. This negative evidence again supports the probable similarity between nuclear behaviour in *R. sexualis* and that described by Cutter for species of *Mucor* and *Zygorhynchus*.

(*c*) *Probable functions of cytoplasmic organelles*

The above detailed study of the developing zygospore of *Rhizopus* sheds some light on the problems of the roles of the various cytoplasmic organelles.

(i) *Endoplasmic reticulum*

Despite numerous published papers on various aspects of fungal ultrastructure our knowledge and understanding of the polymorphic membrane system of the endoplasmic reticulum (E.R.) remains scanty. A feature which has emerged from a number of studies on different fungi is that the form of the E.R. often reflects the metabolic state and development of the cell(s). In *Rhizopus* similar correlations have been observed. In the young progametangia there is a marked association between the E.R. and nuclei before and during the dissolution of the fusion wall (figure 19, plate 7). Examples are readily found of a continuity between E.R. and the outer membrane of the nuclear envelope. Aldehyde fixation shows these membranes to be part of the granular reticulum since they are studded with ribosomes (figure 23, plate 8). The association between E.R. and nuclei persists during gametangial septum formation and during ornamentation of the zygospore wall, though here it is not known whether the E.R. is granular since aldehyde fixation of this stage was unsatisfactory. It is possible that in all cases there is a precise control of the respective processes by the nucleus through the mediation of E.R. Sites for synthesis of materials could be provided by the E.R. which in turn could provide the link for subsequent transport of these materials to various locations in the cell. Vesicle formation by budding or blebbing of E.R. cisternae may be a further important function providing both the means to package material and a 'vehicle' for its transport to specific sites. During fusion wall dissolution, septum formation and wall ornamentation, vesicles are abundant in the

cytoplasm and frequently closely associated with E.R. Furthermore, during septum formation vesicles are alined within a zone delimited by parallel sheets of E.R., indicating that perhaps in this case the E.R. might act as a demarcation factor bringing together the vesicles prior to their fusion to form the septum. Aggregations of E.R. on either side of the gametangial septa produce a membrane system the extent of which is rarely seen in fungi, figures 4 to 8, 24, 28 to 31, (plates 5, 9 and 10). During development there is a marked increase in size of the zygospore, an accumulation of food reserves, an increase in number of organelles by division, and the formation of a complex lateral wall. These processes must involve the passage of water and dissolved materials through the gametangial septa from the suspensors. It is suggested that the role of the E.R. masses on the suspensor sides of the septa might be to provide multiple sites for the enzymic breakdown of complex food substances such as glycogen and lipid and to act as a link for their subsequent transport through the plasmodesmata of the gametangial septa. Similarly, sites along the E.R. membranes in the gametangium/zygospore could allow for the resynthesis of such transported material into macromolecules. In this connexion it is interesting to speculate on the possibility that these sites might act as metabolic sinks or the starting-points for density gradients within the cell and in so doing may in fact initiate local cytoplasmic streaming. To what extent E.R. might mediate cytoplasmic streaming in *Rhizopus* is completely unknown, but there is some evidence that masses of E.R. might themselves be positioned by peripheral cytoplasmic currents (figure 24, plate 9; figure 60). The intimate association between the lipid droplets and E.R. in the zygospore is interesting (figure 56, plate 15). It is possible that lipids may be synthesized within the zygospore in association with E.R. or they may be transported within the extracellular space provided by the lumen of the E.R. system, crossing from the suspensor to the zygospore through the plasmodesmata by way of E.R. connexions. Finally E.R. membranes within the mature suspensors appear to play a part in compartmentalizing mitochondria, whereupon these organelles undergo degeneration. Such a process, involving the breakdown of membranes, could provide further supplies for the pool of reserve lipid which is such a prominent feature of the mature zygospore.

(ii) *Mitochondria*

The regular association of mitochondria with specific sites of activity, (e.g. the fusion wall during dissolution, the ingrowing septa), reflects the need for an energy supply at these locations. As the spore enlarges and its wall develops, mitochondria increase in number and become invaginated and irregularly lobed, their morphology most probably being governed by the increasing anaerobic conditions within the spore.

(iii) *Vesicles*

In all fungi examined critically large numbers of small vesicles have been reported in the cytoplasm of the extreme tips of actively growing hyphae (McClure *et al.* 1967; Bracker 1967, Marchant, Peat & Banbury 1967; Girbardt 1969). It has been concluded that these are associated with primary wall formation and extension.

The possible role of these vesicles not only in the deposition of both primary and secondary wall material, but also in the erosion and ultimate complete dissolution of the fusion wall, has already been discussed (p. 92). One must conclude, therefore, that these vesicles are able to transport enzymes and wall materials or their precursors through the plasmalemma in either direction. The factors determining this direction are unknown. It is not possible at present to suggest particular functions for the different types of vesicle observed (p. 76).

(iv) *'Lomasomes'*

The term lomasome was originally introduced by Moore & McAlear (1961) to denote aggregations of membranes between the plasmalemma and the cell wall first reported by Girbardt (1958, 1961). These structures have since been reported in many fungi and in some algae and higher plants and the term has been used to describe a wide range of extra-plasmalemma membranous, tubular or vesicular complexes. It is not, therefore, surprising that a variety of functions has been ascribed to 'lomasomes'. They have most frequently been associated with secondary wall formation but they are not universally present when such deposition of wall materials is taking place (Wilsenach & Kessel 1965; Bracker 1967; Marchant *et al.* 1967).

Heath & Greenwood (1970), in a study of *Saprolegnia* and related fungi, make a novel distinction between 'plasmalemmasomes' closely associated with the plasmalemma and 'true lomasomes' which are similar structures embedded in the cell wall. The Oomycetes, however, differ from all other fungi in the chemical composition of the cell wall (Bartnicki-Garcia 1968, 1970) and in some other characters. No lomasome-like bodies have been seen actually embedded in the walls of *Rhizopus* although, as stated above (p. 83) they are frequently present between the wall and the plasmalemma.

In *Rhizopus* lomasome-like bodies are present at most sites where secondary wall deposition is proceeding but have not been seen along the gametangial septa at any stage. Thus, although there is some correlation between lomasomes and secondary wall formation this correlation is not complete. In addition, lomasomes have been seen along the edge of the dissolving fusion wall, and thus associated with a process the reverse of wall deposition. Here they are probably involved in either the removal of wall material during dissolution, or the deposition and release of enzymes at the edge of the wall prior to and during its degradation.

Attempts to ascribe a definitive function to lomasomes outside the context of specific experimental subjects is somewhat pointless, since in many instances it is difficult to discern whether they are in fact cause or effect. However, in most instances where lomasomes have been observed, both in *Rhizopus* and in other organisms cited in the literature, their presence is associated with the passage of material(s) across the plasmalemma in one direction or other. This process itself might involve the breakdown or synthesis of membrane at specific points which in turn could account for some of the variety of morphological forms covered by the term 'lomasome'.

(v) *Multivesicular bodies* (*m.v.bs*) (p. 76)

These have been reported in a number of fungi and their presence has been associated with the passage of materials through the plasmalemma. Marchant *et al.* (1967) considered that m.v.bs are concerned in the transport of wall material across the plasmalemma during secondary wall formation. Calonge, Fielding & Byrde (1969) reported that m.v.bs were more numerous in hyphae of *Sclerotinia fructigena* when these were grown under conditions inducing maximum secretion of pectolytic enzymes. M.v.bs are also associated with the formation of extracellular enzymes by yeasts (Lampen 1968).

These bodies are said to be formed from E. R. (Marchant *et al.* 1967) and, in germinating conidia, to follow the utilization of stored phospholipids (Buckley *et al.* 1966). Marchant *et al.* (1967) and Peat & Banbury (1967) suggest that they may be the forerunners of lomasomes. They are not, however, invariably associated with these structures. Thus Heath & Greenwood

(1970) saw no m.v.bs in *Saprolegnia* where lomasomes were commonly present. The Oomycetes, however, unlike most other fungi, possess a well-defined Golgi apparatus (Bracker 1967) which itself gives rise to vesicles and may play the same role as m.v.bs.

The origin and function of m.v.bs are obscure and these bodies could be artefacts. In the present study they can be related to the dissolution of the fusion wall both chronologically and spatially. There is some evidence to suggest their possible formation by the accumulation of single vesicles within a bounding membrane.

(vi) *Ring cisternae*

It has been suggested that groups of ring cisternae may represent the functional equivalent of a Golgi apparatus in those fungi not possessing true dictyosomes (C. E. Bracker, personal communication). Observations on these ring cisternae in *Rhizopus* substantially support this hypothesis. Their origin is unknown, but they are closely associated with wall formation or dissolution and are particularly abundant during ornamentation of the lateral zygospore wall (plate 13). Characteristic electron-transparent vesicles (microbodies)of type (i) γ are apparently formed from these cisternae and appear to fuse with the primary lateral wall between the developing warts (figures 41, 42, 44, plates 13, 14). Gelatinization of the inner layer of the primary wall is thought to occur at this stage and the incorporation of enzymes such as hydrolases might occur in this way.

Summary

The fine structure of the zygospore of *Rhizopus sexualis* (Smith) Callen throughout initiation, development and maturation is described.

The general structure of the zygophore resembles that of an actively growing vegetative hypha, but in the young progametangia the various organelles become more numerous and are rearranged in distinct zones.

The fusion wall, derived from fusion of the apical walls of the conjugating zygophores, soon undergoes dissolution but this process is not completed until after the delimitation of the gametangia. Characteristic microbodies and lomasome-like structures are associated with the dissolving wall.

The gametangial septa are produced by coalescence of previously alined vesicles. Cytoplasmic continuity between suspensor and gametangia is maintained through numerous plasmodesmata even after considerable secondary thickening of the gametangial/zygospore sides of the septa has taken place.

Secondary material is also deposited in rather regularly spaced blocks in the inner side of the lateral wall of the zygospore. Further material is deposited at the rims of these blocks resulting in first saucer-shaped structures and finally the characteristic black warts shaped like inverted flower pots. The rims of these finally meet. Meanwhile, the primary wall becomes gelatinous except for its outer layer. The latter remains as torn fragments adhering to the apices of the warts. Tertiary and quaternary wall layers are laid down within the wart layer, following its shape closely. The mitochondria in the zygospore then show signs of anaerobiosis.

Evidence from light microscopy suggests that the numerous nuclei fuse in pairs immediately after the fusion wall is breached and then undergo rapid and repeated divisions.

Food material, predominantly in the form of lipid droplets, accumulates in the zygospore as it matures.

Examples of abnormal development are described.

The roles of various cytoplasmic organelles and the behaviour of the nuclei are discussed. Probable mechanisms for controlling development of the zygospore are suggested.

We wish to thank the Science Research Council for a grant during the early stages of this work for the employment of a research assistant; the University of Bristol for continuing this grant; Dr M. F. Madelin for helpful discussion of the results; Professor H. E. Hinton, F.R.S., for permission to use the stereoscan electron microscope provided for his use by the S.R.C.; Professor J. P. Hudson, M.B.E., G.M. for use of a similar instrument at the Long Ashton Research Station; Mr R. J. Hendy, Mrs M. A. Gooday and Mrs B. Thomas, former research assistants who carried out preliminary investigations; and Mr R. M. Crawford for assistance in operating the stereoscan electron microscope.

References

Bajer, A. 1968 Fine structure studies on phragmoplast and cell plate formation. *Chromosoma, Berl.* **24**, 383–417.

Banbury, G. H. 1955 Physiological studies in the Mucorales III. The zygotropism of zygophores of *Mucor mucedo* Brefeld. *J. exp. Bot.* **6**, 235–244.

Bartnicki-Garcia, S. 1968 Cell wall chemistry, morphogenesis and taxonomy of fungi. *A. Rev. Microbiol.* **22**, 87–108.

Bartnicki-Garcia, S. 1970 Cell wall composition and other biochemical markers in fungal phylogeny. Chap. 5 in *Phytochemical phylogeny* (ed. J. B. Harborne), pp. 81–103. New York: Academic Press.

Bracker, C. E. 1966 Ultrastructural aspects of sporangiospore formation in *Gilbertella persicaria.* In (ed. M. F. Madelin), *The fungus spore. 18th Symp. Colston Res. Soc. Bristol.* London: Butterworths.

Bracker, C. E. 1967 Ultrastructure of fungi. *A. Rev. Phytopathology* **5**, 343–374.

Bracker, C. E. 1968 The ultrastructure and development of sporangia in *Gilbertella persicaria. Mycologia* **60**, 1016–1067.

Buckley, P. M., Sjaholm, V. E. & Sommer, N. F. 1966 Electron microscopy of *Botrytis cinerea* conidia. *J. Bact.* **91**, 2037–2044.

Buckley, P. M., Sommer, N. F. & Matsumoto, T. T. 1968 Ultrastructural details in germinating sporangiospores of *Rhizopus stolonifer* and *Rhizopus arrhizus. J. Bact.* **95**, 2365–2373.

Callen, E. O. 1940 Morphology, cytology and sexuality of the homothallic *Rhizopus sexualis* (Smith) Callen. *Ann. Bot. Lond.* (N.S.) **4**, 791–818.

Calonge, F. D., Fielding, A. H. & Byrde, R. J. W. 1969 Multivesicular bodies in *Sclerotinia fructigena* and their possible relation to extracellular enzyme secretion. *J. gen. Microbiol.* **55**, 177–184.

Cutter, V. M. 1942*a* Nuclear behaviour in the Mucorales I. The *Mucor* pattern. *Bull. Torrey. bot. Club* **64**, 480–508.

Cutter, V. M. 1942*b* Nuclear behaviour in the Mucorales. II. The *Rhizopus*, *Phycomyces* and *Zygorhynchus* patterns. *Bull. Torrey. bot. Club* **69**, 592–616.

Dangeard, P. A. 1906 Récherches sur le développement du périthèces chez les Ascomycètes. Chap. IV. Les Mucorinées. *Le Botaniste* **9**, 227–253.

Ekundayo, J. A. 1966 Further studies on germination of sporangiospores of *Rhizopus arrhizus. J. gen. Microbiol.* **42**, 283–291.

Girbardt, M. 1958 Uber die Substruktur von *Polystictus versicolor* L. *Arch. Mikrobiol.* **28**, 255–269.

Girbardt, M. 1961 Licht-und elektron-mikroskopische Untersuchungen an *Polystictus versicolor*. 2. Die Feinstruktur von Grundplasma und Mitochondrien. *Arch. Mikrobiol.* **39**, 351–359.

Girbardt, M. 1969 Die Ultrastruktur der Apikalregion von Piltzhyphen, *Protoplasma* **67**, 413–441.

Gooday, G. W. 1967 Sexual reproduction in the Mucorales. Thesis submitted for the Degree of Doctor of Philosophy, University of Bristol, 140 pp.

Grove, S. N., Bracker, C. E. & Morré, D. J. 1970 An ultrastructural basis for hyphal tip growth in *Pythium ultimum. Am. J. Bot.* **57**, 245–266.

Hawker, L. E. 1954 British hypogeous fungi. *Phil. Trans. R. Soc. Lond.* B **237**, 429–546.

Hawker, L. E. & Abbott, P. McV. 1963*a* Fine structure of vegetative hyphae of *Rhizopus. J. gen. Microbiol.* **30**, 401–408.

Hawker, L. E. & Abbott, P. McV. 1963*b* An electron microscope study of maturation and germination of sporangiospores of two species of *Rhizopus. J. gen. Microbiol.* **32**, 295–298.

Hawker, L. E., Abbott, P. McV. & Gooday, M. A. 1968 Internal changes in hyphae of *Rhizopus sexualis* (Smith) Callen and *Mucor hiemalis* Wehm. associated with zygospore formation. *Ann. Bot.* (N.S.) **32**, 137–151.

Hawker, L. E., Bracker, C. E. & Gooday, M. A. 1966 Plasmodesmata in fungal cell walls. *Nature, Lond.* **212**, 635.

Hawker, L. E. & Gooday, M. A. 1967 Delimitation of the gametangia of *Rhizopus sexualis* (Smith) Callen: an electron microscope study of septum formation. *J. gen. Microbiol.* **49**, 371–376.

Hawker, L. E. & Gooday, M. A. 1968 Development of the zygospore wall in *Rhizopus sexualis* (Smith) Callen. *J. gen. Microbiol.* **54**, 13–20.

Hawker, L. E. & Gooday, M. A. 1969 Fusion, subsequent swelling and final dissolution of the apical walls of the progametangia of *Rhizopus sexualis* (Smith) Callen: an electron microscope study. *New Phytol.* **68**, 133–140.

Hawker, L. E. & Hepden, P. M. 1962 Sporulation in *Rhizopus sexualis* and some other fungi following a period of intense respiration. *Ann. Bot.* (N.S.) **26**, 619–632.

Hawker, L. E., Hepden, P. M. & Perkins, S. M. 1957 The inhibitory effect of low temperature on early stages of zygospore production in *Rhizopus sexualis*. *J. gen. Microbiol.* **17**, 758–767.

Heath, I. B. & Greenwood, A. D. 1970 The structure and function of lomasomes. *J. gen. Microbiol.* **62**, 129–138.

Hepden, P. M. 1959 The physiology of reproduction in members of the Mucorales – a study in morphogenesis. Thesis presented for the Degree of Doctor of Philosophy in the University of Bristol, p. 196.

Hepden, P. M. & Hawker, L. E. 1961 A volatile substance controlling early stages of zygospore formation in *Rhizopus sexualis*. *J. gen. Microbiol.* **24**, 155–164.

Hunsley, D. & Burnett, J. L. 1970 The ultrastructural architecture of the walls of some hyphal fungi. *J. gen. Microbiol.* **62**, 203–218.

Jensen, W. A. 1962 *Botanical histochemistry*. W. H. Freeman and Company.

Lampen, J. O. 1968 External enzymes of yeast; their nature and formation. *Antonie van Leeuwenhoek* **34**, 1–12.

Ling-Young, M. 1930 Étude biologique des phénomènes de la sexualité chez les Mucorinées. *Rev. gén. Bot.* **42**, 722–725.

Littlefield, L. J. & Bracker, C. E. 1971 Ultrastructure of septa in *Melampsora lini*. *Trans. Br. mycol. Soc.* **56**, 181–188.

Marchant, R., Peat, A. & Banbury, G. H. 1967 The ultrastructural basis of hyphal growth. *New Phytol.* **66**, 623–629.

McClure, W. K., Park, D. & Robinson, P. M. 1967 Apical organization in the somatic hyphae of fungi. *J. gen. Microbiol.* **50**, 177–182.

Mollenhauer, H. H., Morré, D. J. & Kelly, A. G. 1966 The widespread occurrence of plant cytosomes resembling animal microbodies. *Protoplasma*, **62**, 44–52.

Moore, R. T. & McAlear, J. H. 1961 Fine structure of mycota, 5. Lomasomes – previously uncharacterized hyphal structures. *Mycologia* **53**, 194–200.

Newcomb, E. H. 1969 Plant microtubules. *Ann. Rev. Plant Physiol.* **20**, 253–288.

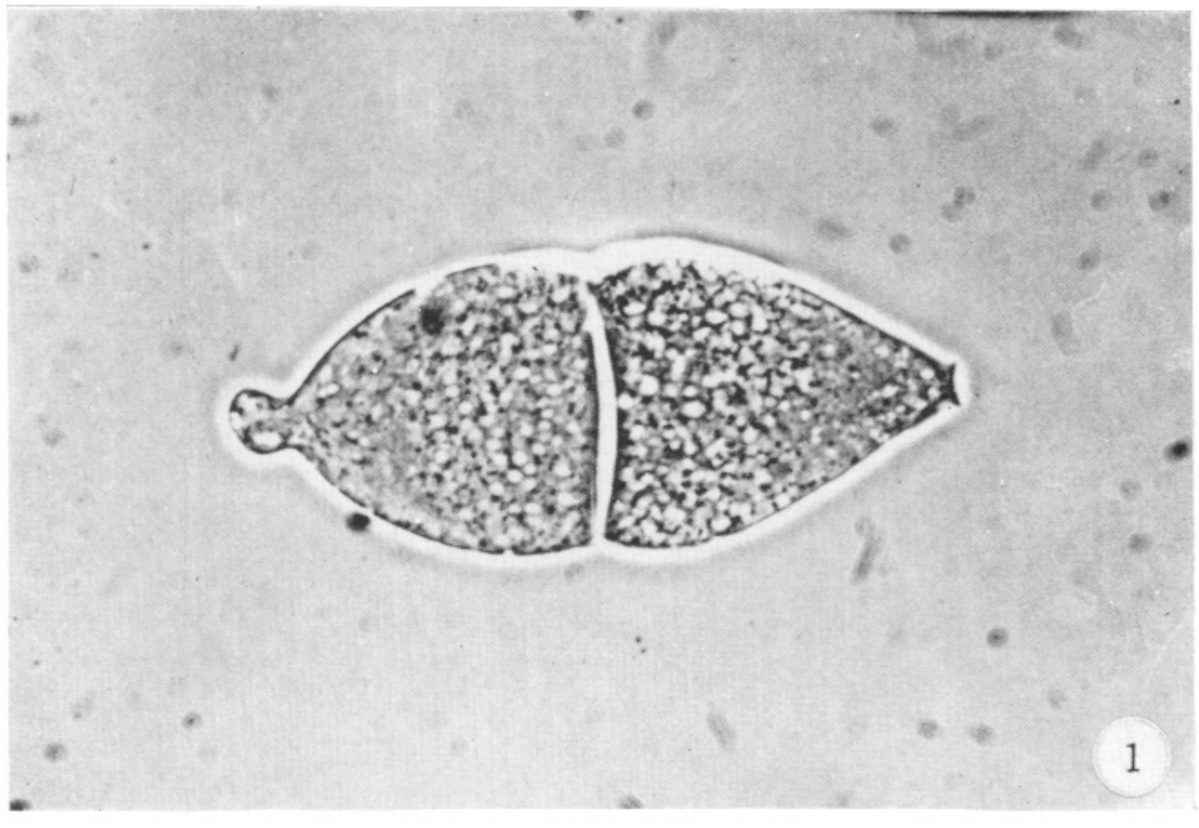
Peat, A. & Banbury, G. H. 1967 Ultrastructure, protoplasmic streaming, growth and tropisms of *Phycomyces* sporangiophores. *New Phytol.* **66**, 475–484.

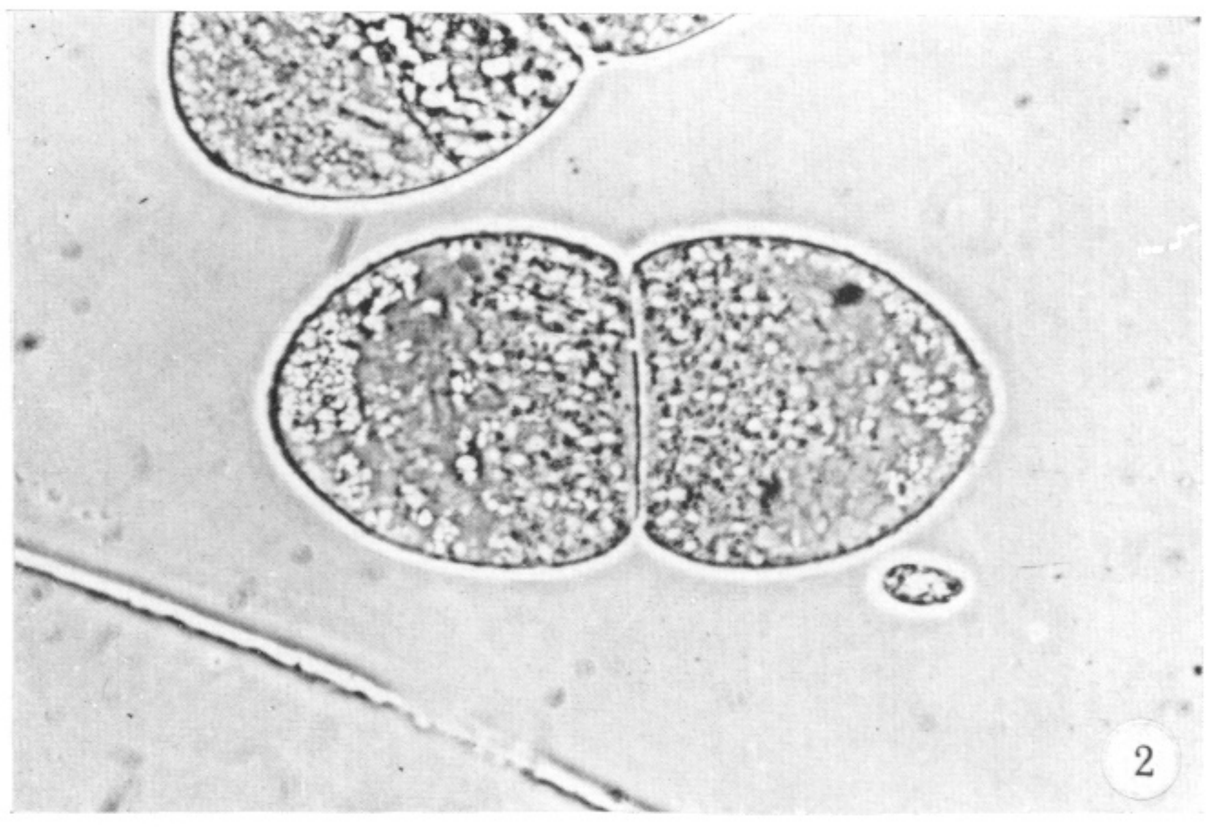
Reynolds, E. S. 1963 The use of lead citrate at a high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208–212.

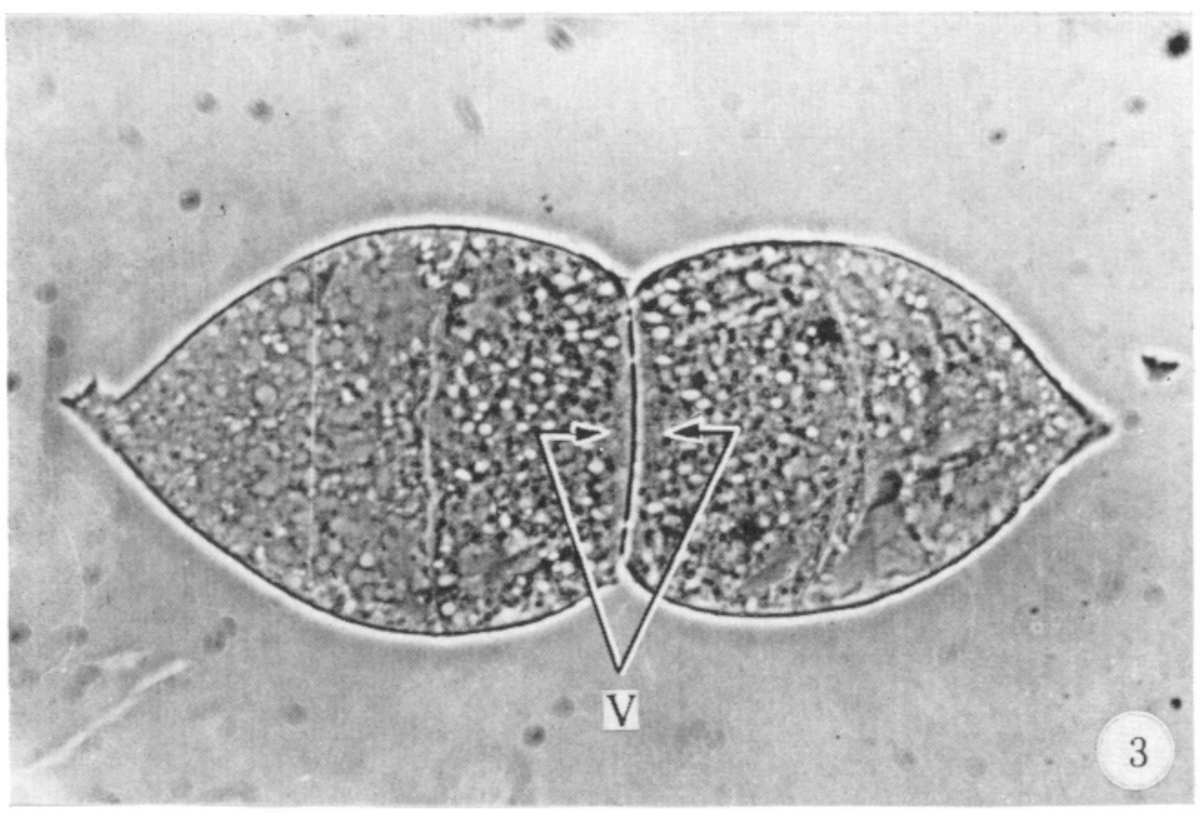
Sassen, M. M. A. 1962 Breakdown of cell wall in zygote formation of *Phycomyces blakesleeanus*. *Proc. K. ned. Akad. Wet.* **65**, 447–452.

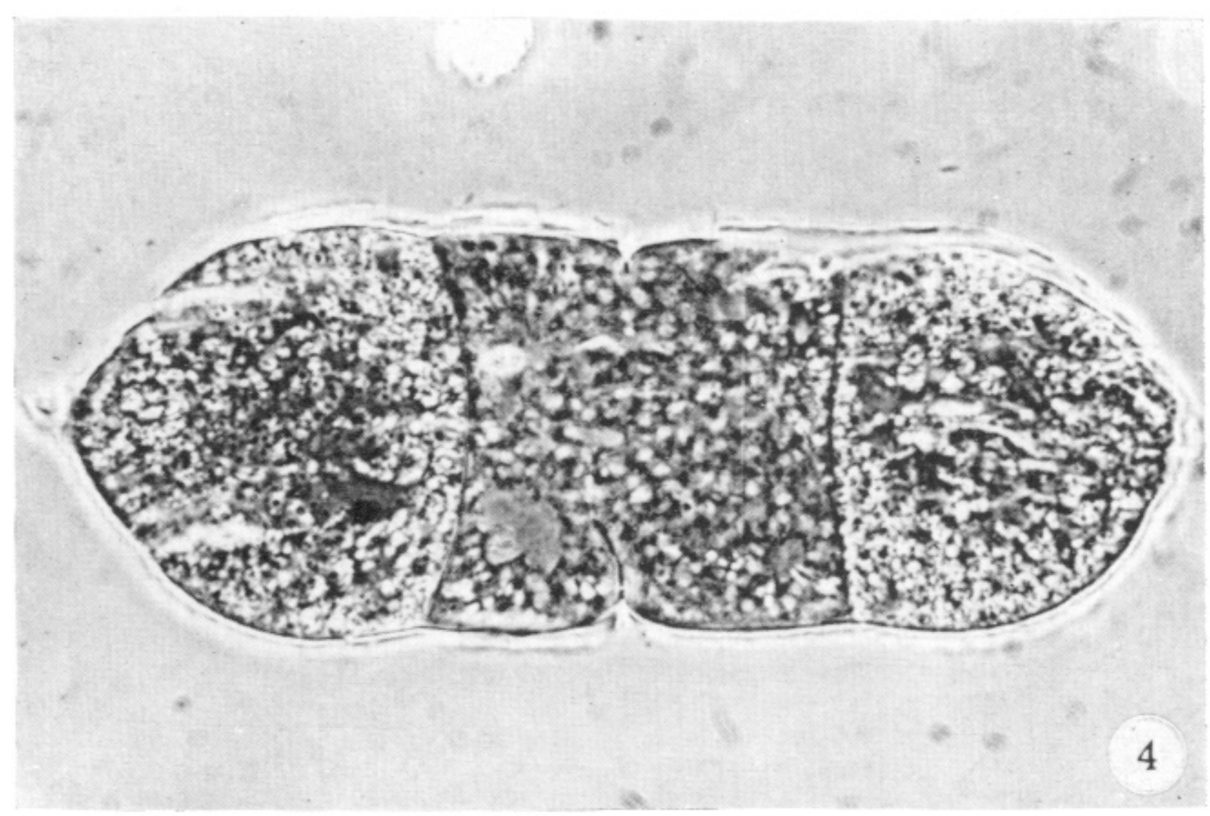
Vuillemin, P. 1904 Recherches morphologiques et morphogeniques sur la membrane des zygospores. *Ann. Mycol.* **2**, 483–506.

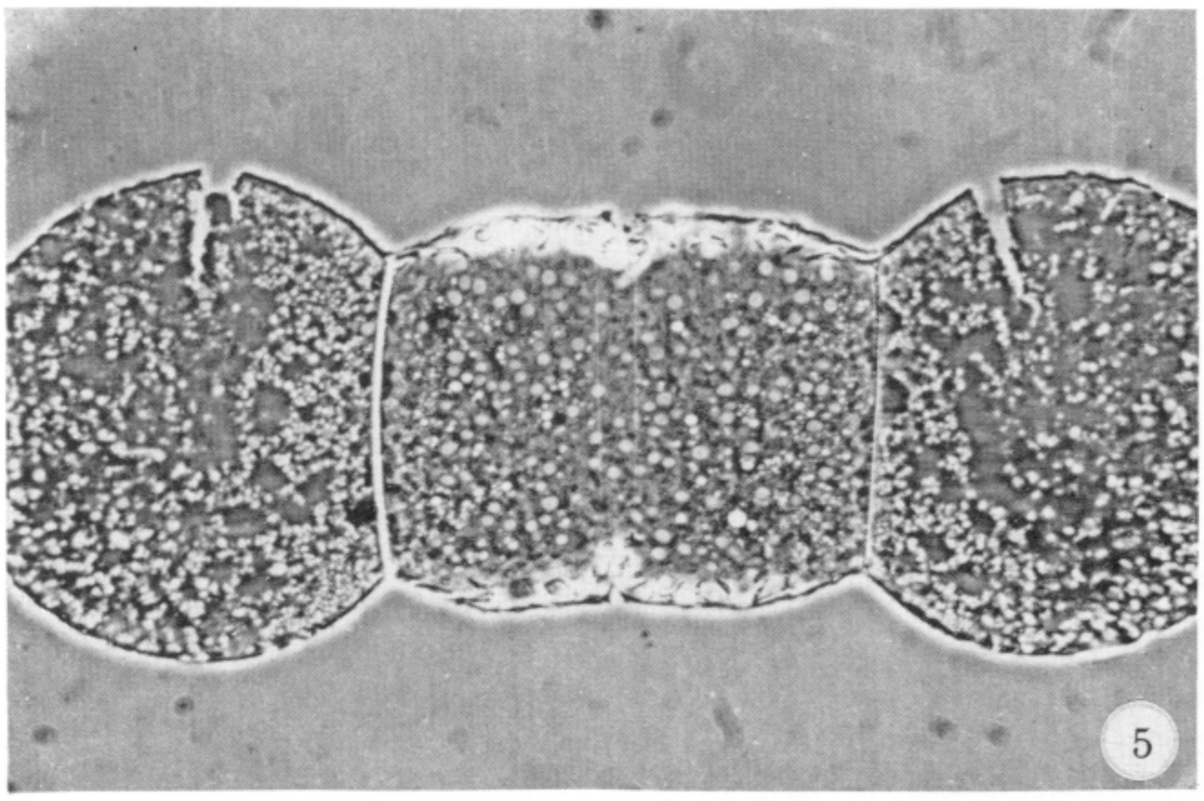
Wilsenach, R. & Kessel, M. 1965 The role of lomasomes in wall formation in *Penicillium vermiculatum*. *J. gen. Microbiol.* **40**, 401–440.

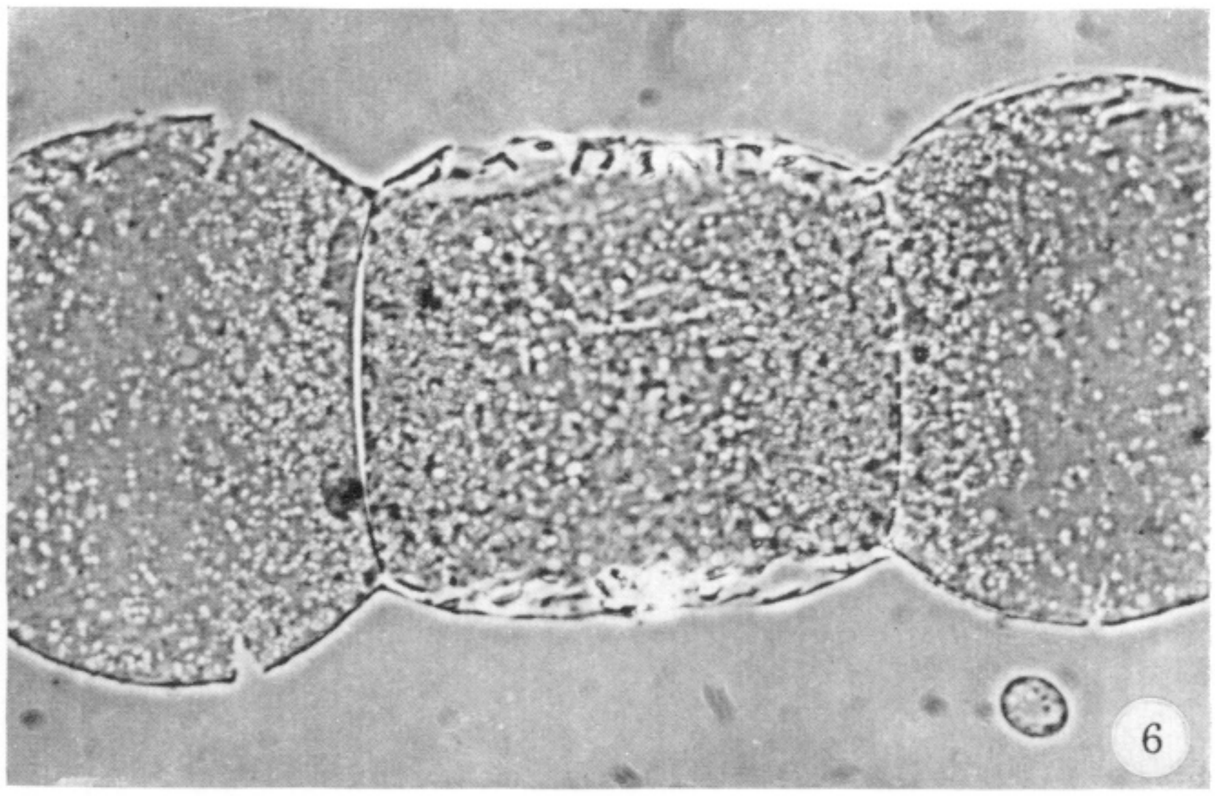


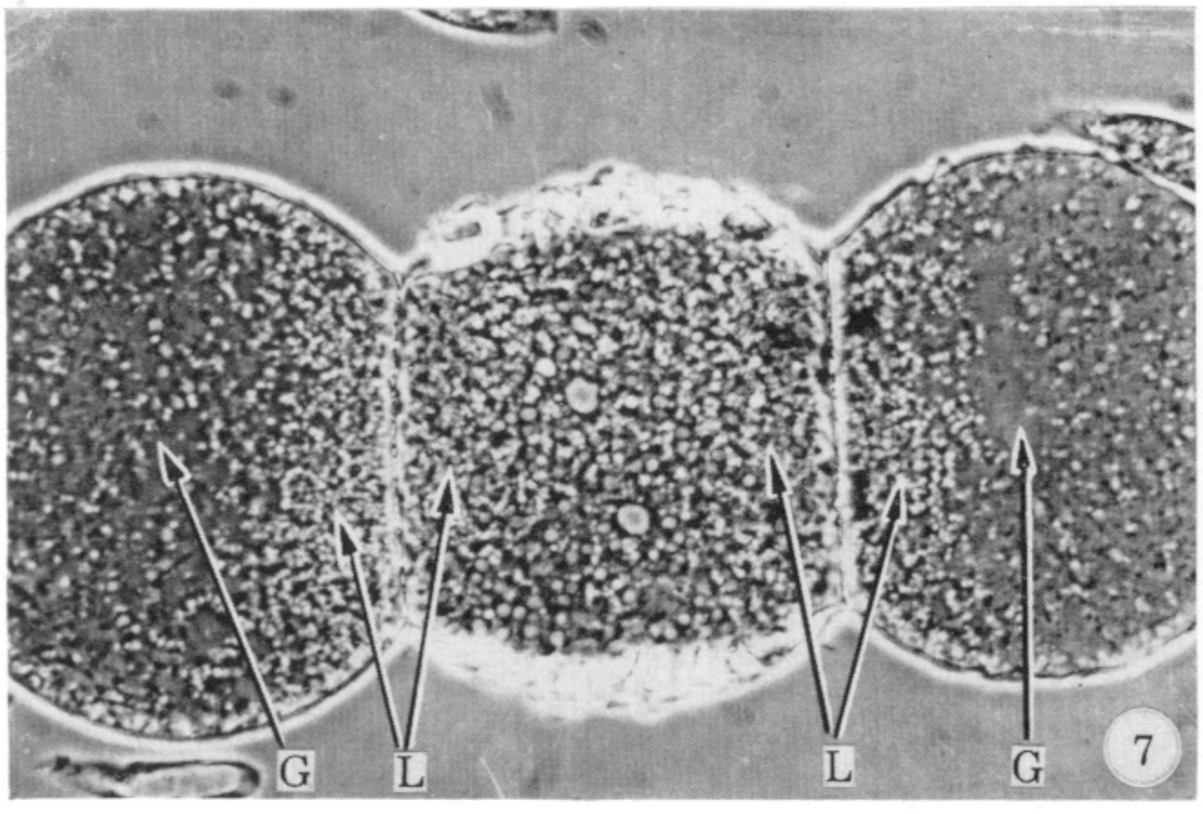


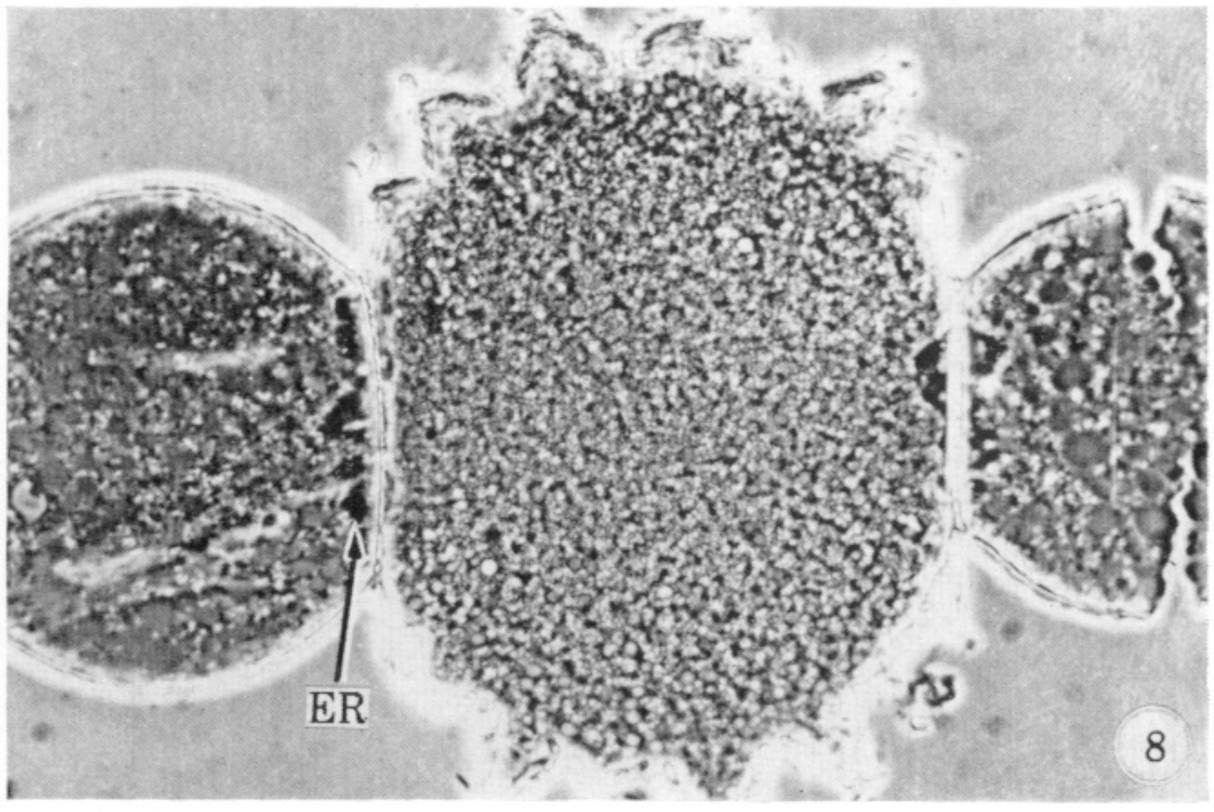




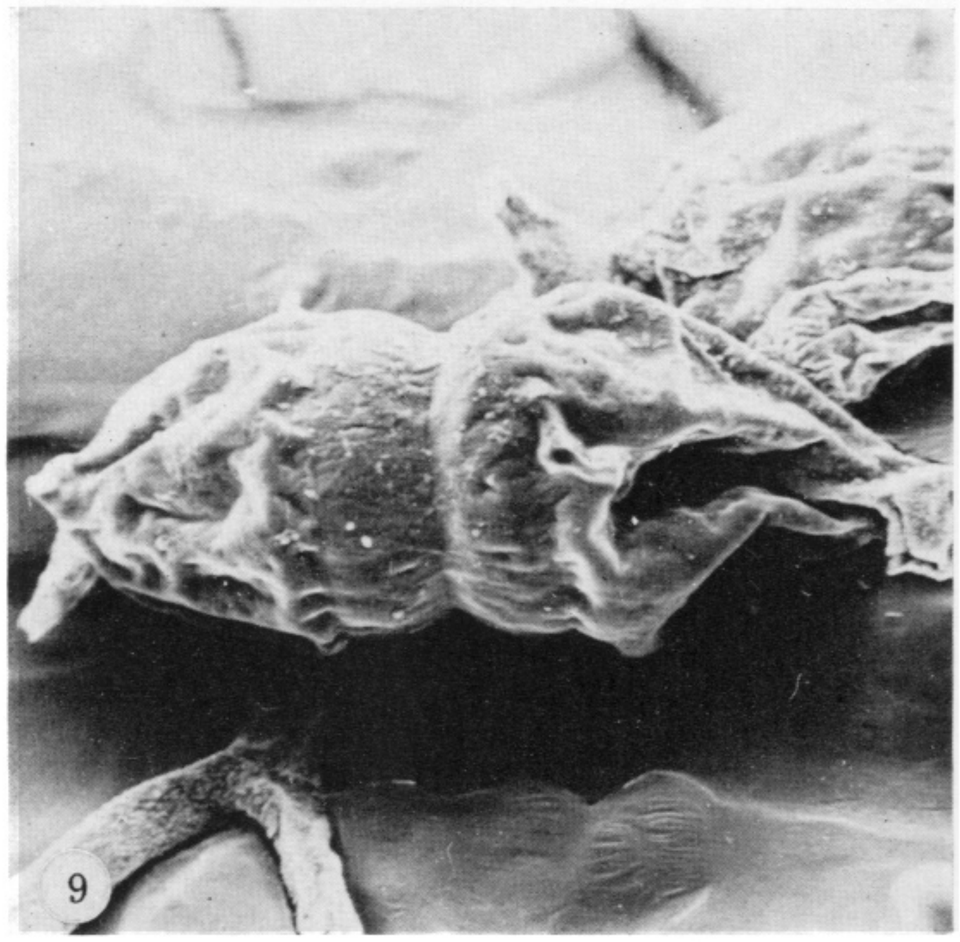


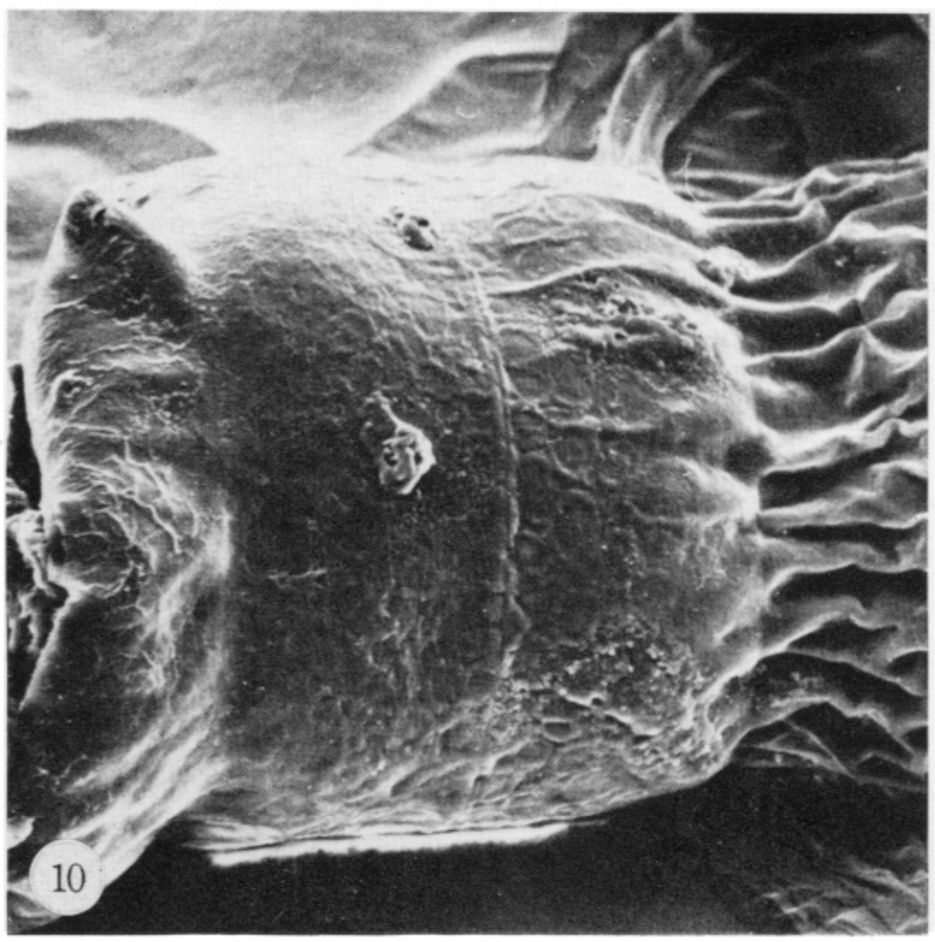


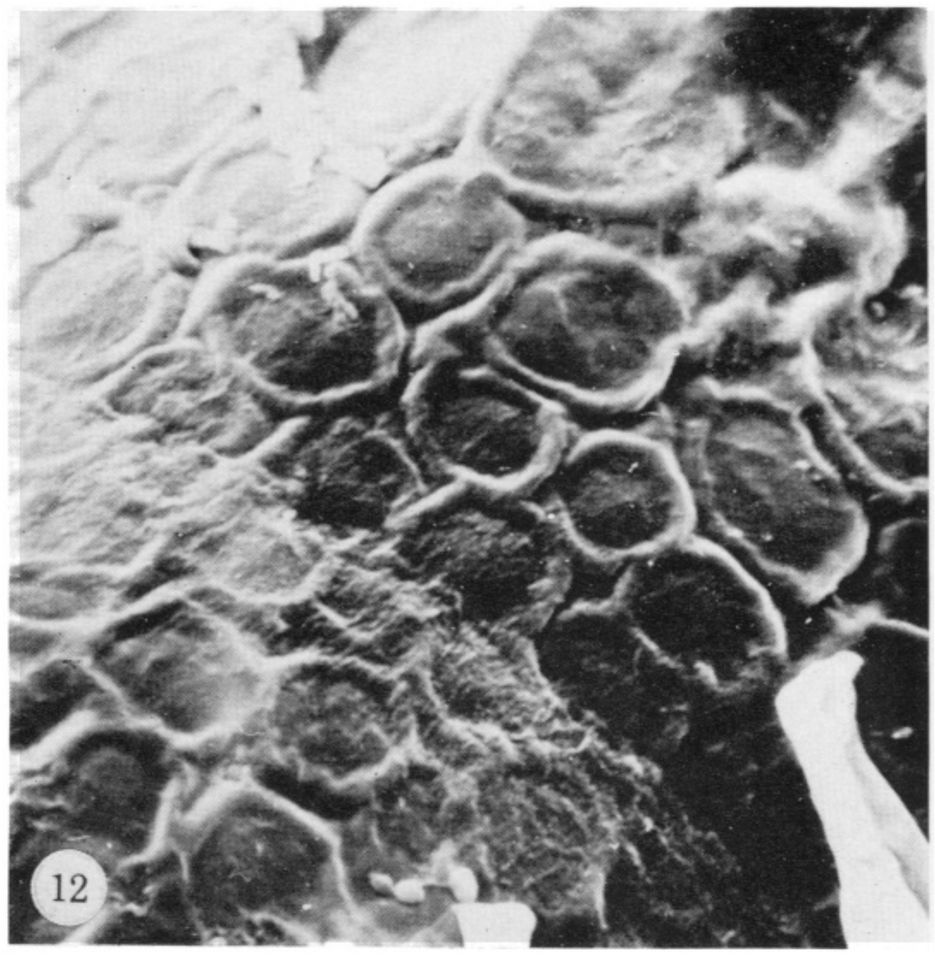


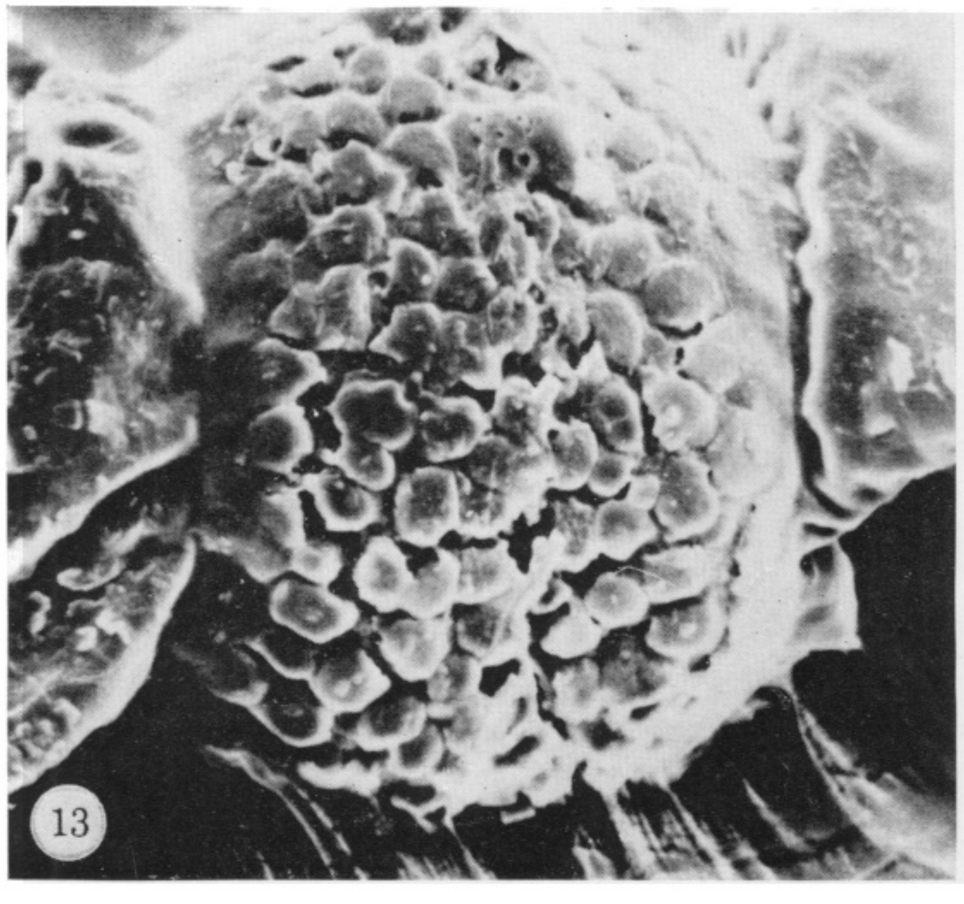


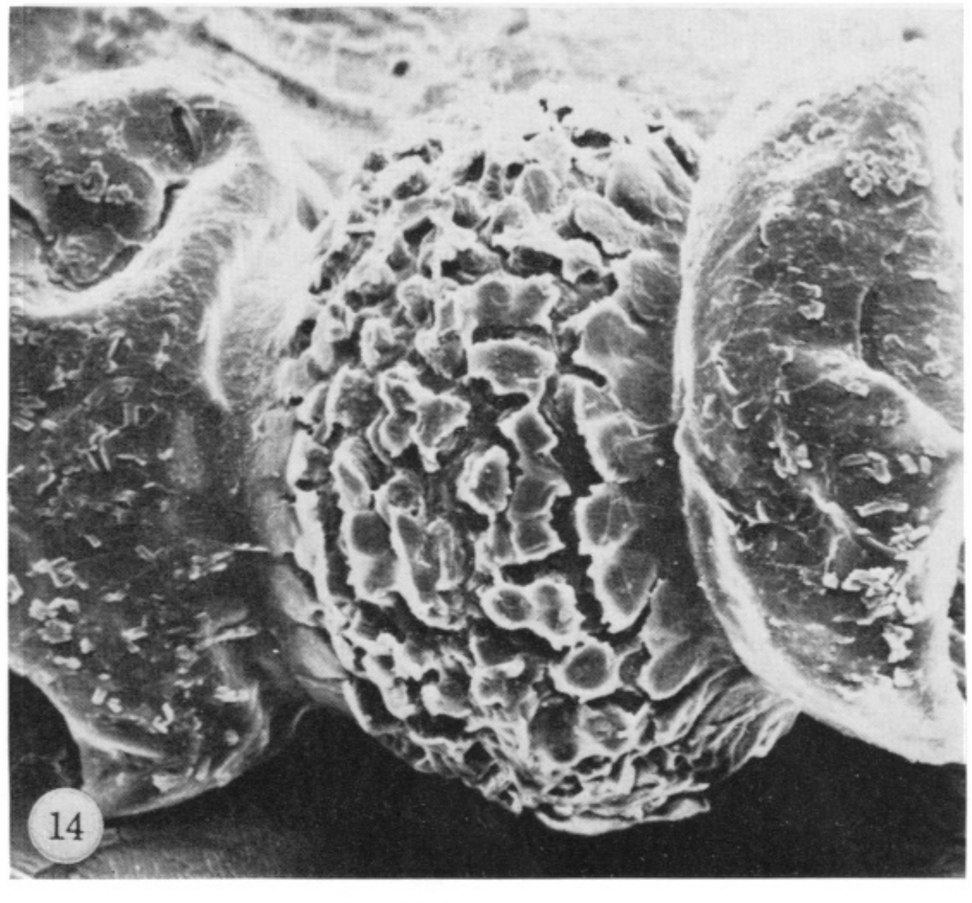
FIGURES 1 to 8. For legends see facing page

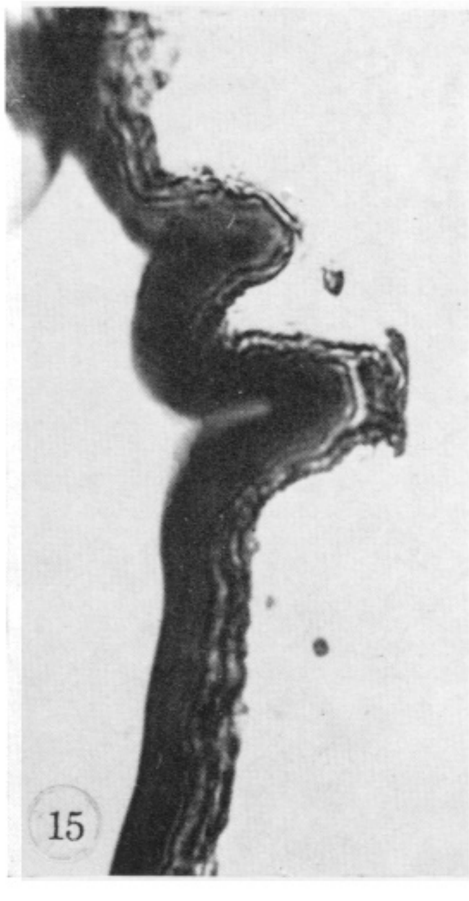




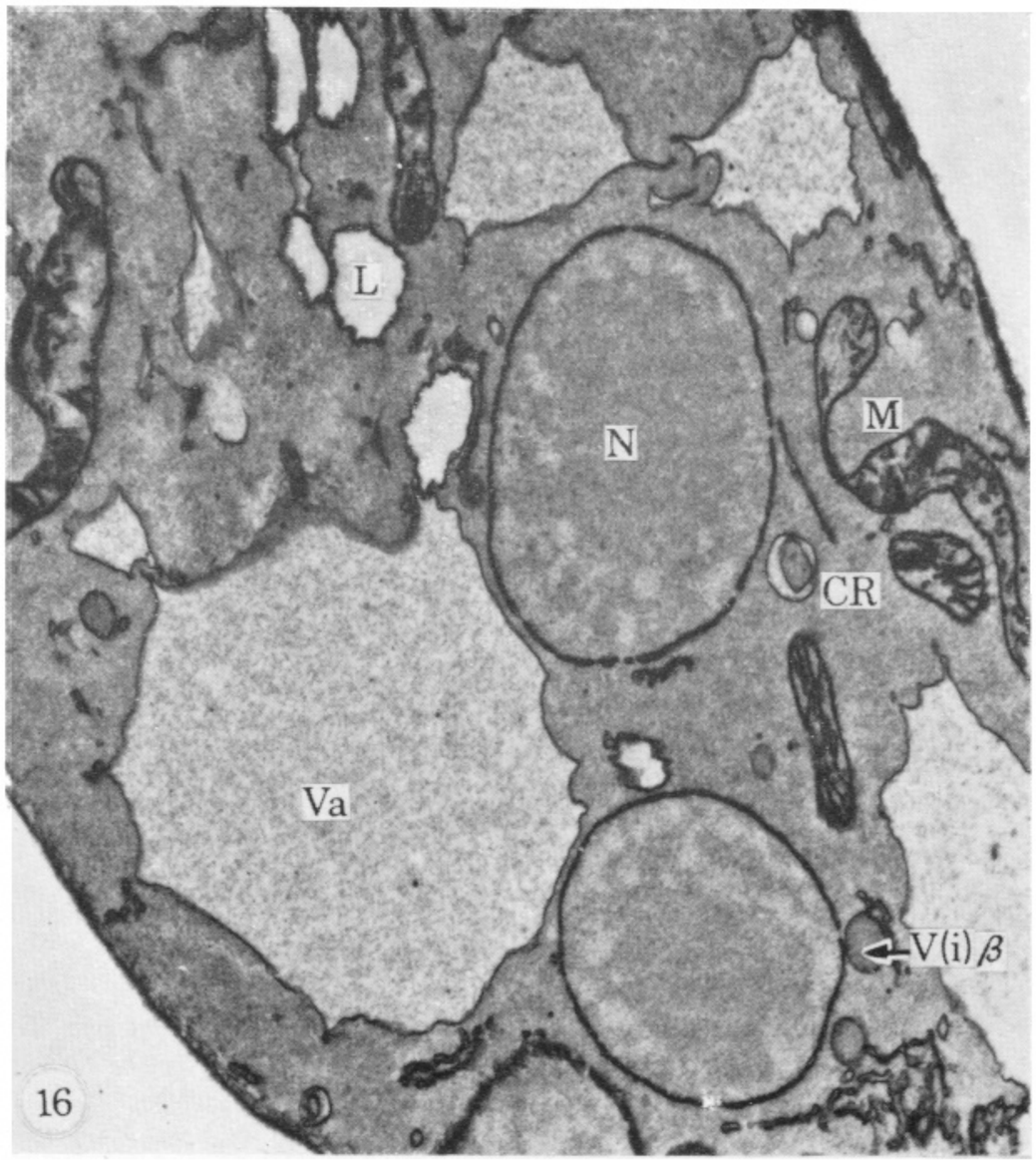




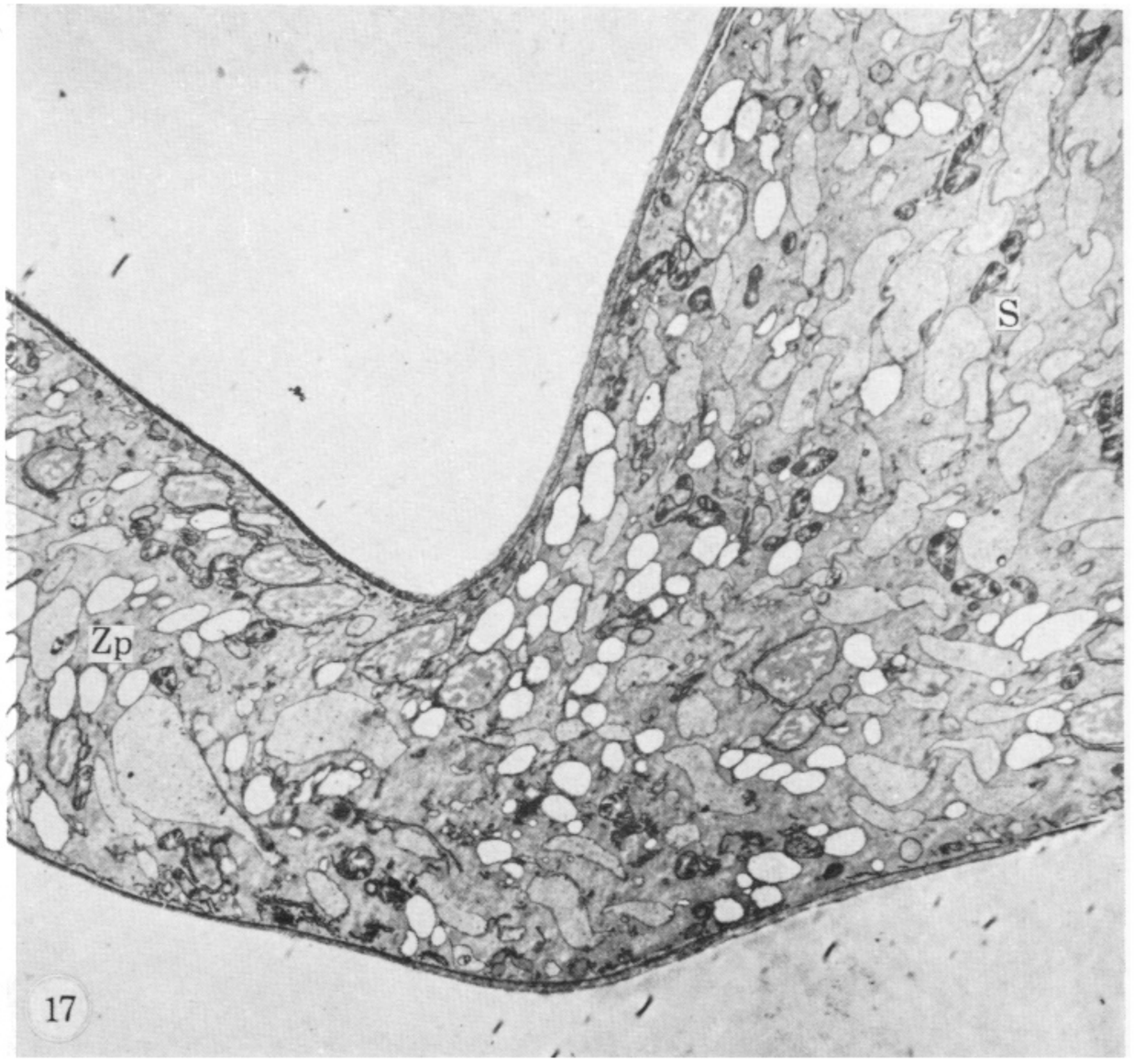




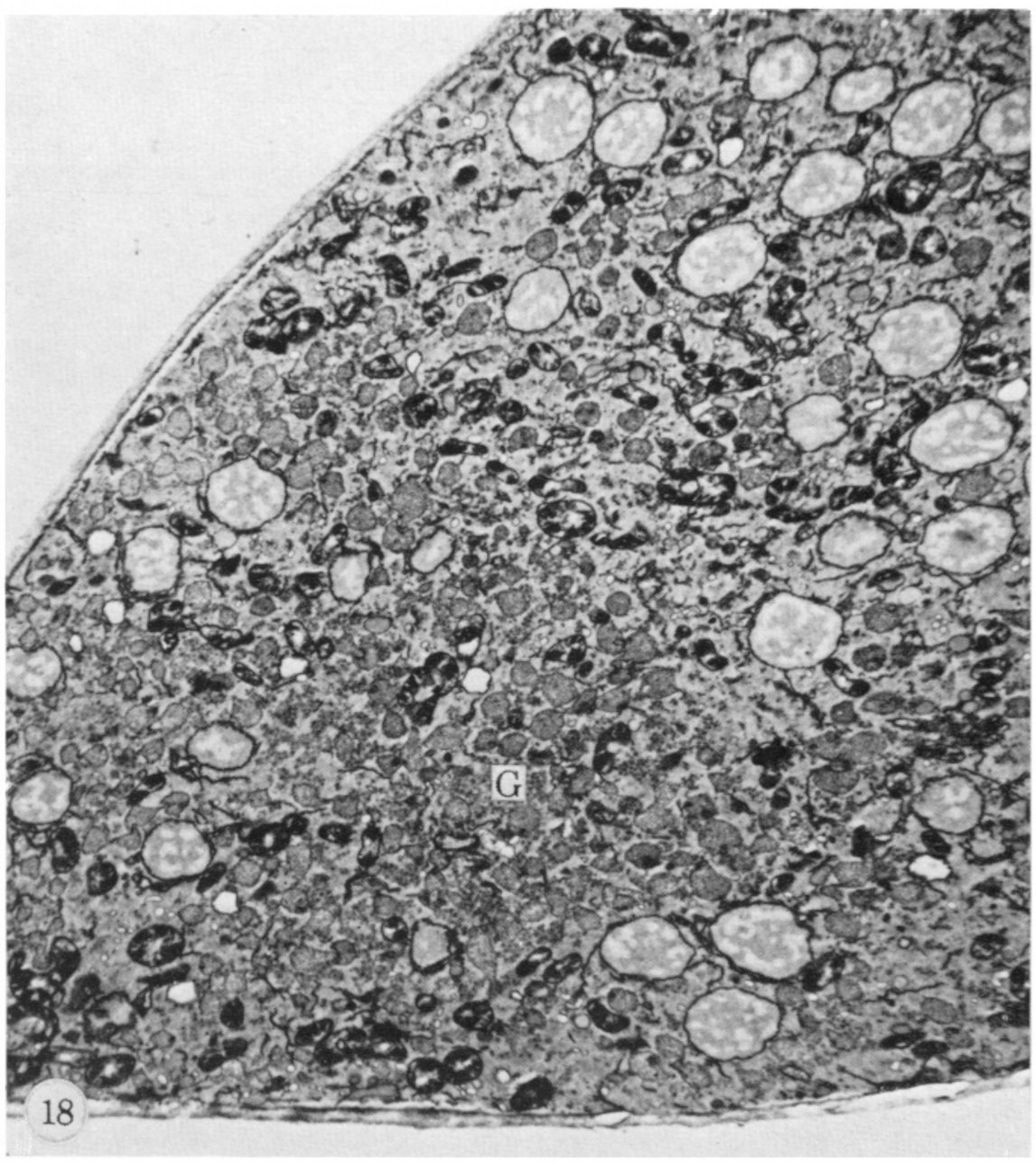
FIGURES 9 to 15. For legends see facing page



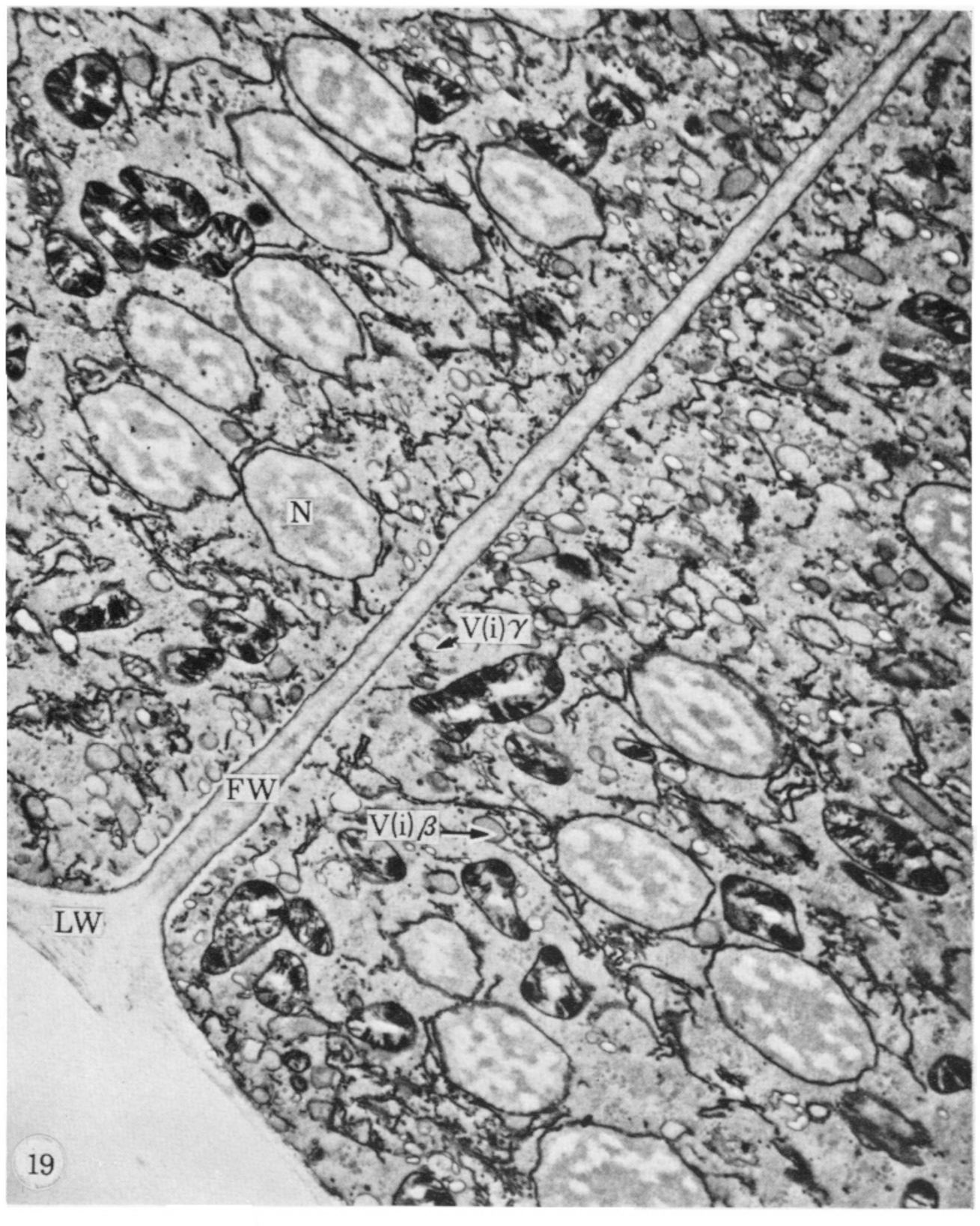
16



17

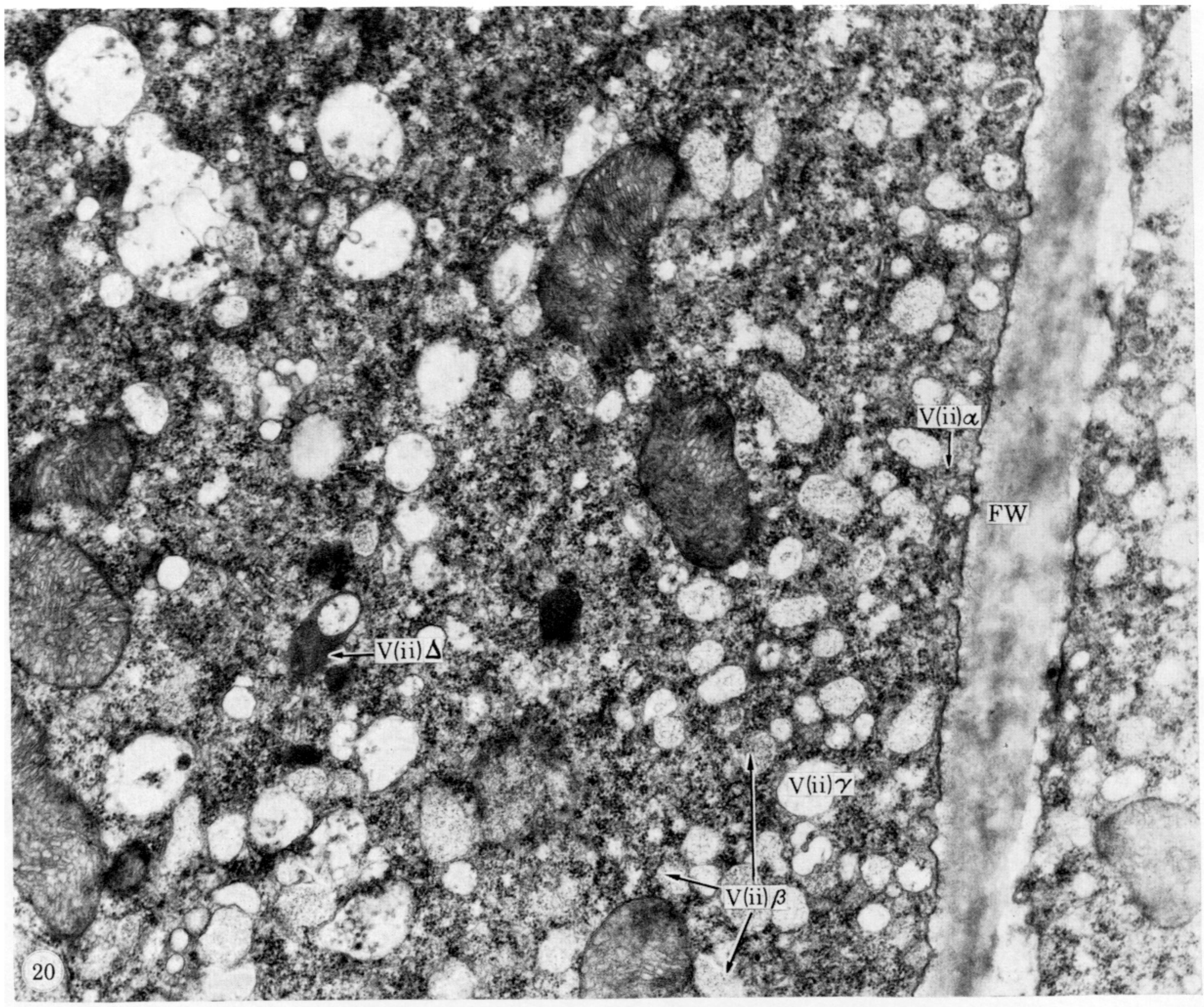


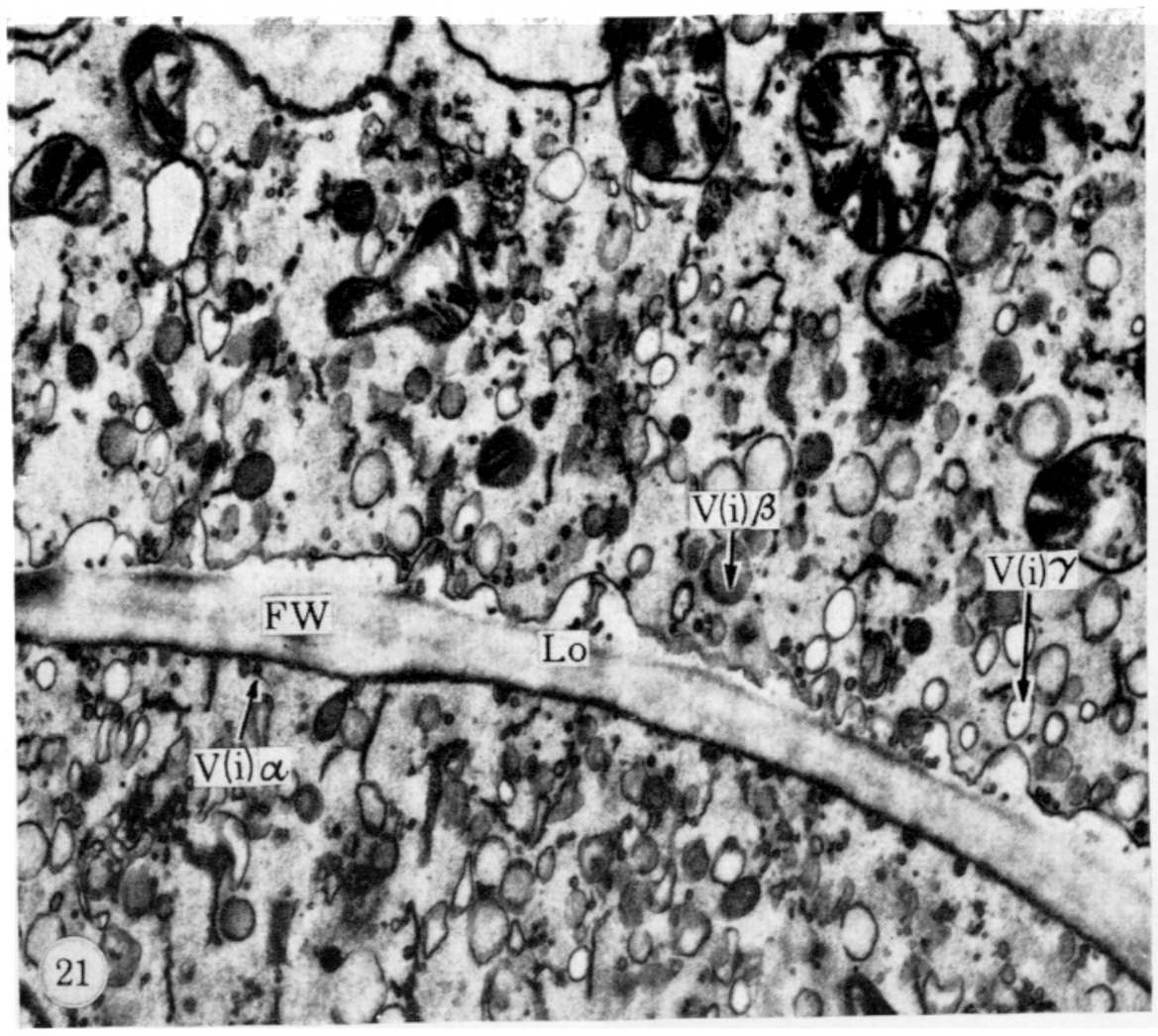
18

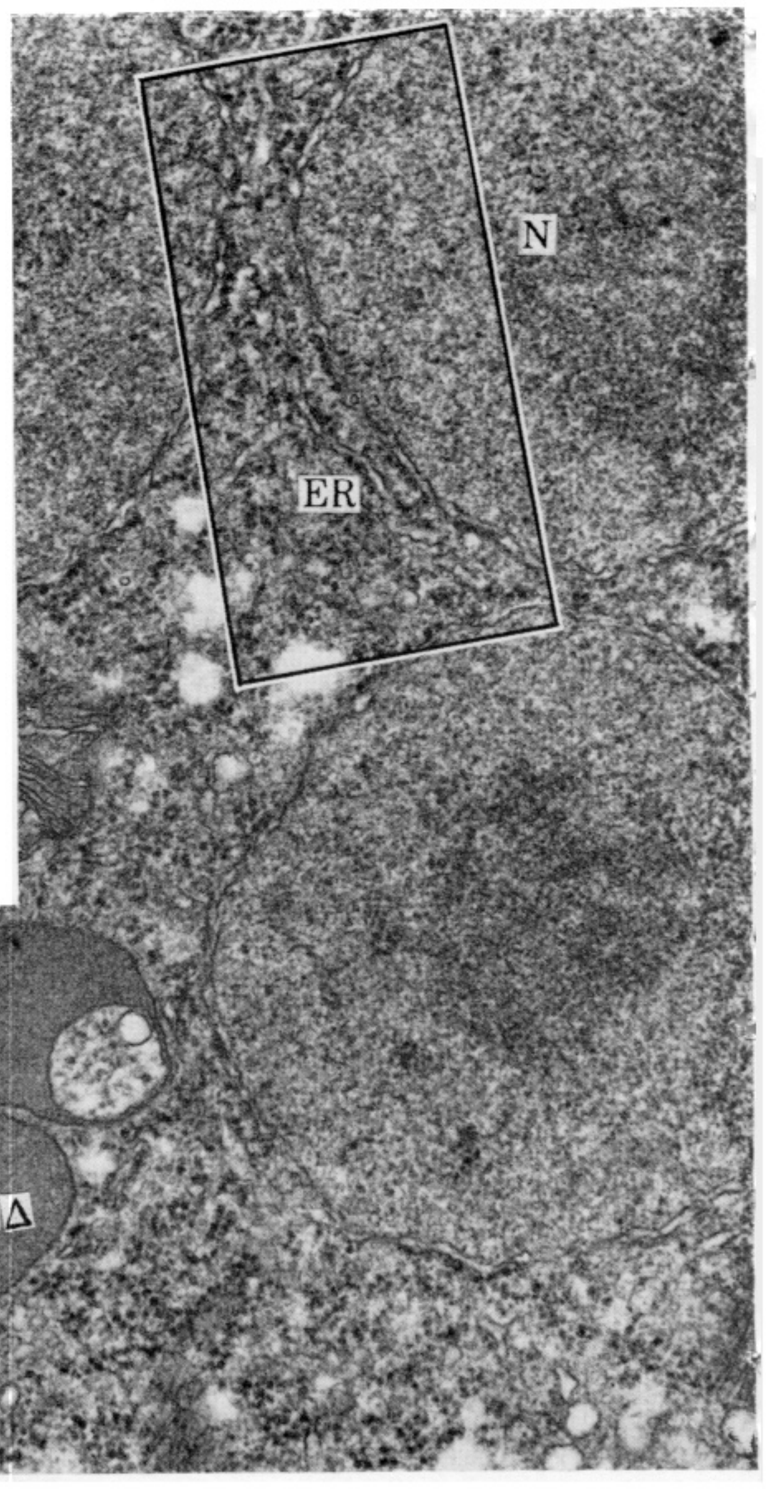


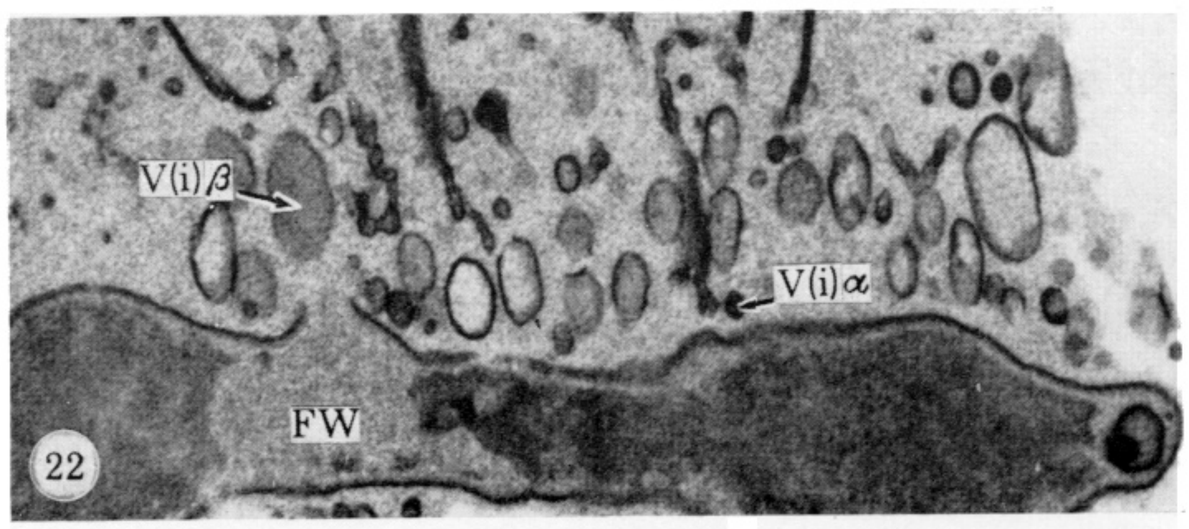
19

FIGURES 16 to 19. For legends see facing page

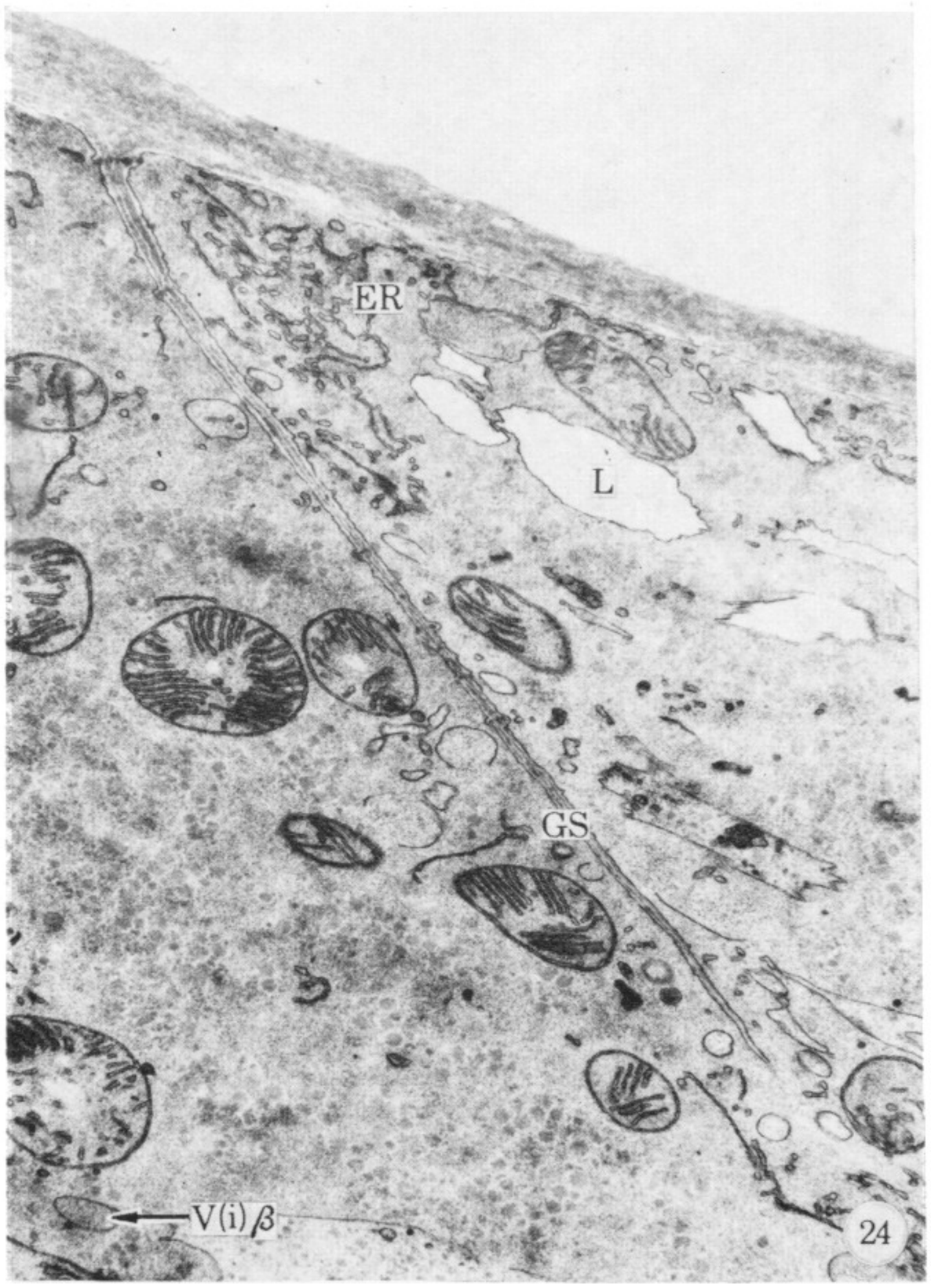


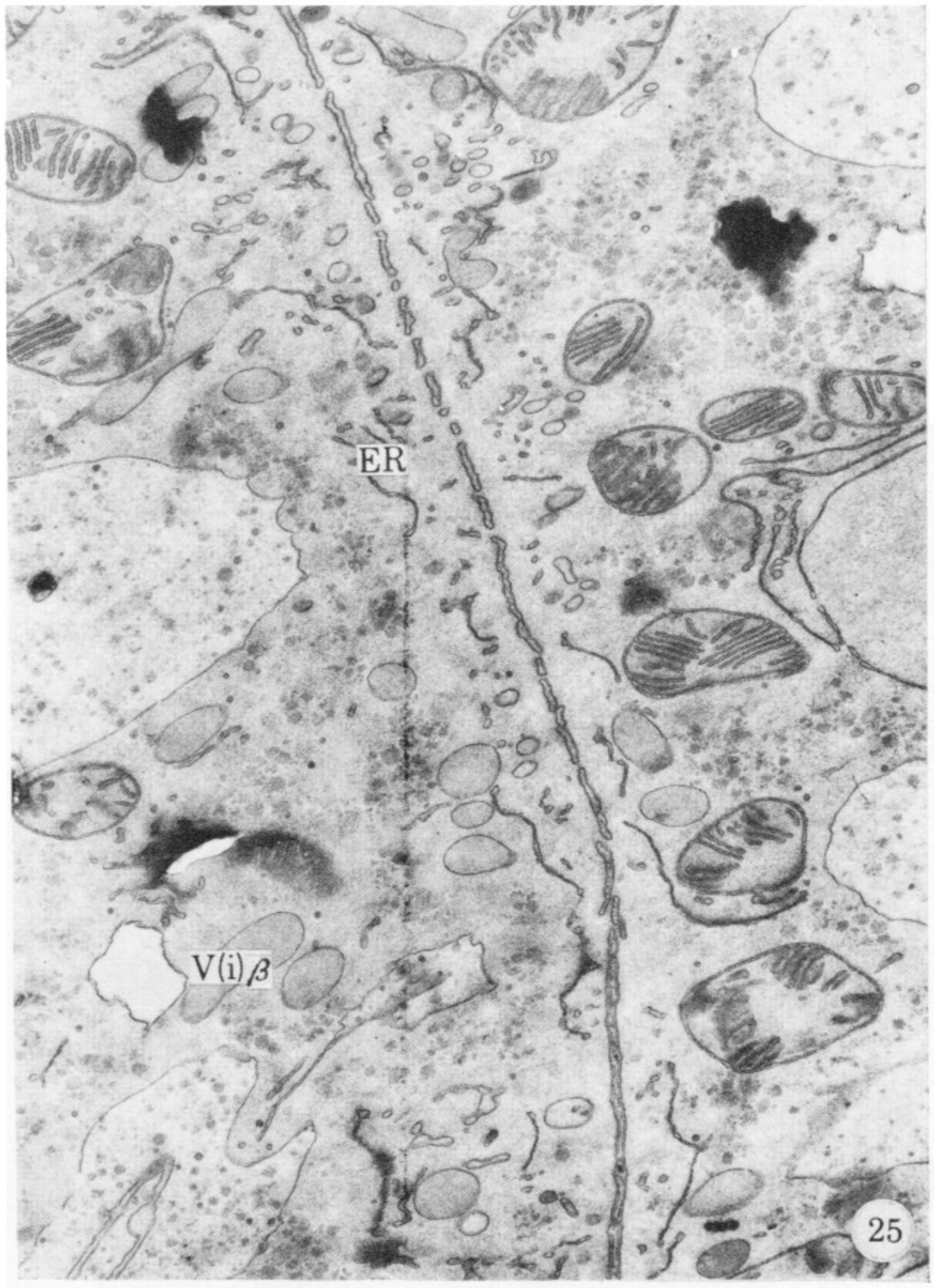


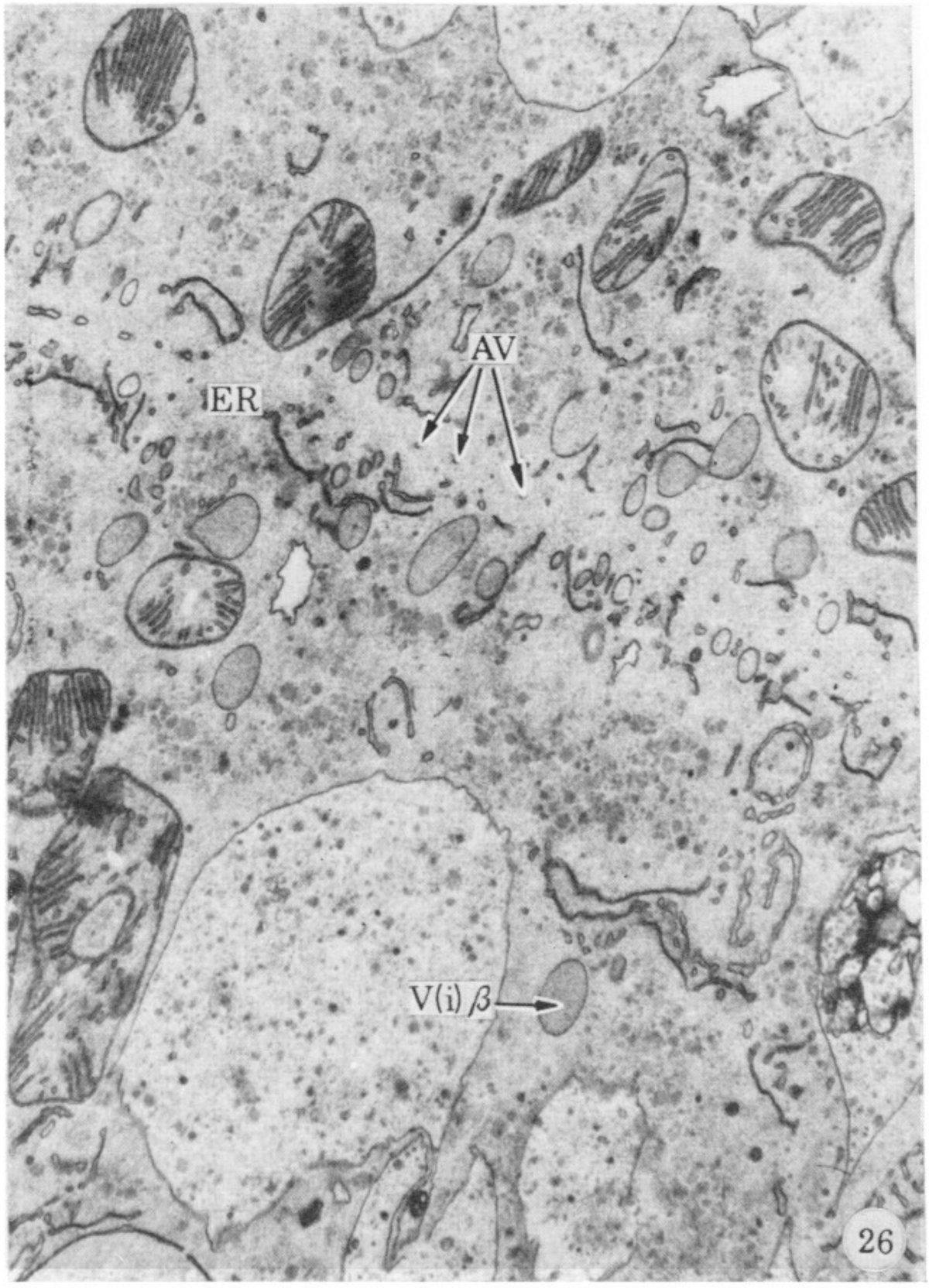


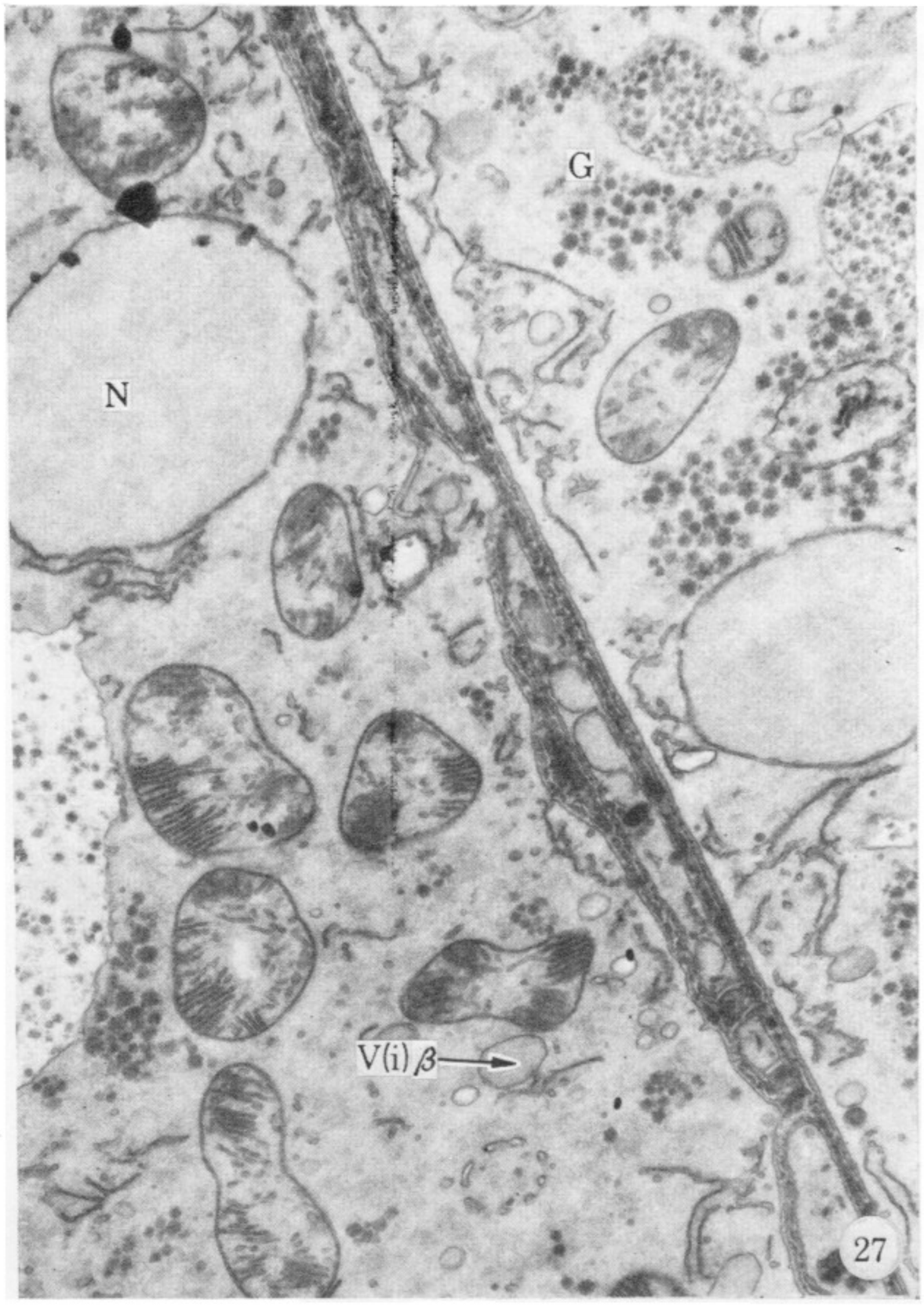


FIGURES 20 to 23. For legends see facing page

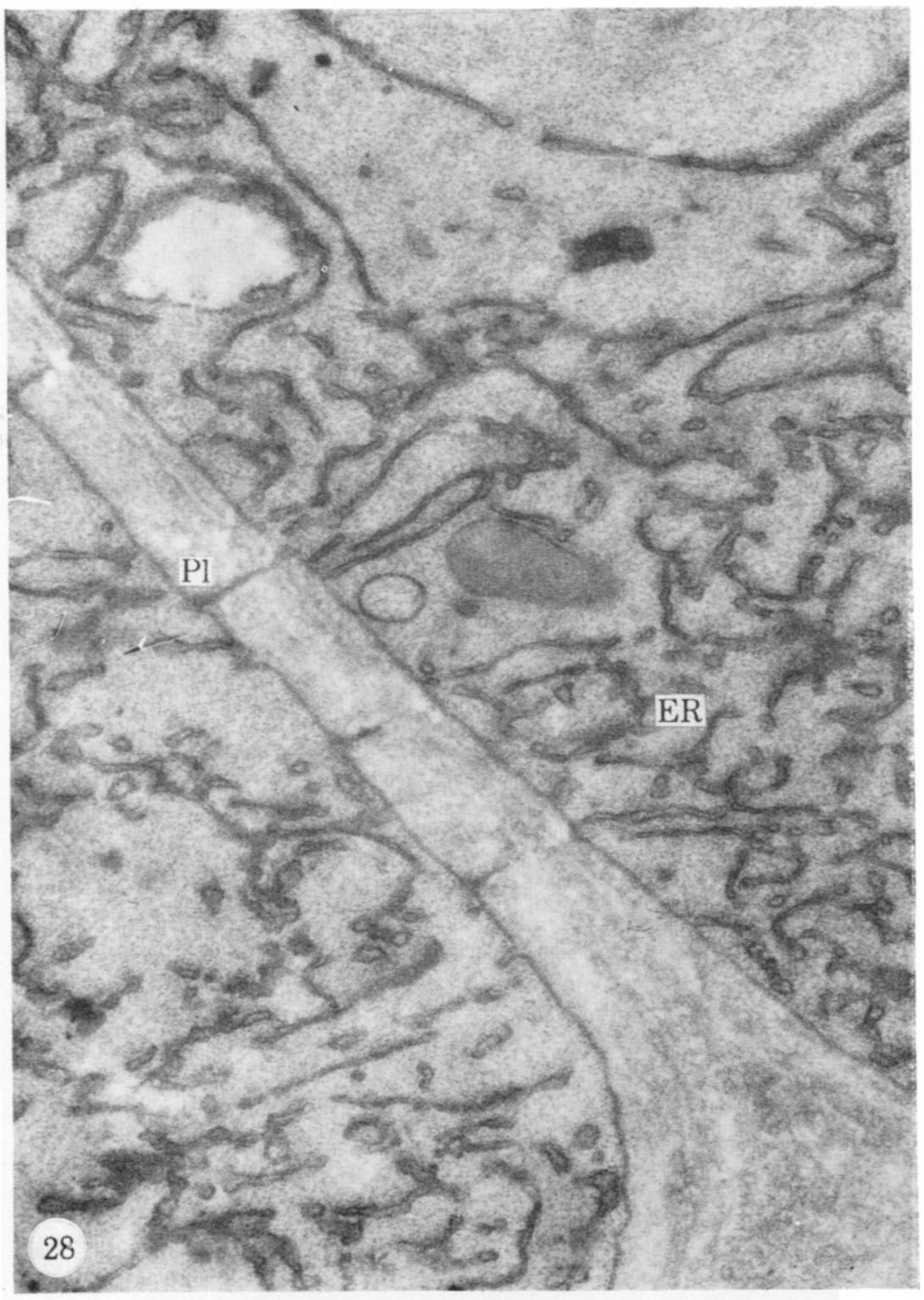


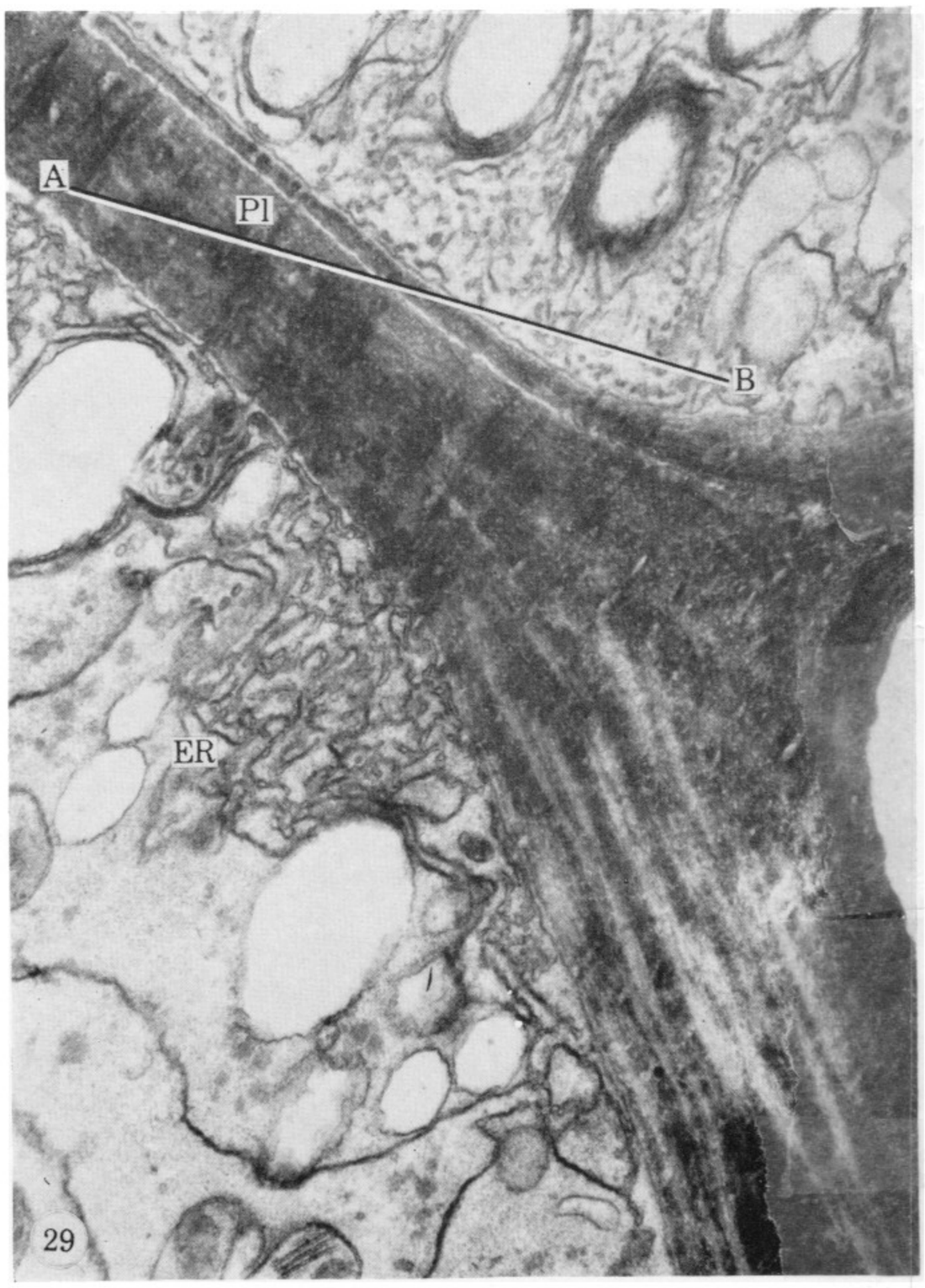


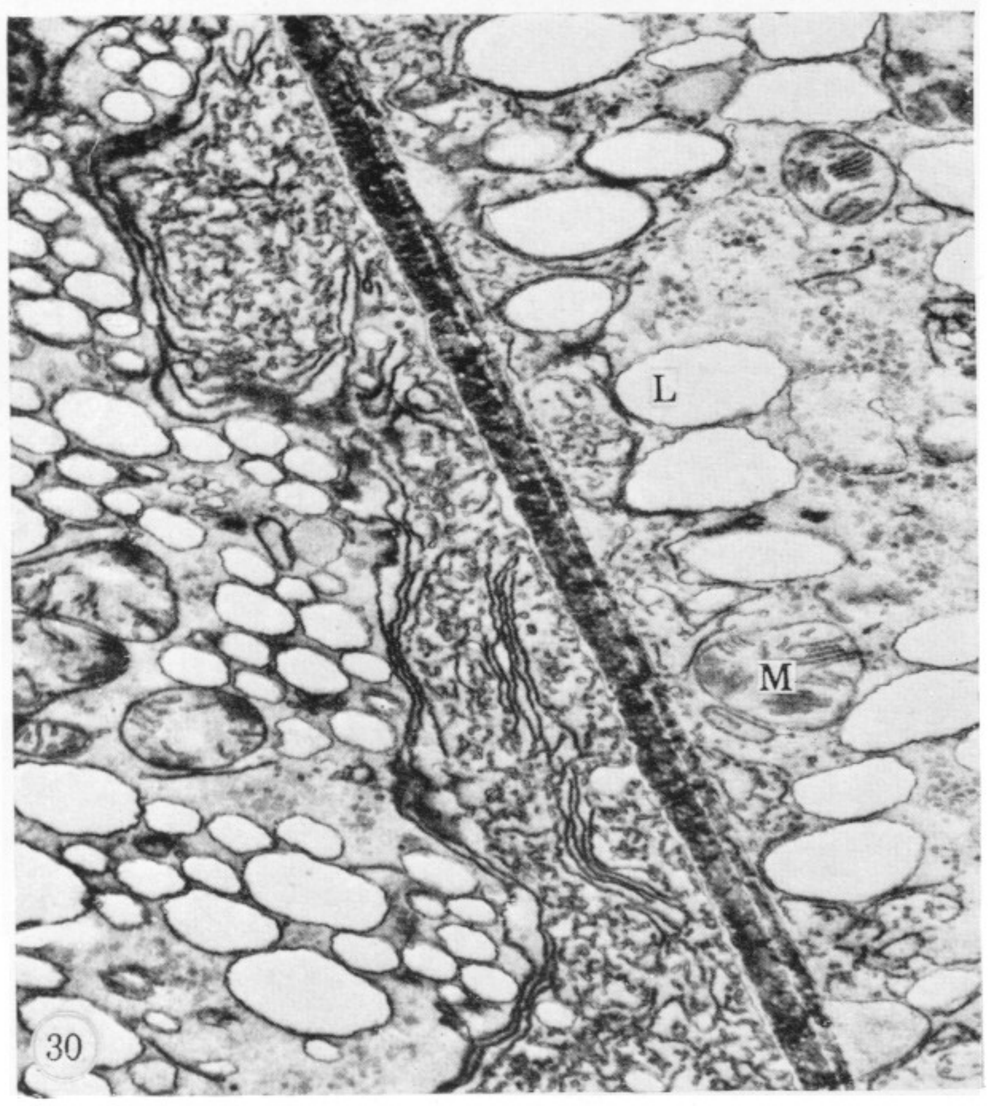


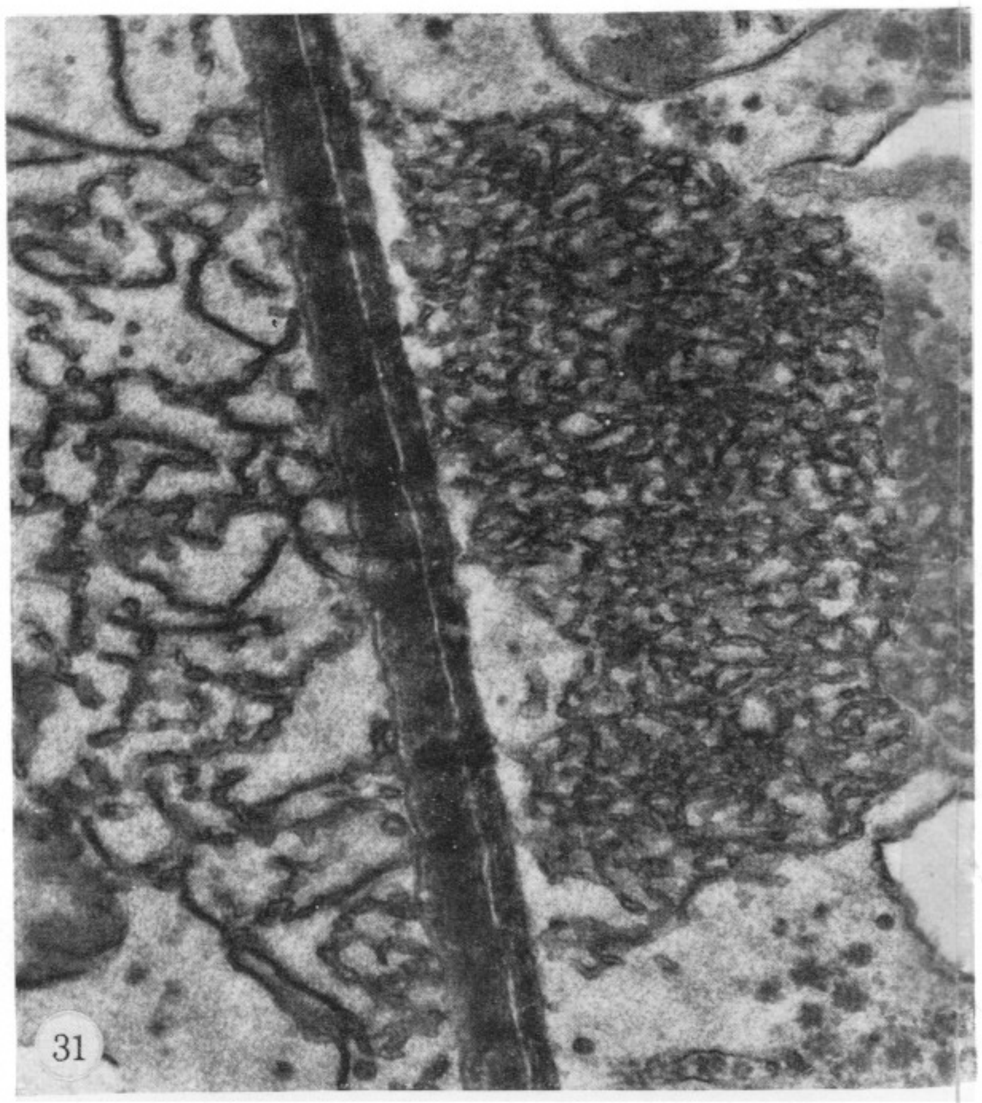


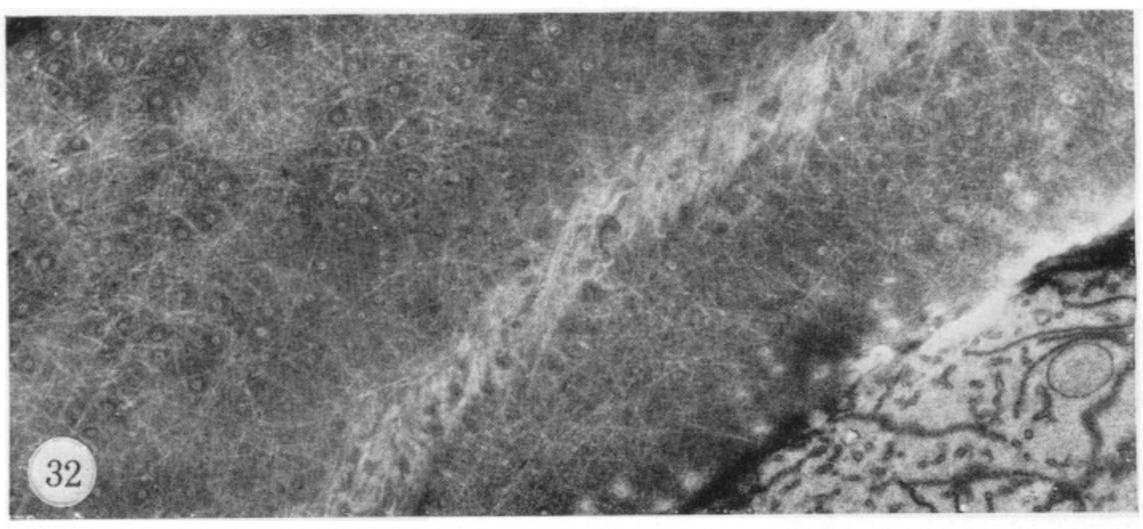
FIGURES 24 to 27. For legends see facing page

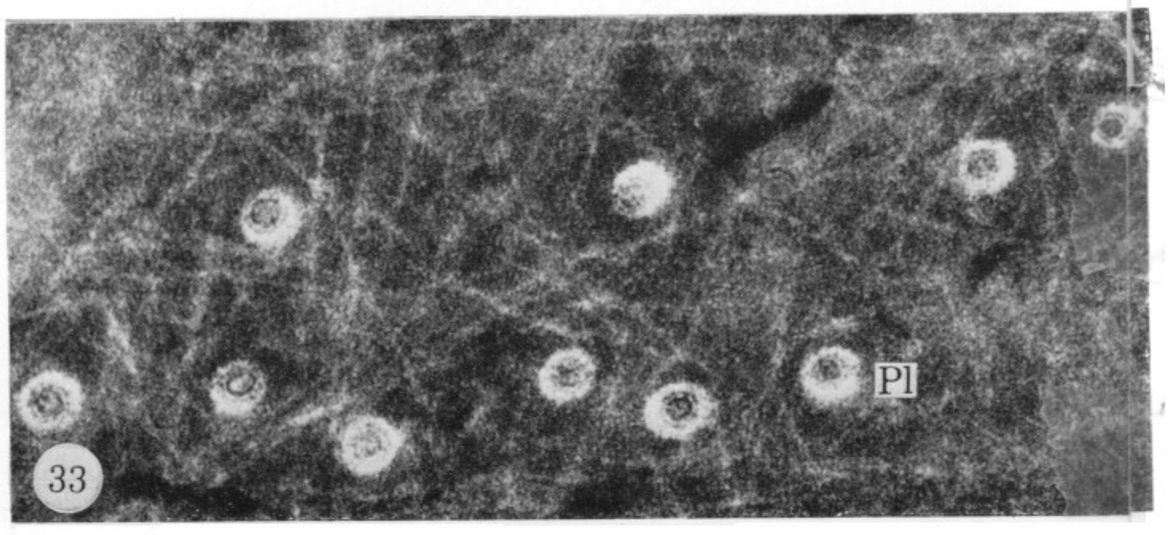




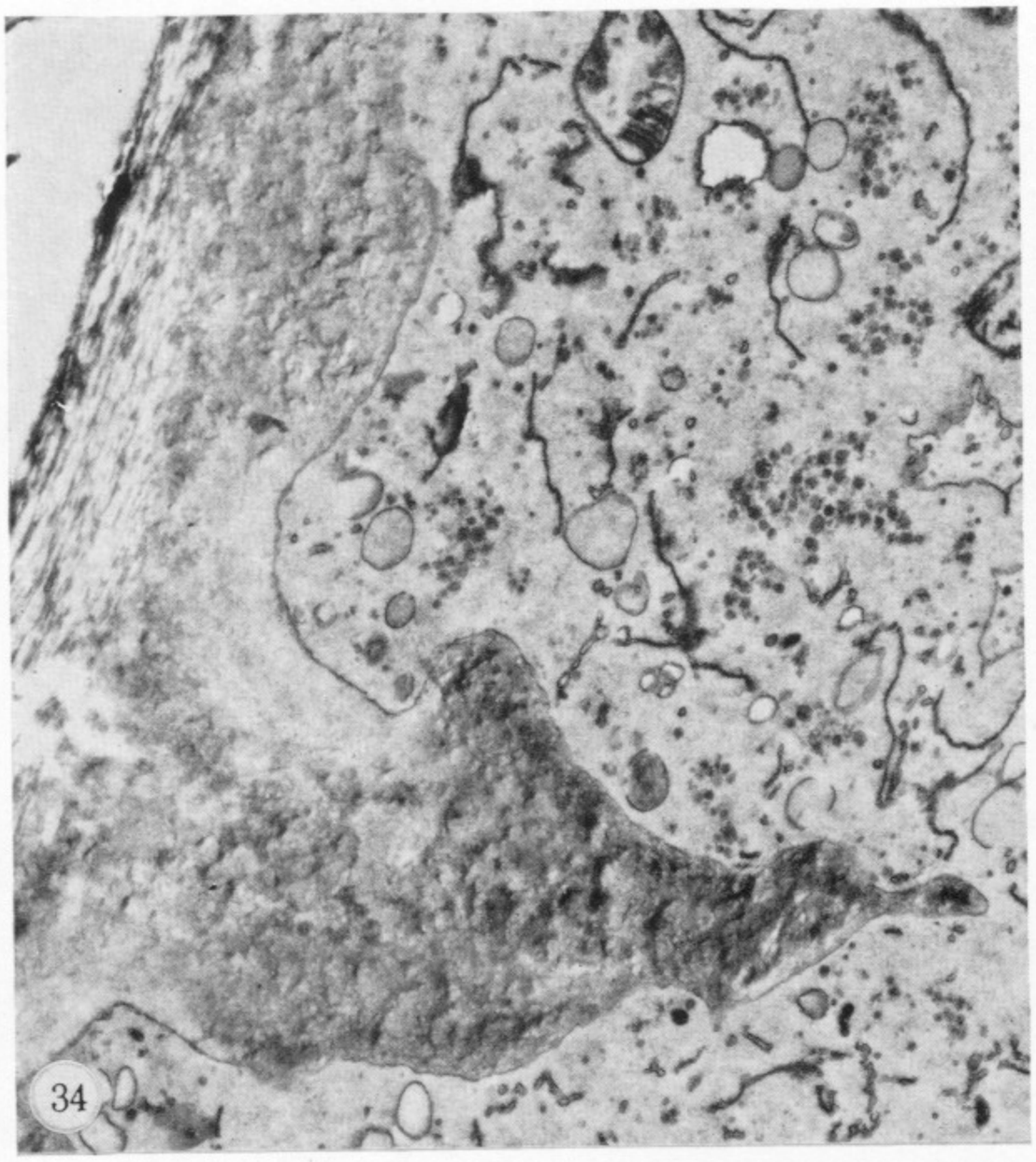


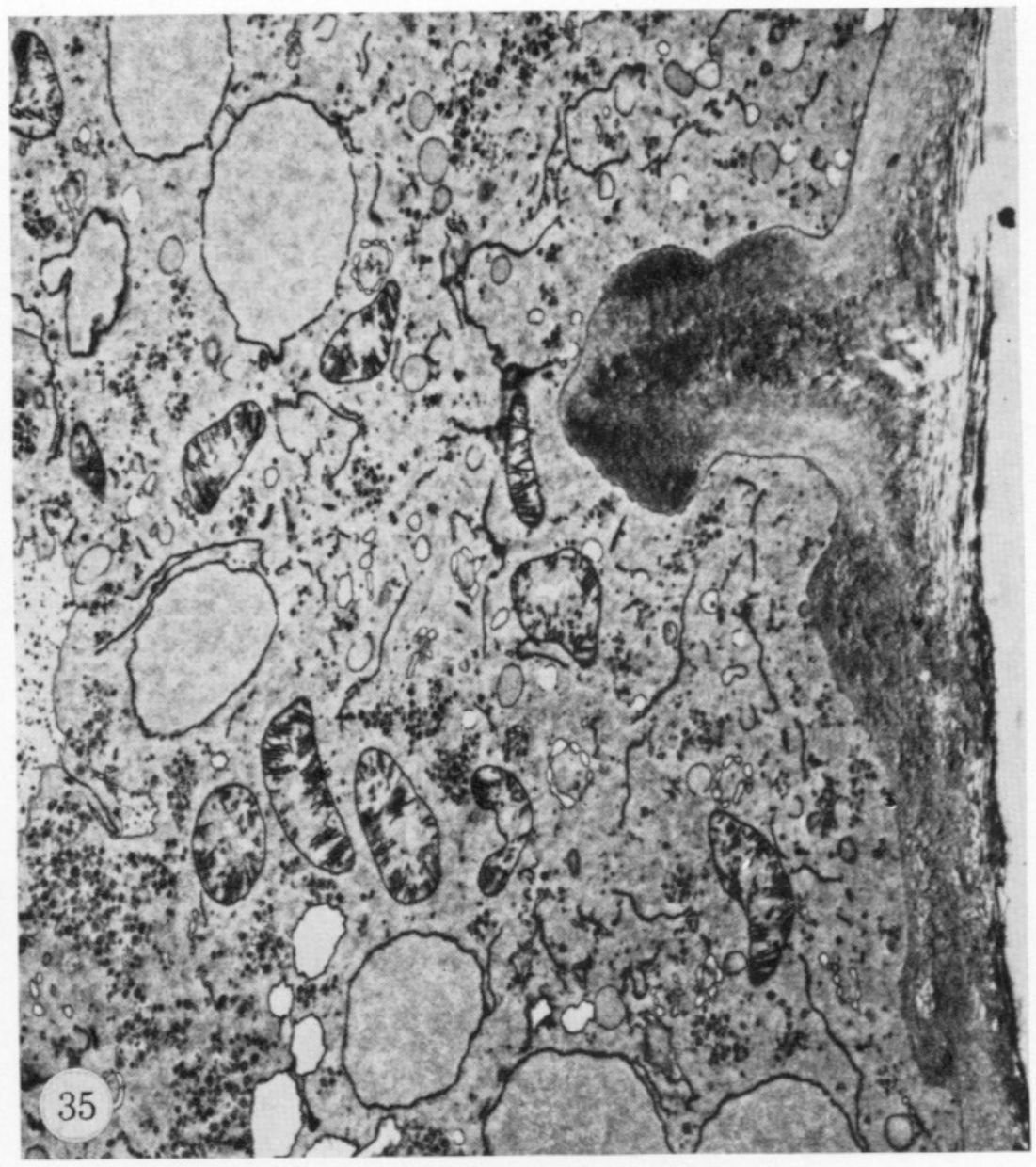


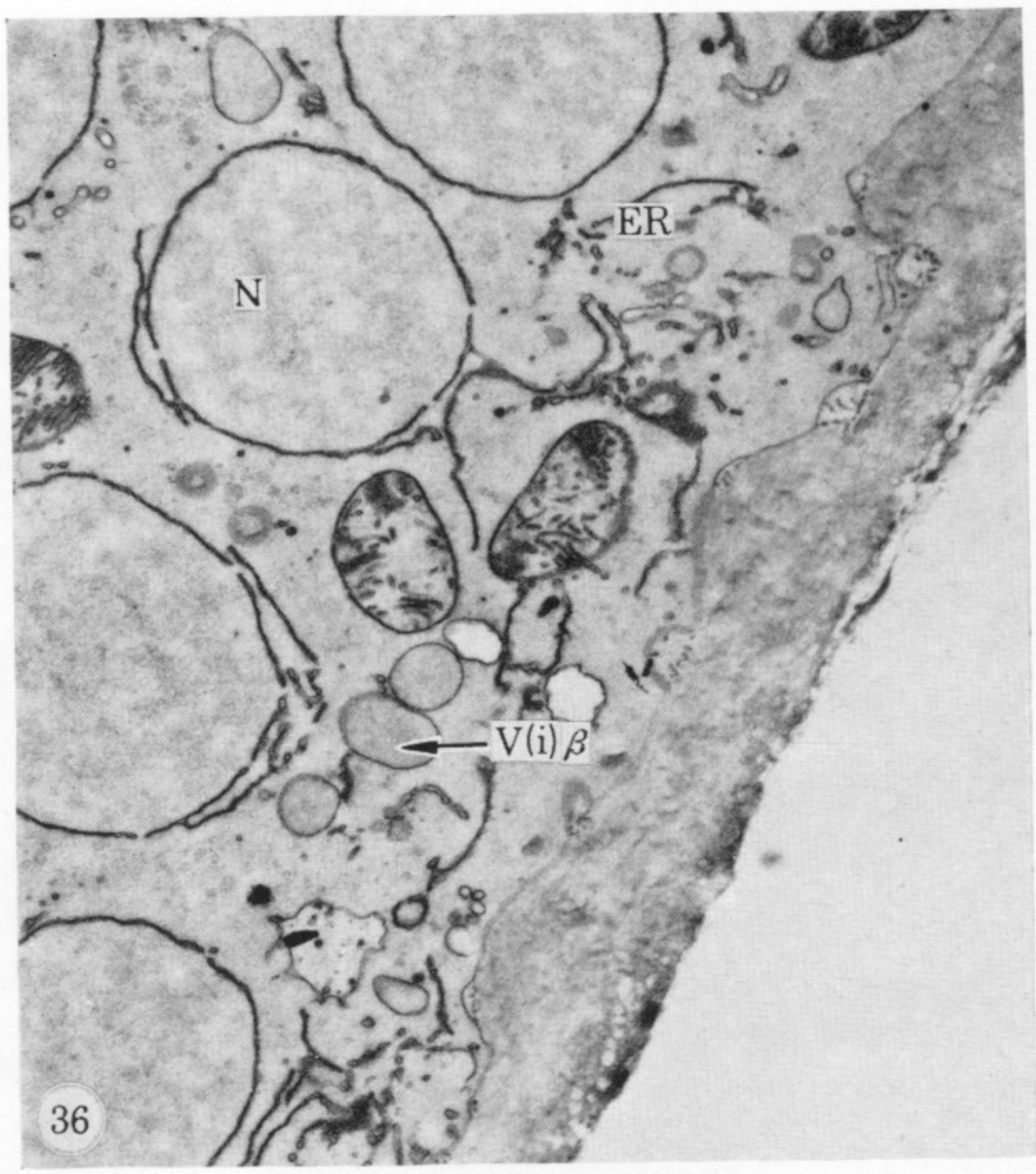


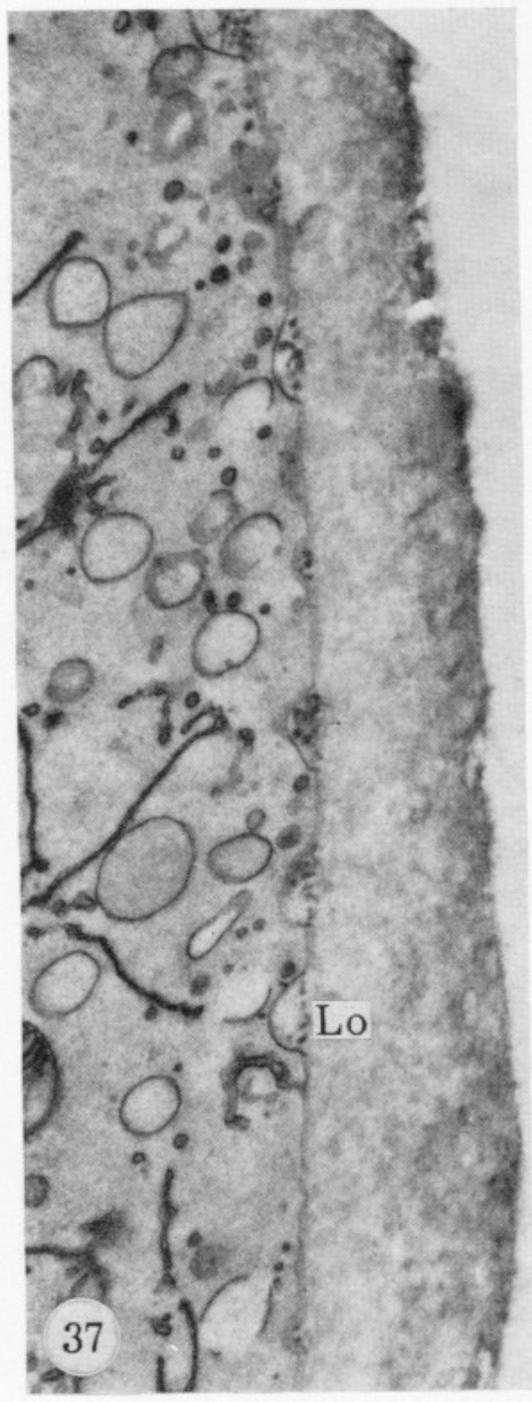


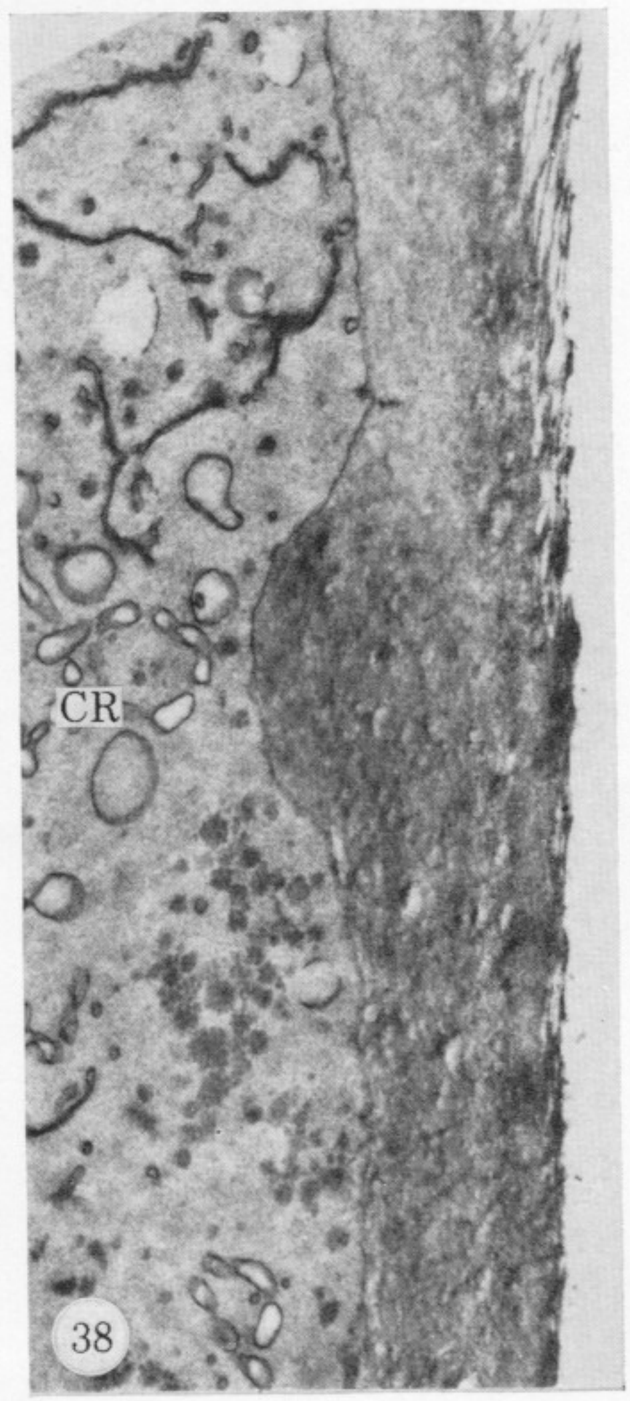
FIGURES 28 to 33. For legends see facing page

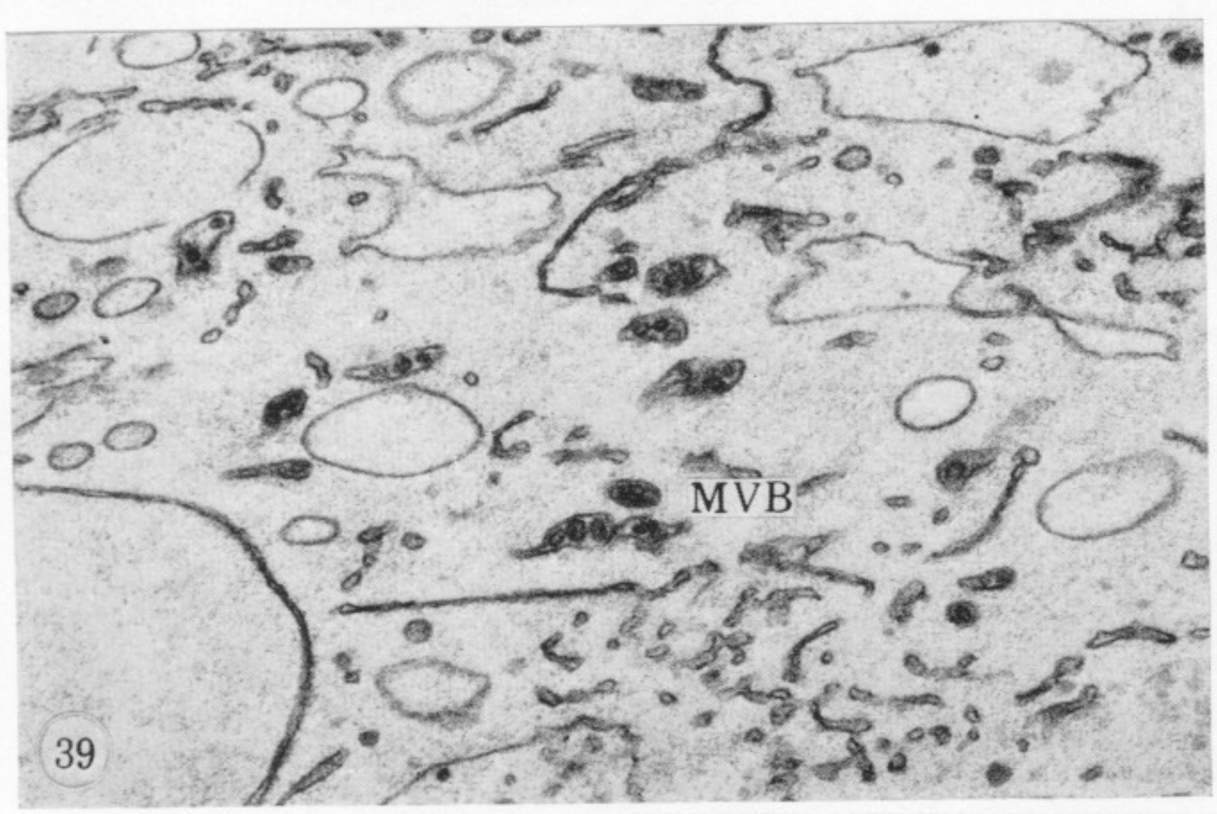


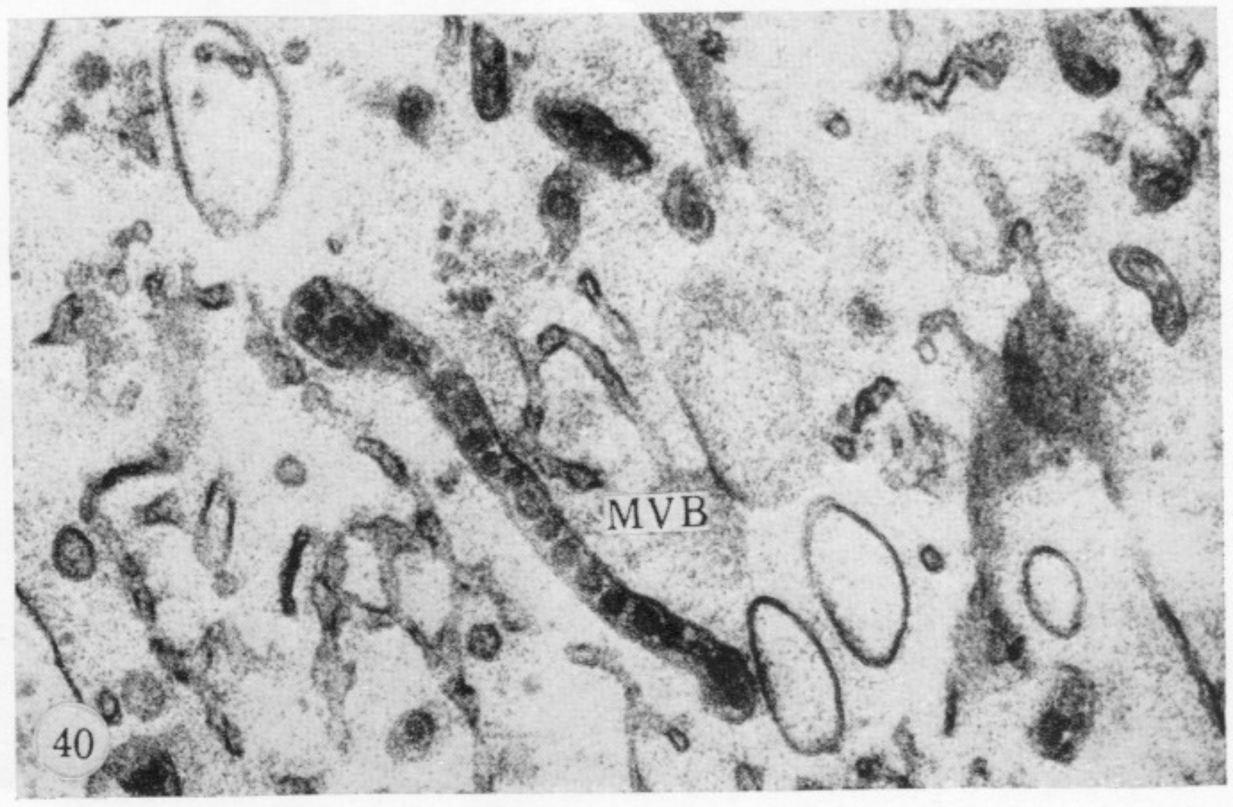




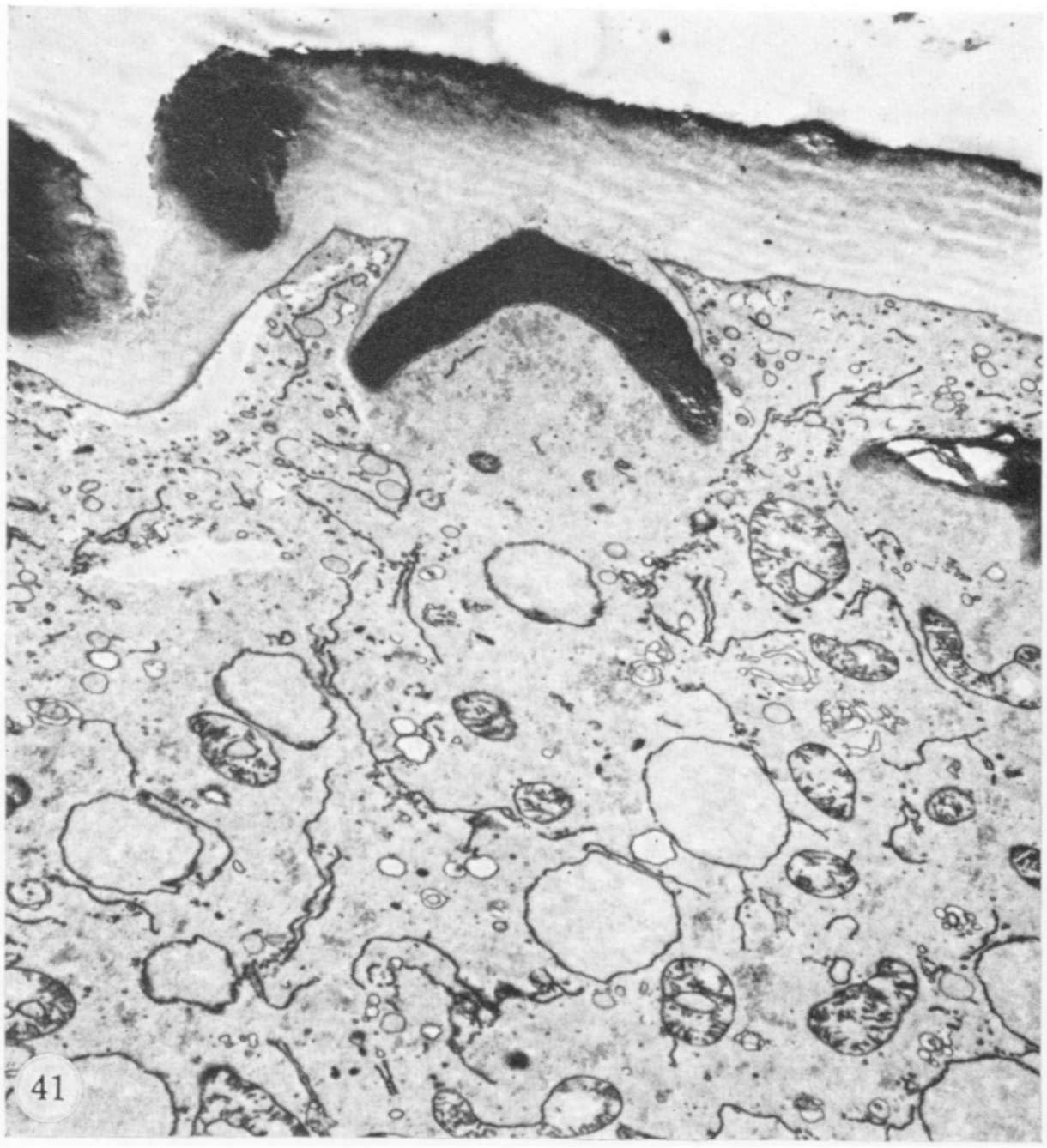




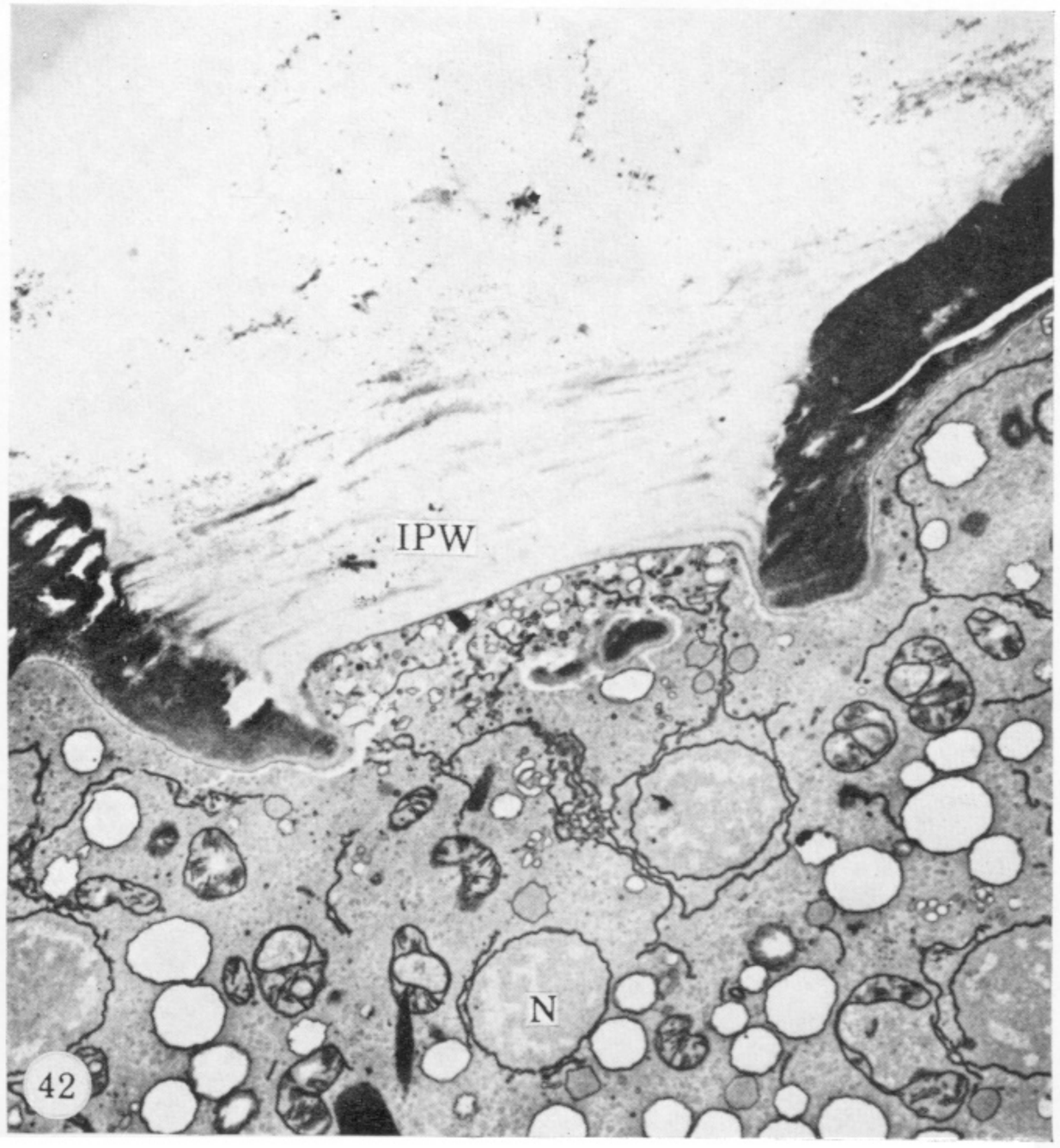




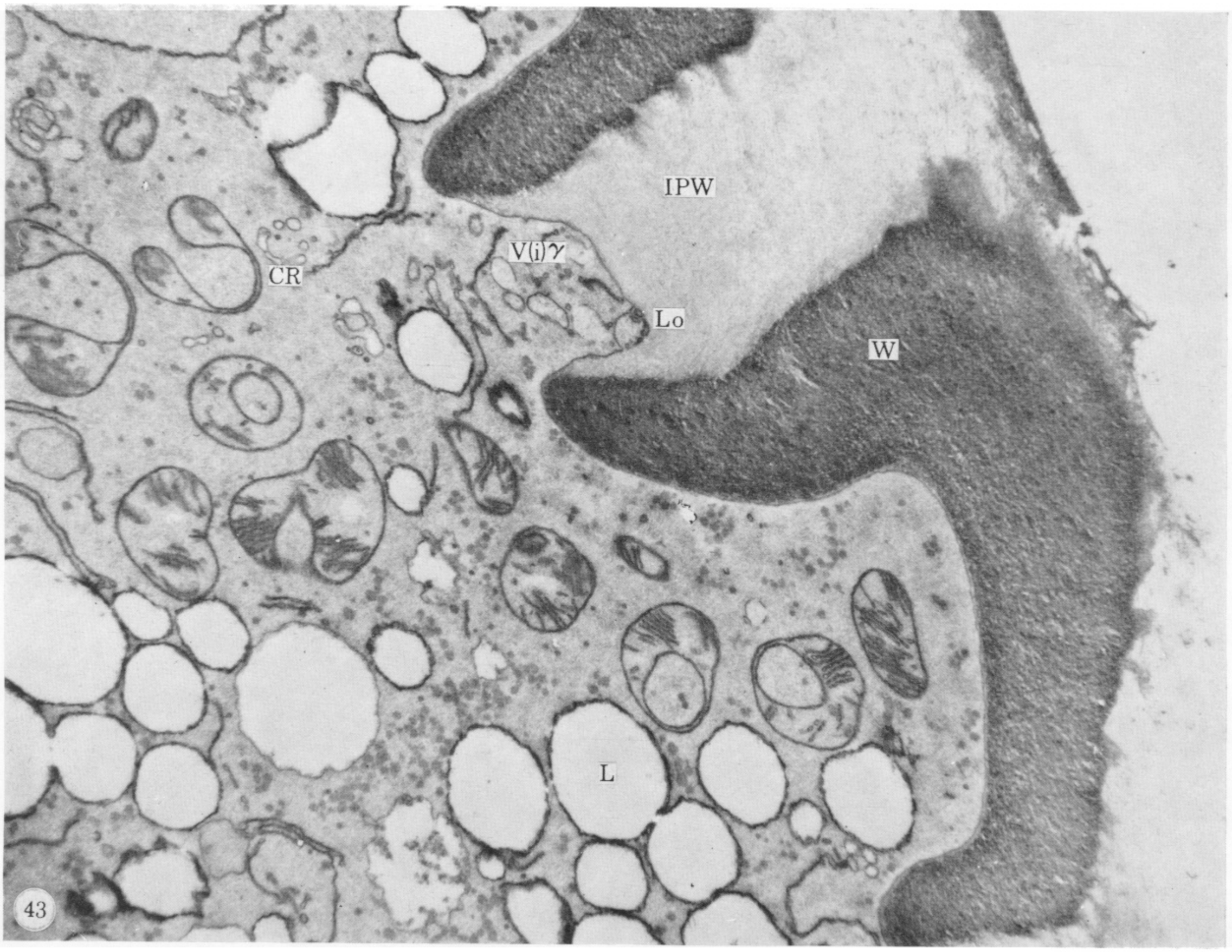
FIGURES 34 to 40. For legends see facing page



41

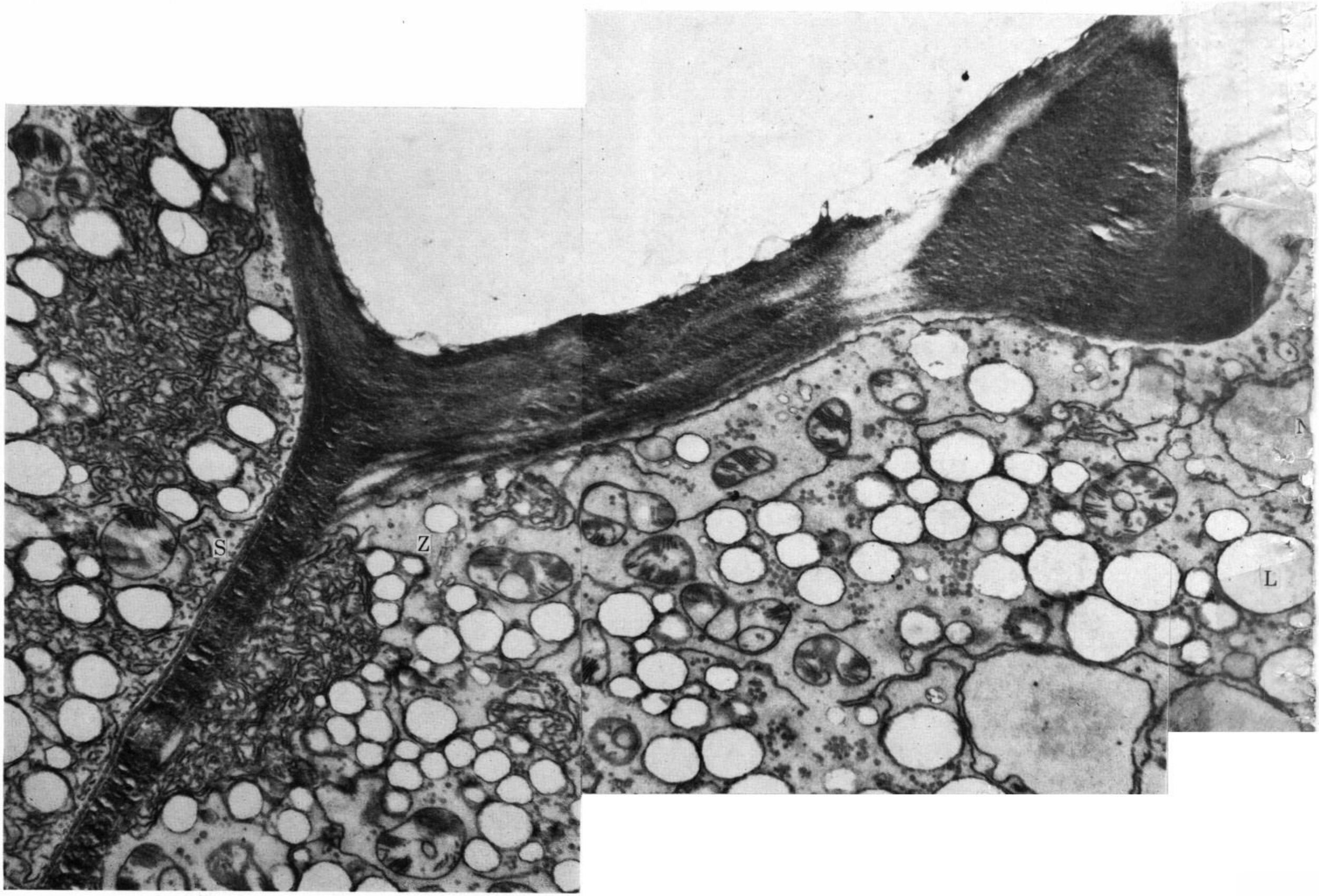


42



43

FIGURES 41 to 43. For legends see facing page


S
Z
L

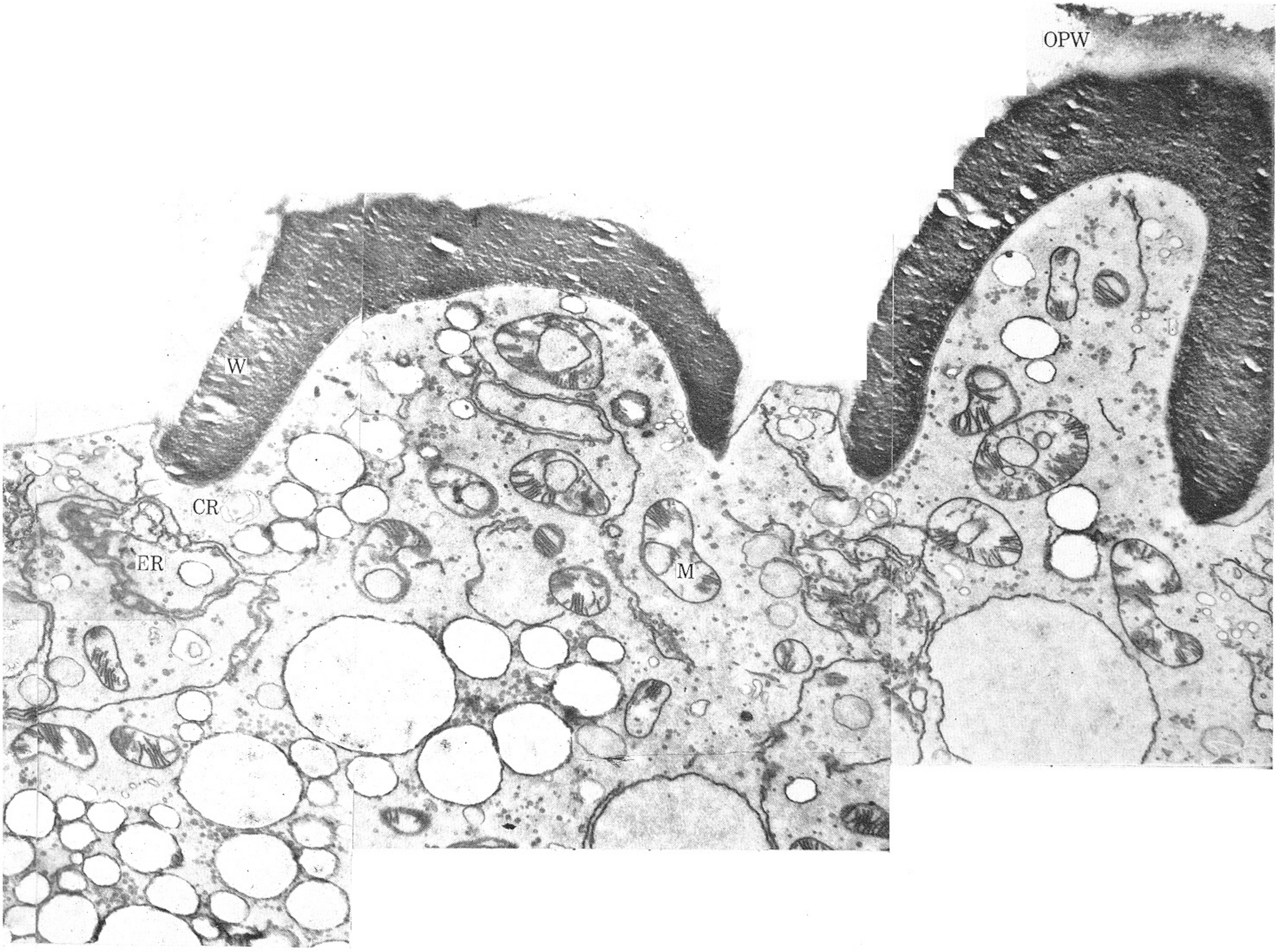
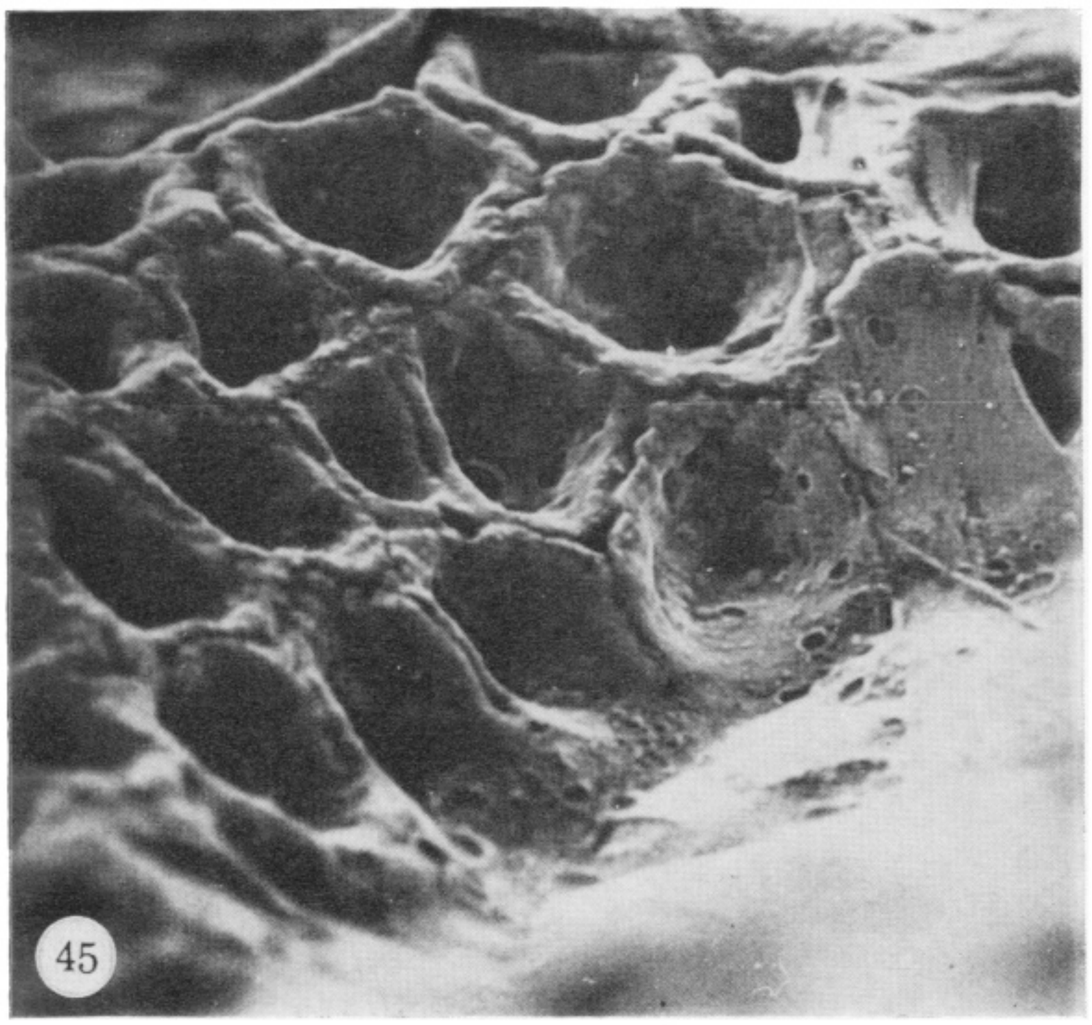
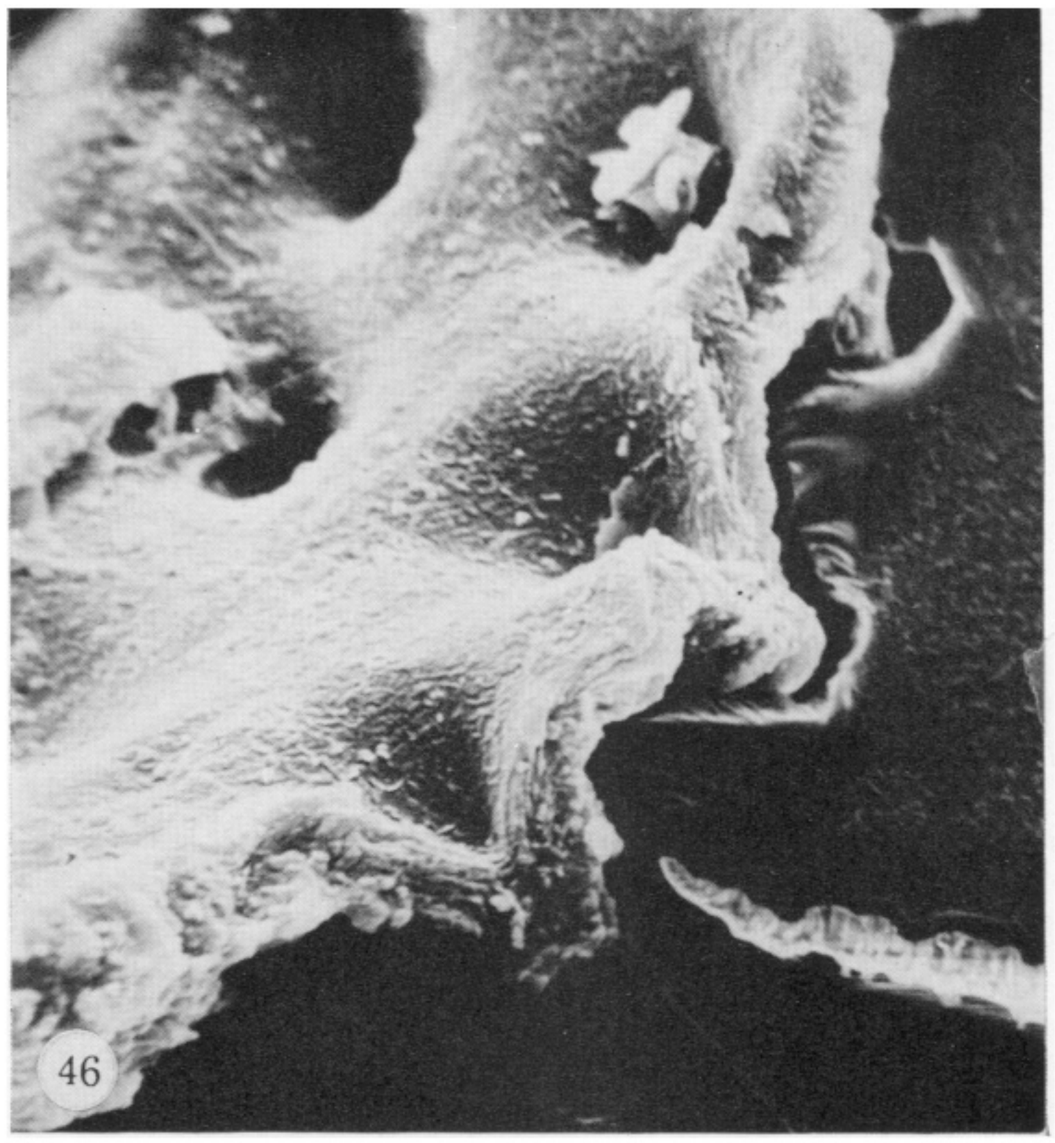
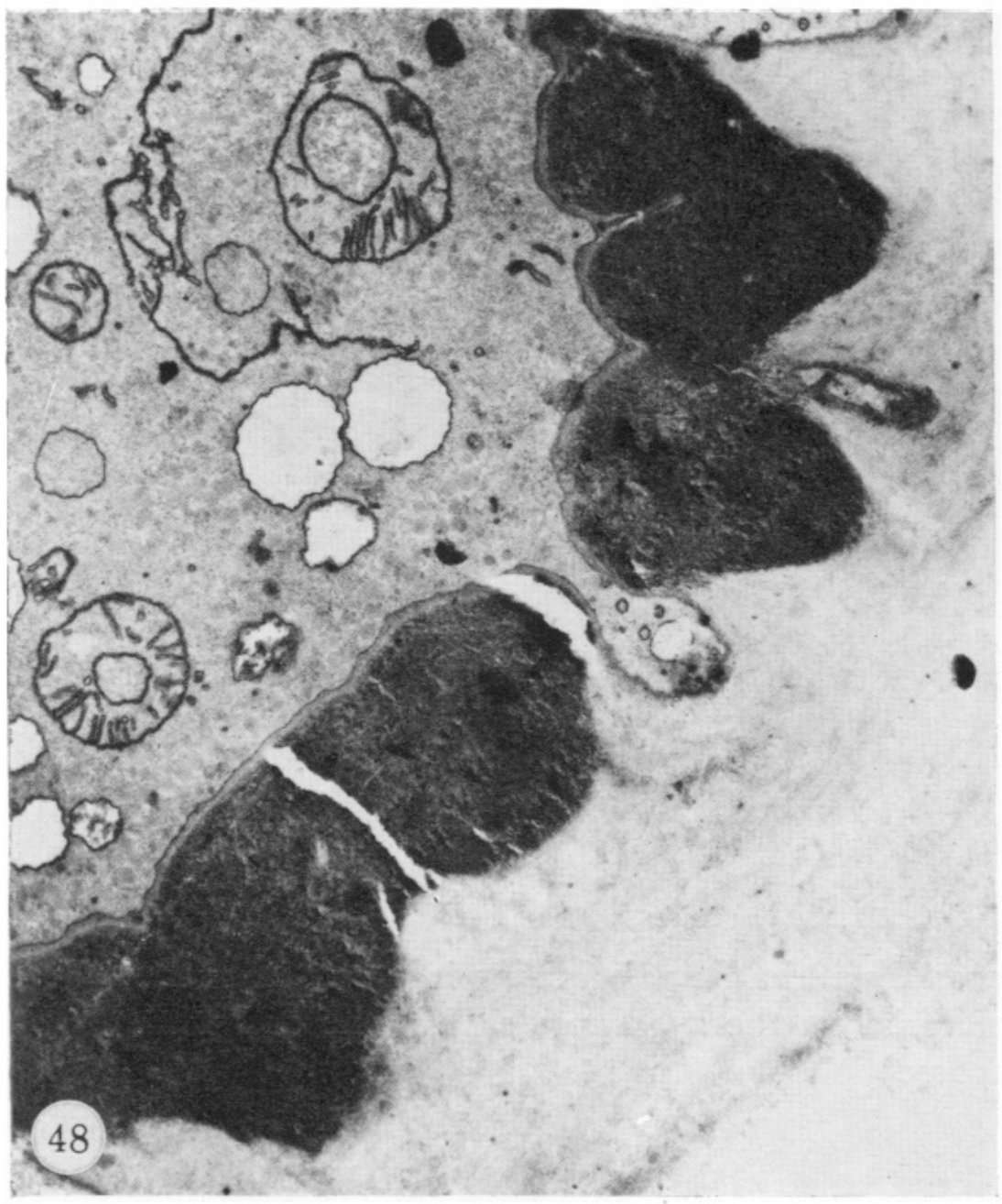
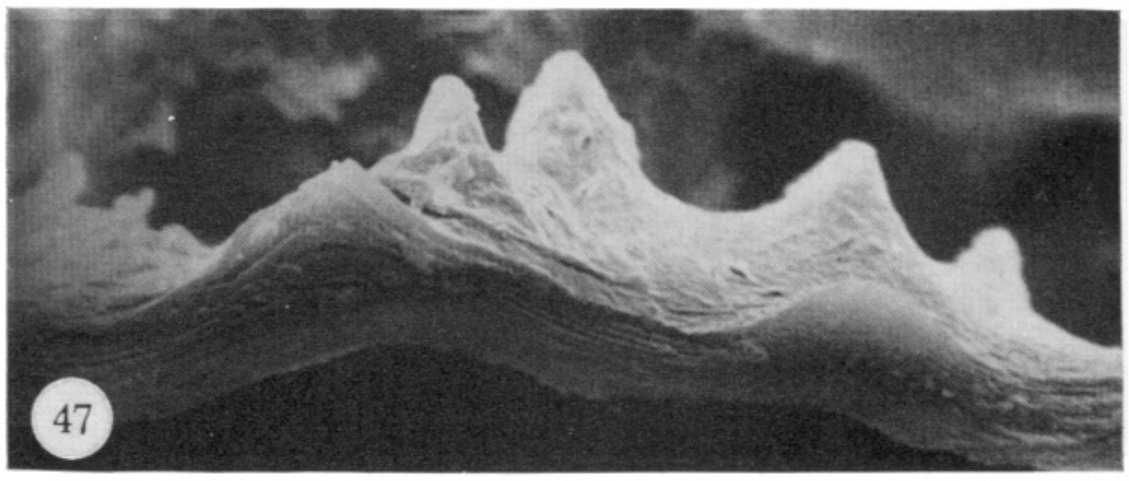


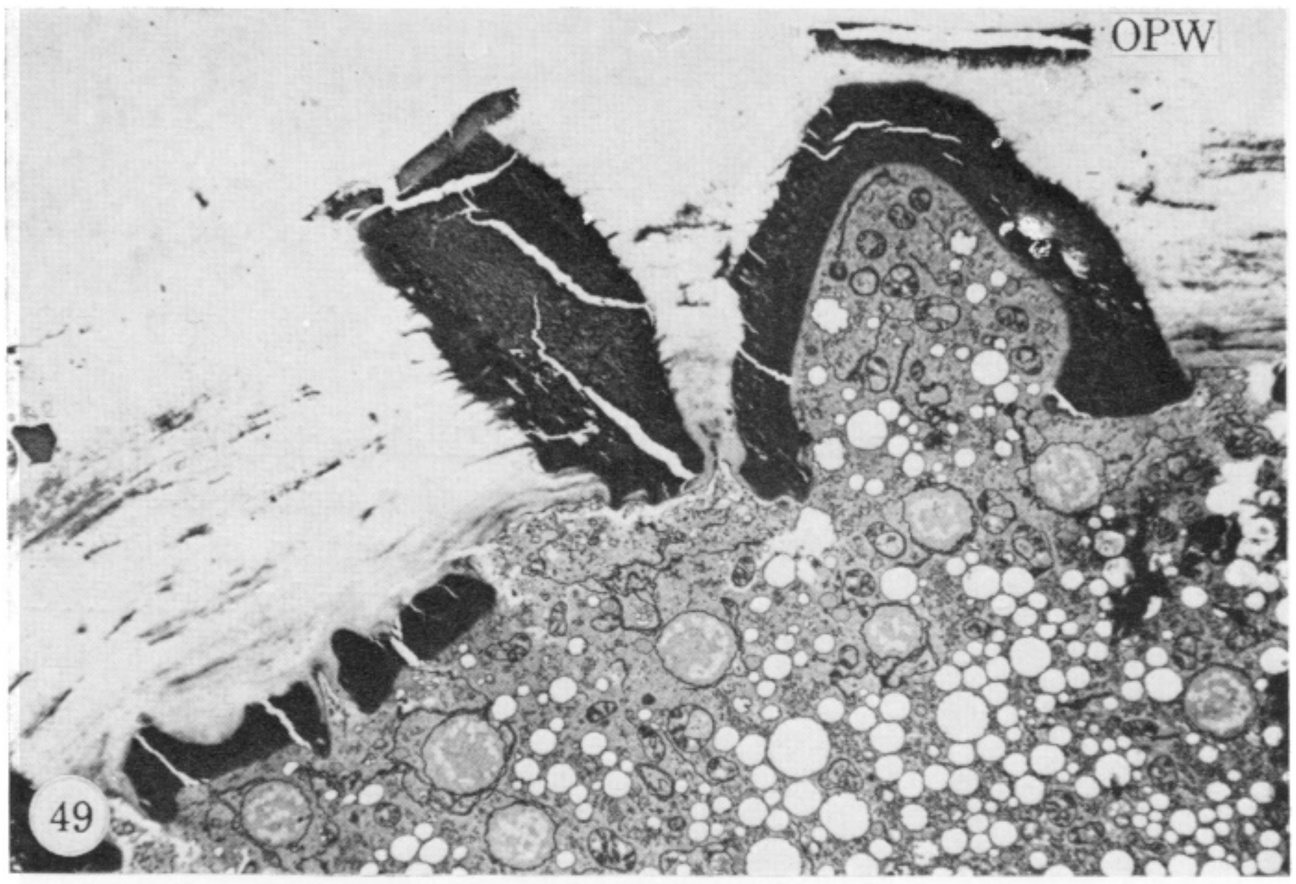
FIGURE 44. Photomontage of a longitudinal section through part of the zygospore wall showing the formation of the wart-like ornamentation by deposition of secondary wall material within the gelatinizing inner layers of the primary wall. Note the uniform deposition of secondary material on the zygospore side of the end wall. $KMnO_4$ fixation ($\times$ 4285). (Micrographs by Mrs M. A. Gooday.)

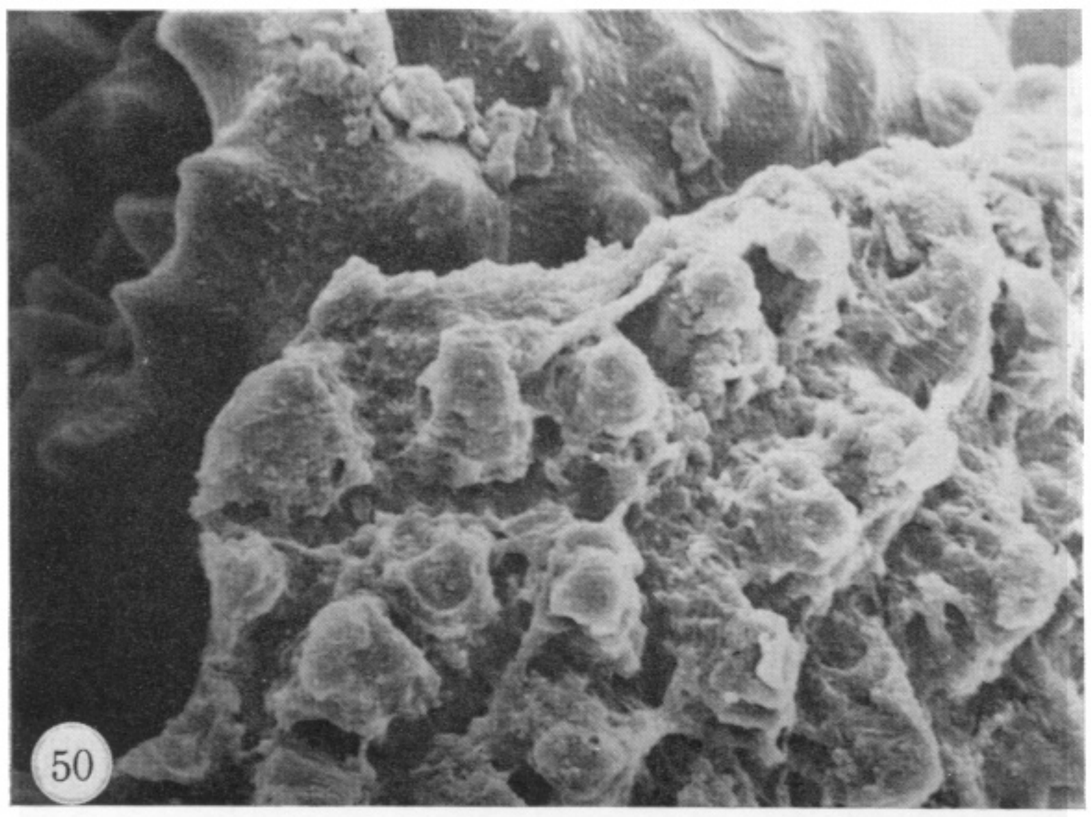


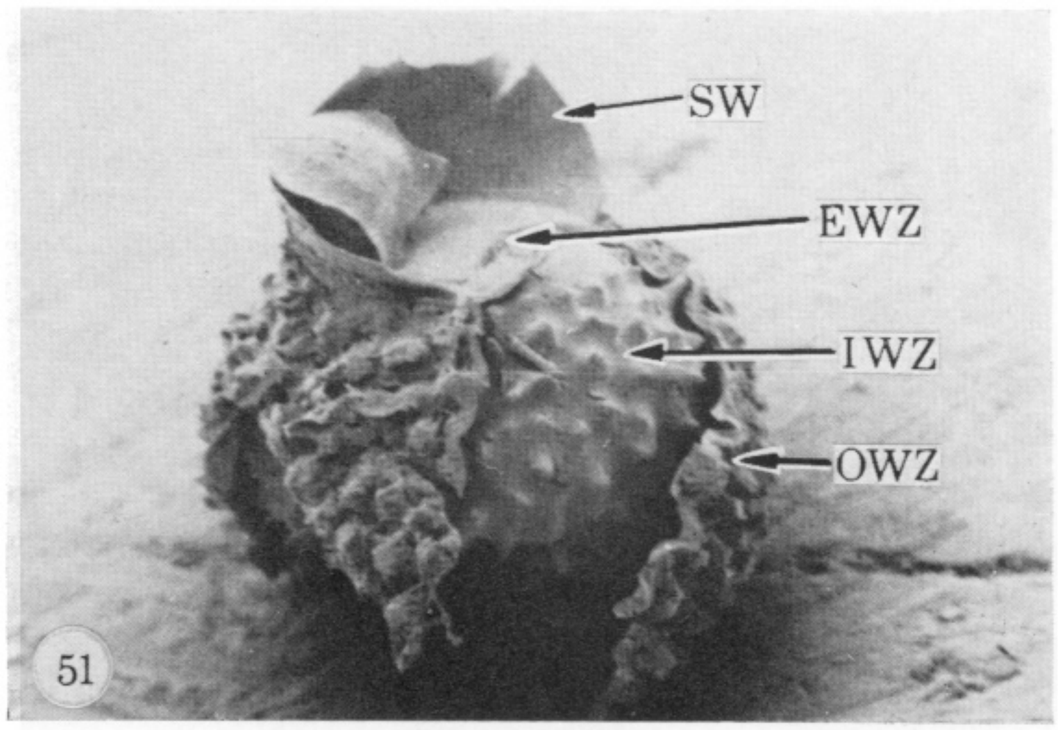




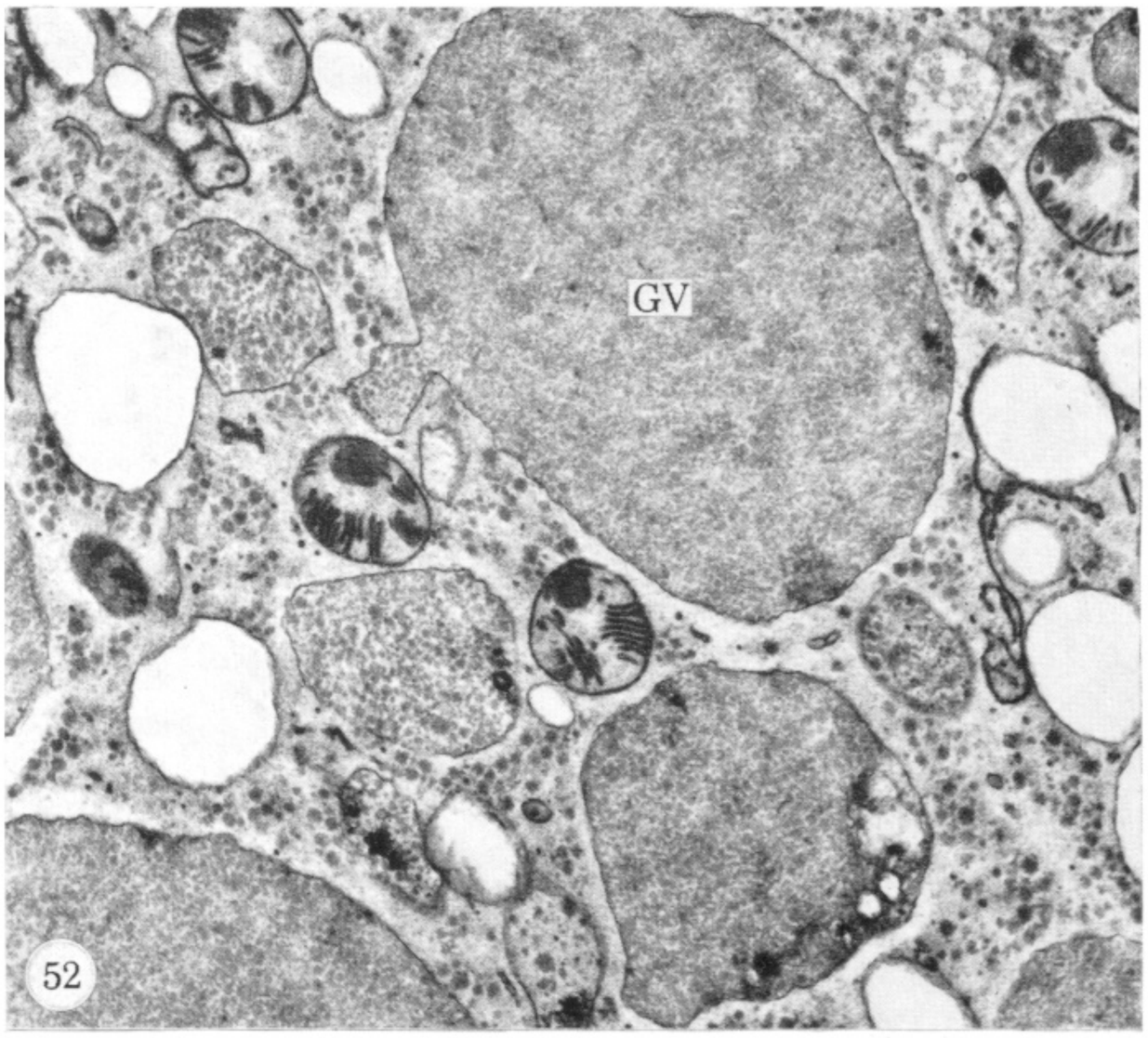


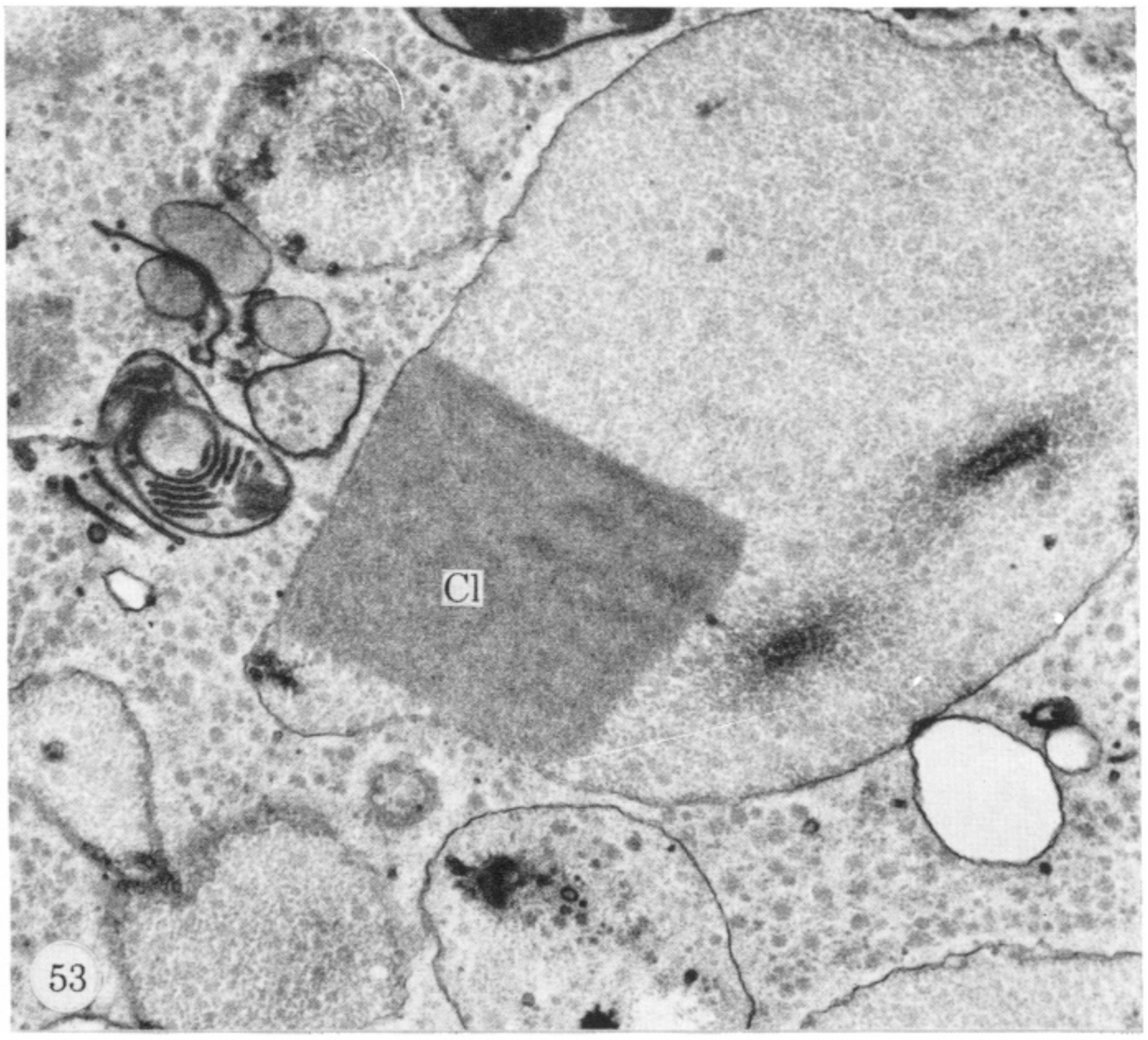


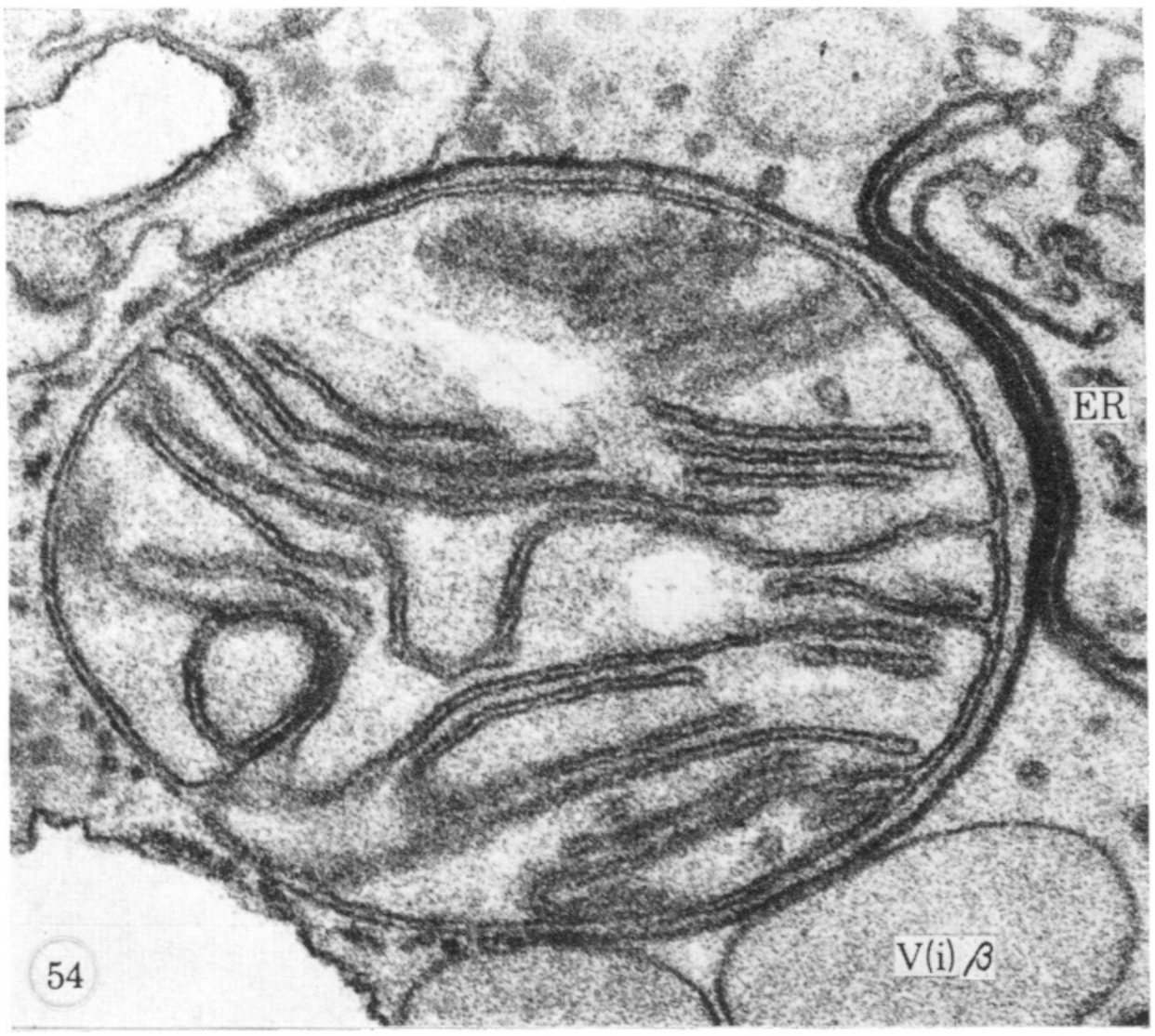


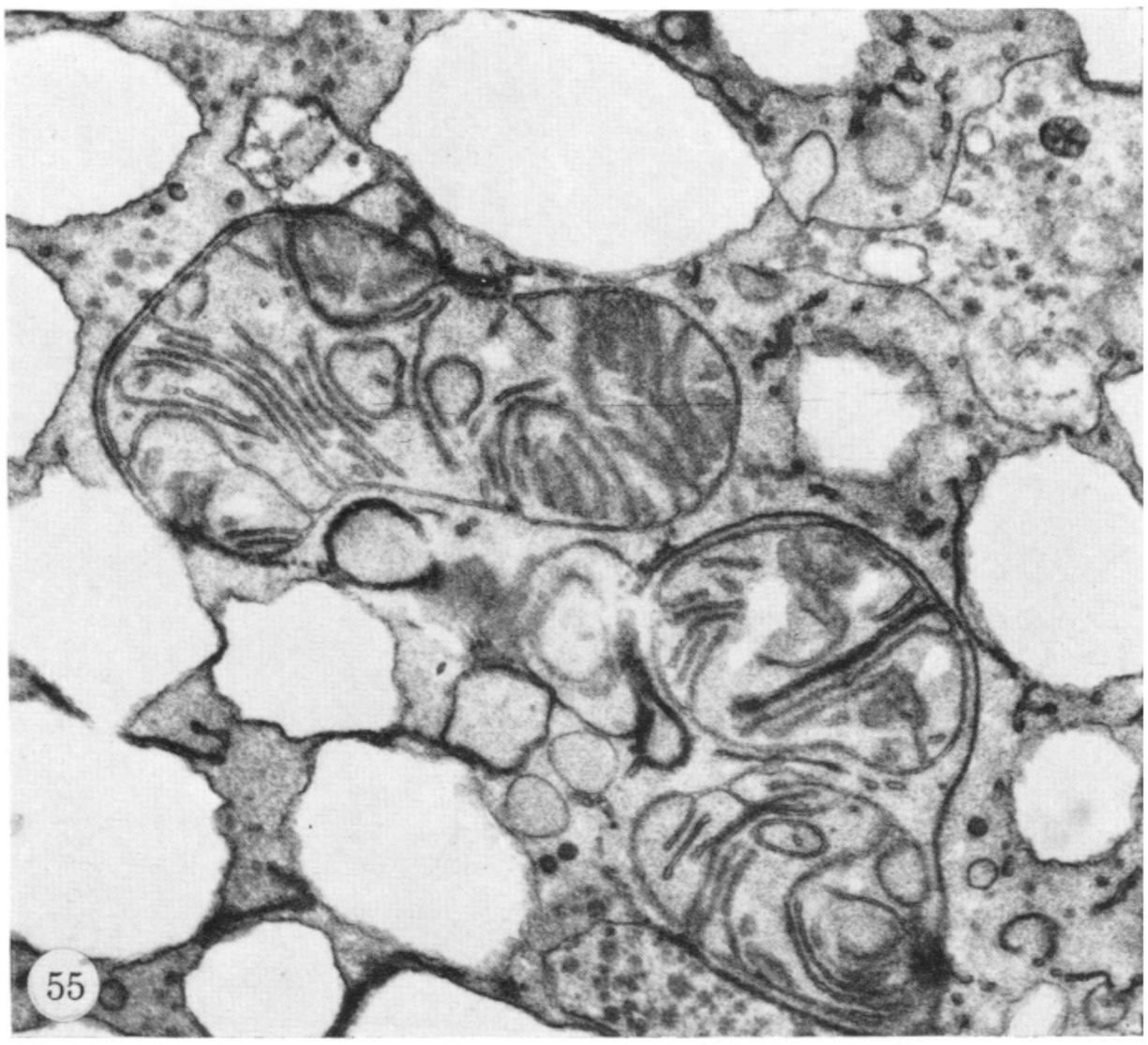


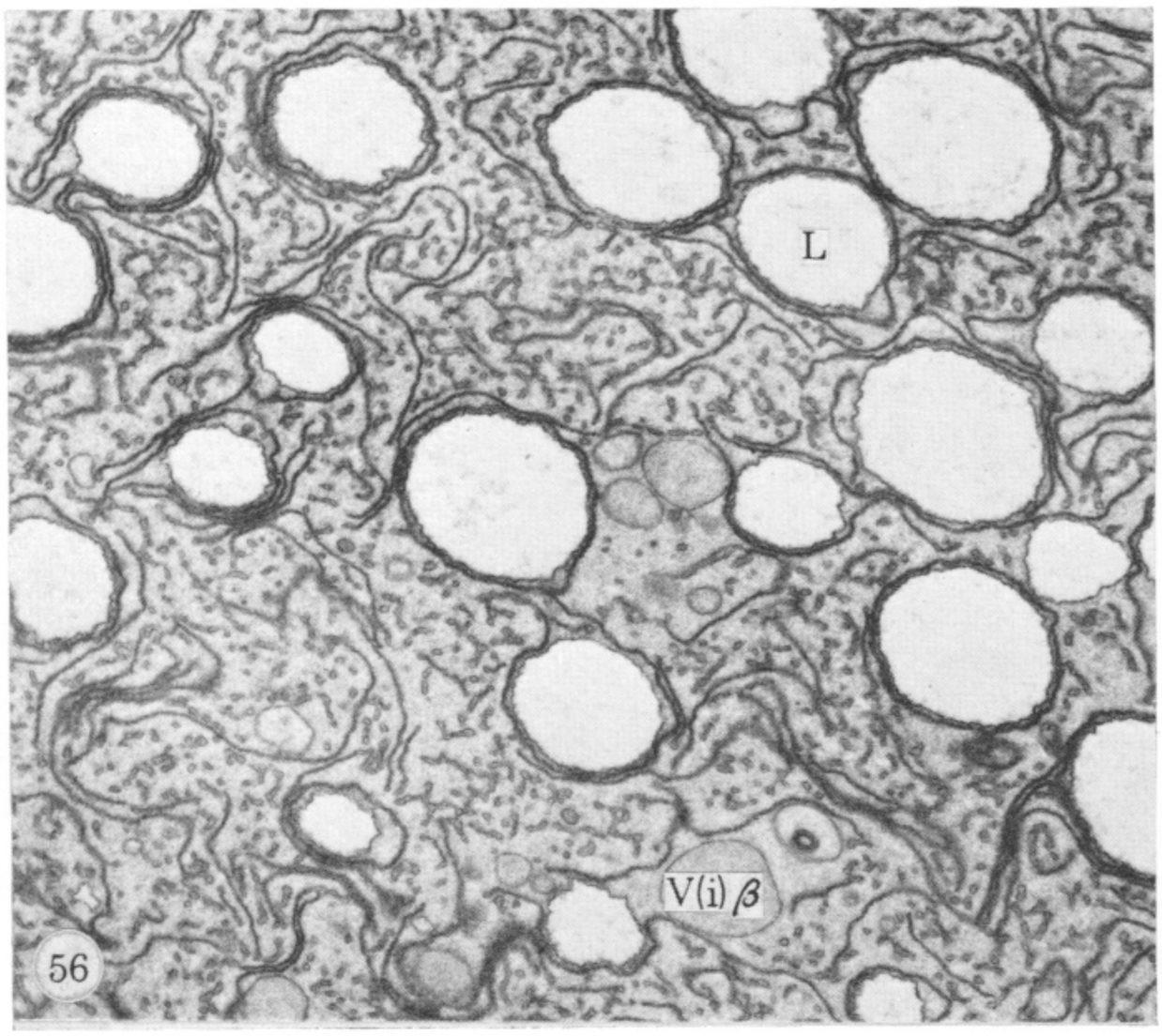
FIGURES 45 to 51. For legends see facing page

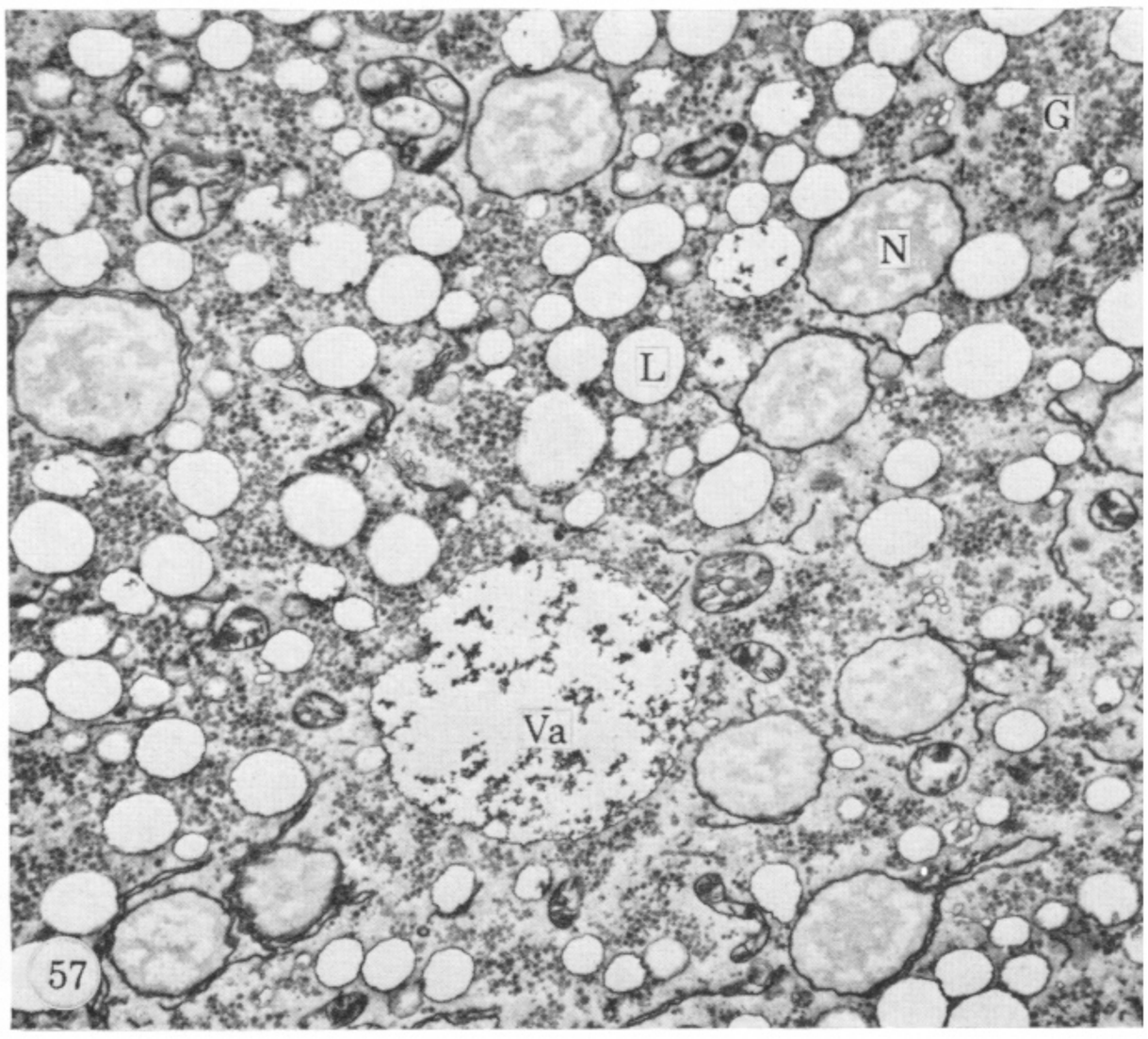












FIGURES 52 to 57. For legends see facing page