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Fine-structural Correlates of Growth in Hyphae of *Ascodesmis sphaerospora*

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Mycelial mats of *Ascodesmis sphaerospora* were fixed and embedded for electron microscopy, and thin sections of 1-mm blocks, taken from the 1st to the 7th mm behind the hyphal tips, were cut parallel to the long axis of the hyphae. The hyphal tip region is characterized by an outer zone of electron-transparent vesicles, 500 to 1,000 A in diameter, and is apparently associated with wall elaboration. Immediately behind this region, dense granules become evident along convoluted membrane systems and along the plasma membrane; in the same region are numerous small lomasomes in the lateral wall. As the hypha grows, septa are laid down at 3- to 7-min intervals at a distance of 200 to 250 μ behind the hyphal tip. A cylinder of endoplasmic reticulum is intimately involved in cross-wall deposition from its earliest stages; as the wall grows in, it becomes increasingly constricted in the pore region, finally assuming a torus-like configuration. Woronin bodies are shown to have a crystalline substructure and to originate in pouch-like membrane systems. Cross-walls from a 7- to 13-hr-old mycelium frequently show highly ordered structures in the vicinity of the pore. These structures may appear either as laminar stacks of discs to one side of the pore or as series of stubby concentric rings within the pore area itself. In the latter case, a mass of granular material is frequently seen plugging the pore. Other unusual organelles and inclusions in 7- to 13-hr hyphae are vesicles containing swirls of beaded or dilated membrane, membrane-enclosed rods, and stacks of unit membranes associated with spherical, electron-transparent vesicles.

Since the time of Woronin (14) and DeBary (2), the chief attributes of ascomycetous hyphae have been generally recognized. They exhibit apical growth; they have perforate cross-walls which render them functionally coenocytic; they contain small, highly refractive spherical or oblong bodies, Woronin bodies, associated with the septa. Recent studies with the electron microscope have revealed the septum to be an annular ingrowth of the lateral cell wall through which the plasma membrane is continuous from one cell to the next via the central pore (7, 8). Under the electron microscope, Woronin bodies appear as electron-dense membrane-bounded spheres which occur near the septa in modest numbers, 3 to 12 per septum (7, 8). No one has yet attempted to correlate the development of these structures, at the fine structural level, with hyphal growth. The following investigation was undertaken to study specifically the ultrastructure of (i) apical growth, (ii) cross-wall and pore development, and (iii) Woronin body formation in the Ascomycete *Ascodesmis sphaerospora*.

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MATERIALS AND METHODS

Growth rate determination. Mycelial plugs from a single spore clone of *Ascodesmis sphaerospora* UO 107 were inoculated into 100-mm petri dishes containing the following sterile medium (CMMY): 17 g of corn meal agar (Difco); 1 g of yeast extract (Difco); 1 g of malt extract (Difco); and 10 g of Difco agar in 1 liter of distilled water. These plates were incubated at 22 C with a 12-hr light-dark cycle. After an initial period of 30 hr, insect needles were stuck in the agar at the advancing edge of the mycelial mat. At intervals of 5 to 15 hr, new needles were placed at the colonial perimeter along radii connecting the previous needles with the center of the colony. Distances were measured between needle points which could be seen from the bottom of the petri dish. Four such measurements were made on single colonies in each of four separate plates over a period of 38 hr, and an average growth rate was established.

Phase microscopy. Mycelium for observation under the phase microscope was grown out on 1-cm squares of dialysis membrane placed on the surface of CMMY

medium. When a colony had almost reached the edge of the square, the membrane, together with the living mycelium, was mounted in water and examined on a Zeiss Standard Universal microscope equipped with Zeiss phase optics.

Isolation and observation of septa. Forty CMMY plates were inoculated with *Ascodesmis* and incubated at 22 C until the colonies reached a diameter of 7.5 cm; the outer portions of these were then cut into five concentric zones, each 3 mm wide, with a flattened, sharpened, dissecting needle mounted in a conventional compass. The first such zone contained only the first 2 mm of mycelium behind the hyphal tips; the surfaces of the remaining four zones were covered entirely by mycelium. Each annulus of agar with mycelium was placed according to its position on the plate in a separate flask immersed in ice to halt further growth. This operation was repeated for all 40 plates, by which time a considerable amount of mycelium of approximately the same age (age in the center of the zone ± 3 hr) had accumulated in each flask.

Septa were isolated from these mycelia by using a modification of the method employed by Reichle and Alexander for *Fusarium* (7). Water was added to the flasks, and they were autoclaved to liquify the agar and kill the mycelia. The clumped, dead mycelia were pulled from the liquid agar with a transfer needle; they were washed several times in distilled water and autoclaved for 20 to 30 min in 23 M KOH. They were then run through several washes with distilled water, a wash in 2% acetic acid, and a final wash in distilled water; after each washing, the mycelia were centrifuged at 30,000 × *g* for 15 min in a Servall automatic centrifuge. After the final wash, each sample of chemically cleaned hyphae was suspended in 2 to 3 ml of distilled water and was sonically treated for 2 to 3 min with a Branson Sonifier.

Suspensions of the free septa and disrupted hyphae thus produced were diluted 1:4 and 1:8 in distilled water, and drops of the dilute suspensions were allowed to dry down on Formvar-coated 200-mesh copper grids. These grids were shadowed at a low angle with a platinum-palladium alloy, and were examined under a Hitachi HS-7 electron microscope.

Electron microscopy, thin sections. Radial segments of agar containing the hyphal tips and the zone of mycelium 2 cm behind the growing edge of the colony were cut from plates and fixed according to one of the following procedures: (i) 2.5% glutaraldehyde buffered at *p*H 7.8 with 0.1 M s-collidine (Polysciences Inc., P.O. Box 4, Rydal, Pa.) for 1 hr at 4 C; washed briefly and postfixed with 1% OsO_4 for 2 hr in the same buffer at 4 C; (ii) 2% $KMnO_4$, unbuffered, for 2 hr at 4 C. The tissue was then washed several times in distilled water, soaked 4 to 6 hr in 0.5% uranyl acetate solution, dehydrated in a standard alcohol series, and embedded in a 3:4 Epon-Araldite mixture with DMP-30 added as a catalyst. Bubbles in the plastic were removed in a vacuum oven, and polymerization of the plastic was carried out at 60 C for several days.

Beginning with the hyphal tip and moving back along the radial axis of the colony, the material was cut into 1-mm segments; these blocks were trimmed of excess plastic and were mounted with the agar face out. The amount of plastic to be cut away to reach the fixed mycelium was measured with the calibrated pinion head of the fine focus adjustment on a phase microscope by focusing first on the surface of the block and then on the material embedded within. The measured amount of plastic was then cut away as thick sections on a Porter-Blum MT-1 ultramicrotome. Thin sections of the material were cut with a diamond knife on the same microtome and were mounted on unsupported 100-mesh copper grids. The sections were poststained with 0.5% uranyl acetate for 20 min, washed with water, and finally stained with Reynolds' lead citrate for 30 min on a microscope slide under a cover slip. Sections were examined on one of three electron microscopes: an RCA EMU 3-D; a Hitachi HS-7; and a Siemens Elmiskop IA. All results are based on sections of glutaraldehyde-OsO_4-fixed tissue, unless explicitly stated otherwise in the legend.

RESULTS

Apical growth. At 22 C, the edge of a colony of *A. sphaerospora* advanced 550 ± 15 μ/hr, a rate equivalent to about 9 μ/min and roughly comparable with growth rates observed directly for living individual hyphae under the light microscope. This hyphal growth and elongation seemed to occur mainly at the hyphal tip and at a 200 μ zone immediately behind. Consequently, the fine structure of this region was studied intensively for evidence of organelles or structures which might be involved in apical growth. A near longitudinal section through a hyphal tip (as determined by serial sectioning) revealed an outer zone of cytoplasm containing numerous electron-transparent vesicles 500 to 1,000 A in diameter; in several places these vesicles appeared to be fusing with the plasma membrane (Fig. 1). To the inside of this zone were many elongate mitochondria (Fig. 1) and an occasional electron-dense body enveloped in a unit membrane system (Fig. 1, 2). Such a body is termed here "crystalloid," because similar structures in older cells revealed a definite internal lattice when sectioned in the appropriate plane (Fig. 20).

In the region immediately behind the hyphal tip, conspicuous microtubules (Fig. 3), as well as several cytoplasmic bodies of unknown significance, appeared. These included electron-dense, membrane-bounded saucer or bowl-shaped vesicles (Fig. 3–5) and crystals which showed a well-defined internal lattice when sectioned perpendicularly to the hexagonal face (Fig. 3, 6). In this same zone numerous very electron-dense granules, 380 to 450 A in diameter, occurred in close association with convoluted membrane systems in the cytoplasm and with the plasma membrane; occasionally they were also seen free in the cytoplasm (Fig. 3, 7–9). A section through

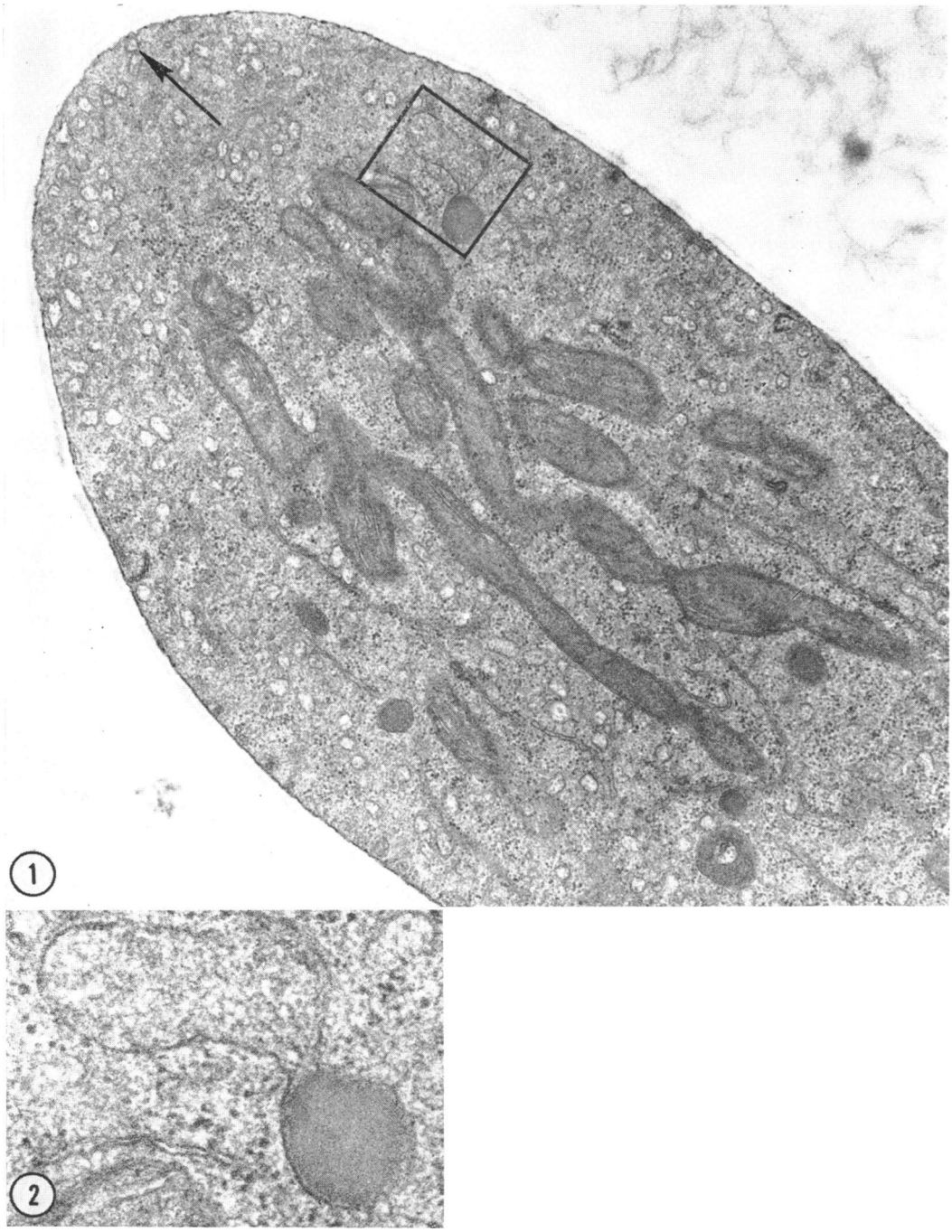

FIG. 1. *Longitudinal section of hyphal tip showing mitochondria, crystalloid formation (box), and small electron-transparent vesicles. Note one of the small vesicles apparently fusing with the plasma membrane (arrow).* × *29,000.*

FIG. 2. *Detail of crystalloid formation.* × *90,000*

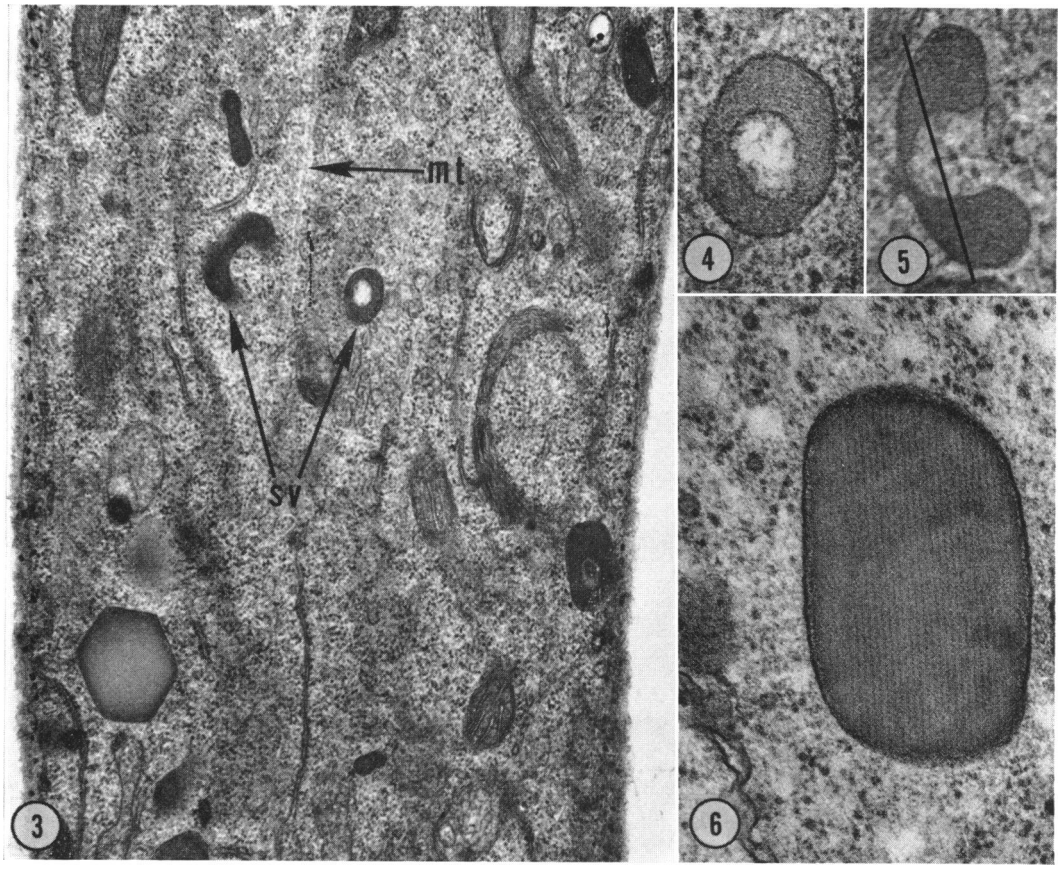

FIG. 3. *Longitudinal section of hypha approximately 100 μ from hyphal tip. Note the saucer-shaped vesicles (sv), microtubules (mt), dense granules along the lateral walls, and crystals. × 28,000.*

FIG. 4. *Transverse section of a saucer-shaped vesicle. × 68,000.*

FIG. 5. *Longitudinal section of a saucer-shaped vesicle; line indicates plane of sectioning for Fig. 4. × 68,000.*

FIG. 6. *Crystalloid from first 0.5 mm of hypha sectioned perpendicularly to its hexagonal face and showing internal lattice structure. × 50,000.*

one of these granules revealed a dark outer layer and a less electron-dense core (Fig. 8, lower left). Toward the basal end of the first cell, these granules disappeared. In addition, numerous small tubular evaginations of the plasma membrane, lomasomes (Fig. 10, 11), as well as outpocketings of cytoplasm (Fig. 12), were evident in the lateral hyphal wall in the region immediately behind the hyphal tip; these were also found occasionally in older hyphae.

Septum formation. In a mature mycelium, septa occur every 35 to 60 *u*. Thus, at a growth rate of 550 μ/hr, a new septum must form in any given hypha on an average of every 4 to 6 min. Observations of living hyphae under a phase microscope revealed this prediction to be valid; a young septum appeared first as a faint line 200 to 250 μ behind the hyphal tip. It became increasingly definite, and within 2 min it acquired the aspect of a cross wall in older hyphae. Septa continued to be laid down in this way in acropetal sequence at intervals of 3 to 7 min.

The rapidity of cross-wall formation made the discovery of intermediate stages difficult in thin sections of fixed material seen under the electron microscope. Nevertheless, several sections of incomplete septa were found. Figure 13 represents an early stage in septum initiation; a small amount of amorphous material occurred close to the young cross wall, and endoplasmic reticulum (ER) can be seen already associated with the advancing pore rim. Figure 14 shows a much later stage in septum formation, in which this association was evident. Here, between the ER and the newly formed septum, a definite zonation of cytoplasm into an outer amorphous region and an

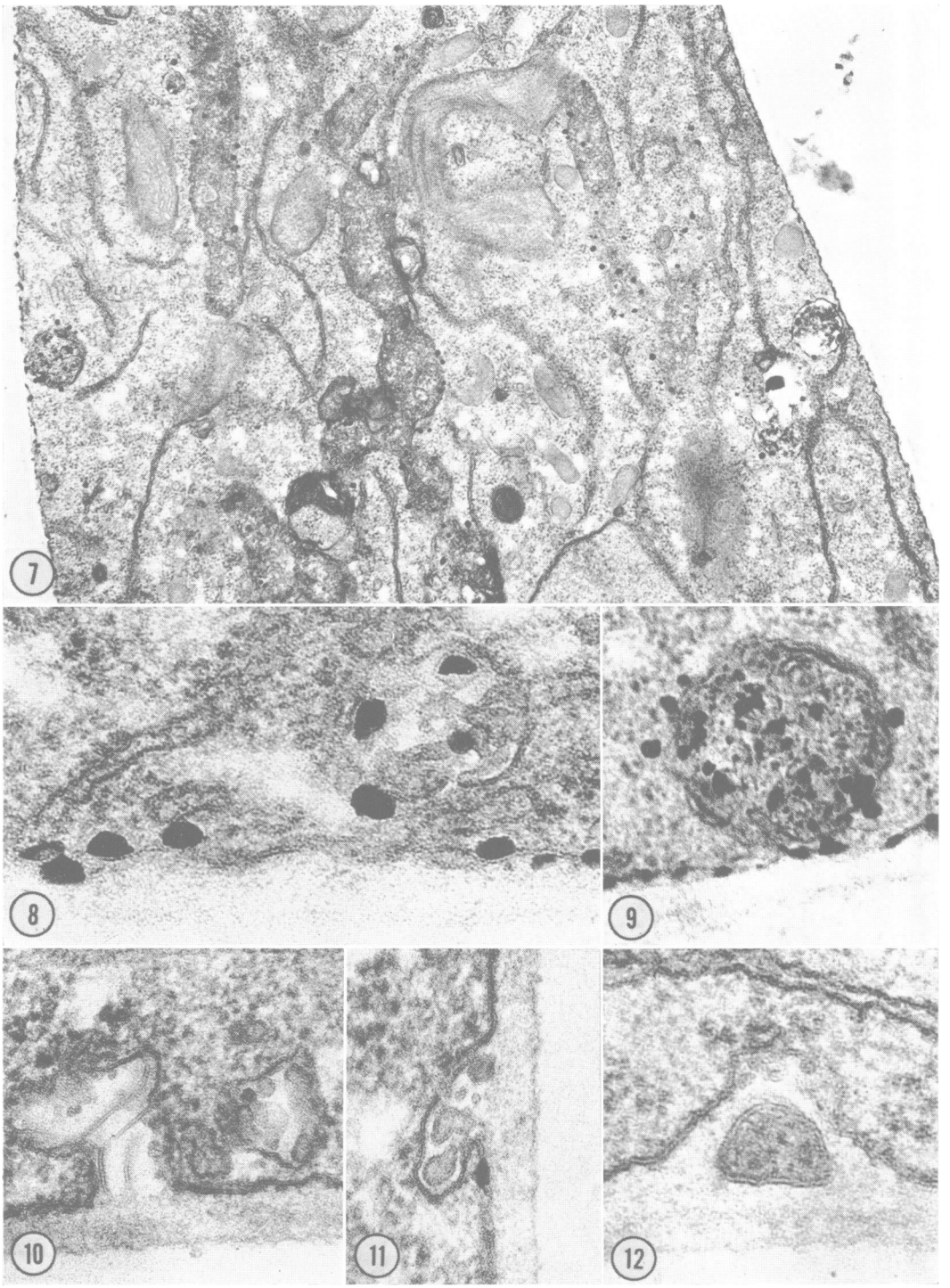

FIG. 7. *Longitudinal section of hypha just behind growing apex. Note dense granules associated with the convoluted membrane systems in the cytoplasm and with the plasma membrane.* × *24,300.*

FIG. 8. *Detail of granule elaboration.* × *133,000.*

FIG. 9. *Detail of granule elaboration.* × *98,000.*

FIG. 10, 11. *Small lomasomes in lateral walls of first hyphal cell, approximately 100* μ *behind tip. Fig. 10,* × *115,000. Fig. 11,* × *133,000.*

FIG. 12. *Small outpocketing of cytoplasm into lateral wall in same region.* × *147,000.*

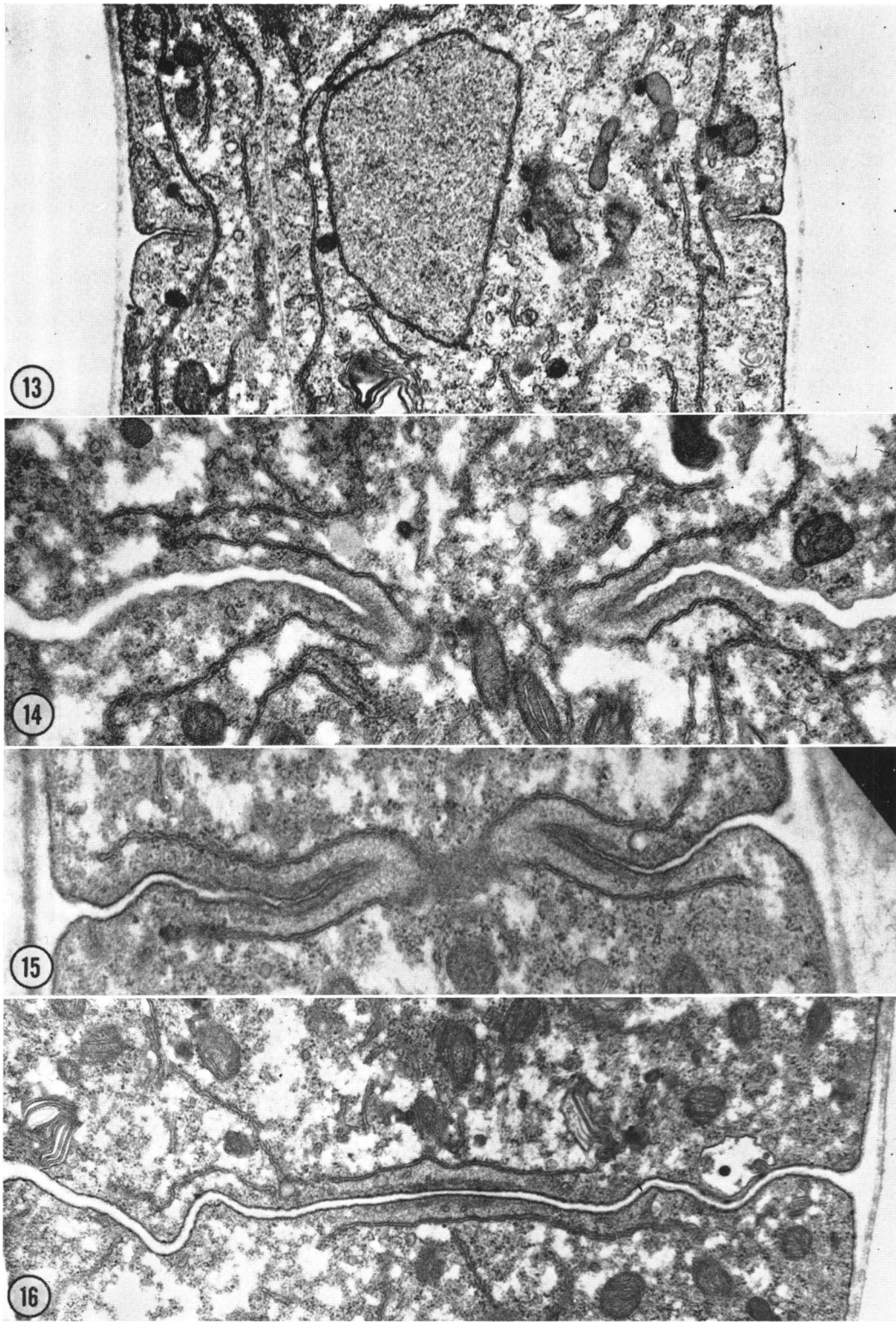

FIG. 13–15. *Successive stages of cross-wall formation. Note endoplasmic reticulum associated with the advancing rim of the septal pore. Fig. 13, × 20,000. Fig. 14, × 19,500. Fig. 15, × 24,700.*

FIG. 16. *Longitudinal section of hypha in the region of a newly formed septum; section has been cut just to one side of the pore. Note lateral extension along the septum of endoplasmic reticulum associated with the pore rim. × 17,200.*

inner, more electron-dense, vesicular region was discerned. The alveolar appearance of the plasma membrane along the septum suggested that small vesicles in the inner region were actually fusing with the plasmalemma. In Fig. 15, a septum of approximately the same age was sectioned to one side of the center of the pore, thus revealing the surface of the enveloping ER system as an apparent pore-plug. In Fig. 16, the plane of sectioning completely missed the pore in the young septum, and, consequently, the associated ER system appeared as two separate cisternae, one on each side of the wall. Taken in sequence, Fig. 13–16 indicate that a cylinder of ER occurred in connection with cross-wall deposition from the earliest stages and that, as the wall grew in, this ER system became increasingly constricted in the pore region, finally assuming a torus-like configuration. Several structures associated with the lateral walls in the zone immediately behind the hyphal tip occurred also in conjunction with septum development. These included electron-dense granules along the plasma membrane (Fig. 16, 17) and prominent lomasomes (Fig. 18).

Attempts to isolate septa from definite zones of hyphal growth were successful, except for those from the 2-mm region directly behind the hyphal tip. This very young mycelium did not agglomerate when autoclaved and proved very difficult to isolate from the melted agar; further, it showed a high susceptibility to dissolution during the chemical cleaning process. Although the septa from the older growth zone showed great variation in size, all of the 1,000 to 1,500 examined were uniformly perforate (see, for example, Fig. 19).

Woronin body formation. Under the phase microscope, Woronin bodies were usually visible as highly refractive spheres 0.6 to 0.8 μ in diameter. In young cells just behind the hyphal tip region, these inclusions appeared as somewhat vague circular outlines dispersed in the cytoplasm and against the lateral walls (Fig. 23). In cells over 2 hr old, they became more refractive and always occurred in close association with the septa (Fig. 24–26). When hyphae are injured, the Woronin bodies apparently coalesce and plug the septal pores, thus sealing off living from nonliving cells (Fig. 27).

Under the electron microscope, Woronin bodies showed a definite internal lattice structure when sectioned in the appropriate plane (Fig. 20, 28). The apparent spacing of lines in the lattice varied greatly from one Woronin body to the next (200 to 400 A) and presumably depended on the angle of the section through the lattice. Putative stages in the development of Woronin bodies were found in almost every age of mycelium examined, from the hyphal tip (Fig. 1, 2) to cells 10 hr old (Fig. 20); thus, Woronin body formation appeared to take place continually in the first 4 or 5 mm behind the hyphal tips. These structures appear to arise in closed membrane sacs, possibly dilated cisternae of the endoplasmic reticulum; Fig. 21 and 22 show successive stages in the pinching-off of Woronin bodies from such membrane systems.

Laminar and striate structures associated with the pore rim. When cross walls from 7- to 13-hr-old mycelium were examined under the electron microscope, various highly ordered structures were frequently seen in the vicinity of the pore rim. In some instances, these appeared as stacks of hollow discs originating at the pore rim and arranged parallel to the cross wall, but extending out into the cytoplasm on just one side of the septum (Fig. 29–33). Whether this structure extends toward, or away from, the hyphal tip has not been determined. Within a single array, the discs are spaced 250 to 280 A apart; close scrutiny revealed lines running perpendicularly to the surfaces of the discs, thus suggesting some three-dimensional periodicity for the entire structure.

The occurrence of organelles in pores completely bracketed by such laminar arrays of discs suggests that they do not obstruct cytoplasmic movement from one cell to the next (Fig. 29, 30). In permanganate-fixed hyphae (Fig. 33), this structure looked basically the same as in hyphae fixed with glutaraldehyde followed by OsO_4 (Fig. 29–32).

More frequently, this organization of material at the pore rim took the form not of a stack of perforate discs, but of a series of stubby concentric cylinders within the pore area itself (Fig. 34–36). The distance between the cylinders was 250 to 280 A. Strands arranged perpendicularly to the surfaces of the cylinders again indicated some sort of three-dimensional periodicity within the structure as a whole. Here, a longitudinal section through the pore suggested individual strands of material emanating from the pore rim joined at regular intervals by electron-dense plaques; it resembled muscle seen in thin section. In the zone of 5 to 8 mm from the hyphal tip, evidence of such striate structures in the septal pores was found; frequently, such structures were enveloped in amorphous, granular material which completely clogged the pore area.

Figures 34–38 show a presumed sequence in the development and disintegration of this material. In Fig. 34 a series of four concentric and progressively smaller rings can barely be discerned lining the pore rim. In Fig. 35 and 36 this structure has developed fully and is associated with voluminous amounts of granular material. In Fig. 37 the rings

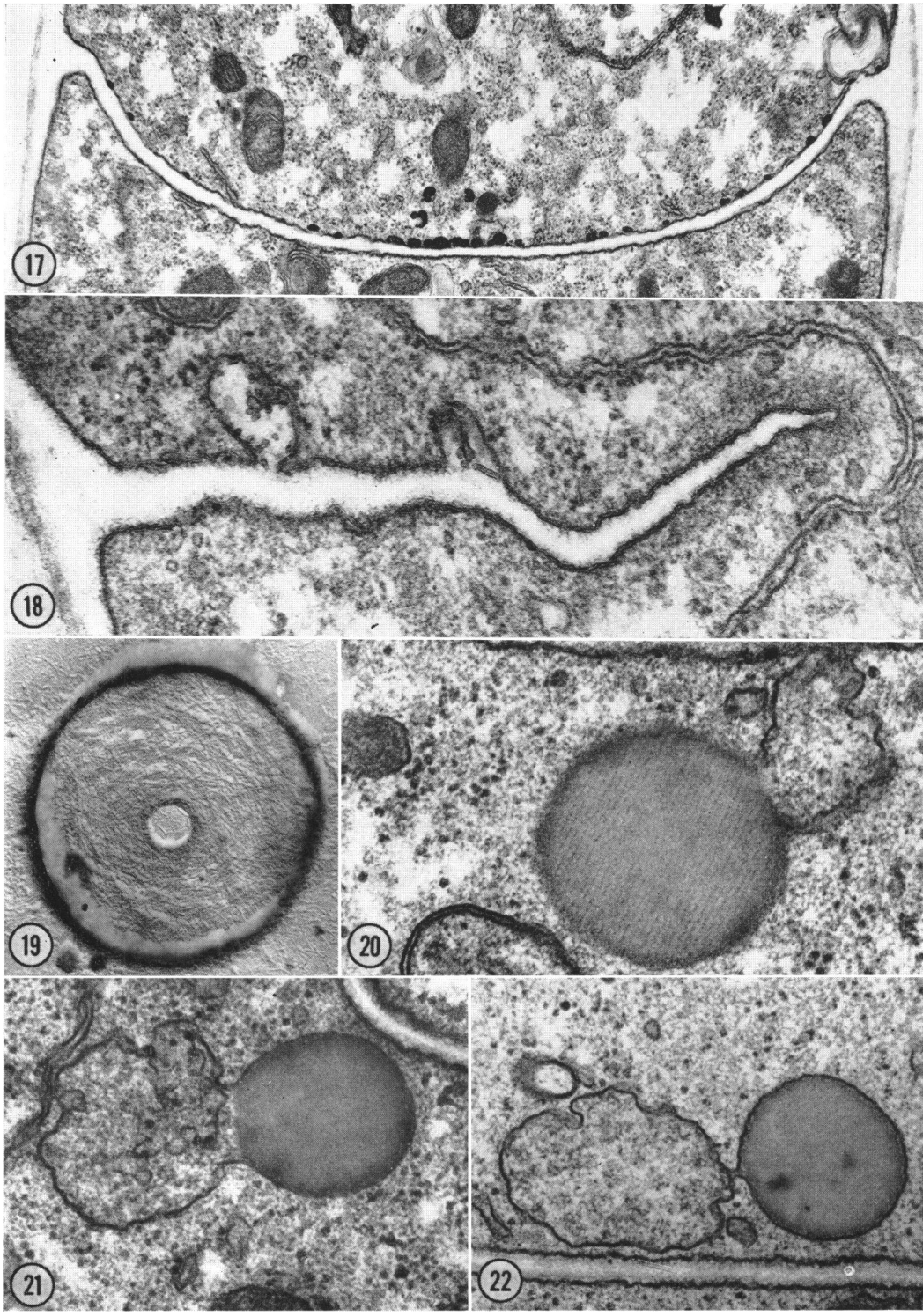

FIG. 17. *Longitudinal section of hypha in the region of a newly formed septum. Note dense granules associated with the septum.* × *21,000.*

FIG. 18. *Lomasomes associated with a forming cross wall.* × *68,000.*

FIG. 19. *Isolated septum shadowed at a low angle. Note fibrils in the wall.* × *12,000.*

FIG. 20. *Woronin body formation. Note internal lattice.* × *77,000.*

FIG. 21, 22. *Successive stages of Woronin body formation.* × *50,000.*

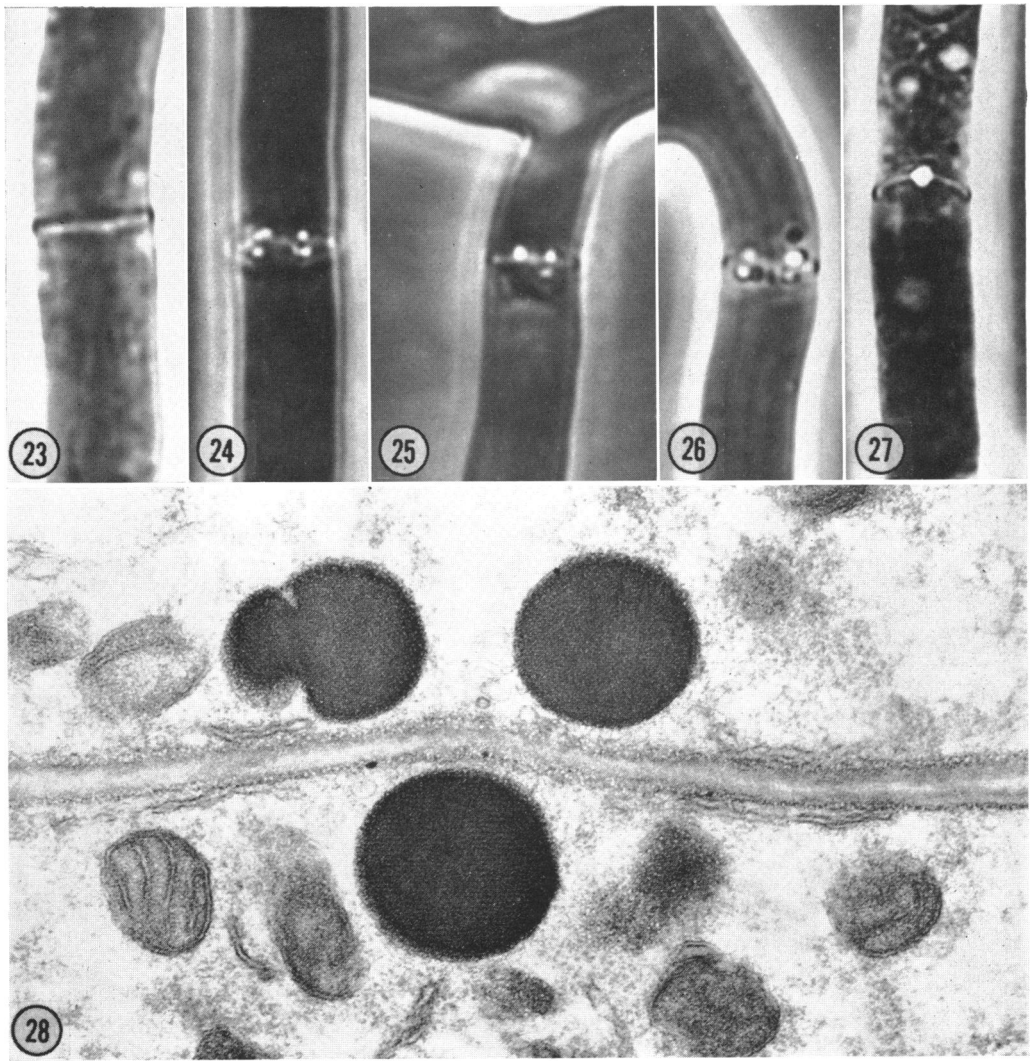

FIG. 23–27. *Phase micrographs of living mycelium.* × *1,700.* FIG. 23. *First septum behind hyphal tip. Woronin bodies appear as inconspicuous light-colored circular areas against the lateral wall. They have not yet formed any definite association with the cross-wall.* FIG. 24–26. *Portions of older hyphae (4 to 8 hr) showing conspicuous Woronin bodies associated with the septum.* FIG. 27. *Woronin bodies plugging a septal pore in an injured hyphal segment.*

FIG. 28. *Electron micrograph of Woronin bodies associated with an older cross wall (approximately 5 hr).* × *52,000.*

in the pore area have all but disappeared, but the granular material persists; Fig. 38 shows a later stage in which the granular material has largely dissipated. Since all of these stages can be found within a given 1-mm segment, the entire sequence of buildup and breakdown probably takes place within a 2-hr period.

Miscellaneous structures. In addition to the derivatives of the pore rim just described, several other unusual inclusions and organelles were seen in hyphae 7 to 13 hr old. Among these were cytoplasmic vesicles containing swirls of beaded or dilated membrane (Fig. 39); such vesicles measured 0.6 to 0.7 μ, and the individual beads within the vesicle were 300 to 450 A in diameter. Other inclusions appeared as rods, 350 A in diameter, each enclosed in a membrane and frequently seen in association with Woronin bodies (Fig. 40), mitochondria, and other organelles. A third sort of organelle consisted of

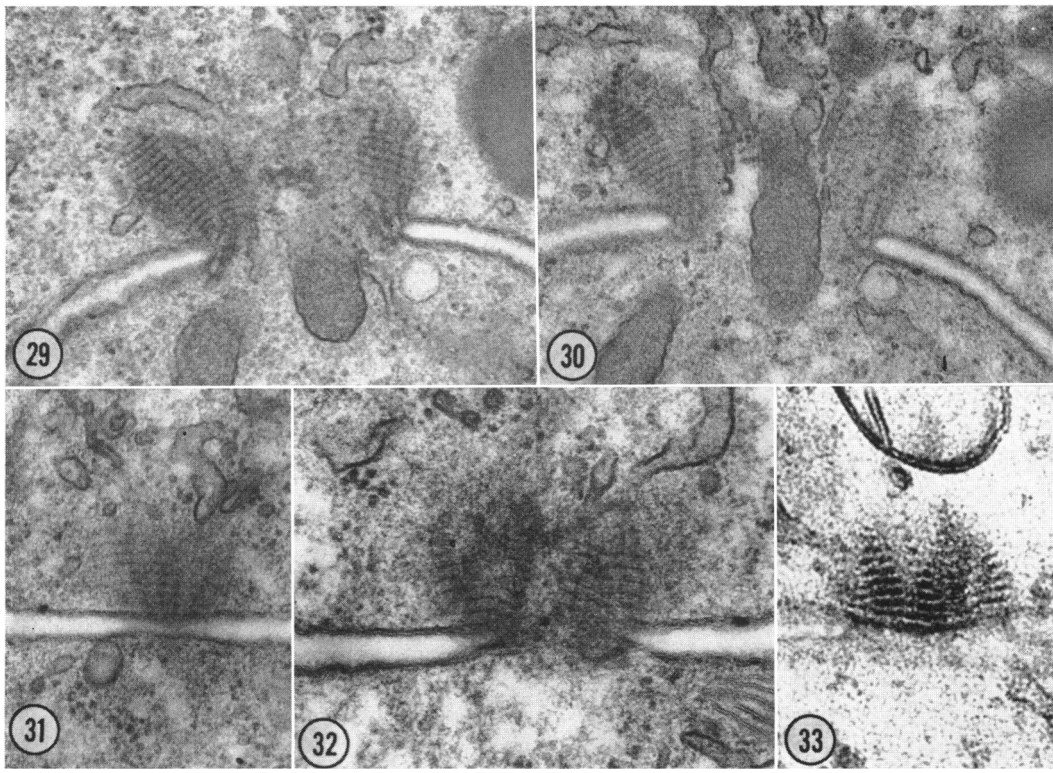

FIG. 29, 30. *Serial longitudinal sections through center of septal pore showing laminar structure on pore rim.* × *39,000.*

FIG. 31, 32. *Serial longitudinal sections through edge of septal pore showing laminar structure on pore rim.* × *50,000.*

FIG. 33. *Oblique section of septum showing laminar structure fixed in 2% $KMnO_4$.* × *45,000.*

stacks of unit membranes apparently involved with the elaboration of spherical, electron-transparent vesicles (Fig. 41–44).

DISCUSSION

The fine structure of fungal hyphal tips has only recently begun to be studied. Grove, Bracker, and Morré (Am. J. Botany **54**:638, 1967) have observed thin sections of the hyphal tip region of *Pythium ultimum*, and found that small vesicles arise from dictyosomes below the apex, fuse to form larger vesicles at the apex, and finally merge with the plasma membrane, releasing their contents to the wall. The situation in hyphal tips of *Ascodesmis* differs in that just one size of vesicle, comparable to the small vesicles seen by Grove et al., is produced. These vesicles seem to fuse directly with the plasma membrane without first coalescing to form larger vesicles. In contrast to *Pythium*, no dictyosomes have been found in any of the *Ascodesmis* hyphae examined; the origin of the apical vesicles here has not been discovered.

Two other systems, not noted by Grove et al. for *Pythium*, appear to function in the elaboration of lateral wall material in *Ascodesmis*. The dense granules below the apical region with associated membrane represent one such system. We suggest that, after their synthesis on convoluted cytoplasmic membranes, these granules migrate through the cytoplasm to the plasma membrane, where they break down and diffuse into the lateral wall. Such a process could account for the addition of a new and different component to the young wall.

Lomasomes represent a third system probably involved in the synthesis of wall components. Lomasomes were first reported by Moore and McAlear (5) from hyphae of a wide variety of fungi. Since then, they have been seen both in fungi and in other organisms, often in situations suggesting a relation with wall synthesis; for instance, in young ascospores they are found only during primary wall elaboration (13; G. C. Carroll, Ph.D. Thesis, Univ. of Texas, 1966).

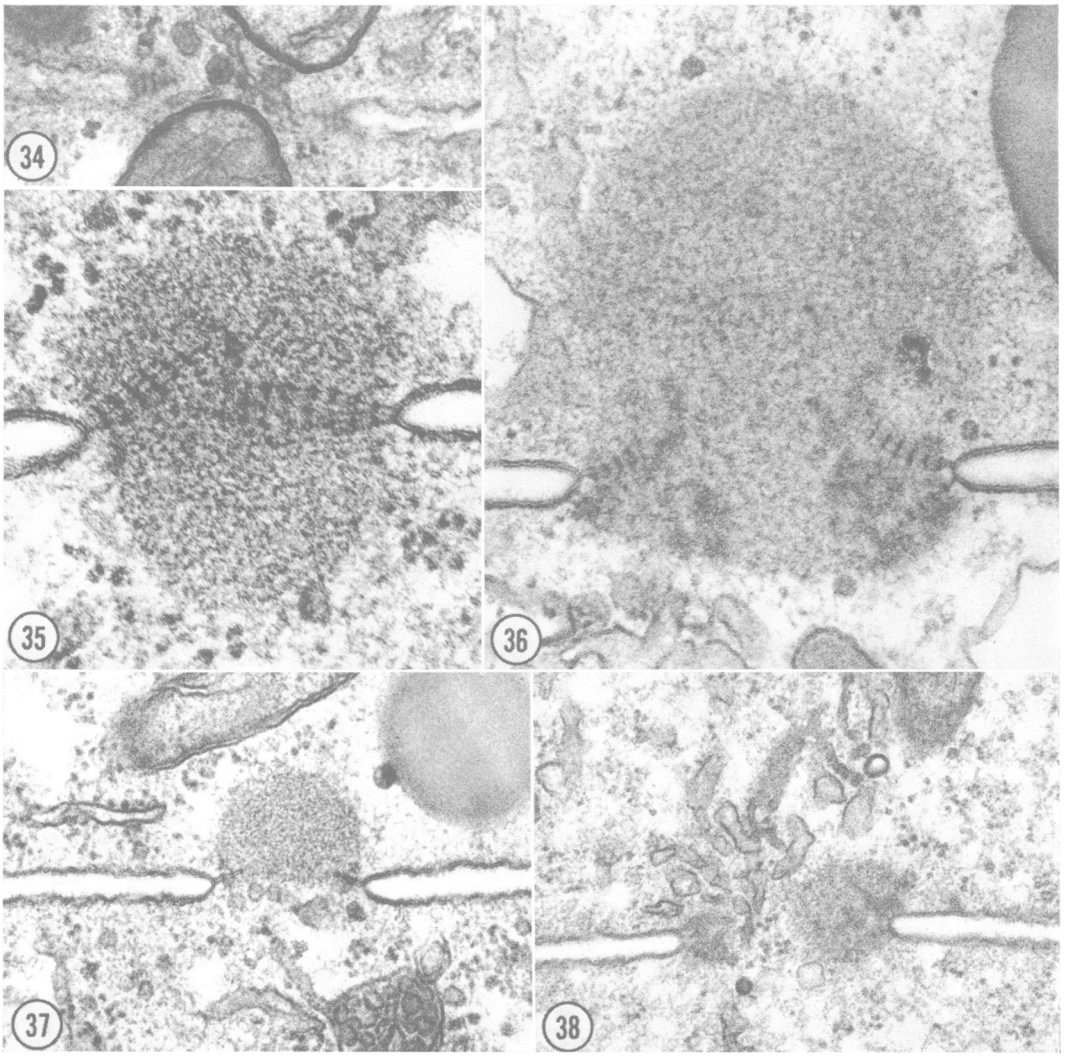

FIG. 34. *Longitudinal section of hypha showing septal pore. Note concentric rings developing in the pore area.* × *55,000.*

FIG. 35, 36. *Longitudinal sections of hyphae showing fully developed striate cylindrical structure associated with the pore rim. Note granular material plugging the pores. Fig. 35,* × *150,000. Fig. 36,* × *75,000.*

FIG. 37, 38. *Presumptive stages in the disintegration of the striate structures and associated granular material. Fig. 37,* × *50,000. Fig. 38,* × *39,200.*

There is very little specific mention of the process of cross-wall formation in the literature. Schweizer (9) believed that hyphal cross walls of *Humaria granulata* were first complete, and that septal pores developed later by swelling and dissolution of the central portions of the cross walls. Recently, Moore (4) suggested, on the basis of electron micrographs of *Ascodesmis* hyphae, that septa are formed by the differential contraction of torus-shaped bodies initially located in the lateral hyphal wall. He believes that subsequent fusion of the advancing edge of the plasma membrane across the pore results in a pore diaphragm which immediately balloons out into the cytoplasm and dissolves. Reichle and Alexander (7) do not discuss septum formation in *Fusarium* hyphae per se, but they do report the occurrence there of highly symmetrical multiperforate septa. Although incomplete lateral ingrowth could account for a uniperforate cross wall, it is difficult to imagine multiperforate septa arising in such a fashion.

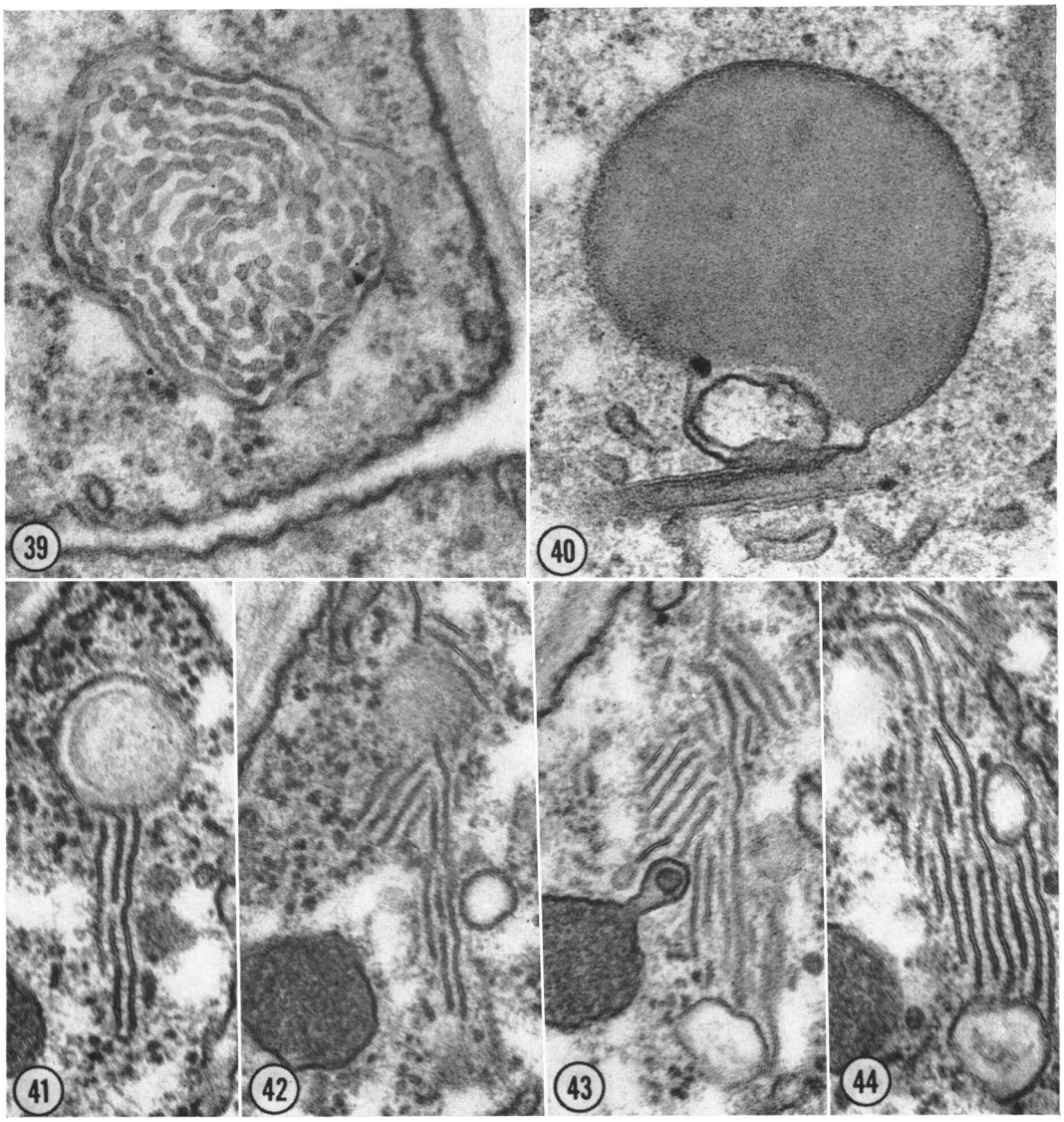

FIG. 39. *Vesicle containing beaded membrane.* × *68,000.*

FIG. 40. *Cylindrical inclusion associated with a Woronin body.* × *88,000.*

FIG. 41–44. *Serial sections through parallel stacks of membrane apparently involved in the elaboration of spherical electron-transparent bodies.* × *73,500.*

Our observations on isolated shadowed septa are in contradiction to the sequence of wall swelling and pore dissolution described by Schweizer (9) for *Humaria*. The discovery of a central pore in all septa examined, regardless of their age, indicates that in *Ascodesmis* the cross wall is never complete, and that pores arise by incomplete annular ingrowth rather than by dissolution of wall material. Although shadowed septa from the 2-mm zone immediately behind the hyphal tips were not seen, a sufficiently high proportion of septal pores were observed in sections of hyphae from this zone to indicate that they are always present there also. In several cases, serial sections of apparently complete septa revealed a pore below or above the plane of the initial section. We did not see any evidence for the fusion of the plasma membranes across the pore and the "blow-outs" of the pore diaphragm described by Moore (4). Occasionally, we saw

multiperforate septa in *Ascodesmis*; the mechanism of their formation, both in this species and in *Fusarium*, remains to be elucidated.

With regard to the initiation and formation of the septum itself, our findings disagree with those of Moore (4). Evidence of tori, which contract differentially and deposit wall substance, was not seen in the lateral wall. Rather, a cylindrical system of endoplasmic reticulum seems to be intimately involved in the formation of septa. It is likely that the small vesicles which appear to fuse with the plasma membrane during cross-wall formation originate from this ER system. The fact that the dark granules and lomasomes seen during lateral-wall production near the hyphal tip also occur in association with septum formation makes it likely that both systems are indeed involved in the elaboration of wall substance.

Since Woronin (14) first saw them, there have been persistent reports in the literature of small, highly refractive, spherical or oblong bodies associated with the septa in ascomycetous hyphae. [See Buller (1) and Reichle and Alexander (7) for list of early references.] Buller (1) coined the term "Woronin bodies" for them, and he concluded that they are always located near the cell vacuole and move in spasmodic jerks along the vacuolar wall. Workers studying the fine structure of ascomycetous hyphae have seen Woronin bodies repeatedly near septa, where they appear as electron-dense membrane-bounded spheres always somewhat larger than the septal pores (3, 7, 8). P. Snider (*personal communication*) recently has implicated Woronin bodies in the sealing-off of dead or injured cells in living mycelium; when a hyphal cell is injured with a microdissection needle under the phase microscope, the Woronin bodies are seen to obstruct the pores instantly. Pictures from the fine-structure literature of electron-dense membrane-bounded bodies plugging septal pores tend to confirm Snider's observations (6, 7, 11).

Our observations with the phase microscope agree with previous reports that Woronin bodies are randomly dispersed in young hyphal cells, that they occur in definite association with the cross walls in older cells, and that they plug septal pores in cross walls adjacent to injured cells. Examination of OsO_4-fixed hyphae under the electron microscope reveals an internal lamellation in these bodies; in fact, the regularity of the lattice justifies use of the term "crystalloid" for them. Their formation in closed membrane sacs is here reported for the first time.

Hexagonal crystalloids have been noted previously in hyphae of *Neurospora*. Shatkin (10) described these as regular structures, 0.2 to 0.3 μ in length, and suggested that they may correspond to the Woronin bodies of Buller. Tsuda and Tatum (12) observed similar crystals in *Neurospora* hyphae under both the light and electron microscopes and concluded, on the basis of cytochemical tests and UV absorption spectra of heptane extracts from crystal pellets obtained by centrifugation, that they are composed of ergosterol.

We saw both Woronin bodies and crystals in *Ascodesmis* hyphae. Because of differences in size and shape between the two groups of inclusions, we believe that they should not be considered synonymous. However, the similarities in electron staining properties, the presence of a lattice in both structures, and the comparable spacing of components in the lattice suggest that they are composed of the same substance. We did not perform microchemical tests, but the similarity of the crystals in *Ascodesmis* to those in *Neurospora* indicates a probable ergosterol composition for both crystals and Woronin bodies in *Ascodesmis*. We did not see crystals being pinched-off from membrane sacs; their mode of formation is a matter for further study.

The occurrence of laminar and ring-shaped structures in association with septal pores of fungal hyphae has not been previously reported. Schrantz (8) published a micrograph of such a structure from hyphae of *Ciliaria hirta*, but did not comment on it. We believe that the laminar and ring-shaped bodies are expressions of a similar activity oriented in a different position with respect to the pore rim in the two cases. The similarity in electron staining properties and spacing of the discs and rings, the suggestions of strands running perpendicularly to these components in both cases, and the simultaneous and restricted occurrence of both organelles to 7- to 13-hr-old cells argue for such an interpretation. The activity concerned may involve the synthesis of components in a restricted zone around the pore rim. If this zone happens to be above or below the actual pore area, a stack of discs results; if it is in the pore area, a series of stubby concentric cylinders is produced. Serial sections leave no question as to the laminar arrangement of components parallel to the septum in the former case. The structures shown in Fig. 34–36 are interpreted as sections of concentric cylinders; this may appear questionable, but it is supported by the following observations. (i) These structures appear essentially the same, regardless of the angle of the section through the longitudinal axis of the hypha; this implies a radial symmetry about the longitudinal axis, and hence, concentric cylinders. (ii) The same number of electron-dense plaques are usually found on each side of the pore rim, thus suggesting their continuity around

the pore out of the plane of the section. No histochemical tests have been performed on these structures, and their composition and function remains to be determined. It seems probable that the masses of granular material seen in septal pores in Fig. 35 and 36 are related to the septal swellings described by Schweizer (9). Similarly, the organelles and inclusions shown in Fig. 39 to 44 must await further study for an explanation of their function.

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