The Ultrastructure of Aphanomyces euteiches During Asexual Spore Formation

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ABSTRACT

The ultrastructure of *Aphanomyces euteiches* was studied to determine what cytological changes occur during the transition from the vegetative state through the formation of the primary spore, its extrusion and subsequent encystment. Cleavage of the sporangial protoplasm commenced with aggregation of the cytoplasm toward each nucleus, and with the withdrawal of the plasmalemma from the hyphal wall to delineate the primary spores. As the spores formed, the central vacuole evaginated and emptied its contents into the space developed between the plasmalemma of the primary spores and the hyphal wall. Much of the tonoplast of the evaginated central vacuole became part of the plasmalemma surrounding the primary spores. Simultaneously, plasmalemmasomes progressively lost their compact nature as the plasmalemma pulled away with the protoplast from the hyphal wall. Certain electron-opaque vesicular inclusions increased in size and became striated after induction of asexual sporulation in the hyphae. Other vesicles with an electron-opaque cortex and center became apparent as the protoplasm was delimited into primary spores. Vesicles concentrated at the developing cyst wall of the extruded primary spore, possibly contributing to its deposition. Centrioles were not observed until after induction of asexual sporulation. Flagella were not observed during the development of the primary spores in the hyphae or in the newly encysted primary spores. This indicates that *A. euteiches* is not diplanetic as previously described.

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Additional key word: zoospore.

Aphanomyces euteiches Drechs. is an important root pathogen of canning peas, often inciting severe root rots which significantly reduce both yield and quality of the harvested crop (18). The pathogen is a unique member of the Saprolegniales, described as diplanetic and exhibiting two separate stages of spore encystment. Under favorable conditions, the juvenile thallus is holocarpic, converting itself entirely to zoospores. The sequence of events in asexual spore formation have been previously described (11, 16). After vegetative hyphae are induced to sporulate, the hyphal protoplasm differentiates to form primary spores which are then extruded through a rupture in the tip of the sporangium. The extruded spores encyst immediately. Later, a zoospore emerges from each encysted primary spore and, in turn, eventually encysts and germinates. Cytological changes during these events are known solely from light microscopic studies (11, 16). Consequently, many of the important events, such as cleavage of the protoplasm into primary spores and development of flagella in the zoospores, are poorly understood. With certain other Phycomycetes, changes in the ultrastructure of sporangia during spore formation have been studied and are better understood (2, 3, 5, 8, 10, 12, 20). In these fungi, a cleavage apparatus derived from coalescing vesicles delimits the developing spores within the sporangium.

In order to better understand the relationship of environment to the formation of zoospores, we examined the ultrastructural changes that occur in *A. euteiches* during the transition from vegetative growth through asexual spore formation, and found that unique and different mechanisms are involved in the delineation of primary spores.

MATERIALS AND METHODS.–*Aphanomyces euteiches* was grown on 2% peptone-glucose agar in petri plates at 20-24 C. Glass cover slips thinly coated with the same agar and 1-cm squares of dialysis membrane were placed on the agar surface near the periphery of an actively growing culture. When three-fourths of the surface of the cover slips and dialysis membranes were overgrown by mycelium, they were removed from the petri plates and washed by submerging them in a flowing sterile basal salt solution (14) in a petri plate. Hyphae in various stages of spore formation were fixed in 1.5% glutaraldehyde in 0.025 M phosphate buffer at pH 6.8 for 1.5 hr. Unwashed vegetative hyphae were similarly fixed. The fixed hyphae were then washed in 0.025 M phosphate buffer for 1.5 hr and postfixed with 1% OsO_4 in the same buffer for 2 hr. After a brief rinse in distilled water, the tissue was stained for 2-3 hr in 0.5% aqueous uranyl acetate. The material was again rinsed in distilled water and dehydrated in a graded series of acetone. Final dehydration was effected by two changes of 100% acetone dried over anhydrous Na_2SO_4. The material was embedded in an Epon-Araldite mixture.

Segments of hyphae in the plastic to be sectioned were first photographed through a light microscope to provide a means of identifying and orienting the selected hyphae during sectioning at a later date. The hyphae were sectioned longitudinally with a diamond knife on a Porter-Blum MT-2 ultramicrotome. Thin sections were stained for 5 min with lead citrate and examined in a JEM-7 electron microscope.

An attempt was made to determine the presence of lipid within certain structures in the hyphae. The fungus grown on agar-coated cover slips was subjected

to extraction with 100% methanol, pyridine, ether, acetone, or a methanol-chloroform mixture (1:3) for 3 hr prior to OsO_4 fixation as previously reported (19, 20).

RESULTS.–*Ultrastructure of vegetative hyphae prior to washing*.–Somatic hyphae appear vacuolate, with a large central vacuole extending the length of the hyphae except for 40 to 60 μm of the growing tip (Fig. 1-A). The cytoplasm is restricted to a cylindrical layer limited on the inside by the tonoplast and on the outside by the plasmalemma (Fig. 1-B, C). Ribosomes, endoplasmic reticulum, mitochondria, and nuclei in *A. euteiches* were described previously (17). The nuclei are frequently elongated, with a Golgi complex (Fig. 1-D) and microtubules located along one side. Centrioles were not observed in vegetative hyphae; however, structures resembling procentrioles (not shown) (10) were noted in some of the hyphae examined.

Large lomasomes or "plasmalemmasomes", similar to those reported by Heath & Greenwood (6), are frequently invaginated 2 μm or more into the cytoplasm, and are irregularly shaped (Fig. 2-A). Each plasmalemmasome consists of a mass of tubules approximately 30 nm in diam. The tubules are formed of a unit membrane, and are often tightly coiled within the enclosing plasmalemma (Fig. 2-B). The space between the tubules is continuous, with the external environment via the cell wall at the point where the plasmalemma is invaginated (Fig. 2-A, C).

Vesicles containing electron-opaque inclusions occur throughout the hyphae. Near the growing tip, these inclusions range from 100 to 200 nm in diam and are surrounded by a unit membrane (Fig. 1-A, 3-A). Other electron-opaque inclusions with diameters of approximately 250 nm are observed in the vacuolate hyphae. The vesicles containing these inclusions are considerably larger than those nearer the hyphal tip, and contain much electron-transparent space. The structure of these latter inclusions subsequently alter markedly.

Lipid bodies are prominent in older hyphae, and vary greatly in size. They are usually spherical, with a distinct interface separating them from the rest of the cytoplasm (Fig. 1-B).

Ultrastructural changes induced by washing.–The ultrastructure of hyphae which were washed for periods of from 1 to 5 hr appear nearly the same as that of vegetative hyphae. No distinct differences are noted in any of the organelles. At this stage, centrioles adjacent to the nuclei were observed (not shown).

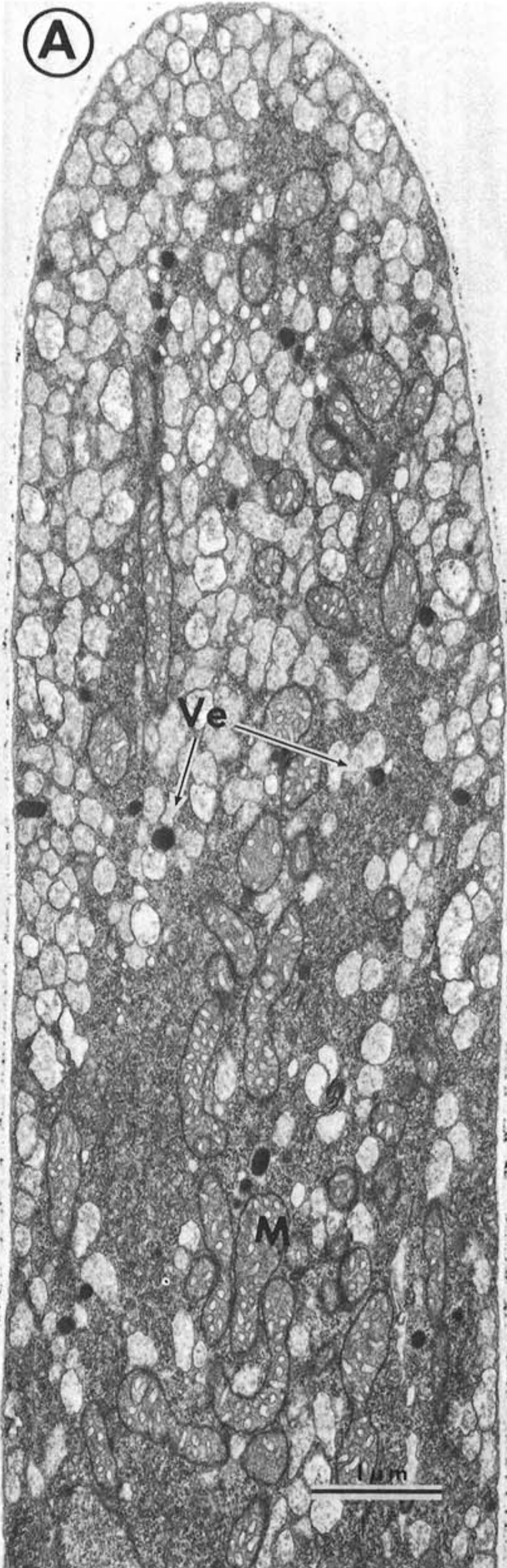
At irregular intervals, cytoplasmic strands extend into the central vacuole from the peripheral cylinder of cytoplasm. Eventually, many of these strands come together, connect with the cytoplasm on the opposite side of the hyphae, and segment the continuous central vacuole into smaller central vacuoles (Fig. 1-C, 4-A). Differences in content of some of these smaller central vacuoles are occasionally observed. Some of them appear clear and electron-transparent, whereas other vacuoles contain amorphous material (Fig. 1-C). Frequently, the two types of vacuolar contents are present in the same central vacuole, but remain distinct as though separated by a membrane. Smaller vacuoles within the segmented central vacuole are also common (Fig. 1-C), many of which are later seen located outside the developing spores (Fig. 2-D, 5).

One of the first changes noticeable by light microscopy in washed hyphae preceding delimitation of primary spores is an increase in refractivity of the cytoplasm. This is followed by an aggregation of cytoplasm toward the nuclei now situated nearer the longitudinal axis of the hyphae (Fig. 4-B, C). Continued aggregation results in a cytoplasmic septum extending across the hyphae that cleaves the central vacuole in the process. Simultaneously, the cytoplasmic layer at the periphery of the hyphae (Fig. 1-B) becomes thinner at points between successive nuclei until presumably the plasmalemma and tonoplast make contact and coalesce. At the points of coalescence, the contents of the vacuole empty into the space next to the hyphal wall, and are observed outside the developing spores (Fig. 2-D, 5). Concurrent with this evagination of the vacuole, the plasmalemma pulls away from the hyphal wall and, with the tonoplast, encompasses the developing primary spores (Fig. 4-D). The primary spores are generally connected with the next spore in a single row by a thin, membrane-bounded cytoplasmic strand (Fig. 4-E). The aggregation and actual differentiation of these spores occurs within 1-2 min immediately prior to their extrusion from the sporangium.

During early stages of spore differentiation, the plasmalemmasomes enlarge and protrude farther into the central vacuole of the hyphae (Fig. 2-C). The tubules become less tightly coiled. Finally, when the protoplast contracts toward the nuclei, the plasmalemmasome tubules are left external to the new spores together with the debris from the central vacuoles (Fig. 2-D, 5).

Several other changes in fine structure are noted in differentiating hyphae. Microtubules (Fig. 6-B) are more numerous, and are oriented toward the

Fig. 1. Ultrastructure of vegetative hyphae of *Aphanomyces euteiches* and of hyphae induced to sporulate. **A)** Near median longitudinal section of a vegetative hyphal tip showing the electron-opaque inclusions (Ve); also see Fig. 3-A (X 18,000). **B)** Cross section of older vegetative hypha showing the central vacuole (V) with a plasmalemmasome (P1) extending into it. (X 20,300). **C)** Longitudinal section through a hypha induced to sporulate. Note the difference in the content of the two vacuoles (V) separated by cytoplasm (X 16,500). **D)** An elongate nucleus (N) with a Golgi complex (G) aligned along one side in a hypha induced to sporulate (X 12,500). Bar scales = 0.1 μm except where noted otherwise. L = Lipid. M = Mitochondria. Nu = Nucleolus.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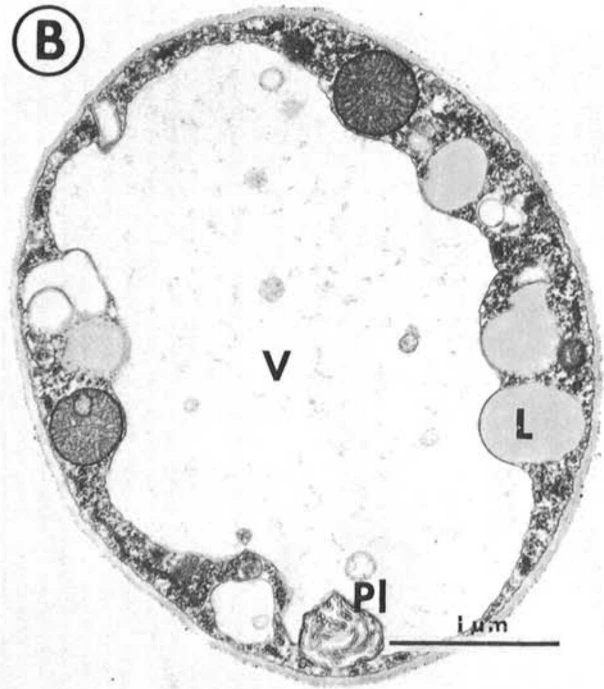
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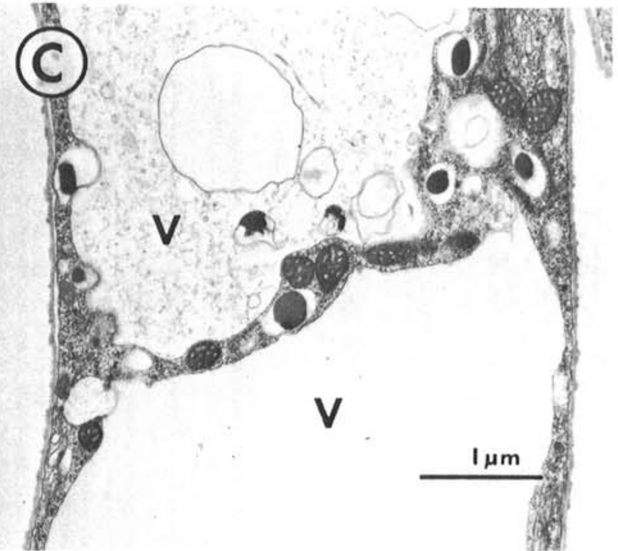
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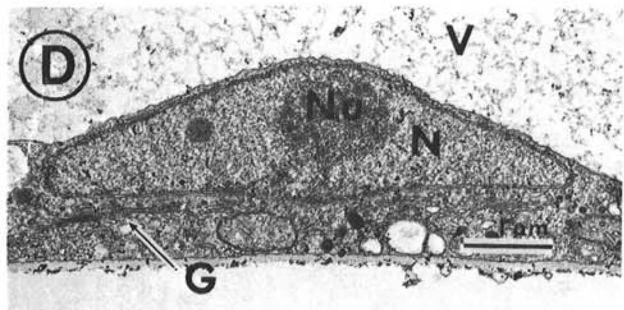
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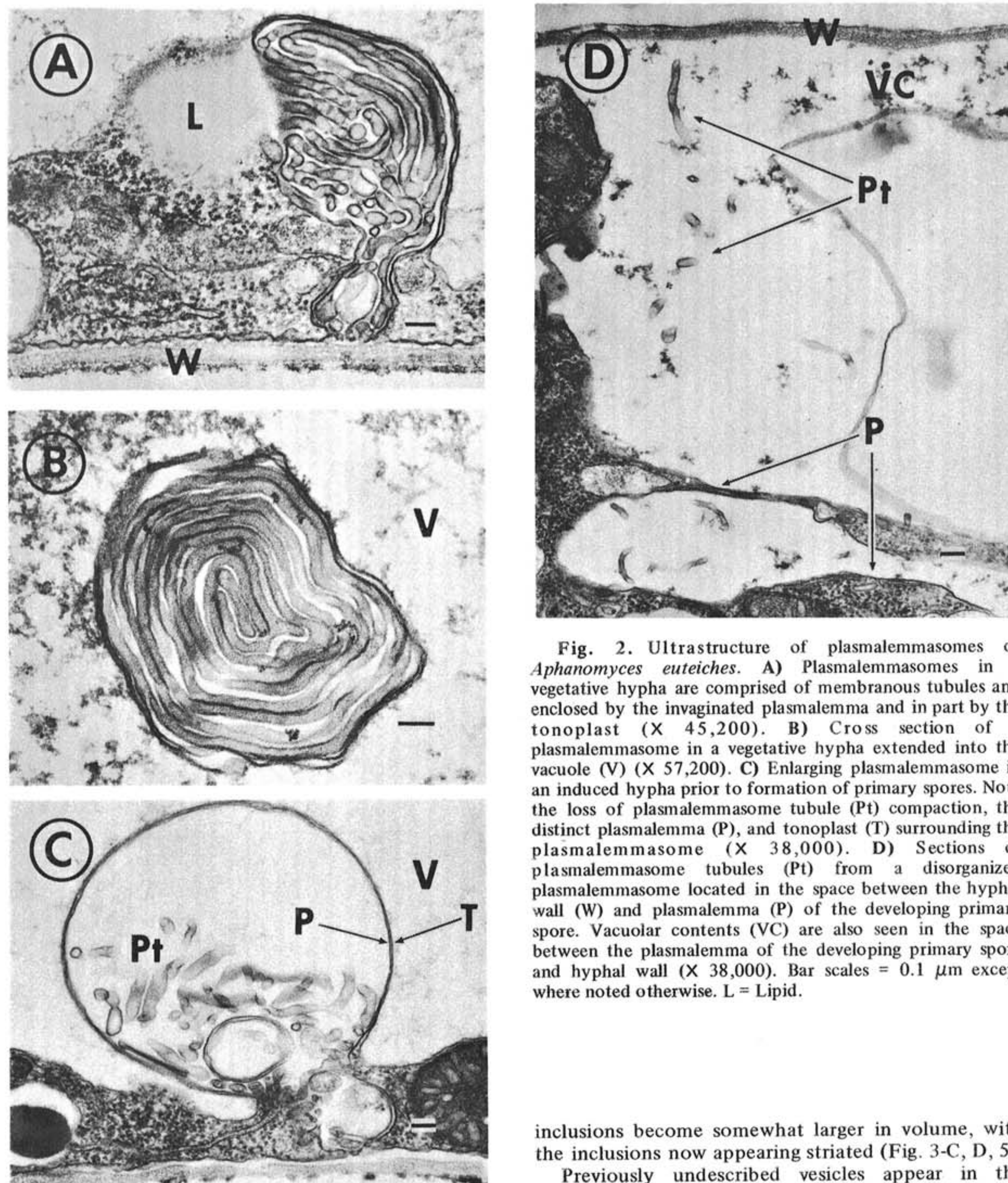

Fig. 2. Ultrastructure of plasmalemmasomes of *Aphanomyces euteiches.* **A)** Plasmalemmasomes in a vegetative hypha are comprised of membranous tubules and enclosed by the invaginated plasmalemma and in part by the tonoplast (X 45,200). **B)** Cross section of a plasmalemmasome in a vegetative hypha extended into the vacuole (V) (X 57,200). **C)** Enlarging plasmalemmasome in an induced hypha prior to formation of primary spores. Note the loss of plasmalemmasome tubule (Pt) compaction, the distinct plasmalemma (P), and tonoplast (T) surrounding the plasmalemmasome (X 38,000). **D)** Sections of plasmalemmasome tubules (Pt) from a disorganized plasmalemmasome located in the space between the hyphal wall (W) and plasmalemma (P) of the developing primary spore. Vacuolar contents (VC) are also seen in the space between the plasmalemma of the developing primary spore and hyphal wall (X 38,000). Bar scales = 0.1 μm except where noted otherwise. L = Lipid.

kinetosomes, generally parallel to the hyphal axis. The two kinetosomes (Fig. 6-B) observed in close proximity with each nucleus exhibit characteristics previously observed in fungi (5, 9, 15, 20), including the nine sets of triplet fibrils (Fig. 6-C) and the terminal plates (Fig. 6-A).

The vesicles which contain the electron-opaque inclusions become somewhat larger in volume, with the inclusions now appearing striated (Fig. 3-C, D, 5).

Previously undescribed vesicles appear in the newly formed primary spores. Spherical vesicles ca. 200 nm in diam with an electron-opaque cortex and center separated by a lighter median zone and bounded by a unit membrane are abundant at the periphery of the spores (Fig. 6-D, E). The contents of these vesicles appear granular. Other membrane-bounded vesicles ranging in size from 20 nm to 35 nm, with relatively electron-transparent contents, are also in the peripheral cytoplasm of the primary spores (Fig. 6-E).

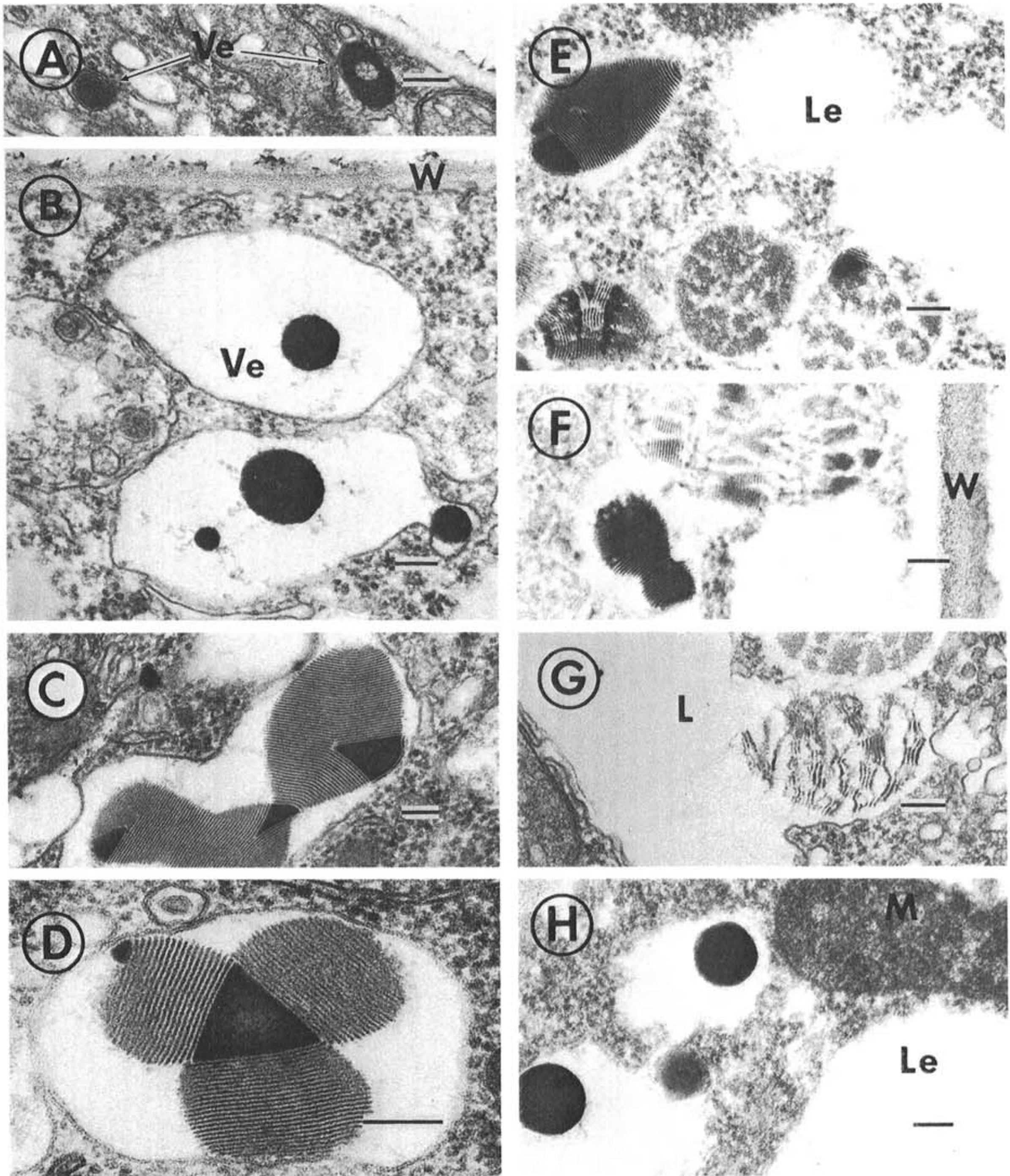

Fig. 3. A-D) Electron-opaque vesicular inclusions observed in the hyphae of *Aphanomyces euteiches.* **A)** Vesicular inclusions in vegetative hyphal tip (X 70,600). **B)** Vesicles with electron-opaque inclusions of an older portion of a vegetative hypha (X 67,700). **C, D)** Vesicular electron-opaque inclusions which have become striated 5-8 hr following initial washing of the hyphae (X 69,000 and X 121,000, respectively). **E-H)** Appearance of the electron-opaque inclusions following extraction with lipid solvents in a hypha induced to sporulate. **E)** Ether extraction. Electron-transparent areas are assumed to be sites corresponding to lipid bodies prior to extraction (Le) (X 70,600). **F)** Acetone extraction (X 66,200). **G)** Methanol extraction. Note the presence of lipid (L) and unit membranes not removed (X 70,600). **H)** Methanol-chloroform extraction. These inclusions apparently were not striated prior to extraction (X 67,700). Bar scales = 0.1 μm except where otherwise noted. W = Hyphae wall. M = Mitochondria.

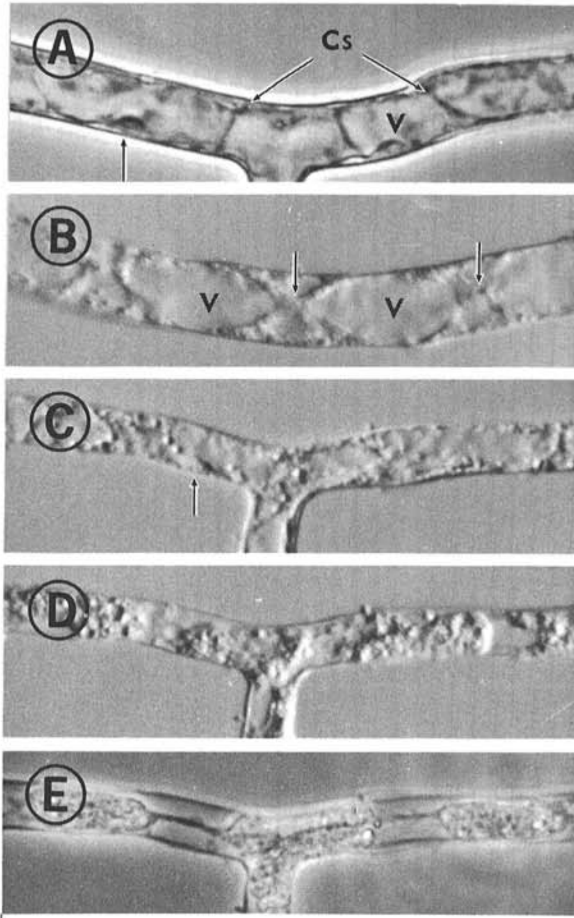

Fig. 4. Hyphae of *Aphanomyces euteiches* in various stages of sporulation. **A)** Cytoplasmic strands (Cs) extended across the central vacuole (V), often cleaving it into smaller vacuoles during the first 5 hr of washing. Arrow indicates position of nucleus (phase contrast). **B, C)** Aggregation of cytoplasm toward the centered nuclei (arrows). The central vacuole (V) is segmented into smaller vacuoles located between the nuclei (Nomarski interference-contrast). **D)** Hyphae with developing primary spores (Nomarski interference-contrast). **E)** Hyphae with primary spore formation complete. Note the membrane-bounded strands interconnecting the spores (phase contrast).

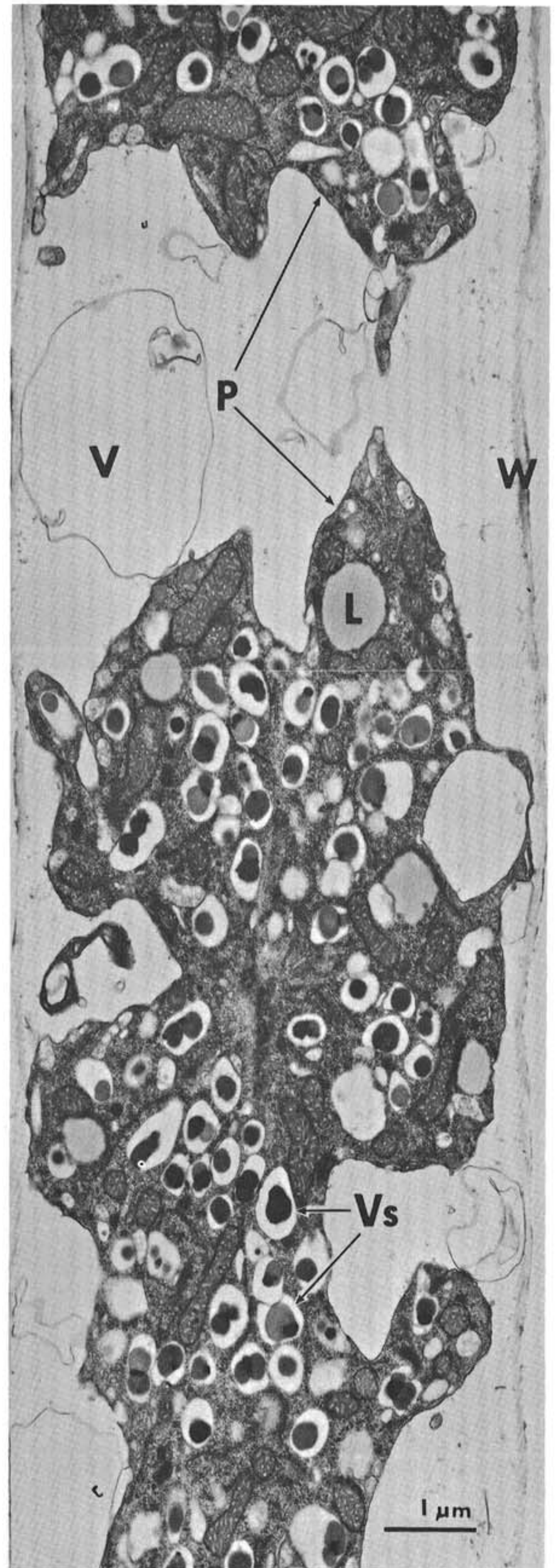

Fig. 5. Ultrastructure of a longitudinal section through a hypha of *Aphanomyces euteiches* containing portions of two developing primary spores. The plasmalemma (P) has retracted from the hyphal wall (W) and envelops the developing spores. Various membranous remmants and small vacuoles (V) evaginated from the central vacuole are situated between the wall and the spores. Note the abundance of vesicular electron opaque striated inclusions (Vs) (× 12,000). Bar scales = 0.1 µm except where noted otherwise. L = Lipid.

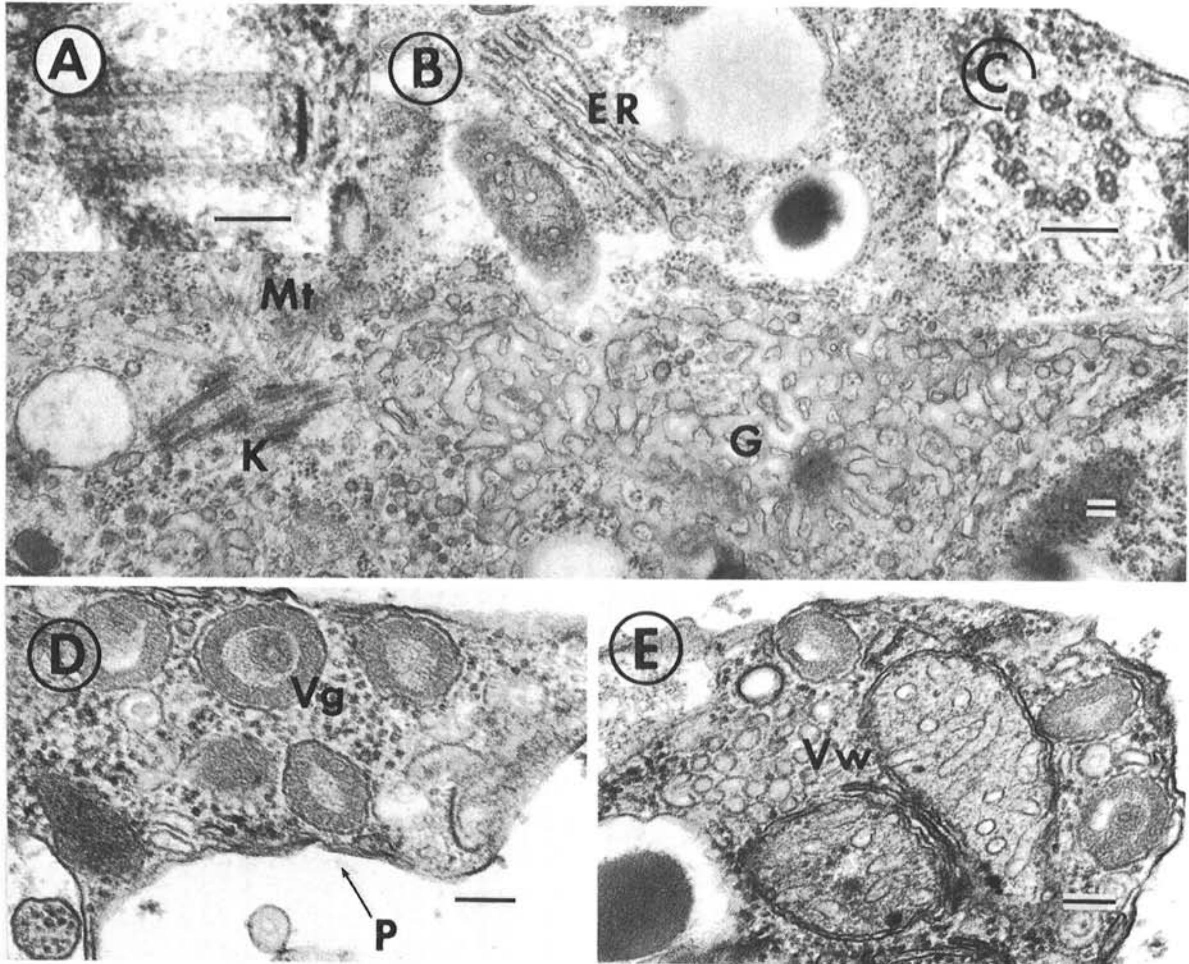

Fig. 6. Ultrastructure of developing primary spore within the hyphae of *Aphanomyces euteiches.* **A)** Longitudinal section through one of two kinetosomes illustrating the terminal plate (X 101,500). **B)** Area tangential to the nucleus (not shown) showing the two kinetosomes (K) with associated microtubules (Mt), a rough endoplasmic reticulum (ER), and a Golgi complex (G) (X 38,000). **C)** Cross section of one of two kinetosomes exhibiting the 9 sets of triplet fibrils (X 101,500). **D)** Vesicles with granular cortex and centers (Vg) (X 80,800). **E)** Grouped vesicles (Vw) are commonly observed in the developing primary spore (X 69,000). Bar scales = 0.1 μm except where noted otherwise.

Extrusion of primary spores.–Primary spore formation is complete within 8 hr after initiation of the washing process. The primary spores are discharged in succession through a rupture in the tip of the sporangium. They are elongated as they emerge from the spore, but become nearly spherical 10 to 20 sec later (Fig. 7-A).

Ultrastructurally, the extruded primary spores are bounded by a single membrane, with several of the inclusion-containing vesicles bulging at the periphery (Fig. 7-B). The membrane-bounded vesicles with the electron-opaque granular cortex and center are prominent in the outer zones of the extruded primary spores (Fig. 7-B). Various other vesicles are also present, including one type not observed in any previous stage (Fig. 7-C). These are flattened vesicles varying in length while averaging 25 nm thick and having an electron-transparent content (Fig. 7-B). They also are observed most commonly near the plasmalemma of the extruded primary spore.

Extruded primary spores developed cellulose cyst walls (16). The wall is characterized by a darker outer zone and a lighter inner zone (Fig. 8-A). What appears to be a thin and newly formed cyst wall with various vesicles perhaps contributing to its deposition is seen in Fig. 8-A, B. Multivesicular membrane-bounded bodies in these encysted primary spores (Fig. 8-A) are not observed in any previous stage of spore differentiation. The plasmalemma is close to the cyst wall except for an occasional plasmalemmasome.

Nuclei in older encysted primary spores are pear-shaped and characteristic of zoospore nuclei (9, 15, 20), with one of the two kinetosomes shown situated near the tapered end (Fig. 9). The cytoplasm of these spores remains extremely dense, with a well-developed rough endoplasmic reticulum close to the nucleus. Examination of serial sections has indicated that flagella are not present in any of the newly encysted primary spores. The tangentially sectioned cisternae of the rough endoplasmic

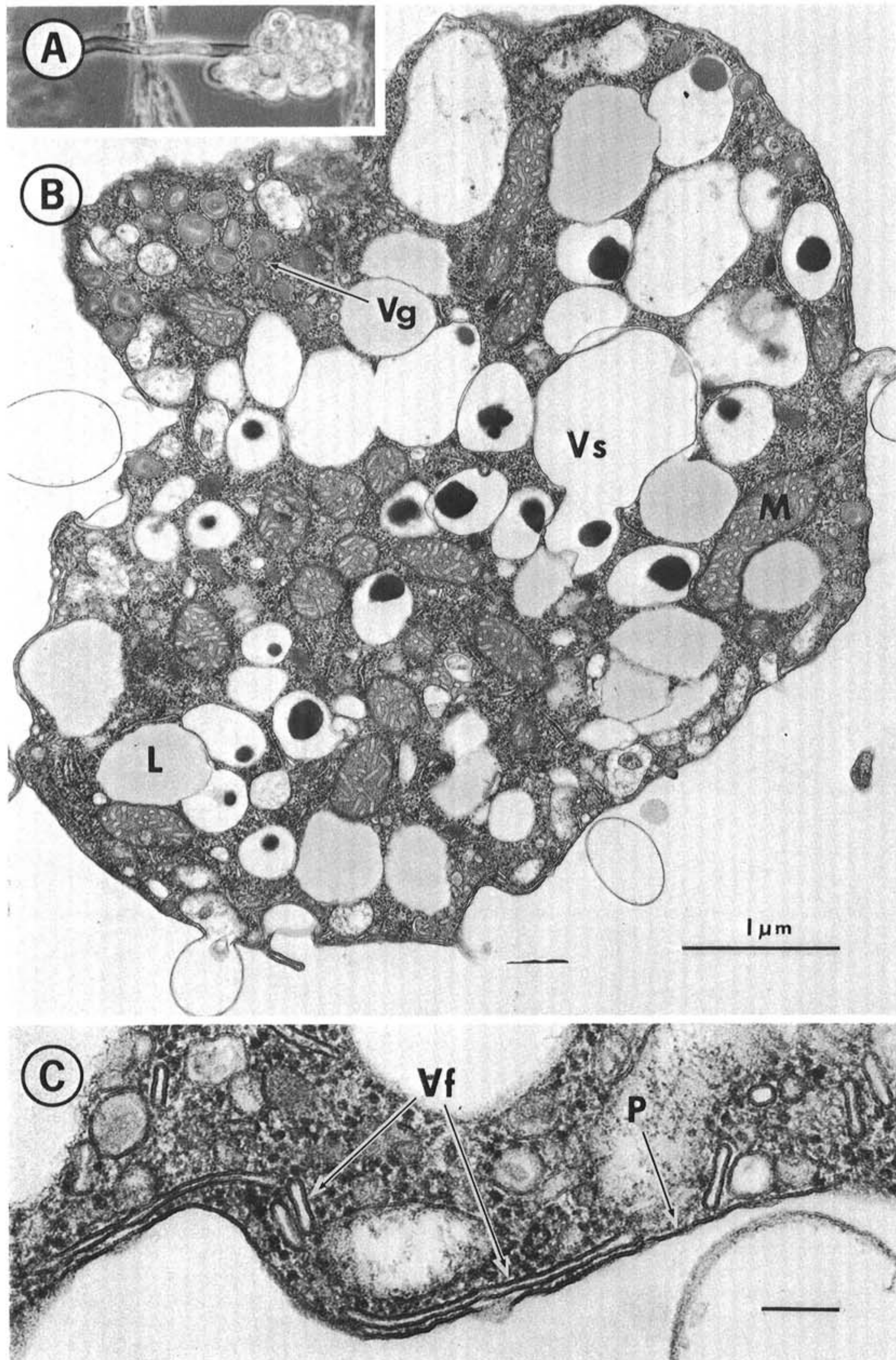

Fig. 7. A) Extrusion of primary spores at the sporangium apex (phase contrast). **B)** Ultrastructure of a recently extruded primary spore from the sporangium of *Aphanomyces euteiches*. Many vesicles containing the electron-opaque striated inclusions (Vs) are present within the dense cytoplasm as well as bulging from the surface of the spore. Cyst wall deposition is not evident in this spore (X 22,400). **C)** Enlarged section of the spore above exhibiting flattened vesicles (Vf) situated near the plasmalemma (P) (X 101,500). Bar scales = 0.1 μm except where noted otherwise. L = Lipid. M = Mitochondria. Vg = Vesicles with granular cortex and center.

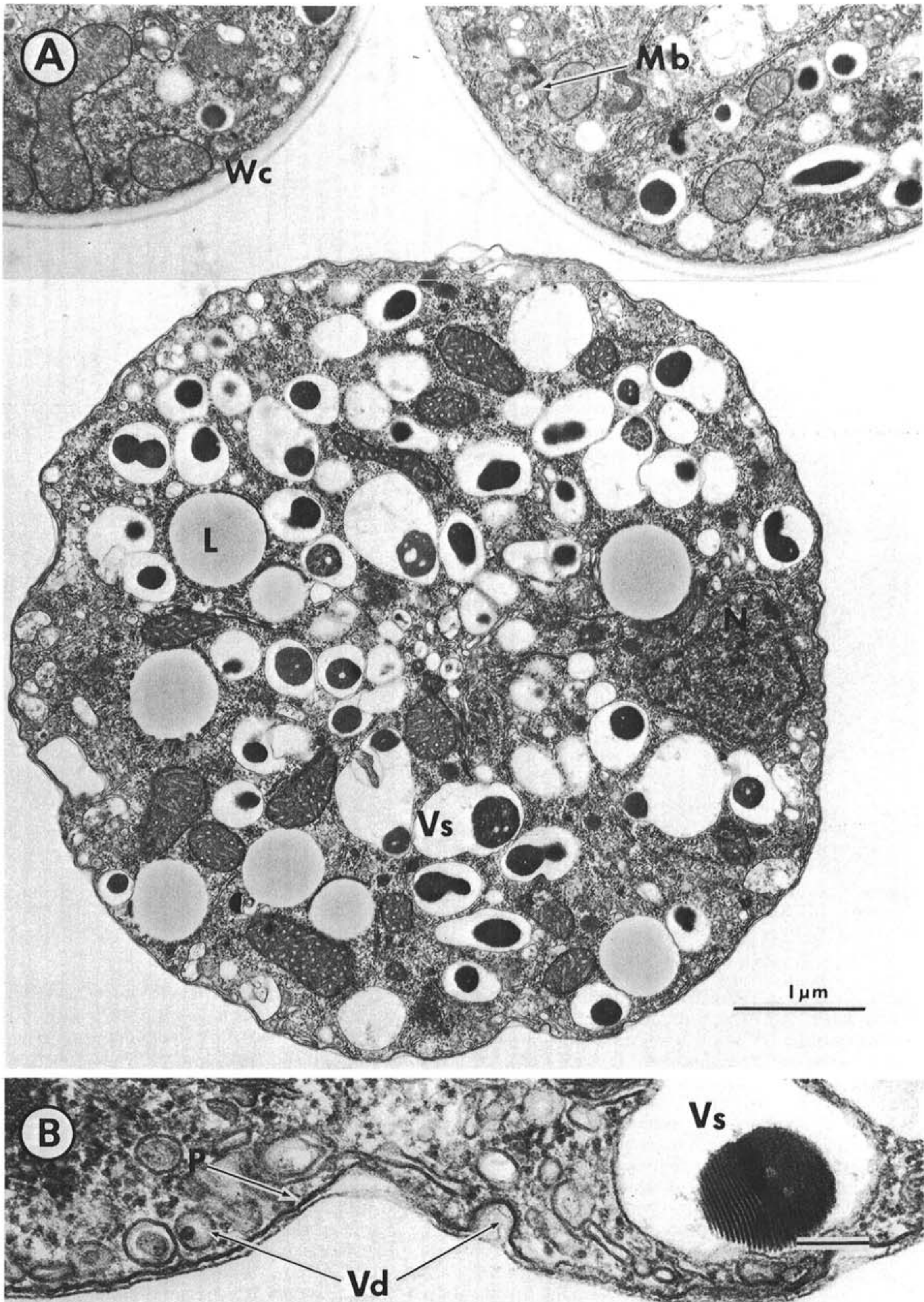

Fig. 8. Recently encysted primary spores of *Aphanomyces euteiches.* **A)** Three primary spores, two of which show a well-developed cyst wall (Wc) with two distinct layers. Note the presence of multivesicular bodies (Mb) within the top two spores (X 22,400). **B)** Enlarged lower portion of the primary spore above. Note the vesicles (Vd), and what appears to be a vesicle coalesced with the plasmalemma (P). Possible deposition of cyst wall material from these vesicles is speculative. Bar scales = 0.1 μm except where noted otherwise. L = Lipid. Vs = Vesicles with electron-opaque striated inclusions.

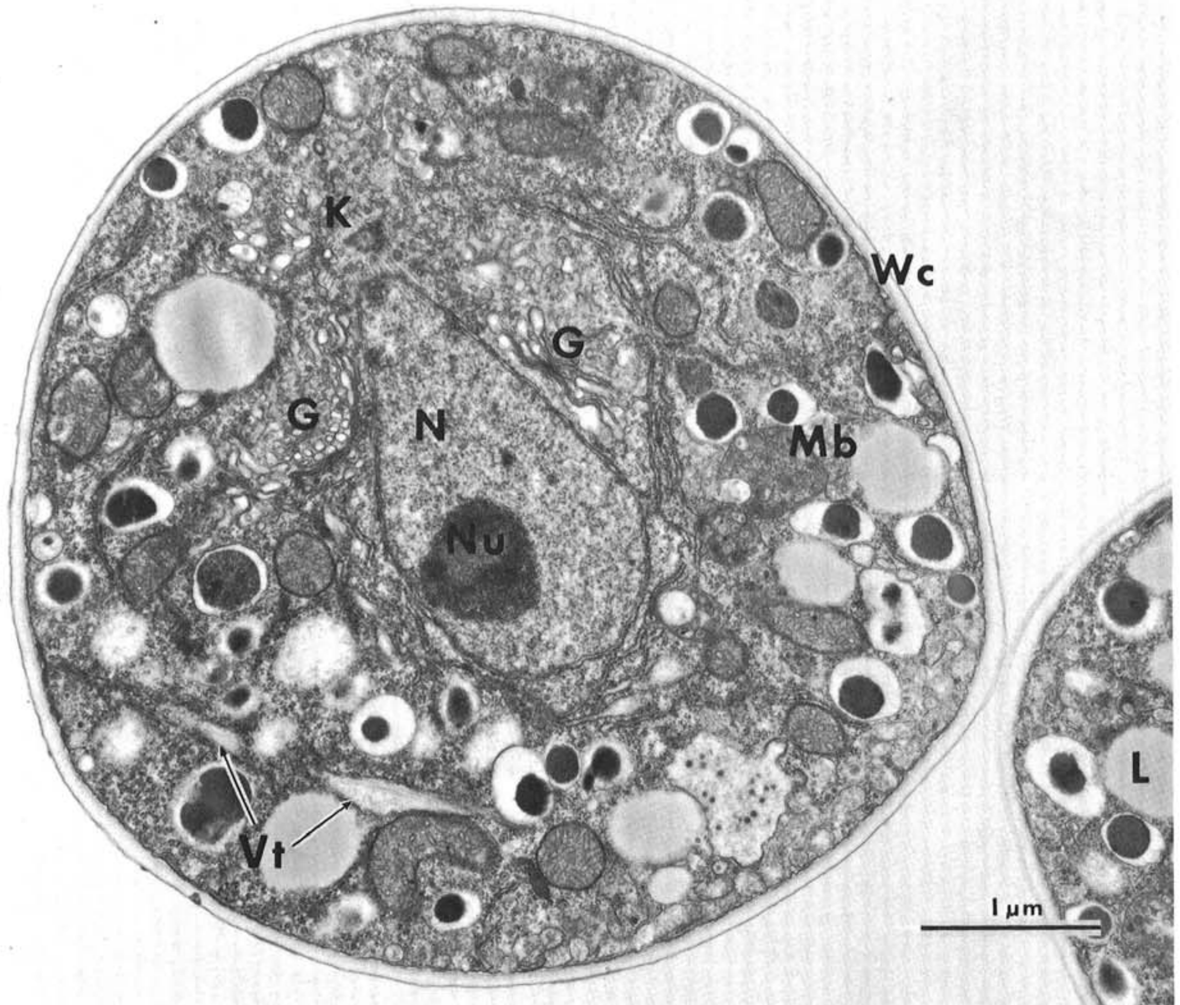

Fig. 9. Encysted primary spores of *Aphanomyces euteiches*. The nucleus (N) is pear-shaped, with one of the two kinestosomes (K) seen near the tapered end. Golgi complexes (G) are located on either side of the nucleus. Note the endoplasmic reticulum cisternae containing microtubules (Vt) (X 23,600). L = Lipid. Mb = Multivesicular bodies. Wc = Cyst wall.

reticulum in Fig. 9 containing microtubules are probably identical to The "Flimmer hairs" observed in *Saprolegnia ferax* (7). Similar structures were observed in zoospores of *Phytophthora capsici* (20), *P. parasitica* (15), and *Rhizidiomyces apaphysatus* (4).

Nature of the electron-opaque vesicular inclusions.–The increase in refractivity of washed mycelium observed by light microscopy appeared at first to have been due to an increase in concentration of lipid bodies. Examination of hyphae using electron microscopy has revealed no significant change in number of lipid bodies. However, the number of electron-opaque vesicular inclusions increases at the reticulum in Fig. 9 containing microtubules are time of primary spore formation. These inclusions, which appear striated in differentiated hyphae (Fig. 3-C, D, 5) are similar to those observed in other Oomycetes (3, 5, 9, 10, 20). Extraction with lipid solvents such as acetone, pyridine, ether, methanol, and a 1:3 mixture of methanol-chloroform failed to remove the inclusions completely (Fig. 3-E, F, G, H). All membranes and obvious lipid bodies were extracted with all the solvents except methanol, where incomplete extraction occurred (Fig. 3-G). In some instances, the inclusions appear markedly altered, but evidence of the characteristic striations remain.

DISCUSSION.–Cytological changes during asexual spore formation in *A. euteiches* indicate that this fungus differs in many ways from other members of the Saprolegniaceae or the Oomycetes that have been studied. The cleavage of spores within the sporangium appears to require no new membrane synthesis to delineate the spores. It has been shown, in other fungi which form sporangia, that newly formed vesicles coalesce to form a cleavage apparatus by which individual spores are delineated (2, 10, 12). Primary spore formation in *A. euteiches* does not involve the

coalescence of cleavage vesicles, but utilizes instead the existing plasmalemma and tonoplast of the hyphae that is present prior to induction of spore formation.

When a median section through a hypha containing primary spores was examined, the total length of membrane surface around the convoluted spores was nearly twice the length of the hypha containing them. This, together with determinations of membrane thickness around the developing primary spores (75-80 A for tonoplast and 90 Å for plasmalemma) and the absence of large quantities of extraneous membranes outside the developing primary spores, indicates that the tonoplast was in fact part of the plasmalemma surrounding the new spores. The possibility that these two membranes possess properties similar enough to fuse and come together as a continuous external membrane was suggested by Gay & Greenwood (5).

The existence of lomasomes in fungi has been controversial (1). Evidence recently has been presented suggesting that they may actually be normal components in living fungal cells (6). Observation of changes in plasmalemmasome configuration during asexual spore formation in *A. euteiches* has lent further evidence for their reality. The sequential change in arrangement of plasmalemmasome tubules from that observed in the vegetative state (Fig. 2-A, B) to that in the completely differentiated sporangium (Fig. 2-D) suggests that they exist in similar configurations in living cells. The "unidentified structures" observed in the study of Shatla et al. (17) appear to be the plasmalemmasomes described in this paper.

Striated inclusions have been previously reported in *A. euteiches* (17). However, we observed that these inclusions are not striated in the vegetative state (Fig. 3-A, B), but become so as the organism is induced to sporulate (Fig. 3-C, D). A similar change in the appearance of these inclusions during zoospore formation in the sporangium of *P. capsici* was reported (20). Other reports citing the striated inclusions have not correlated them with any nonstriated form prior to spore induction in related organisms (3, 5, 9, 10). These vesicular inclusions are probably the small granules we observed by light microscopy to increase in number within washed hyphae. Ho et al. (9) suggested that they are accumulated as waste products in *P. megasperma* var. *sojae*. Williams & Webster (20) indicated that similar structures, presumably lipid, disappeared when tissues were extracted with a 1:3 methanol-chloroform mixture for 1 hr; we have not been able to remove them completely with lipid solvents from *A. euteiches*. The altered appearance of the inclusions may be due to the sudden shift to the solvent systems used for extraction. Disruption or redistribution of the inclusion material also could occur if lipid material were present within the inclusions.

In *A. euteiches*, spores extruded from the sporangium are referred to in the literature as primary zoospores and the hyphae in which they differentiate, the zoosporangia (11, 13, 14). This terminology is erroneous. Zoospores are motile spores, either by flagella or by ameboid movements. Our observations indicated that flagella are not present in these primary spores (Fig. 5, 7, 8). No ameboid movement has been observed by light microscopy, and movement through the hyphae (sporangium) appeared to be passive. The spores formed within the sporangium should be referred to as "primary spores", and those extruded and having developed a cyst wall, as "encysted primary spores". The spore that emerges from the encysted primary spores and becomes motile should then be referred to simply as a "zoospore", and not as a "secondary zoospore". The organism would then be considered monoplanetic and not diplanetic.

The wall structure appears to be of importance in explaining the mechanism of spore extrusion from the sporangium. This extrusion of the spores is a most intriguing event in the sporulation sequence of this fungus. The absence of active motility, and the rapidity with which they move through the sporangia and are extruded, suggest that a positive hydrostatic pressure develops within the hyphae. However, to have a positive pressure in this region, the molecules contributory to this pressure must be large enough not to diffuse through the hyphal wall, as the plasmalemma is no longer adjacent to it. The properties of the wall may be such that it can act for a brief period as a semipermeable barrier to the osmoticum. Such molecules may have been released into this space during evagination of the central vacuole. Release of larger materials into this space is evidenced by the presence of amorphous material as well as membranes of the smaller vacuoles (Fig. 2-D, 5) observed within the central vacuole prior to evagination (Fig. 1-B, C, D, 2-A, B). There is no amorphous lining such as that previously described situated on the inner surface of the hyphal wall of *A. euteiches* (17) during the brief period of differentiation and extrusion.

The newly formed vesicles in the primary spore prior to and immediately after extrusion suggest that new systems are operating. Certainly a most obvious change is the appearance of a new cyst wall around the primary spore (Fig. 8). This would lead one to believe that some of the vesicles observed in the spores are contributing to this process. The vesicles in Fig. 8-B that appear to have coalesced with the plasmalemma have a density similar to that of the cyst wall. The flattened vesicles in the younger primary spores (Fig. 7-B, C) have previously been described lying parallel to the surface membranes of *P. parasitica* zoospores (15).

In this study of *A. euteiches*, some of the cytological changes that occurred during asexual sporulation from the vegetative state to the immature encysted primary spore have been described. The origin, function, and chemical composition of many of the structures observed will remain unknown until further studies, including cytochemical ones, are made. However, several important events in the process of asexual sporulation in this pathogen not previously observed in fungi have been elucidated.

The lack of flagella in the primary spores is an important observation, as this organism has been characterized as being diplanetic (11, 16). The differentiation process involving a withdrawal of the plasmalemma from the hyphal wall and an emptying of vacuolar material into the space between the hyphal wall and the developing spore is in sharp contrast to processes described for other Oomycetes.

Finally, with a better understanding of the events and mechanisms involved in increasing the inoculum potential of this root pathogen, environmental influences involving zoospore production can be dealt with more coherently.

LITERATURE CITED

1. BRACKER, C. E. 1967. Ultrastructure of fungi. Annu. Rev. Phytopathol. 5:343-374.
2. BRACKER, C. E. 1968. The ultrastructure and development of sporangia in Gilbertella persicaria. Mycologia 60:1016-1067.
3. CHAPMAN, J. A., & R. VUJICIC. 1965. The fine structure of sporangia of Phytophthora erythroseptica Pethybr. J. Gen. Microbiol. 41:275-282.
4. FULLER, M. S., & R. REICHLE. 1965. The zoospore and early development of Rhizidiomyces apaphysatus. Mycologia 57:946-961.
5. GAY, J. L., & A. D. GREENWOOD. 1966. Structural aspects of zoospore production in Saprolegnia ferax with particular reference to the cell and vacuolar membranes, p. 95-110. *In* M. F. Madelin [ed.]. The fungus spore. Colston Res. Soc. Paper No. 18. Butterworth Sci. Publ. London.
6. HEATH, I. B., & A. D. GREENWOOD. 1970. The structure and formation of lomasomes. J. Gen. Microbiol. 62:129-137.
7. HEATH, I. B., A. D. GREENWOOD, & H. B. GRIFFITHS. 1970. The origin of Flimmer in Saprolegnia, Dictyuchus, Synura and Cryptomonas. J. Cell Sci. 7:445-461.
8. HEMMES, D. E., & H. R. HOHL. 1969. Ultrastructural changes in directly germinating sporangia of Phytophthora parasitica. Amer. J. Bot. 56:303-313.
9. HO, H. H., K. ZACHARIAH, & C. J. HICKMAN. 1968. The ultrastructure of zoospores of Phytophthora megasperma var. sojae. Can. J. Bot. 46:37-42.
10. HOHL, H. R., & S. T. HAMAMOTO. 1967. Ultrastructural changes during zoospore formation in Phytophthora parasitica. Amer. J. Bot. 54:1131-1139.
11. JONES, F. R., & C. DRECHSLER. 1925. Root rot of peas in the United States caused by Aphanomyces euteiches (n sp). J. Agr. Res. 30:293-325.
12. KING, J. E., J. CALHOUN, & R. D. BUTLER. 1968. Changes in the ultrastructure of sporangia of Phytophthora infestans associated with indirect germination and aging. Brit. Mycol. Soc. Trans. 51:269-281.
13. LLANOS M., CARMEN, & J. L. LOCKWOOD. 1960. Factors affecting zoospore production by Aphanomyces euteiches. Phytopathology 50:826-830.
14. MITCHELL, J. E., & C. Y. YANG. 1966. Factors affecting growth and development of Aphanomyces euteiches. Phytopathology 56:917-922.
15. REICHLE, R. E. 1969. Fine structure of Phytophthora parasitica zoospores. Mycologia 61:30-51.
16. SCOTT, W. W. 1961. A monograph of the genus Aphanomyces. Virginia Agr. Exp. Sta. Tech. Bull. 151. 95 p.
17. SHATLA, M. N., C. Y. YANG, & J. E. MITCHELL. 1966. Cytological and fine structure studies of Aphanomyces euteiches. Phytopathology 56:923-928.
18. WALKER, J. C., & W. W. HARE. 1943. Pea diseases in Wisconsin in 1942. Wis. Agr. Exp. Sta. Res. Bull. 145.
19. WILLIAMS, P. H., N. T. KEEN, J. O. STRANDBERG, & S. S. MCNABOLA. 1968. Metabolite synthesis and degradation during clubroot development in cabbage hypocotyls. Phytopathology 58:921-928.
20. WILLIAMS, W. T., & R. K. WEBSTER. 1970. Electron microscopy of the sporangium of Phytophthora capsici. Can. J. Bot. 48:221-227.