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Development and Ultrastructure of the Marine, Parasitic Oomycete, *Lagenisma coscinodisci* Drebes (*Lagenidiales*): Formation of the Primary Zoospores and Their Release

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With 34 Figures

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Summary

Zoosporogenesis in *Lagenisma* begins after the final nuclear division by the development of "encystment vesicles" which presumably are derived from Golgi vesicles. The sporangial wall is secreted simultaneously. Initially, the encystment vesicles have an internal coat of fine ribs which becomes a uniform mass during the complicated invagination of the vesicles. When the sporangial wall is complete the protoplast cleaves centripetally by means of narrow "cleavage cisternae" apparently coming from the distal face of the dictyosomes and being detached by interposing ER cisternae. The cleavage cisternae fuse with each other and with the plasmalemma to which they are often parallel. Narrow cytoplasmic compartments are then cut off and swell to become "separation vesicles" which lie between the developing zoospores but later disintegrate. Basal bodies develop from procentrioles after the final nuclear division and elongate into flagella (without participation of a flagellar vesicle) when cleavage is complete. The mastigonemes are formed within the ER, mature within the peripheral elements of the dictyosomes near the flagellar bases and appear to be extruded after the elongation of the flagellum. Structurally, especially in the organization of the flagellar root apparatus, the zoospores resemble primary zoospores of other Oomycetes. They become motile within the zoosporangium but seem to be driven out by means of additional unknown forces.—Formation of the encystment vesicles and the manner of cleavage are compared with those of other Oomycetes and general aspects of *Lagenisma* zoosporogenesis are discussed.

1. Introduction

This communication is part of a series on the marine Oomycete, *Lagenisma coscinodisci* Drebes, an intracellular parasite of large planktonic diatoms (*Coscinodiscus, Palmeria*). In the preceding papers we have described the general development of the fungus (Schnepf and Drebes 1977) and ultrastructural aspects of the infection of the host cell (Schnepf *et al.* 1978 a), of the development of the vegetative thallus, and of the formation of the zoosporangium (Schnepf *et al.* 1978 b). In the present paper, we continue with observations on zoosporogenesis.

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The formation of zoospores in Oomycetes has been studied in some *Saprolegniales* (*Saprolegnia:* HAGEDORN and WEINERT 1972, 1974; *Aphanomyces:* HOCH and MITCHELL 1972 a), some *Peronosporales* (*Phytophthora:* HOHL and HAMAMOTO 1967, CHRISTEN and HOHL 1972; *Pythium:* LUNNEY and BLAND 1976 a) and in two species of the *Lagenidiales* (*Lagenidium:* BLAND and AMERSON 1973, GOTELLI 1974; *Blastulidium:* MANIER 1976).

Zoosporogenesis in *Lagenisma* was found to differ considerably from that of the other Oomycetes described previously, especially the mode of cleavage with participation of special "cleavage cisternae" and the development of "separation vesicles" from isolated portions of the cytoplasm.

Also, special "encystment vesicles" which apparently are used during the subsequent encystment of the zoospores to form a primary cyst wall (SCHNEPF *et al.* 1978 c) are formed in a complicated way.

In addition to the ultrastructural studies, we have tried to influence the zoosporogenesis experimentally using cytochalasin B as an inhibitor of contractile processes.

2. Material and Methods

The fungus was cultivated as desribed previously (SCHNEPF and DREBES 1977, SCHNEPF *et al.* 1978 a) using the diatom *Coscinodiscus granii* Gough as the host and the f/2 seawater medium. For electron microscopy we mainly used glutaraldehyde + OsO_4 as fixatives. Details of preparation are given in SCHNEPF *et al.* (1978 a).

Cytochalasin B (CB) was dissolved in dimethylsulphoxide (DMSO); the concentration was 0.1%. This solution was added to the seawater medium to give a final concentration of 5 or 20 µg/ml. Controls were cultivated in the normal f/2 medium or with 5 or 20 µl/ml DMSO.

3. Observations

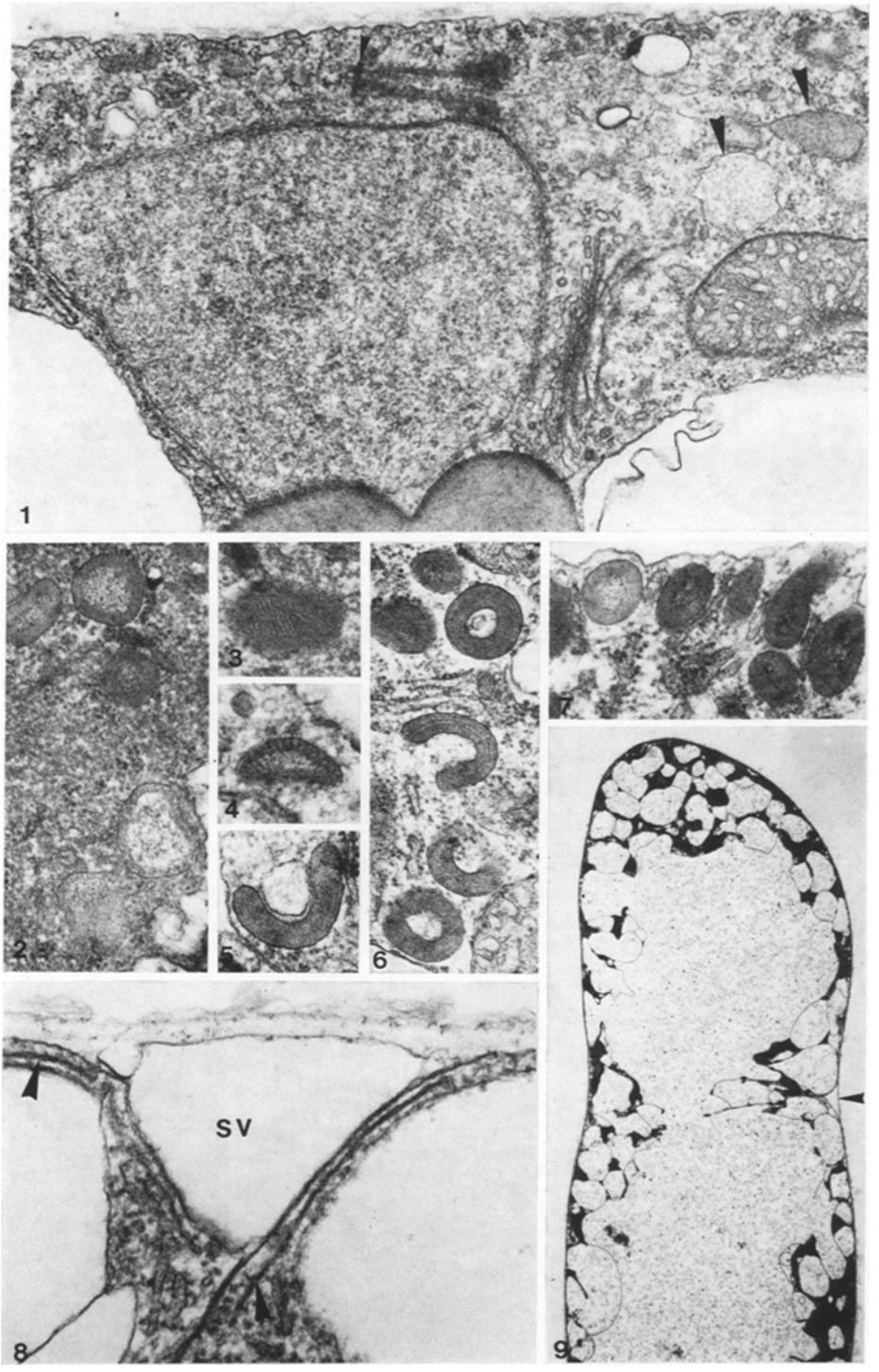
The vegetative thallus is naked. It develops directly and completely into a zoosporangium by forming a wall when the host cell has died. Cleavage of the multinucleate protoplast into single zoospores begins when the sporangial wall has been completed (SCHNEPF *et al.* 1978 b). However, zoosporogenesis has already begun with the formation of encystment vesicles and mastigonemes, and is completed by the formation of flagella and morphogenesis of the swarmers.

Fig. 1. Early stage of zoospore formation. Nucleus with fully grown centriole and "posteriorly" directed microtubules; terminal plate marked by small arrowhead. The dictyosome seems to produce vesicles which could be juvenile stages of encystment vesicles (large arrowheads). ×28,000

Figs. 2–7. Encystment vesicles, in development (Figs. 2–6) and mature (Fig. 7), Fig 2. ×34,000, Fig. 3. ×42,000, Fig. 4. ×52,000, Fig. 5. ×48,000, Fig. 6. ×36,000, Fig. 7. ×43,000

Fig. 8. Separation vesicle (*SV*) and cleavage cisternae (arrowheads). ×66,000

Fig. 9. Young zoosporangium, onset of cleavage (arrowhead). ×2,900



Figs. 1–9

3.1. Formation of Encystment Vesicles

Immediately after the last nuclear division, simultaneous with the formation of the zoosporangial wall (SCHNEPF *et al.* 1978 a), vesicles with an irregular profile and more or less dense, fuzzy, homogeneously distributed contents appear in the region around the nuclear tip (Fig. 1). It is not clear whether they are derived from the dictyosomes which are situated here or from dilating cisternae of the endoplasmic reticulum (ER). Apparently, they soon transform into another type of vesicle since they come to occur rather seldom and seem to be replaced by vesicles with a less irregular outline. In the latter, the

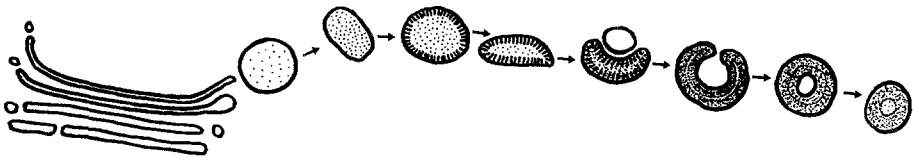


Fig. 10. Development of the encystment vesicles, schematic

vesicle membrane is covered on its inner face by a dense material (Fig. 2). This layer is about 40 nm thick and is composed of rib-like elements with a spacing of 12–18 nm (Fig. 3).

The vesicles then either flatten, elongate or become invaginated (Fig. 4). The invaginations may contain a dilated element of the ER (Fig. 5). The internal coat is now denser and generally no longer shows the substructure. The coats of opposite membrane areas come closely together and are separated only by a space of less than 10 nm in this developmental phase (Fig. 6).

The flattened vesicles also transform to become cup-like, like those which have been invaginated. After cleavage of the protoplast, the aperture of the "cup" closes. The vesicles then contain a central portion of cytoplasm bound by a membrane (see Fig. 10).

During the morphogenesis of the zoospores this membrane eventually disintegrates. The mature encystment vesicles (Fig. 7) have a core which is the remnant of the cytoplasmic inclusion and a peripheral, dense, more or less homogeneous layer which is derived from the two, now fused, coats. They

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Figs. 11–16. Details of cleavage

Fig. 11. Ingrowing cleavage furrow and separation vesicle (*SV*). ×22,000

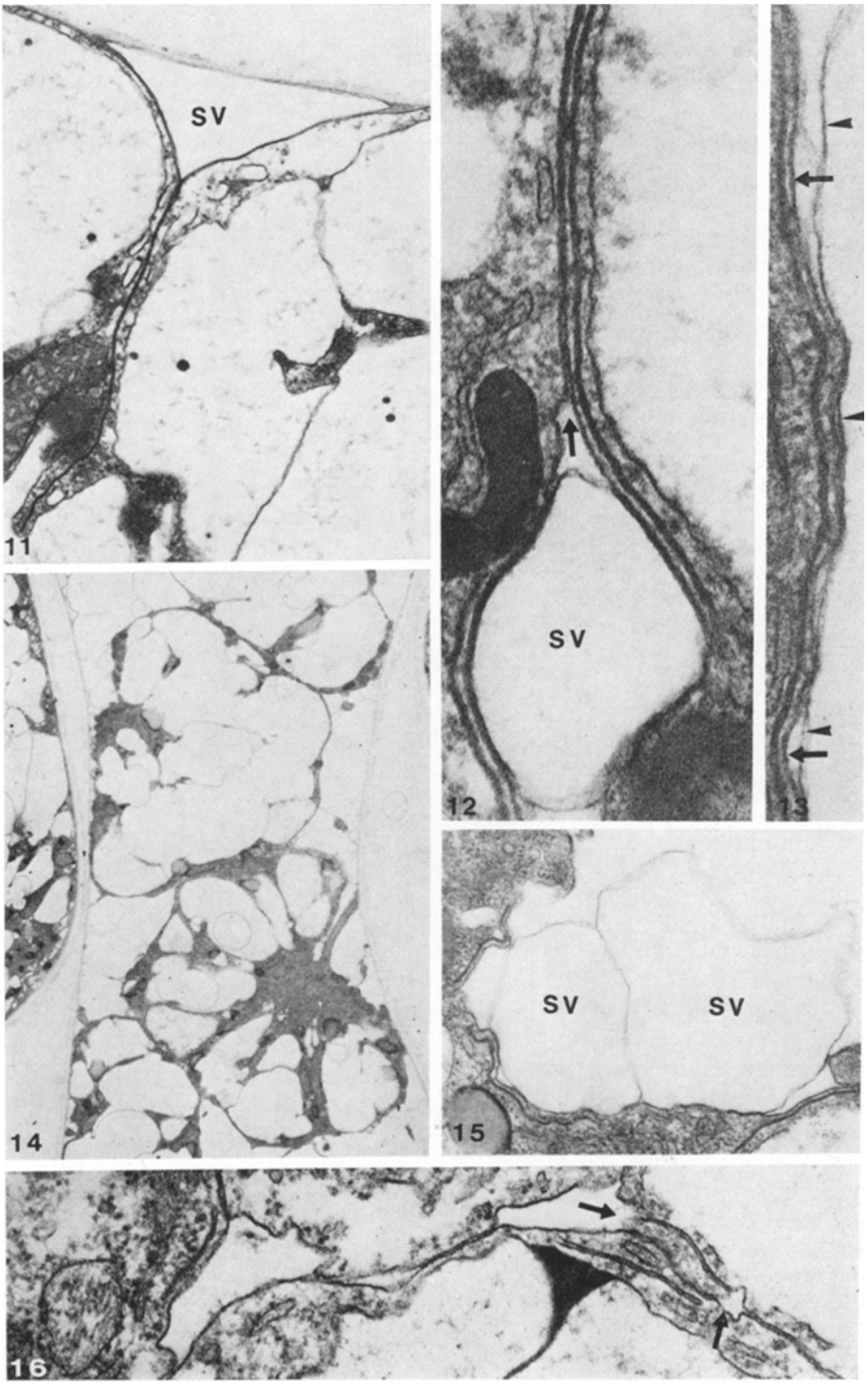
Fig. 12. Cleavage cleft (arrow), cleavage cisternae and separation vesicle (*SV*). ×88,000

Fig. 13. Plasmalemma (arrows), subplasmalemmal cleavage cisterna and membrane of a separation vesicle (arrowheads). ×100,000

Fig. 14. Zoosporangium after cleavage, separation vesicles between the zoospores. ×5,000

Fig. 15. Separation vesicles (*SV*) and subplasmalemmal cleavage cisternae. ×33,000

Fig. 16. Fusions of a cleavage cisterna with the plasmalemma after cleavage (arrows). ×47,000



Figs. 11–16

remain concentrated around the flagellar bases but are found also in the other peripheral regions of the zoospores. Usually they are closely associated with irregular profiles of the ER. The function of these vesicles becomes evident during the encystment of the primary zoospores ("encystment vesicles") (see SCHNEPF *et al.* 1978 c).

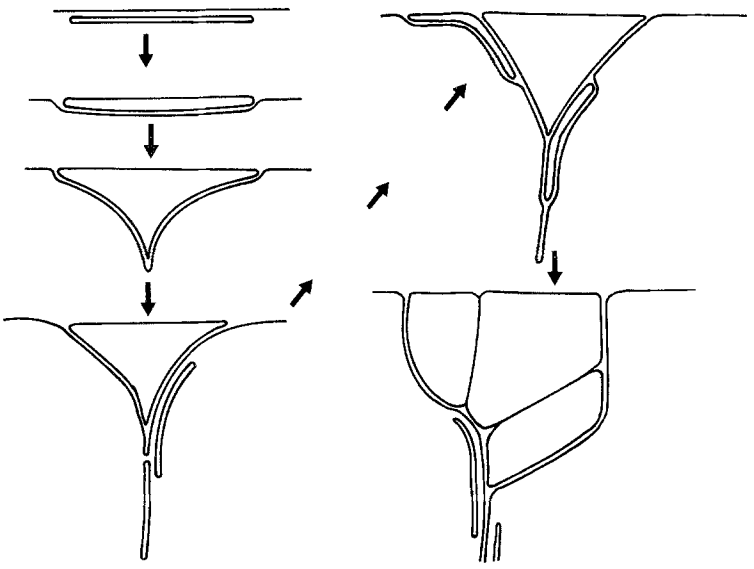


Fig. 17. Scheme of proposed course of cleavage and the development of separation vesicles

3.2. Cleavage

Before the cleavage phase the sporangial hyphae have large central vacuoles and peripheral, equidistantly distributed nuclei, each embedded in a portion of cytoplasm, and a set of organelles, especially two procentrioles at the tip of the pear-shaped nucleus and some dictyosomes around the nuclear tip.

Fig. 18. Cleavage cisternae and cisternae of the endoplasmic reticulum. ×83,000

Fig. 19. Dictyosome apparently releasing cleavage cisternae; at the distal face, cisternae of the ER between Golgi cisternae (arrow); encystment vesicles below the plasmalemma. ×38,000

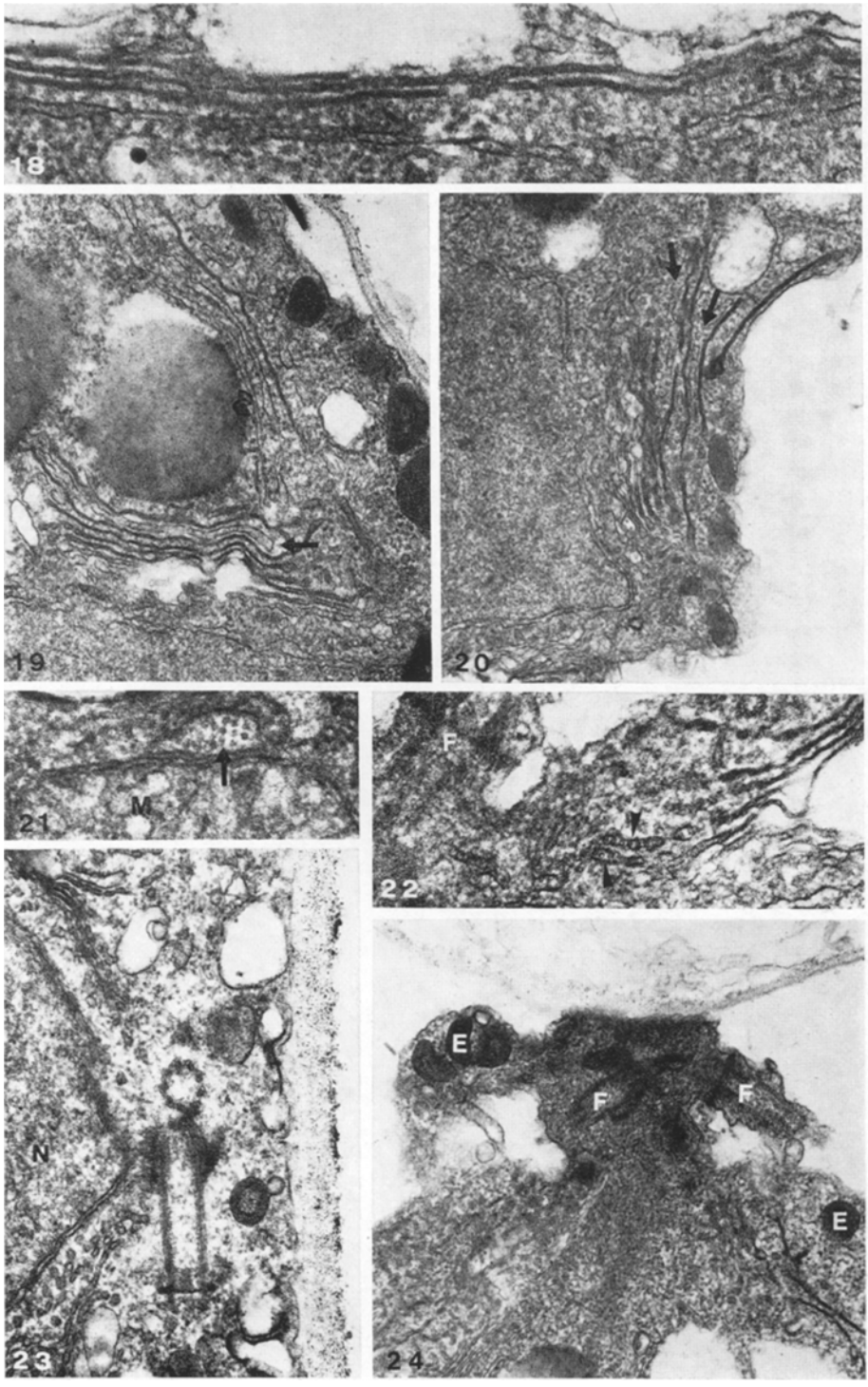
Fig. 20. As Fig. 19. ×34,000

Fig. 21. Developing mastigonemes within a cisterna of the ER (arrow) close to a mitochondrion (*M*). ×77,000

Fig. 22. Mastigonemes (arrowheads) in the periphery of a dictyosome near the flagellar base (*F*). ×61,000

Fig. 23. Nuclear tip (*N*) and two centrioles near the sporangial wall. ×33,000

Fig. 24. Flagellar pole (flagellum and flagellar base = *F*) of a developing zoospore closely appressed to the wall of the zoosporangium; encystment vesicles (*E*). ×29,000



Figs. 18–24

At the beginning of cleavage (Fig. 9) the central vacuole becomes more and more subdivided into smaller ones by thin protoplasmic sheets. Then the subplasmalemmal dilated tubules, characteristic for thalli with thickening walls (SCHNEPF *et al.* 1978 b), disappear.

An initiating centripetally growing cleavage furrow is shown in Fig. 11. We failed to detect any association of microtubules or microfilaments with the furrow.

At the periphery, the cleavage furrow does not run straight and anticlinally toward the sporangial wall but branches, thereby including a wedge-like "vesicular" profile between its two arms (Fig. 8). This "vesicle" is in fact an isolated portion of cytoplasm surrounded by the plasmalemma, but appears to be rather empty and swollen.

Such "vesicles" seem to participate in the separation of the individual cells ("separation vesicles"). At first they are found mainly in peripheral parts of the sporangial hyphae, later on also more centrally between the zoospores (Fig. 14).

Both cleavage and the formation of the separation vesicles appear to be achieved by narrow cisternae ("cleavage cisterna") which are found below the plasmalemma in the periphery of the cleaving thallus and parallel to the cleavage furrows. Their membrane is similar to the plasmalemma in thickness and structure (Fig. 12) and they have almost no lumen. They seem to fuse with each other and with the plasmalemma. In this way they deepen the cleavage furrow but the fusions also cut off narrow cytoplasmic islands (Fig. 16) which then swell and develop into the separation vesicles, as shown in Fig. 17 which represents schematically our hypothetical interpretation of the electron micrographs of cleavage.

The membranes of the separation vesicles become extremely thin (up to 5 nm) in the course of swelling (Fig. 13) and their triple-layered substructure disappears. The membranes of adjacent separation vesicles (Fig. 15) stick together so intimately that they form an integrated unit with a thickness of about 6 nm which cannot be resolved into its constituents. In the same way the membranes of swollen separation vesicles can be associated with the plasmalemma. Nevertheless, this form of pairing is only transitory because the separation vesicles finally disintegrate.

In some cases the fusions are so irregular that membranous debris, lipid droplets or myelin figures arise. Pseudopodium-like cytoplasmic strands and non-swelling portions of cytoplasm can also result.

It cannot be excluded that the cleavage cisternae are derived from peripheral cisternae of the ER, as suggested by Fig. 18, but their structure rather suggests a direct origin from the dictyosomes. During the cleavage phase and during the separation phase the dictyosomes produce almost no vesicles (with the exception of mastigoneme vesicles, see below). The cisternae at the distal face bear a close resemblance to the cleavage cisternae. They aparently move away

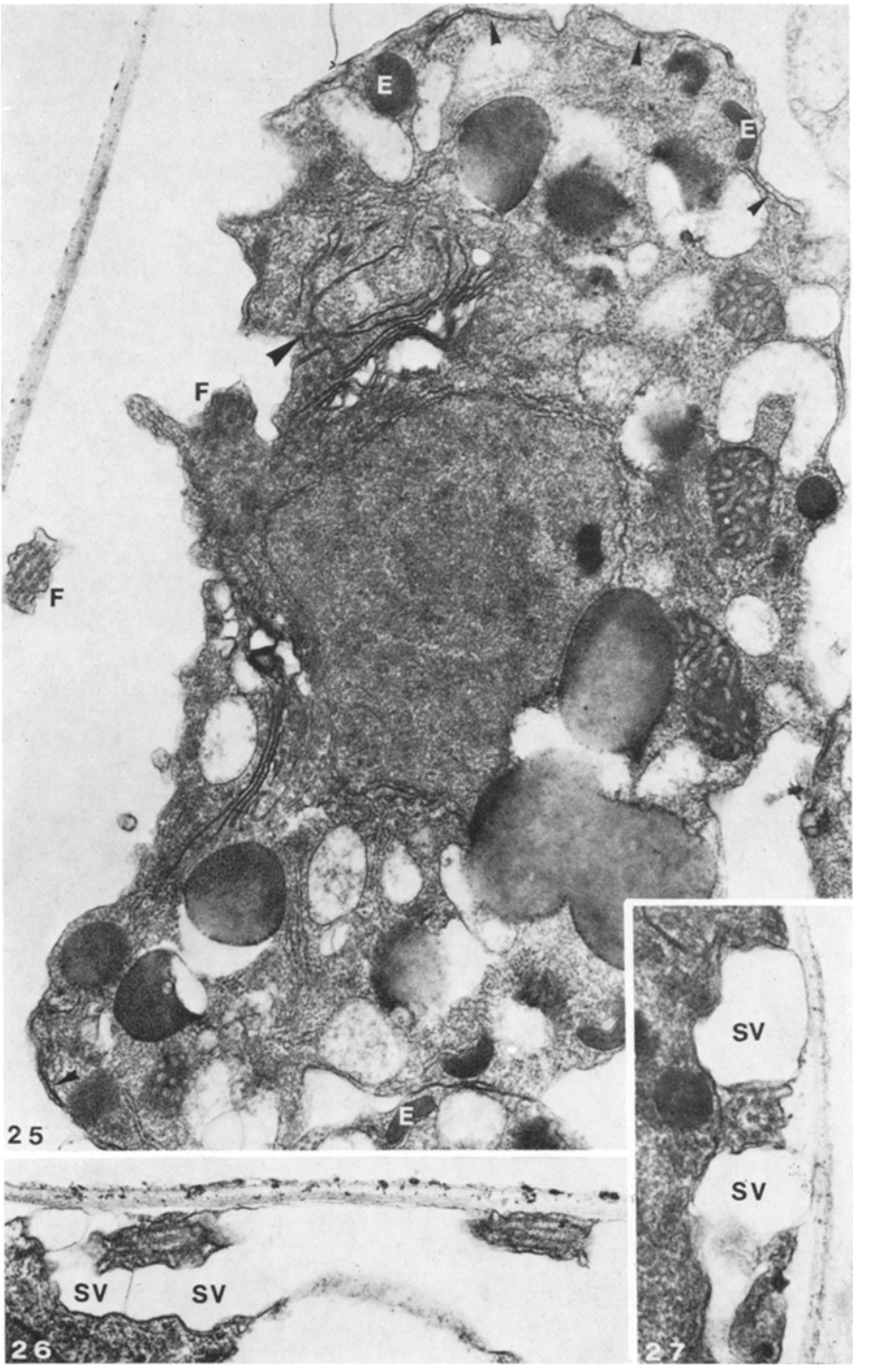


Fig. 25. Semi-mature zoospore; encystment vesicles (*E*); flagella (*F*); cleavage cisternae (small arrowheads) apparently produced by the dictyosomes (arrowhead). ×26,000

Fig. 26. Flagellum (in oblique longitudinal section) of a semi-mature zoospore between separation vesicles (*SV*) and cell wall. ×25,000

Fig. 27. As Fig. 26, flagellum in cross section. ×36,000

from the dictyosomes. Figs. 19 and 20 show cisternae of the ER, easily recognized by their thinner membranes and by their larger lumens, between the distal dictyosomal cisternae. It seems possible that these ER cisternae participate in detaching the latter which then are transported to other parts of the cell periphery and eventually fuse with the plasmalemma. After the end of the separation phase, Golgi cisternae seem no longer to leave the dictyosomes. The peripheral cleavage cisternae have disappeared almost completely in free-swimming zoospores.

3.3. Formation of the Flagella

The zoospores of *Lagenisma* have a whiplash and a flimmer flagellum. The mastigonemes of the latter are formed in bundles within the cisternae of the ER which always are closely associated with a mitochondrion (Fig. 21). The first mastigonemes are already to be seen when production of the sporangial wall begins. Later, after cleavage, smaller groups of mastigonemes can be observed in the dictyosomes (Fig. 22). They occur only in the peripheral vesicles of the Golgi cisternae and only in the region near the flagellar bases.

During this time the flagellar bases develop. After the last mitosis the two procentrioles elongate from about 0.2 μm (SCHNEPF *et al.* 1978 b) to 0.7 μm (Figs. 1 and 23) and become centrioles. Their proximal ends become surrounded by an osmiophilic fibrillar material which also connects them basally. In contrast to younger stages they now form an angle of 90°, with one centriole laterally attached by its base to the base of the other (Fig. 23) from which microtubules extend into the cytoplasm "backward" in the direction of the centriolar axis (Fig. 1). Distally the terminal plate is formed with associated structures and the triplets of the basal bodies appear instead of the singlets of the procentrioles in earlier stages (see SCHNEPF *et al.* 1978 b). Later on the flagellar rootlets (see below) become elaborated.

In the region around the base of the centrioles or of the kinetosomes, respectively, most, if not all, cytoplasmic microtubules originate. They radiate from here into the interior of the cell, mainly along the nuclear tip.

Fig. 28. Nearly mature zoospore; flagella in longitudinal section. ×26,000

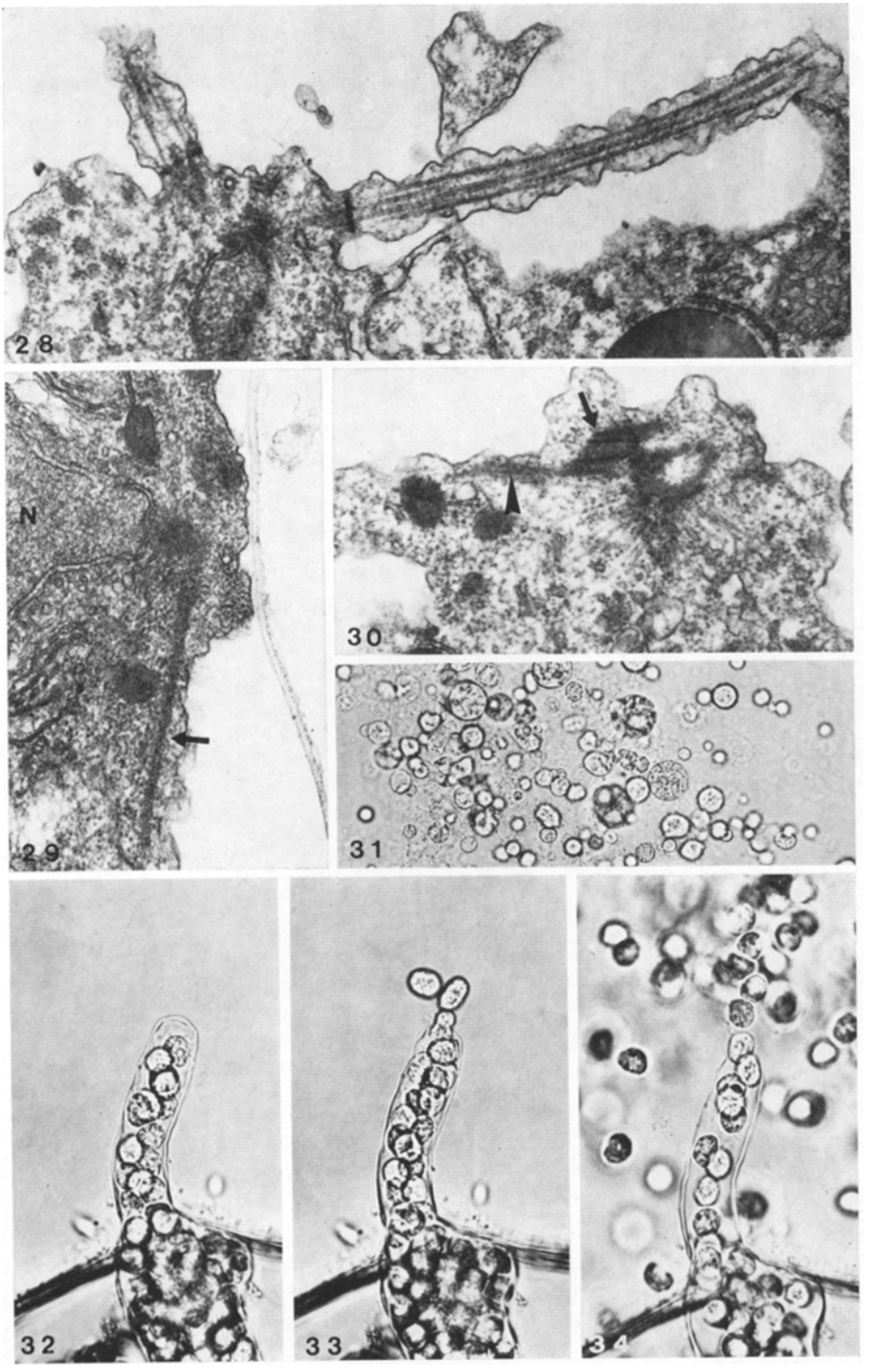
Fig. 29. Semi-mature zoospore, band of the flagellar root apparatus (arrow), nucleus (*N*). ×37,000

Fig. 30. Mature zoospore, flagellar root apparatus with a long (arrowhead) and a short, striated band (arrow) and radiating microtubules. ×34,000

Figs. 31–34. Light micrographs, *in vivo*

Fig. 31. Released products of incomplete cleavage after treatment with cytochalasin B. ×220

Figs. 32–34. Release of zoospores, different stages of the same zoosporangium. In Fig. 33 the deformation of the zoospore during passage through the initially small opening is seen. ×1,070



Figs. 28–34

The development of the flagella is interrupted and does not continue until cleavage has been completed. At the onset of the separation of the zoospores a papilla is formed around the nuclear tip, the flat distal part of which remains closely appressed to the sporangial wall because separation vesicles do not occur here (Fig. 24).

When the flagellar root apparatus has been nearly completed, the two basal bodies grow out directly to form the flagella. As far as we could observe, vesicles are not involved in this process. The two young flagella insert laterally in the papilla. At first they run over the periphery of the cell, forming an angle of about 225° to each other. They are wedged between separation vesicles (Figs. 26 and 27).

During this stage the mastigonemes are found within the dictyosomes. We were, however, unable to detect them on the surface of young flagella. If they had been there, the surrounding separation vesicles should have protected them during the preparation. In mature and nearly mature zoospores, mastigonemes attached to a flagellum were observed; their preservation, however, was poor.

3.4. Structure of the Zoospores

In the apex of the discharge tube, zoosporogenesis often is delayed. In the final stages of zoospore development the separation vesicles disintegrate. The flagellar papilla is removed from the sporangial wall and is reduced. The contours of the zoospore become more and more regular (Fig. 25). Some cytoplasmic fragments are found even in mature zoosporangia. The flagella of motile zoospores form an angle of about 100° (Fig. 28).

The most conspicuous elements of the flagellar root apparatus are two fairly long fibres (Fig. 29) which are each attached to the proximal end of one basal body and extend nearly parallel to the corresponding flagellum under the plasmalemma. The basal bodies are connected by a dense mass of fibrils which distally and slightly laterally continues into a broad short band with a longitudinal striation (Fig. 30). A more homogeneous dense material surrounds both basal bodies proximally and joins them and, therewith, the flagella to the tip of the nucleus.

The nuclear tip is surrounded by 4 dictyosomes, which now seem to be inactive and reduced in size, and by groups of microtubules. They radiate from the dense material which covers the proximal ends of the basal bodies (Fig. 30).

The small vacuoles which are formed just before cleavage are distributed among the zoospores. During the maturation of the zoospores they become smaller. In this way the zoospores reduce their volume considerably and become motile within the sporangium.

Mitochondria, numerous large lipid droplets, the remaining vacuoles and the ER seem to have no fixed position in the cell. The zoospores of young freshly infected *Coscinodiscus* cultures generally encyst and develop secondary zoo-

spores which also encyst to form finally an infection tube. In old cultures, the zoospores may represent zoomeiospores (SCHNEPF *et al.* 1978 b) and encyst to become gametangia directly (SCHNEPF and DREBES 1977). How far there are subtle differences between zoomeiospores and zoomitospores remains to be investigated.

3.5. Release of the Zoospores

The zoospores move intensively within the mature zoosporangia. Eventually they are released through an opening in the tip of the discharge tube. Here the sporangial wall is thinner and apparently differs also qualitatively from the side walls (SCHNEPF *et al.* 1978 b). An operculum is not formed. During their release the zoospores are driven out of the sporangium vigorously by an unknown force. Especially the first zoospore, but sometimes even the following ones, may be squeezed through a still narrow opening so that the cells are deformed considerably during the passage (Figs. 32–34). Observing the process *in vivo* (see the film of DREBES 1969) one gets the impression of a high internal pressure which pushes the zoospores out even when in exceptional cases the flagella are immobile. In small zoosporangia even the last zoospore from the opposite end of a hypha seems to be ejected. We were, however, unable to detect the cause of this pressure. There is no swelling slime or similar material within the mature sporangium. Separation vesicles also have disappeared at this time, unless they are destroyed artificially during the fixation in mature zoosporangia.

In large zoosporangia some zoospores may remain in the zoosporangium and encyst there. In old cultures the discharge mechanism occasionally seems to fail so that all or nearly all zoospores encyst within the hyphae and the discharge tube.

3.6. Experiments With Cytochalasin B

5 and 20 µg/ml CB reduces the number of new infections considerably but does not inhibit them completely. The developing hyphae are rather irregular, the zoospores formed with CB and the resulting cysts are very different in size (Fig. 31). The division of the *Coscinodiscus* cells is completely, but reversibly, inhibited by 20 µm/ml of the drug. 5 µm/ml and 20 µm/ml DMSO have no or almost no inhibitory effects, respectively.

4. Discussion

The formation of the primary zoospores of *Lagenisma* reveals some unique features. Based upon their own work on *Pythium* and on data and electron micrographs of *Aphanomyces*, *Pythium*, *Phytophthora*, and *Lagenidium* from other authors, LUNNEY and BLAND (1976 b) have listed the various types of vesicles found in mature and encysting zoospores of Oomycetes. In developing

and motile primary zoospores of *Lagenisma*, "phospholipid vesicles", microbodies, "cell wall vesicles", "U-bodies" and "bar-like structures" could not be identified. The "encystment vesicles" of *Lagenisma* could be an equivalent of the "peripheral vesicles" in the list of LUNNEY and BLAND (1976 b), as well as of the "primary bars" in primary zoospores of *Saprolegnia* (HEATH and GREENWOOD 1970, HOLLOWAY and HEATH 1977) and the "parastrosomes" in *Phytophthora capsici* zoospores (WILLIAMS and WEBSTER 1970). However, these components differ considerably in structure and, apparently, also in mode of formation from the encystment vesicles of *Lagenisma*.

The mature encystment vesicles superficially resemble the "vesicles with a granular cortex and a center (Vg)" of *Aphanomyces euteiches* (HOCH and MITCHELL 1972 a, b) in the concentric organization of their contents which also seem to be used in the primary phase of the encystment (a fact not mentioned by the authors, but the vesicles do not appear in electron micrographs of encysting zoospores; for *Lagenisma* see SCHNEPF *et al.* 1978 c). A certain type of vesicle in *Blastulidium* zoosporangia seems also to be similar (MANIER 1976, Fig. 14, it is however said to be limited by two membranes).

The physiological meaning of the structural transformations of the *Lagenisma* vesicles remains an open question. The "parastrosomes" of *Phytophthora capsici* zoospores (WILLIAMS and WEBSTER 1970) and the "primary bars" in *Saprolegnia* (HOLLOWAY and HEATH 1977) have a complicated internal structure. Their genesis is unclear but there are no indications that they develop as in *Lagenisma*. The same is true for the "Vg"-vesicles of *Aphanomyces*.

Another unique detail of zoosporogenesis of *Lagenisma* is the mode of cleavage. Usually, in Oomycetes (*e.g.*, in *Phytophthora:* HOHL and HAMAMOTO 1967, WILLIAMS and WEBSTER 1970, *Saprolegnia:* GAY *et al.* 1971, *Lagenidium:* BLAND and AMERSON 1973, GOTELLI 1974, and *Pythium proliferum:* LUNNEY and BLAND 1976 a) "cleavage vesicles" fuse with each other and with the plasmalemma to delineate the cells. Typical cleavage vesicles are rather large (0.5–1.0 μm in diameter) and often even vacuole-like in appearance (*Lagenidium:* BLAND and AMERSON 1973). In *Pythium* they contain fibrous material which is released into the space between the sporogenic cytoplasm and the sporangial wall (LUNNEY and BLAND 1976 a). In *Aphanomyces*, the central vacuole is directly involved in the cleavage (HOCH and MITCHELL 1975).

In *Lagenisma* we could not identify cleavage *vesicles*. The distribution of narrow cisternae in cleaving sporangia rather suggests the process depicted schematically in Fig. 17. Fusions of the "cleavage cisternae" result in deep furrows which eventually separate the cells. It cannot be excluded completely that infoldings of the plasmalemma are also involved in furrow formation. In the zoosporangia of *Blastulidium* (MANIER 1976) the cleavage process in

part seems to proceed in a similar way. The mechanisms which transport and direct the cisternae in *Lagenisma* are not known. The results of the experiment with cytochalasin B suggest that microfibrils, not seen in the electron micrographs, could be involved. A participation of microtubules in the delineation of zoospores in *Saprolegnia* has been deduced by HOCH and MITCHELL (1975) from the experiments of SLIFKIN (1967) with colchicine. We were, however, unable to detect any indication that microtubules are directly involved in cleavage.

Our observations strongly suggest that the cleavage cisternae of *Lagenisma* are Golgi cisternae which have removed from the distal face of the dictyosomes. The cleavage vesicles of other fungi seem, at least in part, also to be produced from the dictyosomes (HOHL and HAMAMOTO 1967, WILLIAMS and WEBSTER 1970, LUNNEY and BLAND 1976 a) or their equivalents (BRACKER 1968). Thus *Lagenisma* may be only a special modification of a more generally occurring process, due to the fact that secretion of wall material or slime is not necessary. With respect to the manner of cleavage, *Pythium middletonii* seems to have a position between *Lagenisma* and the other Oomycetes. HEINTZ (1970) reports that cytoplasmic vesicles *and* cisternae produced by the dictyosomes coalesce and fuse with the plasmalemma during the division of the protoplast.

The presence of alternate cisternae with different membrane structure within the distal portion of dictyosomes was observed by HEMMES and RIBEIRO (1977) in developing oogonia of *Phytophthora*. The authors suggest that they form the two types of vesicles underlying the expanding oogonial wall. The dictyosomes of the developing *Lagenisma* zoospores have a similar structure. It cannot be excluded that the two different types of cisternae within the stack both have the same origin. However, our electron micrographs rather suggest that the cisternae with a thinner membrane are derived from the ER and have moved in between the real cisternae of the dictyosomal stack. This process is possibly related to the detachment of the Golgi cisternae.

"Flattened vesicles" or "peripheral cisternae", similar to the cleavage cisternae of *Lagenisma*, are common in oomycetous zoospores, as shown in the list of LUNNEY and BLAND (1976 b). In some cases they are also associated with cleavage furrows (WILLIAMS and WEBSTER 1970); LUNNEY and BLAND (1976 a) even found indications for a fusion of such structures (thought to be derived from the ER) with the plasmalemma. The fusions of cleavage cisternae with the plasmalemma as suggested in Fig. 17 take place when the membranes seem to be identical.

Fig. 17 represents our explanation of the mode of cleavage and also of the formation of the separation vesicles which are very common in maturing *Lagenisma* zoosporangia. Similar vesicles can be observed in the electron micrographs of cleaving sporangia of *Lagenidium* (BLAND and AMERSON 1973, GOTELLI 1974), *Aphanomyces* (HOCH and MITCHELL 1975) and *Pythium*

(LUNNEY and BLAND 1976 a) but are not mentioned in the study of MANIER (1976) on *Blastulidium* zoosporogenesis. It cannot be excluded that some of them are formed in a similar way.

The great structural modifications of the membranes of the swelling separation vesicles are most remarkable. The molecular architecture of a pair of membranes 6 nm in thickness which may finally result must deviate considerably from the common organization of biomembranes.

In *Lagenisma* the flagella develop later than in *Phytophthora* (HOHL and HAMAMOTO 1967, WILLIAMS and WEBSTER 1970), *Lagenidium* (BLAND and AMERSON 1973) and *Pythium* (LUNNEY and BLAND 1976 a). In the latter they grow out into an "axonemal vacuole" (or "flagellar vesicle") (for *Albugo* see BERLIN and BOWEN 1964) before cleavage has occurred, in *Lagenisma* they are wedged between separation vesicles. In *Blastulidium* cleavage similarly precedes axoneme formation (MANIER 1976).

The mastigonemes are produced as usual in the ER (HEATH *et al.* 1970) and mature in the dictyosomes (BOUCK 1971). The close association of the mastigoneme-containing ER cisternae with mitochondria is known also from other Oomycetes (LUNNEY and BLAND 1976 a: *Pythium*; for *Lagenidium* see BLAND and AMERSON 1973, Fig. 7 and GOTELLI 1974, Fig. 6). It is unclear how the mastigonemes of *Lagenisma* are distributed along the flagellum for they seem to be extruded after the flagellum has grown out. BOUCK (1971) suggests for the mastigonemes of *Ochromonas* that they are pulled up the flagellum as the axoneme elongates. A later transport of the mastigonemes would imply considerable membrane flow or flow of the anchorages of the mastigonemes within the plasmalemma. Such a case in known from zoidogenesis of brown algae (LOISEAUX 1973).

Although the primary zoospores of *Lagenisma* are kidney-shaped they resemble the pyriform primary zoospores of *Saprolegnia.* The organization of their flagellar root apparatus is simpler than that of the secondary zoospores. Primary zoospores of both genera have two main root bands consisting of fibrils whereas in the kidney-shaped secondary zoospores of *Saprolegnia* bands of microtubules are the dominating elements (HOLLOWAY and HEATH 1977). The zoospores of *Lagenidium* (BLAND and AMERSON 1973) belong to the primary type of zoospores whereas those of *Aphanomyces* (HOCH and MITCHELL 1972 a, b), *Phytophthora* (REICHLE 1969, BIMPONG and HICKMAN 1975) and *Pythium* (LUNNEY and BLAND 1976 b) have a root apparatus which corresponds rather to that of the secondary type of zoospores. Thus electron microscopy supports the general view (GÄUMANN 1964, ESSER 1976) that the latter group of zoospores are equivalent to the secondary type.

It is enigmatic how the zoospores are released. Apparently they are pushed out vigorously (by an internal pressure?) and are even deformed while they pass through the small opening of the discharge tube. There are no indications

for a swelling slime within the sporangia. It is also improbable that balooning separation vesicles drive them out; if this were the case more zoospores should remain in the hyphae. BORKOWSKI (1970), studying the release of *Saprolegnia* and *Aphanomyces* zoospores, suggested that electrical forces are involved in their discharge.

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