THE UNIVERSITY OF MANITOBA

A DEVELOPMENTAL STUDY OF THE CHYTRIDIOMYCETE *CATENARIA ANGUILLULAE* EMPLOYING LIGHT AND ELECTRON MICROSCOPY

by

ANTHONY A. HOPKIN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

MASTER OF SCIENCE

DEPARTMENT OF BOTANY

WINNIPEG, MANITOBA

FALL, 1983

A DEVELOPMENTAL STUDY OF THE CHYTRIDIOMYCETE *CATENARIA ANGUILLULAE* EMPLOYING LIGHT AND ELECTRON MICROSCOPY

by

Anthony A. Hopkin

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ABSTRACT

The development of selected stages in the life cycle of Catenaria anguillulae Sorokine were studied employing light and electron microscopy. These stages were: a) the late stages of germination; b) the production of a swelling on the germ tube which developed into a prosporangium; c) the production of zoosporangia from hyphal elements of the prosporangia; and d) the production of zoospores.

The study emphasized an examination of the gross morphological and ultrastructural changes associated with the aforementioned stages of the life cycle. Distinct changes occurred in the protoplasm during each of these developmental stages. Most notable of these were: a) an increase in the amount of glycogen and in the number of lipid bodies, nuclei and mitochondria as the zoosporangia matured; b) the development of an association between microbodies and lipid bodies was noted to become more frequent and complex prior to zoospore production; and c) the microbodies and mitochondria appeared to enlarge as the thallus approached maturity.

The enlarging zoosporangium was characterized by a zone of smooth tubular endoplasmic reticulum which occupied a significant portion of the cytoplasm but was present for only a short period of time. Bundles of tubules composed of a number of thick-walled tubules in direct contact with each other were observed in close association with the cell wall, particularly in regions of rhizoid development.

The mature zoosporangium contained a large number of nuclei, each associated with several lipid bodies. A flagellum was produced from

each of the nuclei by the elongation of one of the centrioles into an elongating primary flagellar vesicle. The method of flagellum production was typical of that reported for other members of the Blastocladiales. After flagellum production the zoosporangial cytoplasm segregated by the development of cleavage furrows that fused with the primary flagellar vesicles, thus dividing the cytoplasm into uninucleate portions. The possible role of microtubules in the control of this cleavage was also discussed.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

CF	- cleavage furrow
CS	- cisternae
ER	- endoplasmic reticulum
F	- flagellum
G	- glycogen
GT	- germ tube
GZ	- germinated zoospore
IS	- isthmus
K	- kinetosome
L	- lipid
M	- mitochondrion
MB	- microbody
MT	- microtubule
N	- nucleus
NU	- nucleolus
PH	- primary hypha
PS	- prosporangia
R	- rhizoid
S	- septum
SW	- swelling
TB	- tubular body
TER	- tubular endoplasmic reticulum
V	- vacuole
VE	- vesicles
VZ	- vesiculated zone of ribosome exclusion
ZS	- zoosporangium

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to his supervisors, Dr. J. Reid and Dr. J. Dowsett, for their continual guidance, patience and financial support during this study.

He would also like to express his thanks to the other members of his advisory committee, Dr. D. Harder and Dr. L. Van Caeseele, for their valuable advice and assistance.

The author is also indebted to Mrs. R. Gillespie for providing him with training in the techniques of electron microscopy, and to Ms. B. Lindsay for her patience during the typing of this thesis.

INTRODUCTION

An isolate of the aquatic fungus Catenaria anguillulae Sorokine was obtained from a soil sample collected from a wooded site in Assiniboine Park, Winnipeg, Manitoba, Canada, during the summer of 1980. The collection was made as part of a survey for nematode trapping fungi. The isolate of C.anguillulae used in this study was one of the fungi found to be parasitic on nematodes. One of the earliest descriptions of the fungus (Butler and Buckley 1927) was made of an isolate found living as a parasite on liver fluke eggs. Subsequent to this, the fungus was grown in artificial culture in a concentrated fluke extract (Butler and Humphries 1932). A number of other workers have reported C.anguillulae as a parasite of nematodes and liver flukes, and as a saprophyte on various substrates, as discussed by Sparrow (1960). Couch (1945) described the development of C.anguillulae in agar culture. His work concentrated on the development of the thallus, from the time at which a septate hypha was produced to the development of the mature zoosporangium and resting sporangium.

Catenaria anguillulae is a member of the Chytridiomycetes, a class generally made up of aquatic fungi. The thallus of members of this group is coenocytic, holocarpic, eucarpic, monocentric, polycentric or mycelial. The reproductive unit of these fungi is a posteriorly uniflagellate zoospore. The Chytridiomycetes are composed of four orders: 1) Chytridiales, 2) Harpochytridiales, 3) Blastocladiales, 4) Monoblepharidales. Catenaria anguillulae belongs to the Blastocladiales, an order

characterized by zoospores bearing a single posterior whiplash flagellum, and a prominent nuclear cap. The Blastocladiales also produce dark-coloured, thick-walled, resting spores. In addition to this, the thallus has well-developed tapering rhizoids.

The life cycle of C.anguillulae is very brief but during this period the fungus will undergo some dramatic changes. When grown on peptone yeast glucose agar at 25^{o}C, the fungus will develop from a motile zoospore into a mature zoosporangium containing dozens of zoospores. After approximately seventy-two hours the sporangia will undergo autolysis. The fungus may also produce a thick-walled resting sporangium that upon germination releases motile zoospores that will develop into a saprophytic mycelium. This, however, requires the transfer of the fungus to a separate medium, yeast soluble starch agar, and this aspect of its life cycle is not reported in this study.

The purpose of this thesis was to repeat the work of Couch (1945) using optical microscopic techniques in order to observe the early stages of germination and development that Couch did not cover in any detail in his study. It was also the intention of the study to examine portions of the life cycle using transmission electron microscopy, as only limited work has been accomplished on C.anguillulae by this method. The use of electron microscopy adds a new dimension to the study of the fungal life cycle. As Bracker (1967) stated, "we can now think of morphogenesis not only in terms of shape, size and arrangement of cells, but also as a multitude of minute but significant integrated changes in subcellular components". Electron microscopy is also an important tool in the study of this group of fungi, due to the extremely small size of the organisms

involved. By using the combined approach of light and electron microscopy, it was hoped that a better understanding of the life cycle and the general biology of C.anguillulae could be developed.

The bulk of the relevant literature was reviewed by Heath (1976) and, in comparison to other groups such as the Basidiomycetes and Ascomycetes, little ultrastructural work has been done. The Chytridiomycetes are a primitive group of fungi, and perhaps a better understanding of their structure may suggest phylogenetic trends, as well as increase the present knowledge of fungal ultrastructure in general.

While C.anguillulae is not of any economic importance, it is representative of an interesting group of fungi that exist both as parasites and saprophytes in the aquatic environment and in moist soils. Sparrow (1960) points out that the study of the aquatic fungi is necessary for any comprehensive account of the interrelated society of aquatic plants and animals. Study of the saprophytic aquatic fungi will provide information as to their "necessary functions in the reduction, reworking and transformation of organic materials". As previously mentioned, C.anguillulae is also of interest as a potential parasite of nematodes. The original description by Sorokine (1876) was of the fungus existing within nematodes. Some authors have more recently considered the role of the fungus as a biological control agent of nematodes (Stirling and Platzer 1978; Mankau, pers.comm.). Certainly if this organism is to have any success in the control of nematodes, more information concerning its life cycle will be of benefit to future workers.

LITERATURE REVIEW

Catenaria anguillulae was first described in 1876 by Sorokine from an association with nematodes. Sorokine suggested that C.anguillulae resembled Achlygeton spp. in its development and because of this, the fungus was placed in the Ancylistales (Dangeard 1885). Later workers placed it in the Chytridiales (Fischer 1892; Schroter 1897; Von Minden 1911; Fitzpatrick 1930; Karling 1932; Sparrow 1943; Whiffen 1944), on the basis of the posterior flagellum of the zoospore, the small amount of mycelium present, and the polycentric arrangement of the zoosporangia.

Butler and Buckley (1927) also reported C.anguillulae in the eggs of sheep liver flukes while other workers have observed C.anguillulae existing as a facultative parasite in nematodes (Boolis and Mankau 1965; Sayre and Heely 1969; Stirling and Platzer 1978). Couch (1945) described the development of the fungus in agar culture from the time the mycelium first developed a septate hypha to the development of the mature zoosporangium. Couch (1945) transferred C.anguillulae to the Blastocladiales primarily because of the similarity of its zoospores to those of Blastocladiella spp., but a number of other characteristics, including the method of zoospore discharge and the life cycle were also important in his decision.

The general ultrastructure of the zoospore and the zoosporangium of a species of Catenaria, believed to be C.anguillulae, living parasitically in an unidentified cyclopod, was described by Manier (1977). Chong and Barr (1974) observed the fine structure of the zoospore of C.anguillulae and compared it with the zoospores of Rhizophydium patellarium Scholz and

Entophylctis confervae-glomeratae (Cienkowski) Sparrow. On the basis of the comparative ultrastructure they proposed that there were taxonomic implications in the ultrastructural similarities of zoospores suggesting that close ultrastructural similarities would indicate a close relationship. Olson et al. (1978) re-examined the ultrastructure of the zoospore of C.anguillulae and noted cytoplasmic similarities between the zoospores of members of the Chytridiales, Blastocladiales, Monoblepharidales, and Harpochytridiales. Olson and Reichle (1978) described the life cycle of C.anguillulae as a "Brachyallomyces type of life cycle" as defined by Emerson (1941). In this type of life cycle there is a regular formation of zoosporangia, and resting sporangia, but gametes are not formed nor does plasmogamy occur. However, in their investigation of the fine structure of the fungus, Olson and Reichle observed meiotic division occurring in the resting sporangia as evidenced by the presence of a synaptonemal complex. They also noted that both the zoospore and meiospore were haploid, however neither spore type acted as gametes. They postulated that the diploid level of the fungus was re-established by diploidization during early development of the resting sporangia.

Ichida and Fuller (1968) studied the ultrastructure of mitosis in the zoosporangia of C.anguillulae, and reported that the nuclear membrane remained intact during nuclear division. They also noted that the centrioles were associated with, but not directly connected to, the intranuclear spindle. However, later workers (Olson and Reichle 1978) suggested that as Ichida and Fuller (1968) grew their cultures on YpSs agar which results in the formation of resting sporangia, they were actually observing meiotic division.

The ultrastructure of other members of the lower fungi has been investigated by numerous workers.

An excellent review of much of the work carried out on the "Ultrastructure of Fresh Water Phycomycetes" is provided in a paper of the same name by Heath (1976). The majority of the ultrastructural work on this group seems to have focused on the subjects of zoospore morphology, encystment, germination, and zoosporogenesis.

Although germination is one aspect not covered in any detail in this study of C.anguillulae, some of the mechanisms involved and the morphological changes that occur are helpful in understanding the early development of the prosporangia of C.anguillulae.

Hoch and Mitchell (1972) reported on the germination of the spore of the Oomycete Aphanomyces eutiches Drechs. The onset of germination is signalled by a "localized bulge in the cyst wall" and an accumulation of vesicles beneath this bulge. These vesicles appear similar in kind and relative abundance to those associated with the extending hyphal tips, described by Grove et al. (1970). The bulge then elongates and a germ tube develops. As germ tube elongation progresses, a large central vacuole is formed within the spore proper, presumably as a result of fusion of smaller vesicles. This vacuole eventually extends into the germ tube. The zoospore of Phytophthora parasitica (Datusr) Waterh. displays a similar accumulation of vesicles beneath the cyst wall as well as an increase in the number of lomasomes during germination (Hemmes and Hohl 1971).

Bimpong and Hickman (1975) noted that the enlargement of the central vacuole in the germinating cyst of Phytophthora palmivora Butler corresponded

with the coalescence and breakdown of lipid bodies, and vesicles containing dense staining bodies. They also reported an increase in the amount of endoplasmic reticulum, and in the number of dictyosomes and lomasomes during germination.

According to Gay et al. (1971), when the zoospores of Saprolegnia ferax (Gruith) Thuret germinated, dense body vesicles appeared to fuse, and contributed to the initial vacuole of the germlings. These authors believed that the dense body vesicles are involved in two functions: 1) the production of additional membrane for tonoplast development and 2) "the differential movement of fluid into the expanding vacuole".

Heath et al. (1971) have presented evidence that cell wall formation in the Saprolegniales is accompanied by the formation of membrane-bound vesicles. Both the cell wall and vesicles reacted in a similar manner to periodic acid-silver hexamine staining, implying the presence of polysaccharides. These authors speculated on the Golgi origin of the wall vesicles but their evidence was not conclusive and although they reported that many of the wall vesicles contained a dark staining globule, all vesicles in direct assocaition with the wall had a conspicuously clear lumen. This would imply that either the vesicles had released their contents into the wall or the dark staining vesicles were not involved in wall synthesis. It is apparent that the dense body vesicles were confined to the more central regions of the cell.

Many of the zoosporic fungi apparently undergo extensive wall production without the presence of wall vesicles (Heath 1976). In the Chytridiales the only member to exhibit wall vesicle production is Blastocladiella emersonii Cantino and Hyatt. Barstow and Lovett (1974b)

observed vesicles of 80-90 nm clustered in the elongating rhizoids of the germinating zoospores of this fungus, as well as microtubules oriented parallel to the rhizoidal wall; however, all vesicles were not observed outside of the rhizoid apex in B.emersonii. These authors also reported the absence of a typical Golgi apparatus and proposed that a cluster of vesicles which occurred in a clearly defined zone of ribosome exclusion could be the functional equivalent to the Golgi. These vesiculated zones of ribosome exclusion have been reported by Lessie and Lovett (1968) in B.emersonii and in two Basidiomycetes by McLaughlin (1973). Other authors have speculated as to the possible functional equivalent to the Golgi. Truesdell and Cantino (1970) proposed that the gamma particles present in the germinating zoospores may fulfil the role of the Golgi. Gamma particles are membrane-bound osmiophilic organelles present in the cytoplasm. They were first observed in the zoospore of B.emersonii by Cantino and Horenstein (1956) and subsequently, gamma bodies have been reported in the zoospores of C.anguillulae (Chong and Barr 1974, Olson et al. 1978) and in the zoosporangium of C.anguillulae (Manier 1977, Barstow 1979), B.emersonii (Barstow and Lovett 1975) and Allomyces macrogynus (Emerson) Emerson and Wilson (Robertson 1972). The role of the Golgi apparatus has not usually been assigned to the gamma body; however, it does appear to be responsible for the deposition of the new cell wall during encystment (Truesdell and Cantino 1970, Myers and Cantino 1974, Mills and Cantino 1979).

The germinating zoospores of the Chytridiomycetes either develop directly into a sporangium or develop a swelling on the germ tube that develops into the sporangium. Whiffen (1944) proposed that "the genera

of monocentric chytrids be grouped into two families on the basis of whether the zoosporangium develops from the encysted zoospore (Rhizidiaceae) or from an enlargement on the germ tube (Entophlyctaceae)". Sparrow (1960) considered this an artificial classification and proposed that the division created by Braun (1856) dividing the chytrids into two groups based on the presence or absence of an operculum, was a more appropriate system. The system proposed by Whiffen has more recently received support, since both Roane and Paterson (1974) and Barr (1975) feel that a taxonomic scheme similar to Whiffen's could be based on thallus development. However, Barr pointed out that one important consideration is the function of the enlarged portion of the rhizoid or germ tube, generally referred to as the apophysis. Barr made the observation that there is so much morphological variation in the rhizoid shape that the term becomes meaningless. The term apophysis, or more correctly prosporangium, should apply to swellings of the rhizoids which form during early development and contain a nucleus.

Porter and Smiley (1980) employing SEM and TEM methods, noted the production of the germ tubes from the encysted zoospores and the swelling of the proximal portion of the young rhizoidal system. The swellings enlarged as did the rhizoidal system concomitant with the enlargement of the encysted zoospores to form a sporangia. Sykes and Porter (1980) observed the development of a swelling along the infection tube of C.allomycis Couch, within its fungal host. The swelling occurred within the host and a nucleus migrated from the spore into this swelling; microtubules were noted accompanying the migrating nuclei. A similar pattern of development is exhibited by a Phlyctochytrium sp. (Kazama 1972). In the latter case, a germ tube penetrated the algal host and produced an

endobiotic swelling. However, this swelling did not enlarge to any extent and was anucleate, and therefore would not fit Barr's defintion of a prosporangium; and might best be described as a subsporangial swelling. In the case of Phlyctochytrium spp., the zoosporangium is produced through the enlargement of the germinated zoospore, which is the pattern of development of many of the chytrids.

There have been few studies of the ultrastructural changes that occurred during the enlargement of the sporangia. Lessie and Lovett (1968) examined the developing sporangium of B.emersonii and termed this the log phase. During this phase the cytoplasm was observed to be unorganized, and contained a number of nuclei, lipids, mitochondria, and various other organelles. The most interesting of these were structures composed of tubules each with a 45 nm diameter and referred to as "macrotubules". These have also been reported by Barstow and Lovett (1975) as being associated with the endoplasmic reticulum cisternae that contained the granules believed to be the precursors to the gamma bodies. Similar tubules were later observed in the developing zoosporangium of C.anguillulae (Barstow 1976) and were described as tubular smooth E.R. Again they were only present in association with the granule-containing cisternae. However, Barstow (1979) noted that the developing sporangium of Allomyces macrogynus contained the granule-containing cisternae but without the associated macrotubules.

One of the most peculiar organelles observed during the growth phase of the sporangium of the aquatic fungi were lomasomes.

Marchant and Robarts (1968) discussed the occurrence of lomasomes and plasmlemmasomes, under the term "paramural bodies", in the fungi and proposed that these bodies may be involved in wall synthesis. However,

Heath and Greenwood (1970a) suggested that lomasomes and plasmalemmasomes in S.ferax and Dictyuchus sterile Coker are produced when plasmalemma production is not balanced by cell expansion, and lomasomes are the result of aggregations of excess membrane. Hemmes and Hohl (1971) endorsed this belief as they felt that it is the Golgi apparatus and not the lomasomes that act in wall synthesis in the fungi.

Microbodies are a major class of organelles in the fungi that have interested various workers, though there is still little known about their diverse functions (Maxwell et al. 1975, 1977).

Mills and Cantino (1979) followed the ultrastructural and biochemical changes occurring in the microbodies of B.emersonii. They noted the presence of a single, large, lobed microbody termed a "symphomicrobody", that fragmented into smaller units after germination of the zoospore. The same authors also demonstrated the presence of catalase, malate synthetase and isocitrate lyase in the microbodies, the latter two enzymes being involved in the glycolate cycle. The authors believed that the microbodies might function as glyoxysomes in the metabolism of triglycerides, the main constituent of lipid bodies. Powell (1976) confirmed the presence of glyoxysomes by cytochemical tests in Entophlyctis variabilis Powell and Koch. Powell comments on the microbody lipid association, explaining the possible role of microbodies in the conversion of lipids into structural components of the cell. She also discussed the confusion that has arisen in the use of the terms peroxisome and glyoxysome in fungi where microbodies contain some, but not all, of the enzymes of the glyoxylate cycle. As the term microbody is a structural term for an organelle with a granular matrix bounded by a single membrane, it will suffice for this study of

C.anguillulae.

From serial sections Powell (1979) determined the probable three dimensional arrangement of microbodies and discussed their association with other organelles in the zoosporangia of E.variabilis. Through examination of the serial sections it was found that many of the oval microbodies that appeared discrete were actually continuous with one another, forming a lobed symphomicrobody. Powell noted the association of microbodies with the endoplasmic reticulum and suggested that the latter may be the point of origin for microbodies. Microbodies have also been found to be associated with the nuclear membrane in the zoosporangium (Chong and Barr 1973; Powell, 1976, 1979). This association may exist to ensure that a portion of the microbody-lipid complex is distributed to each zoospore during cytoplasmic cleavage (Powell 1976).

The microbody-lipid complex (MLC) is unique to the Chytridiomycetes and has been proposed as a unifying phylogenetic marker. Powell (1978) noted that four basic patterns of the MLC are recognized, each corresponding to one of the four orders of the Chytridiomycetes.

When the zoosporangium has reached its mature size a number of changes occur. Cross wall formation is observed at the junction of the zoosporangium and any hyphal extensions such as rhizoids. The formation of cross walls generally signals the initiation of zoosporogenesis. Associated with the formation of septa is the migration of cytoplasm and attendant organelles into the developing sporangium in both S.ferax (Gay and Greenwood 1966) and B.emersonii (Lessie and Lovett 1968), resulting in vacuolate rhizoids. According to Taylor and Fuller (1980) the rhizoids of Chytrium confervae (Wille) Minden exhibited the same cytoplasmic migration.

These authors observed a swift demarcation between the ribosome-rich sporangia and the ribosome-poor rhizoid after induction of sporogenesis. Concomitant with the migration of the cytoplasm and cross wall formation was the occurrence of a number of microtubules and associated fibres lining what they termed the port region connecting the rhizoid and zoosporangia. They also believed that the microtubules may be responsible for the movement of nutrients as well as organelles from the rhizoids. Heath and Heath (1978) have also discussed the possible role of microtubules in organelle movement in the rust fungus Uromyces phaseoli (Pers.) Wint.var.vignae (Barcl.) Arrh.

Taylor and Fuller's study may also show the possible role of microtubules in the function of the septa. In plant cells, wall deposition and orientation seem to be associated with microtubules (Hepler and Palevitz 1974). Taylor and Fuller also noted the occurrence of multivesicular bodies and endoplasmic reticulum at the site of the developing wall. Powell (1974) also found similar organelles associated with developing septa in Entophlyctis variabilis, while Morrison (1977) discussed the possible role of multivesicular bodies with septa in Allomyces macrogynus. The septa of the chytrids appears to develop centripetally (Gull 1978). In Phlyctochytrium arcticum Barr a thickening of the wall was repeatedly observed between the rhizoids and the zoosporangium (Barr 1970) and a similar situation has been observed in B.emersonii (Lessie and Lovett 1968), and in both cases there was an inward growth of this thickening to produce a septum. The situation differs somewhat in some of the Oomycetes where a basal plug composed of a homogeneous matrix is found to be analagous to the septa and is observed to form in several

records. In Phytophthora parasitica the plug "is perforated with a complex, anastomosing network of fine channels filled with electron-dense material" (Hohl and Hamamoto 1967). The septa of Chytridium confervae (Taylor and Fuller 1980) is perforated with plasmodesmatal-like channels. Similar channels have also been found in the septa of E.variabilis and Rhizophydium sphaerotheca Zopf (Powell 1974), while Allomyces macrogynus (Morrison 1977) and B.emersonii (Lessie and Lovett 1968) have imperforate septa.

While cross wall development is occurring in the zoosporangia, papilla formation is occurring simultaneously. This is one of the last major changes noted to occur in the sporangium and occurs just prior to zoospore production. The discharge apparatus of E.variabilis (Powell 1976) is formed through the fusion of vesicles containing fibrillar material. This material is deposited between the membrane and the outer wall, creating an expanding fibrillar plug. The wall at the apex of the plug becomes diffuse and leaves an inoperculate discharge pore. The plug exudes through this pore and produces a sphere that rests at the end of the discharge tube. There is an accumulation of granular material and endoplasmic reticulum at the interface of the discharge plug and plasmalemma and at this point a dense layer is formed, referred to as an endo-operculum. Phytophthora parasitica also produces an endo-operculum separating the cytoplasmic from the plug region. Structures similar to the endo-operculum have also been reported in: Rhizophlyctis rosea (de Bary and Woronin) Fischer (Chambers and Willoughny 1964) and Phlyctochytrium irregulare Koch (McNitt 1974). Chong and Barr (1973) in their study of Phlyctochytrium arcticum observed an inoperculate papilla, filled with an undefined fibrous material. Lunney

and Bland (1976) observed the formation of a septum at the base of the sporangium simultaneous to the initiation of the apical papilla, and noted a large number of wall-forming vesicles associated with both sites. Prior to discharge of the zoospores, the vesicles within the papilla coalesced and the papilla enlarged. The apex of the papilla became thickened with a fibrillar material, similar to that reported in a Phytophthora sp. by Chapman and Vujicic (1965).

Concomitant with this thickening of the papilla, cytoplasmic cleavage was initiated. Cleavage vesicles at the periphery of the sporangium discharge their contents to form a loose, fibrous interface between the sporangial cytoplasm and the wall. This "spongy" material was also reported in Nowakowskiella profusa Karling (Chambers et al. 1967), running beneath the sporangial wall, forming a matrix between the cleaved zoospores. Hohl and Hamamoto (1967) reported that zoospore production by Phytophthora parasitica was signalled by the coalescence of small Golgi-derived vesicles, that form a central vacuole. This was followed by production of flagella at the periphery of the cytoplasm. Subsequently cleavage vesicles line up along the periphery of the cytoplasm, in planes equidistant from neighbouring nuclei, and from the central vacuole to the peripheral vesicles. This was followed by a fusion of cleavage vesicles. The formation of the central vacuole during cytoplasmic cleavage was also reported in S.ferax (Gay and Greenwood 1966; Gay et al. 1971) and in Phytophthora capsici (Williams and Webster 1970). Cleavage furrows in Phlyctochytrium irregulare are formed by flattened vacuoles in the cytoplasm (McNitt 1974). Some of the furrows also appear to be formed as inward projections of the plasma membrane. When a cleavage furrow nears the centrioles, the functional one

elongates and grows into a flagellum. This is typical for those fungi that produce their flagella after cytoplasmic cleavage as is also the case with Harpochytrium hedinii Wille (Trulind and Whisler 1971), Olpidium brassicae (Woron.) Dang. (Temmink and Campbell 1968) and Aphonomyces euteiches Drechs. (Hoch and Mitchell 1972). In those species where flagella developed prior to cleavage, the axenome grew into a sheath-like vesicle that enlarged by the progressive fusion of small vesicles from an unknown source (Heath 1976); these species include B.emersonii (Lessie and Lovett 1968), Allomyces arbusculus Butler and Phytophthora capsici (Williams and Webster 1970).

The mechanism controlling the positioning of the cleavage furrows is uncertain. However, there is evidence of skeletal microtubules and their possible role in establishing zoospore initial boundaries. Heath and Greenwood (1971) reported an "array of microtubular roots around each nucleus" in a Saprolegnia sp. that may separate the nuclei and produce relatively weaker areas of cytoplasm between the nuclei. It is through these areas of weaker cytoplasm that the cleavage furrows may develop, taking the line of least resistance. Olson et al. (1981) reported a similar situation in the Blastocladiales and Barr (1978) reports "skeletal microtubules in the zoospores of the Chytridiales. However, it is uncertain whether the microtubules reported by Barr are produced during cleavage or during the last stages of zoospore organization (Olson et al. 1981). Barron and Hill (1974) reported that a number of cleavage vesicles originated as a result of pinching off from the sporangium membrane. The same authors note that at least two distinct types of vesicles exist in the zoosporangium of an Allomyces sp. Those in the

pre-cleavage sporangium exhibited similar staining properties to that of the nuclear cap and the flagella vesicle. The other type of vesicle did not appear until cleavage was induced, and appeared to be responsible for partitioning the cytoplasm. In contrast to this, the cleavage, flagellar and nuclear cap membrane vesicles were indistinguishable in B.emersonii (Lessie and Lovett 1968). Zoosporogenesis in Lagenidium callinectes Couch as described by Bland and Amerson (1973) was initiated by the fusion of small vesicles. Other small vesicles with dark staining bodies were also scattered throughout the cytoplasm, but the authors believed them to be analagous to gamma bodies. A number of papers have been published on the subject of zoospore production and portions of some have been mentioned. An excellent review on the subject is provided by Heath (1976) and more recently by Olson et al. (1981) who also proposed a model system for zoospore production in the Chytridiomycetes.

MATERIALS AND METHODS

Organisms: Zoosporangial cultures of Catenaria anguillulae were obtained by isolating the fungus from a soil sample obtained from a low lying area of Assiniboine Park, Winnipeg, Manitoba. The soil was stored at 5°C for several months until it was tested. At the time of testing, an aliquot of soil was placed in a 3 cm diameter glass Petri dish. In addition 10 ml of a heavy suspension of the nematode, Caenorhabditus elegans Nigon was added. The Petri dishes were then incubated at 25°C for 7 days. Catenaria anguillulae is known to be an endoparasite of nematodes and therefore had the potential to parasitize some of these nematodes. At the end of 7 days, worms were isolated from the soil using the Baermann funnel technique (Goodey 1957). The collected worms were left in a covered beaker at room temperature for a further 72 h. Samples of the collected worms were placed in well slides and examined with the aid of a Leitz Lm-Lux stereomicroscope. When an infected worm was observed, it was transferred by means of a micropipette to a glass slide and placed under a coverslip. The infected worm was then observed until the zoosporangia within the worms discharged their zoospores. Periodically water was added to the edge of the coverslip to prevent dehydration of the mount; the use of a coverslip seemed to hasten the ejection of spores from the worm.

After the zoospores were released, the coverslip was removed and the zoospores collected by running a wire loop through the water left in the slide. The liquid was then streaked onto a plate of peptone-yeast extract-glucose agar (PYG) consisting of 0.6 gm peptone; 0.4 gm

yeast extract; 1.8 gm glucose; and 16 gm agar in 1 L of distilled water. In order that contamination be kept at a minimum the medium also contained 5 gm each of streptomycin sulphate and penicillin G (Sigma Chemical Co., St.Louis, Mo.).

At selected intervals the plates were observed under a dissecting microscope and within 24 h the spores had produced sufficient mycelium that the thalli could be noted under 16 x's magnification. Mycelium was then transferred to fresh plates of PYG agar without antibiotics, and these were subsequently maintained at 25°C. Transfers were then made every 72 h by flooding selected plates with sterile distilled water and transferring the zoospore suspension obtained in this way to fresh PYG agar plates.

A set of stock cultures was also maintained by inoculating spore suspensions onto yeast-starch agar (YpSs) which consisted of: 4 gm yeast extract; 1 gm K_2HPO_4; 0.5 gm $MgSO_4.7H_2O$; 2 gm soluble starch; and 16 gm agar in 1 L distilled water. YpSs agar induced the production of resting sporangia that could be maintained for several months at 5°C while cultures maintained on PYG agar for more than 4 days at 25°C would undergo autolysis.

Caeonorhabditus elegans was grown in Petri dishes containing NG agar (Brenner 1974) on which colonies of Escherichia coli were growing. The worms were collected by washing the plates with sterile distilled water and pipetting off the water containing the worms.

Transmission Electron Microscopy (TEM): Cultures of C.anguillulae, growing on PYG agar were examined under a stereomicroscope at 100 X magnification to determine their stage of development. Due to the fact

that the sporangia mature successively it was not possible to establish synchronous cultures in which all sporangia were at a similar stage of development. Often two adjacent sporangia would differ markedly in their maturity. Therefore it was often necessary to determine the state of development of the specimens by observing them at the ultrastructural level.

One mm^2 agar cubes of selected material were excised from the culture dishes and fixed in 2.5% gluteraldehyde in 0.1 M sodium cacodylate buffer pH 7.2, for 2 h at room temperature and postfixed with buffered osmium tetroxide for 2 h at $5^{o}C$ according to Hess (1966). Some samples were also stained during post-fixation by adding ruthenium red to the osmium tetroxide, according to Luft (1971); this aided in distinguishing certain structures associated with the cell wall. When ruthenium red was used, a control in which the stain was omitted was also fixed and embedded concurrently. All samples were stained overnight in 0.5% aqueous uranyl acetate, then dehydrated in an ascending series of ethanol and infiltrated with Spurr's medium (Spurr 1968).

Sections were cut on a Reichert OMU2 ultramicrotome using glass knives. Sections in the silver grey and light gold range of interference colours were mounted on copper grids and stained with lead citrate (Reynolds 1963) for various lengths of time in a nitrogen atmosphere. The time required for staining was dependent on the relative maturity of the specimen; the more mature specimens required the shortest staining time.

Sectioned material was examined using either an AEI 6B or AEI 801 transmission electron microscope operated at an accelerating voltage of 60 Kv. When appropriate material was noted, photographs were taken using

Kodak electron microscope film #4489.

Light Microscopy: Cultures were grown in the manner already described, but, depending on the relative age of the portion of the thallus being observed, one of two procedures was followed in preparation for light microscopy. In the first procedure younger material, which was comprised of germinating spores through to developing young sporangia, was excised from the culture dishes and mounted in 0.1% lactophenol toluidine-blue stain on a slide and placed under a coverslip. The slide was then carefully heated over a flame to disperse the agar prior to microscopic examination. In the second procedure, that mycelium exhibiting the developmental stages bearing hyphal swellings through to mature zoosporangia was fixed with 2.5% glutaraldehyde and then mounted in 0.1% lactophenol toluidine-blue stain and gently heated. The more mature material was found to be fragile and difficult to separate, and had not produced satisfactory mounts by the first procedure. However, in the latter process, first employing fixation made the material more rigid and allowed for separation of the material with minimal damage to the hyphal elements. In order to examine nuclei and nuclear migration in the specimens, samples were treated according to Lu and Raju (1970) and stained using 2% iron haemotoxylin stain as presented by the same authors.

The prepared mounts were examined and photographed using a Zeiss standard Universal microscope with phase contrast illumination. Material stained with iron haemotoxylin was examined using light field illumination. Photographs were taken employing Kodak Panatomic-X 35 mm film.

Results and Discussion

Catenaria anguillulae begins its life cycle as a uniflagellate zoospore (Fig. 1a). The zoospores of the isolate used in this study were similar to those described by Couch (1945), being 6.5-8.0 um long x 3.5-5.5 um wide and somewhat tapered at both ends, though sometimes the spores took on an oval appearance. The zoospore will swim in a water film for various periods of time varying from several minutes to three or more hours, loses its flagellum, rounds up, and undergoes encystment. The encysted spore germinated by producing a germ tube that elongates to several times the length of the zoospore (Figs. 1b, 1c). According to Couch (1945), the zoospore germinates by first sending out a delicate rhizhoid, and then produces a tubular growth at "the opposite end which may form a dwarf sporangium". However this pattern of germination was not seen on any occasion in this study. This observable difference may well be an expression of environmental plasticity, similar to that displayed by different isolates of Entophlyctis variabilis (Powell and Koch 1977).

After germination was initiated the spores of C.anguillulae were generally observed to be pyriform to rounded in shape (Figs. 1b, 1c) with an elongate germ tube. Occasionally, some of the germinating spores produced a swelling immediately beneath the spore, from which a germ tube arose (Fig. 1b, arrow). As well, it should be noted that rhizoids may develop directly from this swelling. Several hours after germination had commenced, a swelling was produced at some point along the germ tube (Fig. 1d, arrow). The protoplasmic contents of the zoospore migrated

into this continually enlarging swelling, which will be referred to as the "prosporangium". The term was adopted from Whiffen (1944), who used it to describe a unique swelling that contributed to the development of the zoospore into a zoosporangium. Barr (1973) proposed that the term prosporangium be used to describe a swelling on the rhizoid containing a nucleus during the early developmental stages. As C.anguillulae produces a nucleus-containing swelling on the germ tube which subsequently enlarges and produces other sporangia in a polycentric manner, the use of the term sporangium appears to be justified. Eventually the zoospore was reduced to an empty sac (Fig. 1e) and the developing prosporangium contained the contents of the original zoospore. Figure 1e shows the prosporangium as being approximately twice the size of the zoospore at this stage, and containing lipid bodies and a nucleus. Rhizoids were also evident, as they developed from the sides of the swelling; as well, a hyphal element developed from the posterior end of the prosporangium.

The production of the prosporangium may be analagous to the production of the endobiotic sporangium produced by C.allomyces in its fungal host (Couch 1945; Sykes and Porter 1980). The zoospore of C.allomyces germinates on the surface of its host and produces an appressorium from which an infection tube arises. Eventually the infection tube breaks through the cell wall, enters the host and produces a swelling (similar to that seen in Figure 1d) within the host along the infection tube. Subsequent to this, there is a migration of protoplasm out of the zoospore into the swelling, which eventually produces other sporangia within the host. It would appear then that the early development of the fungus on agar is similar to that which occurs when it is existing as a

LEGEND

Figure 1a-f. Phase contrast photomicrographs of C.anguillulae depicting the free swimming zoospores, germination and subsequent production of a pro-sporangium.

Figure 1a. Free swimming uninucleate zoospores, displaying side bodies of L, x800.

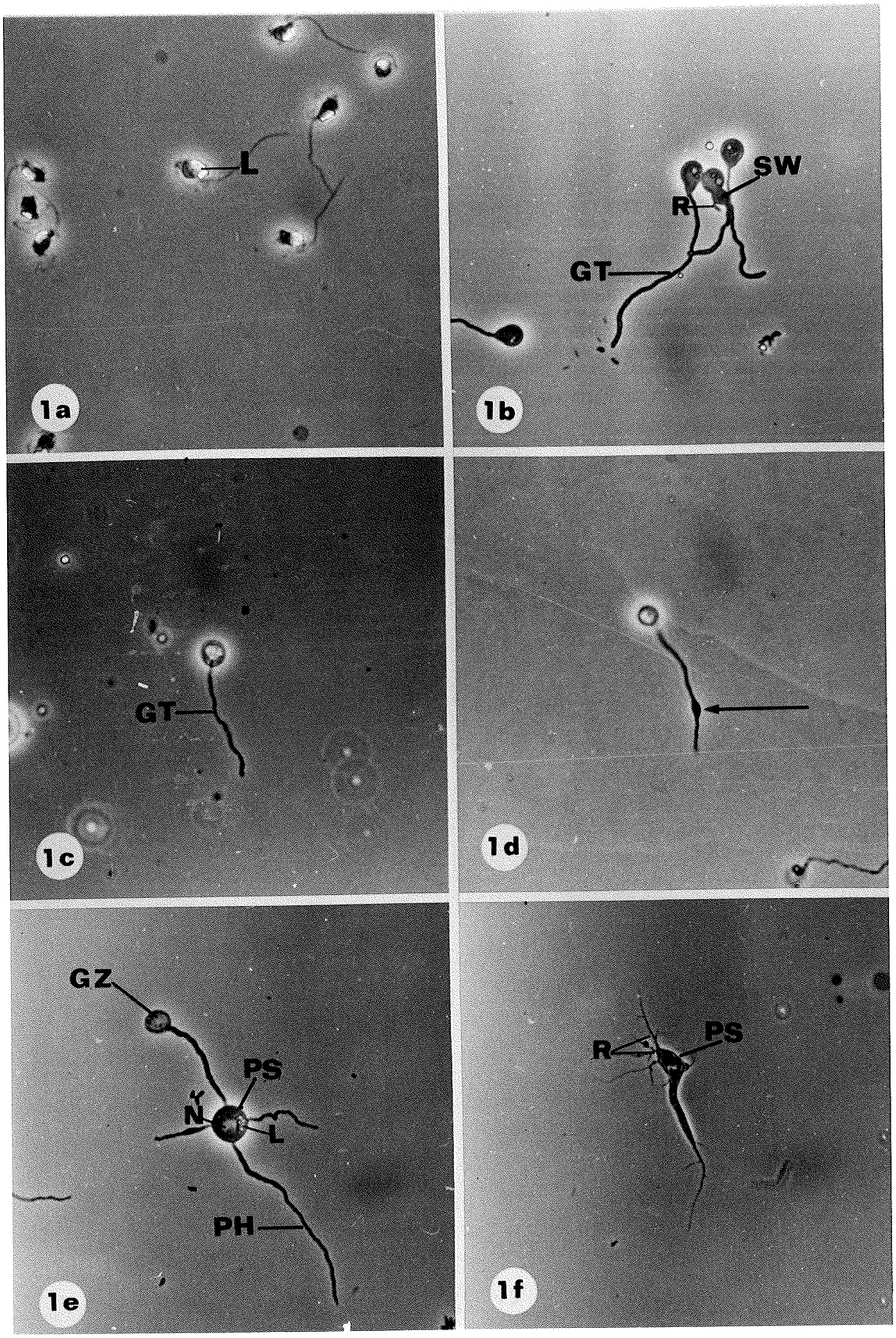
Figure 1b. Germination of zoospores, noting their pyriform shape and elongate GT. Also note the occasional production of a SW immediately beneath the zoospore, x800.

Figure 1c. Germination of spherical-shaped zoo-spore, x800.

Figure 1d. Initial production of PS on the GT (arrow), x800.

Figure 1e. Depiction of empty GZ, enlarging PS and developing PH. Also note the inclusion of N and L in the PS, x800.

Figure 1f. Young PS with well developed R, x315.


L
1a
SW
R
GT
1b
GT
1c
1d
GZ
PS
N
L
PH
1e
R
PS
1f

parasite. As the prosporangium developed, it enlarged and produced a number of rhizoids (Fig. 1f). The primary hyphal element produced from the swelling thickened, and rhizoids were produced from it as well.

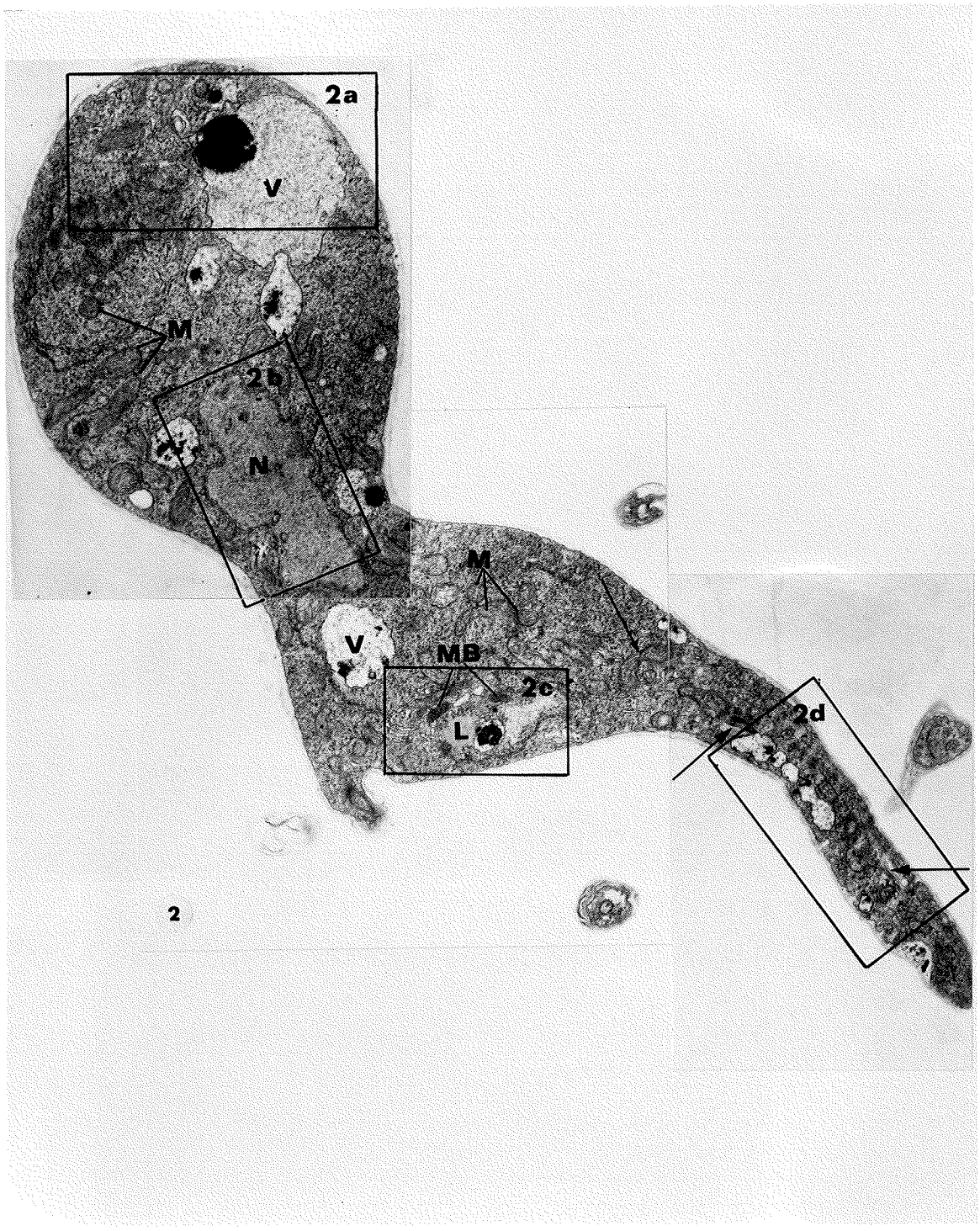
The section shown in Figure 2 was taken through the longitudinal axis of a spore that had produced a swelling similar to that depicted in Figure 1b. It appears that the microbody lipid complex had broken up and was no longer recognizable. Smaller microbodies were evident, and were too distantly separated to be lobes of the same structure. By examining serial sections of this structure, it was apparent that the microbodies were scattered throughout the cytoplasm (Fig. 2a, 2c). The insets shown (Figs. 2a-2d) are from a different plane of section than that depicted in Figure 2. It is apparent that this section bears little resemblance to the structural details of the zoospore of this fungus as reported by Chong and Barr (1974) and Olson et al. (1978). Olson et al. reported that the zoospore had contained a single elongate, lobed microbody that was closely appressed to a linear arrangement of lipid bodies.

The situation regarding the dispersal of the microbodies appears to be similar to that of the germinating zoospore of Blastocladiella emersonii. Mills and Cantino (1979) noticed that upon germination of the zoospores of that species, a single large microbody fragmented into smaller microbodies and lost their affinity for the lipid bodies. The lipid bodies then remained independent of the microbodies for fifteen hours of growth, whereupon they became reassociated with the lipids. The fragmented microbodies in C.anguillulae generally appeared to be independent; however, direct association between a microbody and a

LEGEND

Figure 2. TEM micrograph showing an overall view of a young germling of C.anguillulae.

Figure 2. A number of M are present in the germling, some of which appear to be dividing (small arrow). A migrating N is evident and TB can also be seen in the GT (large arrow), x14,000.



2

LEGEND

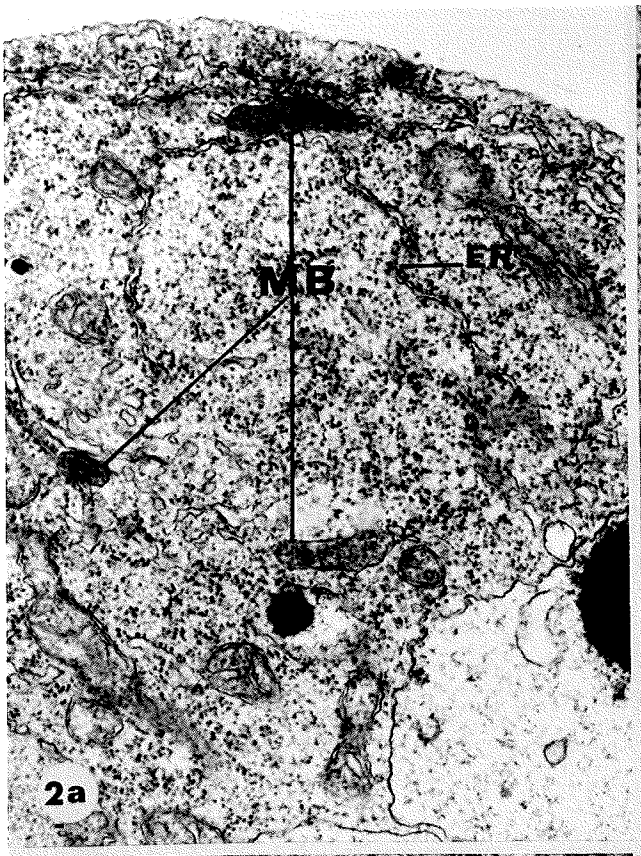
Figures 2a-d. TEM micrographs of enlargements of regions of the cytoplasm deposited in Fig.2, but in different planes of section.

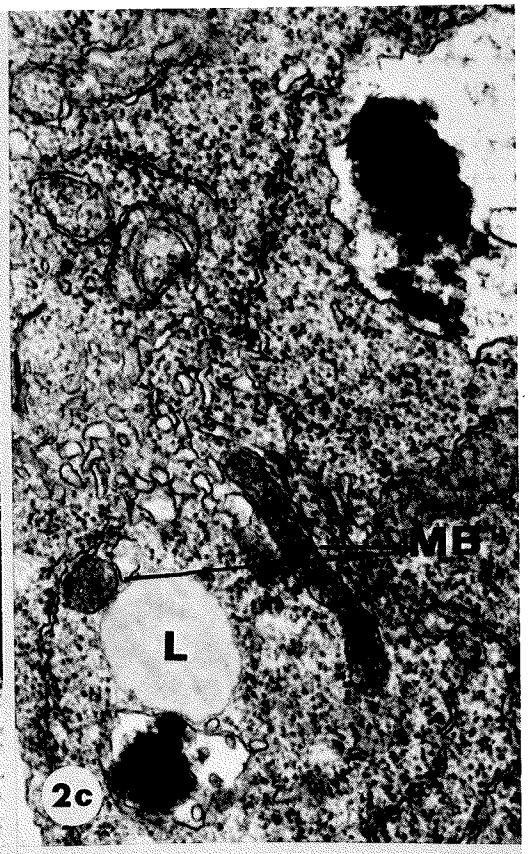
Figure 2a. MB randomly arranged in the cytoplasm and frequently associated with ER, x34,500.

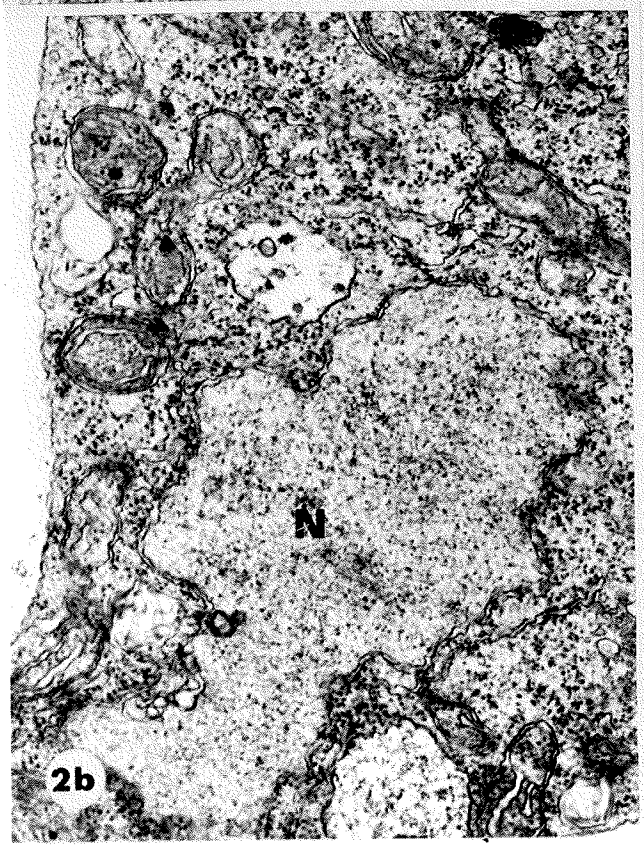
Figure 2b. Elongate migrating N, x34,500.

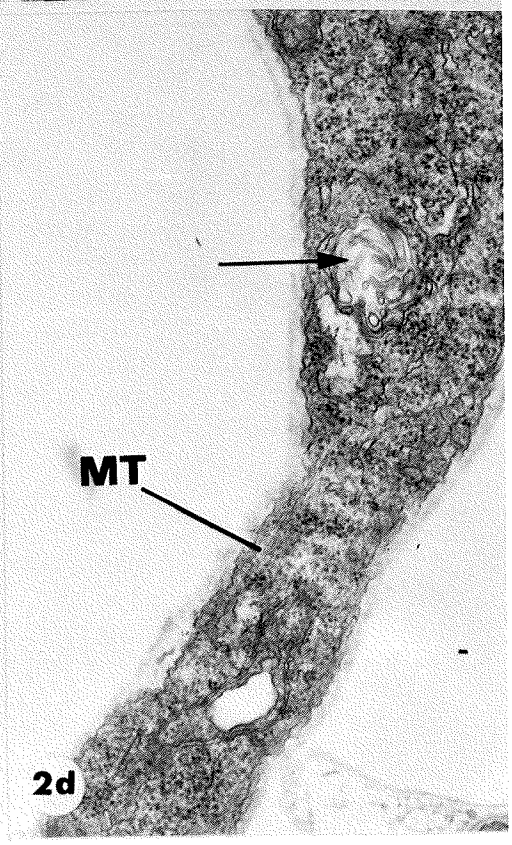
Figure 2c. Two MB, one associated with L, x47,800.

Figure 2d. Tubular body (arrow) and MT present in the germ tube. Note that orientation of MT is parallel to the wall, x34,500.


MB
ER
2a


MB
L
2c


N
2b


MT
2d

lipid body was still observed at this stage (Fig. 2c), possibly implying that a physical connection between the microbody and the lipids that was present in the zoospore may have prevented portions of the microbody from breaking away. Such connections or "bridges" between organelles have been reported in zoospores of a Chytridiomyces sp. (Doward and Powell 1982).

Powell (1976) has shown that the microbodies of Entophlyctis variabilis, another Chytridiomycete, contain the enzymes catalase, malate synthetase and isocitrate lyase. As the latter two enzymes are involved in the glyoxylate cycle, Powell (1976) concluded that the microbodies were functioning as glyoxysomes involved in the metabolism of lipids. If this is the case, then after germination occurs and the fungus is taking up nutrients from its substrate, the use of exogenous storage products, e.g. lipids, would not be necessary. At this stage one would expect to see a disassociation of the lipids and microbodies.

Some of the microbodies in the germinating zoospore also appeared to be associated with the endoplasmic reticulum (Fig. 2a). The origin of microbodies from endoplasmic reticulum has been documented by several workers (Fredrick et al. 1968; Maxwell 1977; Powell 1979). According to Powell (1979), the spatial association of microbodies and other organelles may have some functional implications, one being their possible origin from endoplasmic reticulum.

According to Chong and Barr (1974), the zoospores of C.anguillulae possess a single large mitochondrion. However, from Figure 2, it is clear that this mitochondrion was replaced by a large number of smaller mitochondria. These smaller mitochondria can produce other mitochondria

through a process of division as discussed by Hawker (1965) and Temmink and Campbell (1968) and such divisions may be represented in Figure 2 (small arrows).

There also appeared to be a proliferation of endoplasmic reticulum throughout the cytoplasm, much of it associated with ribosomes, implying that protein synthesis was occurring. Protein synthesis would be expected as protein is known to increase during germination (McLeod and Horgen 1979). It is also apparent from Figure 2 that the cytoplasm of the germling was extremely rich in ribosomes, again indicating protein synthesis. Much of the ribosomal material was probably due to the breakdown of the nuclear cap; the nuclear cap being an aggregation of ribosomes that form a cap over the nucleus in zoospores of members of the Blastocladiales.

A number of vesicles containing dark staining products were present in the cytoplasm of the germinating zoospore of C.anguillulae (Fig.2). These vesicles appeared very similar to those found in the oogonium of Saprolegnia furcata (Gay et al. 1971) and the sporangium of S.ferax (Gay and Greenwood 1966). According to Gay et al. (1971) these dense body vesicles seem to be involved in two major functions: 1) the production of membrane for tonoplast development; and 2) the differential movement of fluid into the vesicles which results in their enlargement. The latter authors also presented evidence suggesting that such dense staining products could be composed largely of phosphotidyl choline, a constituent of membranes. According to Armbruster (1982) the dense body vesicles present in Saprolegnia spp. may be produced from an association of endoplasmic reticulum with mitochondria, with endoplasmic reticulum swelling

at the point of contact with the mitochondria, and producing the amorphous material found in the vesicles in question. However, although dense body vesicles were present and frequently observed in the germling stage of C.anguillulae, their method of production was not noted.

The germ tube illustrated in Figure 2 and 2d, contained endoplasmic reticulum, mitochondria, microbodies, vesicles, and microtubules. A number of tubular bodies were also evident in close association with the germ tube wall. These structures were enclosed in a membrane (Fig. 2, 2d, large arrow) and resembled similar structures observed in the thallus of the thraustochytrid, Ulkenia amaeboidea (Bahnweg and Sparrow) Gaertner by Raghu Kumar (1982). The previous author did not ascribe a function to the structures, but it is possible that they were plasmalemmasome-like structures. Hemmes and Hohl (1971) noted that there was a numerical increase in both lomasomes, and the amount of endoplasmic reticulum in the germinating cysts of Phytophthora parasitica. This reinforces the belief that these tubular structures were plasmalemmasomes.

The microtubules in the germ tube appear to be oriented parallel to the cell wall (Fig. 2d). The orientation of the microtubules may be of functional significance, as it has been suggested that microtubules may be involved in the control of cell wall formation, as discussed in a review by Hepler and Palevitz (1974). Heath and Heath (1978) have also presented evidence that the microtubules observed in the developing portions of the rust fungus Uromyces phaseoli were "typically oriented parallel to the direction of cytoplasmic migration" and were found to be non-randomly associated with mitochondria, implying a possible role in organelle motility. Bundles of microtubules were also observed in

close proximity to the wall of the germinating spore (not illustrated). Most of the cytoplasmic microtubules observed probably served a cytoskeletal function as suggested by Barr (1978).

The fine structure of the germ tube was very similar to that of the young rhizoid of B.emersonii (Barstown and Lovett 1974a) and the germ tube of a Phlyctochytrium sp. (Porter and Smiley 1980). However, in C.anguillulae there was a distinct absence of apical vesicles in the germ tube as compared to B.emersonii. Apical or wall vesicles as described by Grove et al. (1970) were not present in the growing regions of C.anguillulae. This is not unusual for members of this group; as Heath (1976) points out, many of the chytrids enlarge without evidence of wall-vesicles. Even in B.emersonii, the only wall vesicles observed at any point during the life cycle were those located in the elongating rhizoid. Sykes and Porter (1980) have also reported a heavy concentration of vesicles in the apex of the infection tube of C.allomycis, noting that these vesicles contained material similar to that found adjacent to the apical cell wall. It is interesting that wall vesicles are not observed in many of the Chytridiomycetes. However, in these fungi it is also possible that the composition of the wall vesicles is such that they are not preserved by normal fixation techniques (Barstow, personal communication).

After production of the germ tube the nucleus appeared to elongate as it migrated down into the germ tube (Fig. 2, 2b). The occurrence of microtubules associated with the migrating nucleus was not observed. Such an occurrence was reported by Raudaskoski (1972) in the Basidiomycetes, and he suggested that the microtubules function in the migration

of nuclei. Other protoplasmic contents such as lipids, mitochondria, microbodies and cytoplasm obviously migrated into the growing regions as well. This migration may be partially due to the development of cytoplasm by the enlarging vacuoles apparent in the germling (Fig. 2).

After the nucleus had migrated into the prosporangium, the latter appeared to have enlarged (Fig. 1e). The ultrastructure of the prosporangium (Fig. 3) was basically the same as that of the germling in that the structure contained a single nucleus, lipid bodies, a number of mitochondria, vesicles, endoplasmic reticulum, and microbodies. The cytoplasm, like that of the germling, was rich in ribosomes, but differed from the germling in the deposits of glycogen now evident. It is evident from this that the fungus was no longer relying upon its endogenous sources of nutrients, i.e. lipids. Instead, the thallus was probably taking up nutrients from its substrate and producing storage products. According to Suberkropp and Cantino (1972), the polysaccharide pool decreased sharply after the encystment of the zoospores. Following germination, nutrients were taken up and storage products such as glycogen were formed.

A moderate amount of rough endoplasmic reticulum was observed throughout the cytoplasm. Some of the endoplasmic reticulum being closely associated with the tonoplast of the vesicles, gave them the appearance of having a double membrane (Fig. 3). In plant cells, endoplasmic reticulum is occasionally seen lying parallel to the tonoplast, and this is suggestive of the intermediate role of endoplasmic reticulum in the transport of materials (Gunning and Steer 1975).

The vacuoles present at this stage of development appeared to be of two types, one appearing much larger and having an extremely dense matrix

(Fig.3, VI) and occasionally containing dense staining bodies. The nature of these bodies was unclear but they may be related to those present in the germinating spore, and therefore may be involved in expansion of the vacuole. The other form of vacuole appeared to contain only a small amount of fibrillar material (Fig. 3, V2).

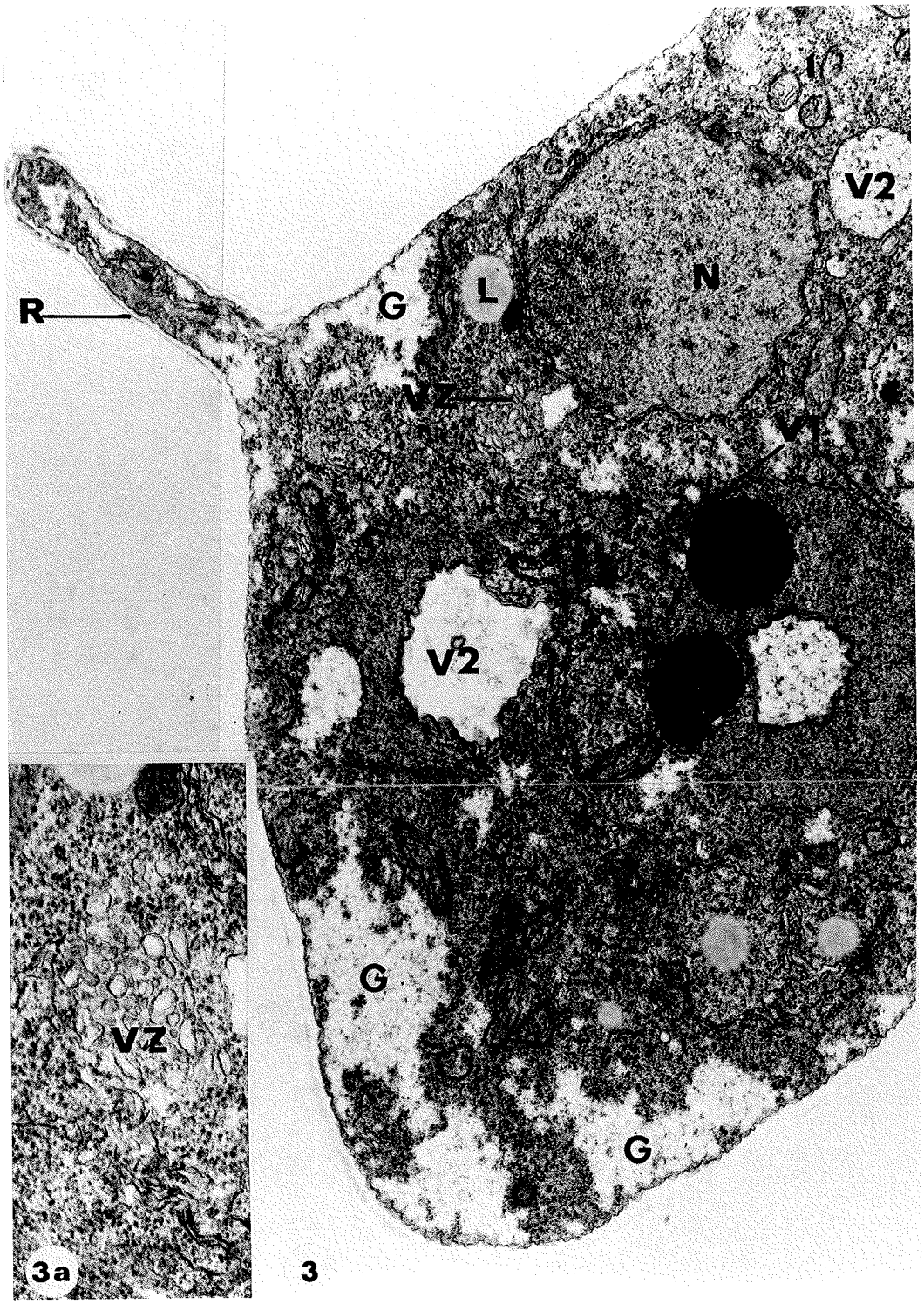
An aggregation of small vesicles was noted in the prosporangium. This aggregation (Fig. 3a, inset) was similar to the "vesiculated zone of ribosome exclusion" observed by Barstow and Lovett (1974a) in B.emersonii. In the absence of a typical Golgi apparatus this zone is of great interest. These authors postulated that these zones may represent the functional equivalent to the Golgi. One of the major reasons for designating these zones as Golgi equivalents was the paucity of ribosomes in the cytoplasm surrounding the vesiculated region which is one of the criteria for a Golgi. McLaughlin (1973) had also observed these zones in the Basidiomycete Coprinus cinereus (Schaeff. ex Fr.) S.F.Gray and Boletus rubinellus Peck. One difficulty with assigning the role of the Golgi to this zone was the problem of explaining the transport of packaged materials from this zone to their final destination. In C.anguillulae vesicles were not noted emanating from this region, therefore the function of this region was unclear.

Rhizoid development also occurred early in the development of the prosporangium (Fig. 1e). The young rhizoids were aseptate and the protoplasm was continuous with the rest of the thallus (Fig. 3). As the prosporangium enlarged there was an increase in the number and length of the rhizoids (Fig. 1f).

A number of other events also occurred as the prosporangium enlarged.

LEGEND

Figures 3 and 3a.	TEM micrographs of a uninucleate prosporangium.
Figure 3.	Two types of vacuoles, V1 and V2, are indicated as well as G deposits, VZ and a developing R, x19,800.
Figure 3a.	Enlargement of VZ as depicted in Fig. 3, x45,000.



3

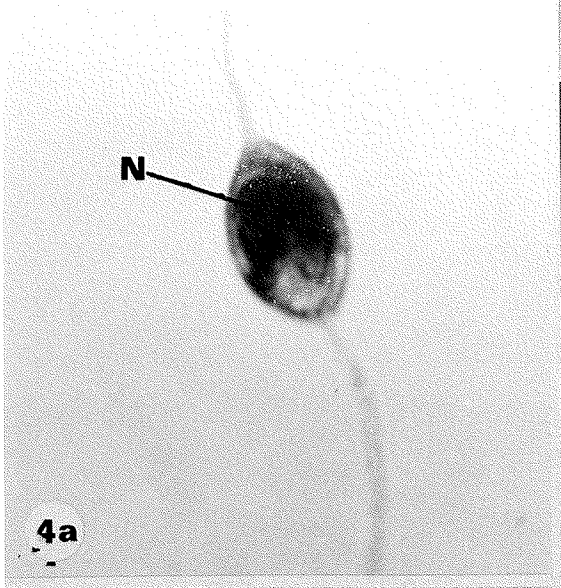
Nuclear division was evident for the first time in the life cycle. The nucleus did not divide until it had migrated into the prosporangium from the germinating zoospore. Figure 4a shows a young uninucleate prosporangium stained with iron haemotoxylin; the prosporangium was at a stage of development similar to that displayed in Figure 1e. Development of the centriole provided evidence of the first mitotic division (Fig. 5, arrow). The prosporangium at this point was approximately 10-15 um in length and uninucleate (Fig. 4a).

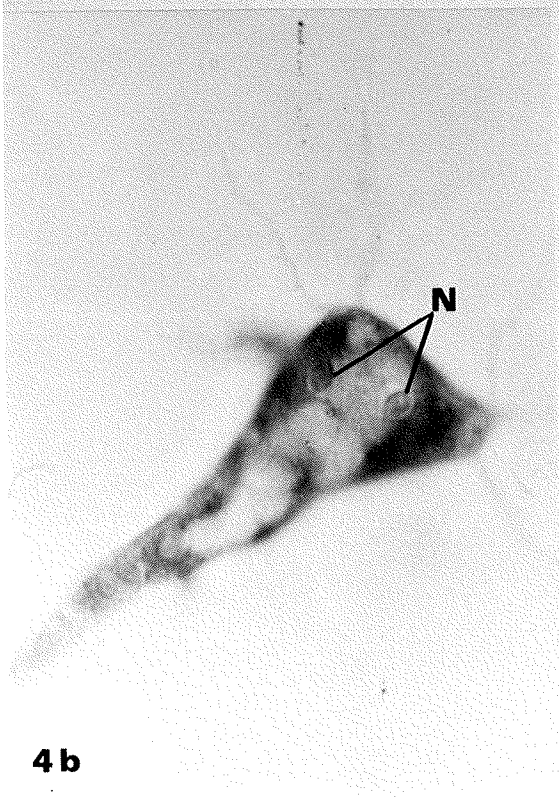
Concomitant with the first mitotic division there appeared to be a further increase in the amount of endoplasmic reticulum present in the prosporangium (Fig. 7). Much of the endoplasmic reticulum was smooth, some portions associated with ribosomes, as well, a number of cisternae were present (Fig. 6). Portions of the endoplasmic reticulum appeared to be associated with microbodies (Fig. 7, open arrows), possibly indicating the synthesis of microbodies at this stage. As well, microbody-lipid associations were observed (Fig. 7, small arrow); however, it was not possible to determine whether this represented the reassociation of lipids and microbodies as discussed by Mills and Cantino (1979) or remnants of the microbody-lipid complex in the zoospore.

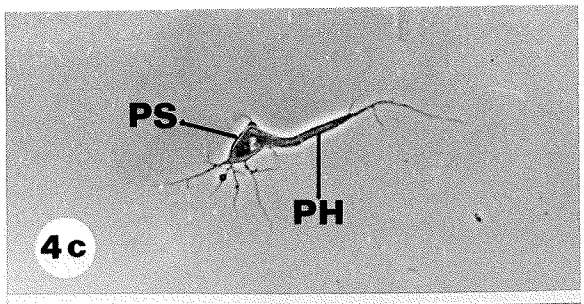
As the prosporangium continued to enlarge, the hyphal strand produced at its proximal end thickened (Fig. 4c), and subsequently protoplasm migrated into the hypha which continued to elongate (Fig. 4d). As this migration continued the binucleate prosporangium showed evidence of a heavy concentration of mitochondria in the hyphal strand and at the opposite end of the prosporangium (Fig. 8). The mitochondria

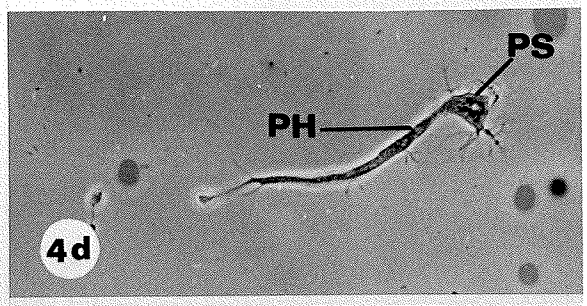
LEGEND

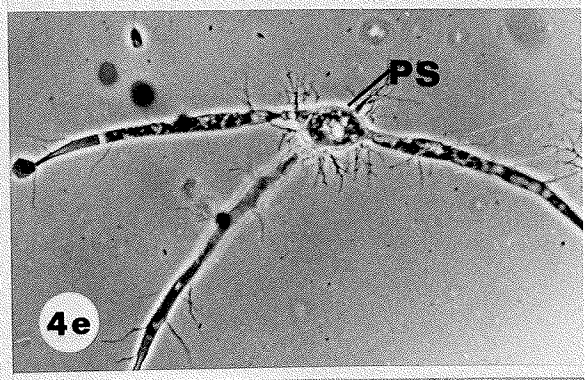
Figures 4a and 4b.	Bright field photomicrographs showing the nuclear conditions at different stages of prosporangial development.
Figure 4a.	Uninucleate (N) prosporangium, x1,800.
Figure 4b.	Binucleate (N) prosporangium, x1,800.
Figures 4c-4f.	Phase contrast photomicrographs depicting the development of prosporangia and primary hyphae.
Figure 4c.	Young PS and thickened PH, x315.
Figure 4d.	Later stage of development of PS with elongating PH, x280.
Figure 4e.	Older PS showing development of secondary hyphal elements, x280.
Figure 4f.	Presence of S separating the PS and PH, x640.

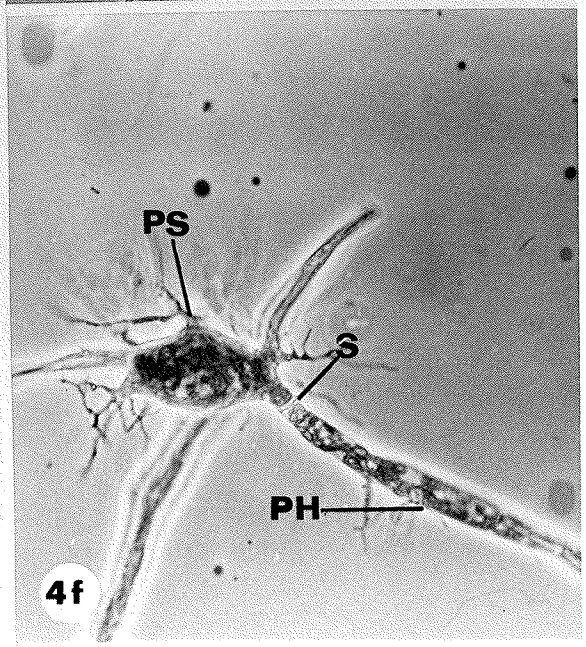

N
4a


N
4b


PS
PH
4c

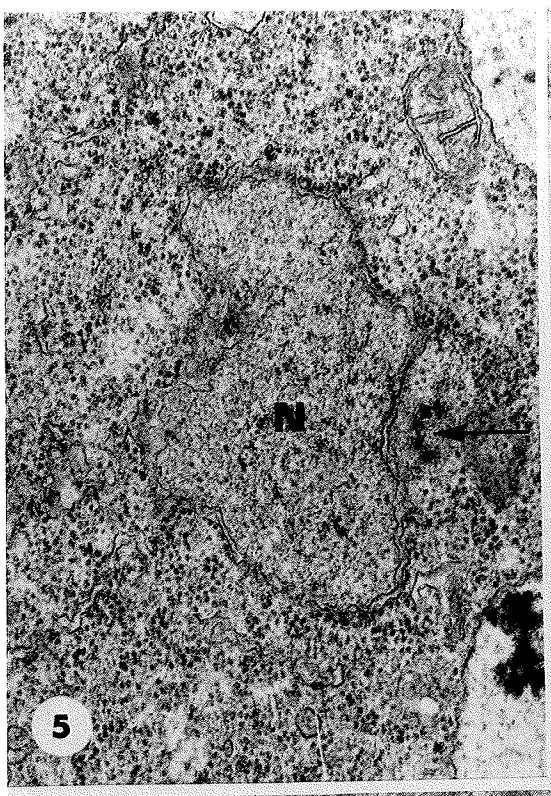

PS
PH
4d

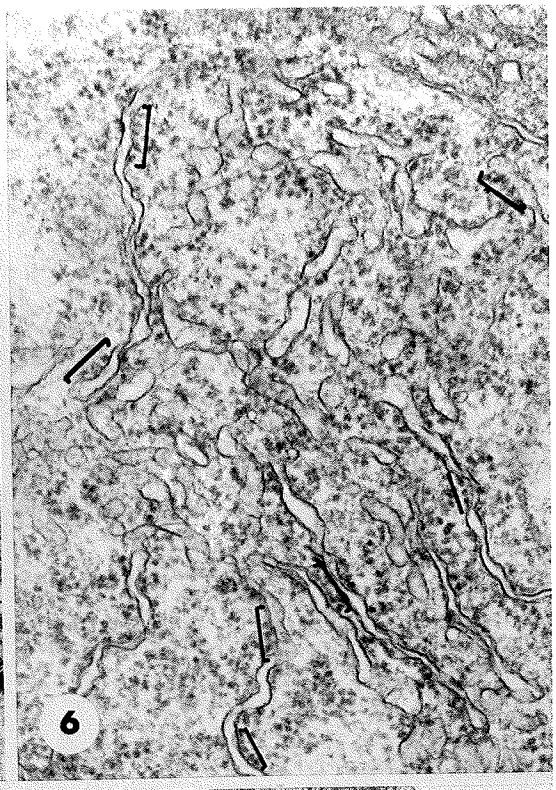

PS
4e

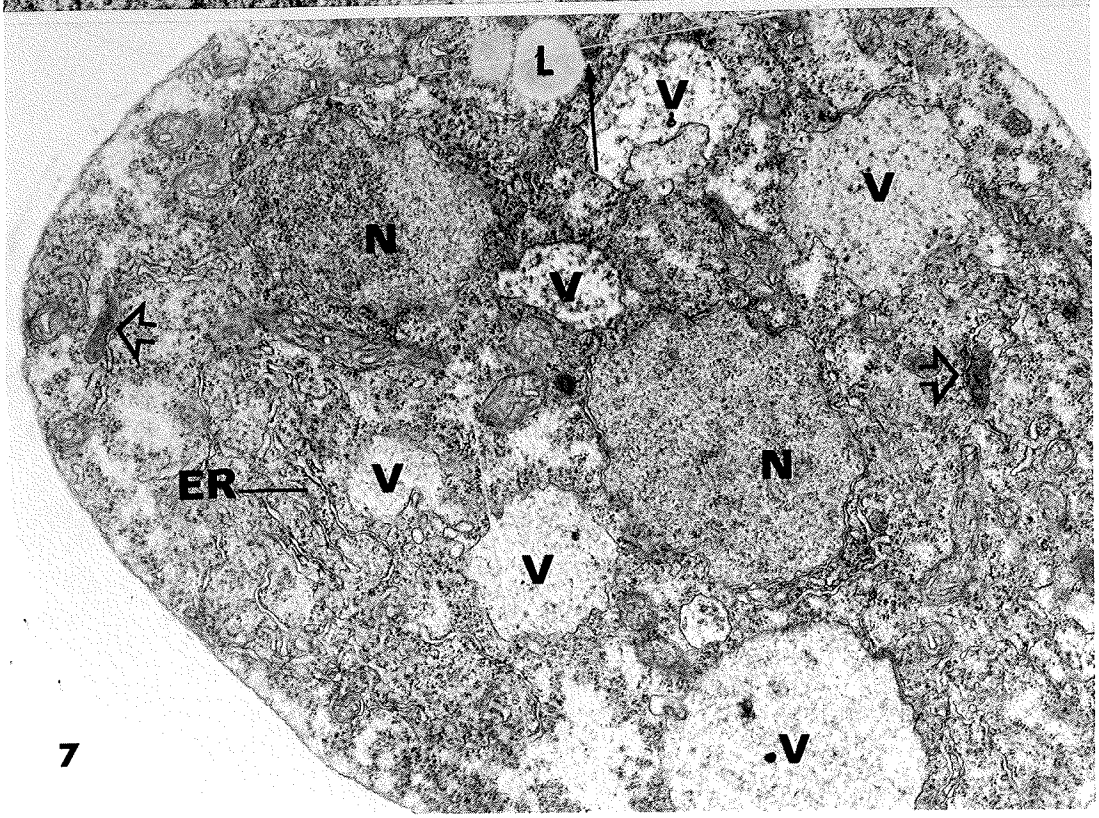

PS
S
PH
4f

LEGEND

Figures 5-7.	TEM micrographs of prosporangial development.
Figure 5.	The presence of a centriole (arrow) is noted, indicating the first stages of nuclear division, x49,000.
Figure 6.	A section through a region of smooth ER; bars indicate regions of ER with associated ribosomes, x17,500.
Figure 7.	Binucleate condition (N) of the PS showing abundant ER as depicted in Fig. 6, also MB associated with ER (open arrows), an MB, L association (small arrow) and numerous V, x53,300.


N
5


6


L
V
V
N
V
ER
V
N
V
.V
7

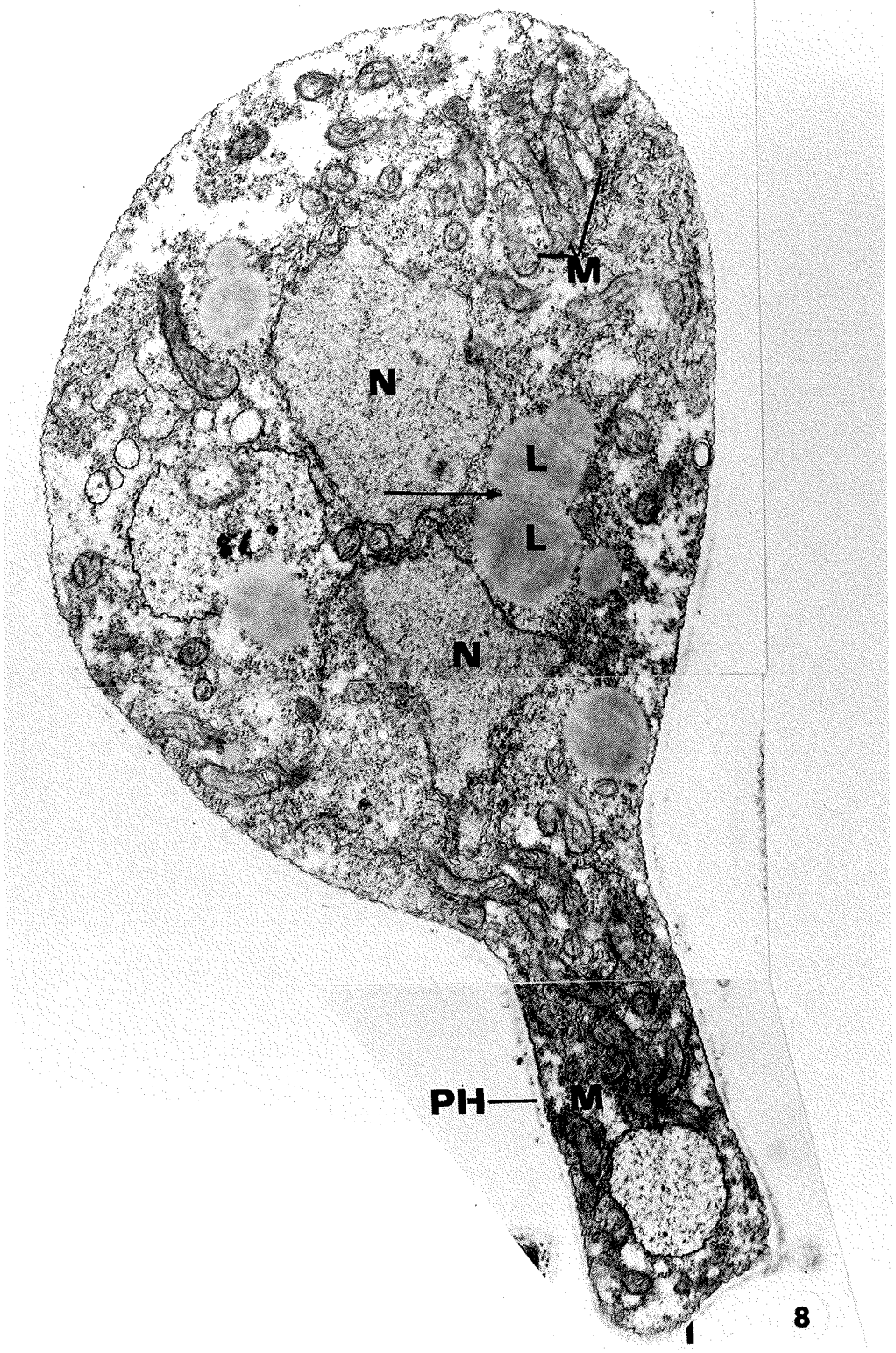
observed migrating into the hypha generally appeared to be oriented parallel to the longitudinal axis of the hypha. The polarization of mitochondria is an interesting phenomenon and has been observed and discussed by other workers. Heath (1976) reported that the motility of Oomycete mitochondria was independent of cytoplasmic streaming, which may also be the case in C.anguillulae, as it is obvious that not all the mitochondria were migrating in the same direction. It was also possible that these concentrations of mitochondria may be an indication of hyphal development. Grove et al. (1970) noted that the growing regions of fungal hyphae contained a large number of vesicles. They also noted that while mitochondria were generally lacking at the growing tip, the subapical zone immediately behind it contained abundant mitochondria. The same situation has been reported in Phytophthora sp. (Hemmes and Hohl 1971; Hoch and Mitchell 1972). As previously mentioned C.anguillulae appeared to enlarge without the benefit of wall vesicles. However it is interesting to note that subsequent to the appearance of aggregations of mitochondria, the prosporangium produced other hyphal elements (Fig. 4e) endorsing the belief that the mitochondria are associated with regions of growth.

In the binucleate prosporangium a number of large lipid bodies were apparent, some of which appeared to coalesce (Fig. 8, arrow). The situation regarding lipids at this stage of development is uncertain. It was previously noted that in earlier stages of development, e.g. uninucleate prosporangium (Fig. 3), lipids were not present in any quantity; and excess carbohydrates taken up by the fungi seemed to be channeled into glycogen production. However, as lipid production is a common

LEGEND

Figure 8. TEM micrograph of the binucleate prosporangium.

Figure 8. The PS shows the polarization of M at this stage, as well as their migration into the PH. The binucleate condition (N) as well as the fusion of L (arrow) indicated, x17,500.



8

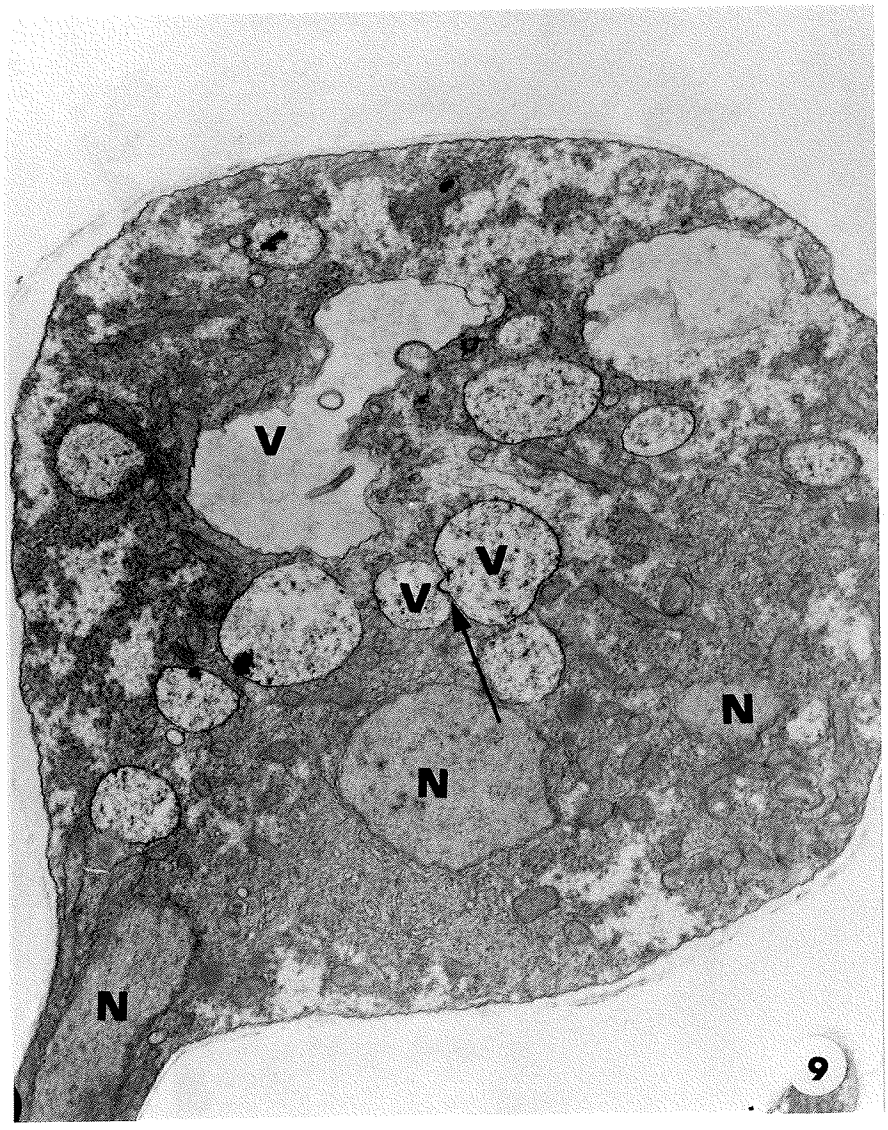
feature of a rapidly growing thallus on a sugar-rich medium, the appearance of lipid globules should not be unexpected (Burnett 1976). It is possible that after nuclear division occurs, some of the products of glycolysis are used in the biosynthesis of fatty acids resulting in the production of lipid bodies which are dispersed into developing zoospores. It is also likely that in C.anguillulae the concentration of lipids will vary from thallus to thallus at this stage of development. As mentioned earlier, zoospores swim for various periods of time before encysting and the amount of lipid material observed in the young thalli was probably dependent on the amount of time elapsed from spore discharge to encystment. Those zoospores that underwent encystment soon after release would probably retain much of their lipids; thus more lipid bodies should be observed in young thalli derived from such spores.

As the prosporangium matured, further nuclear division occurred and nuclei began to migrate from the prosporangium into the primary hyphal element (Fig. 9). The multinucleate prosporangium as depicted by Figure 9 appeared highly vacuolate; it is possible that the enlargement of these vacuoles through fusion (Fig. 9, arrow) may contribute to nuclear migration by displacement of the cytoplasm. Soon after migration began, an imperforate septum developed at the junction of the prosporangium and the hyphal element (Fig. 4f); this precluded further migration of protoplasm into the primary hyphal element. However septa were not yet formed in the secondary hyphal elements derived from the prosporangium, and prosporangial cytoplasm and organelles were now channeled into these. Imperforate septa have also been reported in Phytophthora parasitica (Hohl and Hamamoto 1967) and B.emersonii (Lessie and Lovett 1968) as well

LEGEND

Figure 9. TEM micrograph of prosporangium depicting nuclear migration into germ tube.

Figure 9. N migrating out of multinucleate PS, possibly due to displacement of cytoplasm by enlarging V; enlargement apparently occurring through fusion of V (arrow), x12,000.


V
V
V
N
N
N
9

as other members of the lower fungi. Conversely Powell (1974) demonstrated the occurrence of plasmodesmata in the septa of Entophlyctis variabilis and Rhizophydium spherotheca, but these are the only reported cases of such septa in the zoosporic fungi.

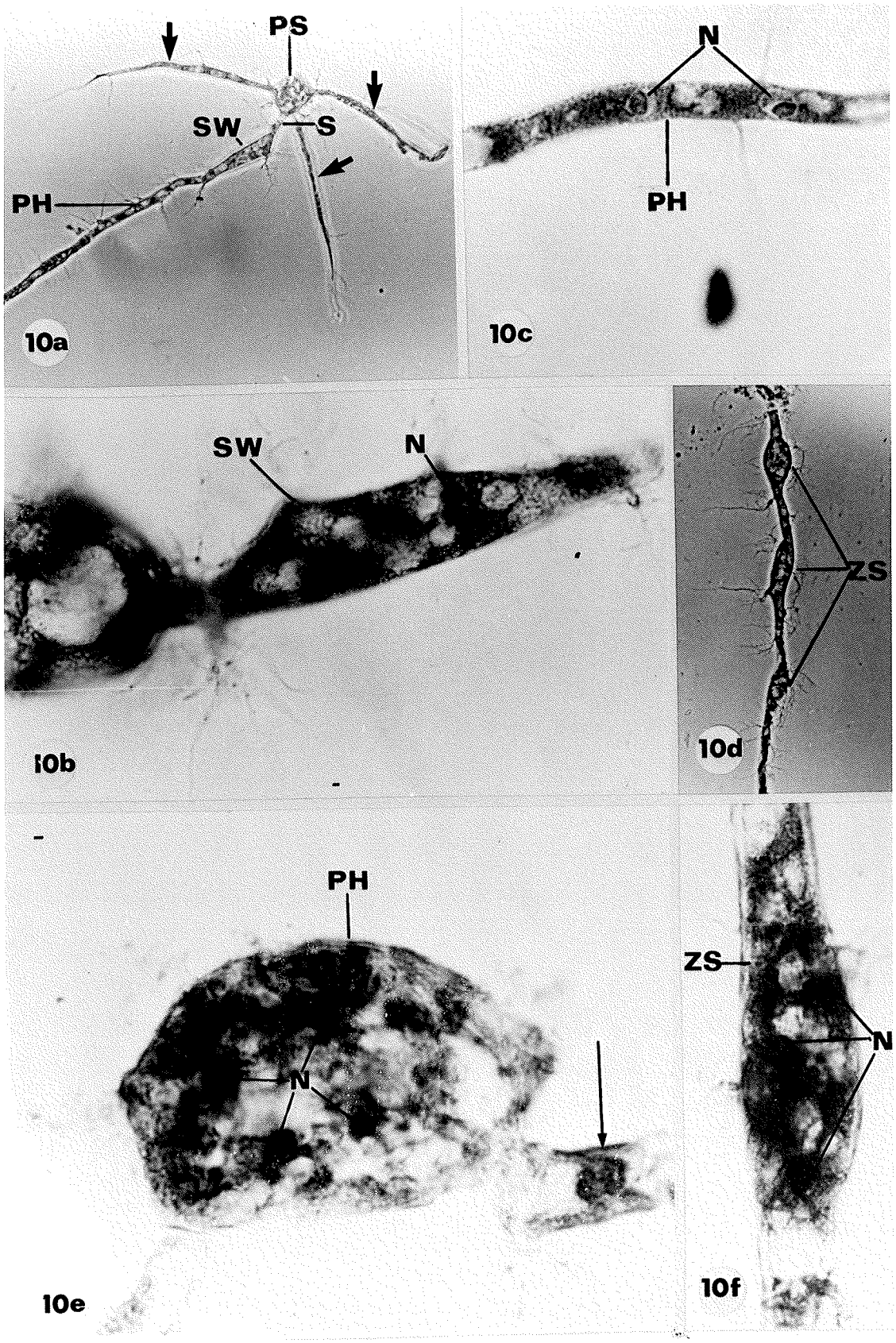
After the formation of a septum separating the prosporangium and primary hyphal element, swellings began to develop on the latter (Fig. 10a). The first such swelling developed at the proximal end of the hypha relative to the prosporangium. The swellings only appeared to develop at points along the hyphae where nuclei were present (Fig. 10b) in a fixed location; these swellings developed into zoosporangia. The fact that zoosporangia will be produced by a young thallus wherever a nucleus is located is well known, and has been recorded by earlier authors (Karling 1937; Hillegas 1940), and it is these swellings that will develop into zoosporangia in C.anguillulae.

As nuclear division proceeded within these swellings in individual hyphae, some of the nuclei migrated into the younger portions of the elongating hypha (Fig. 10c); this led to further development of the zoosporangium along the hypha in a successive manner (Fig. 10d). Concurrently nuclear division was occurring in the prosporangium and some of the nuclear progeny, as well as other protoplasmic constituents, migrated into those hyphae not yet compartmentalized by a septum (Fig. 10e, arrow).

As the young zoosporangium increased in size, it was evident that even at stages of early growth it contained several nuclei (Figs. 10f, 11). The central region of the cytoplasm became increasingly vacuolate, with large vacuoles occupying much of the cytoplasm. Based on their

LEGEND

Figure 10a.	Phase contrast photomicrograph of prosporangium with primary and secondary hyphal elements.
Figure 10a.	An S has developed between the PS and PH, and subsequently an SW develops on the PH; note also the production of secondary hyphae (arrows), x250.
Figures 10b and 10c.	Bright field photomicrographs of the nuclear condition of the swelling and more apical regions of the primary hyphae.
Figure 10b.	Uninucleate condition (N) of SW on PH as depicted in Fig. 10a, x1600.
Figure 10c.	N occurring in more apical regions of PH prior to development of SW, x1400.
Figure 10d.	Phase contrast photomicrograph of young zoosporangium.
Figure 10d.	Illustration of successive arrangement of young ZS that develop from SW, x250.
Figures 10e and 10f.	Bright field photomicrographs of nuclear condition of prosporangium and zoosporangium.
Figure 10e.	Multinucleate condition (N) of PS and migration of N into a hyphal element, x1600.
Figure 10f.	Multinucleate condition (N) in young ZS, x1400.


PS
SW
S
PH
10a
N
PH
10c
SW
N
10b
ZS
10d
PH
N
10e
ZS
N
10f

staining characteristics, at least three distinct types of vacuoles and vesicles were recognized (Fig. 12). Those vacuoles designated VI had a fairly granular matrix while the second major form (V2) displayed a much coarser matrix. The vesicles (V3) had a matrix that for the most part was electron lucent. While speculation should not be excessive, it seems reasonable to assume that a distinct difference in morphology could be an expression of differing functions.

As diagnostic histochemical techniques were not used in this study, it is only possible to speculate as to the function of the vacuoles and vesicles in the developing thallus of C.anguillulae. One of the major functions of vacuoles in the enlarging thallus could be to maintain turgidity, as it appeared that the production of protoplasm may lag behind the increase in the size of the enlarging sporangia, although vacuoles are known to have a number of other functions.

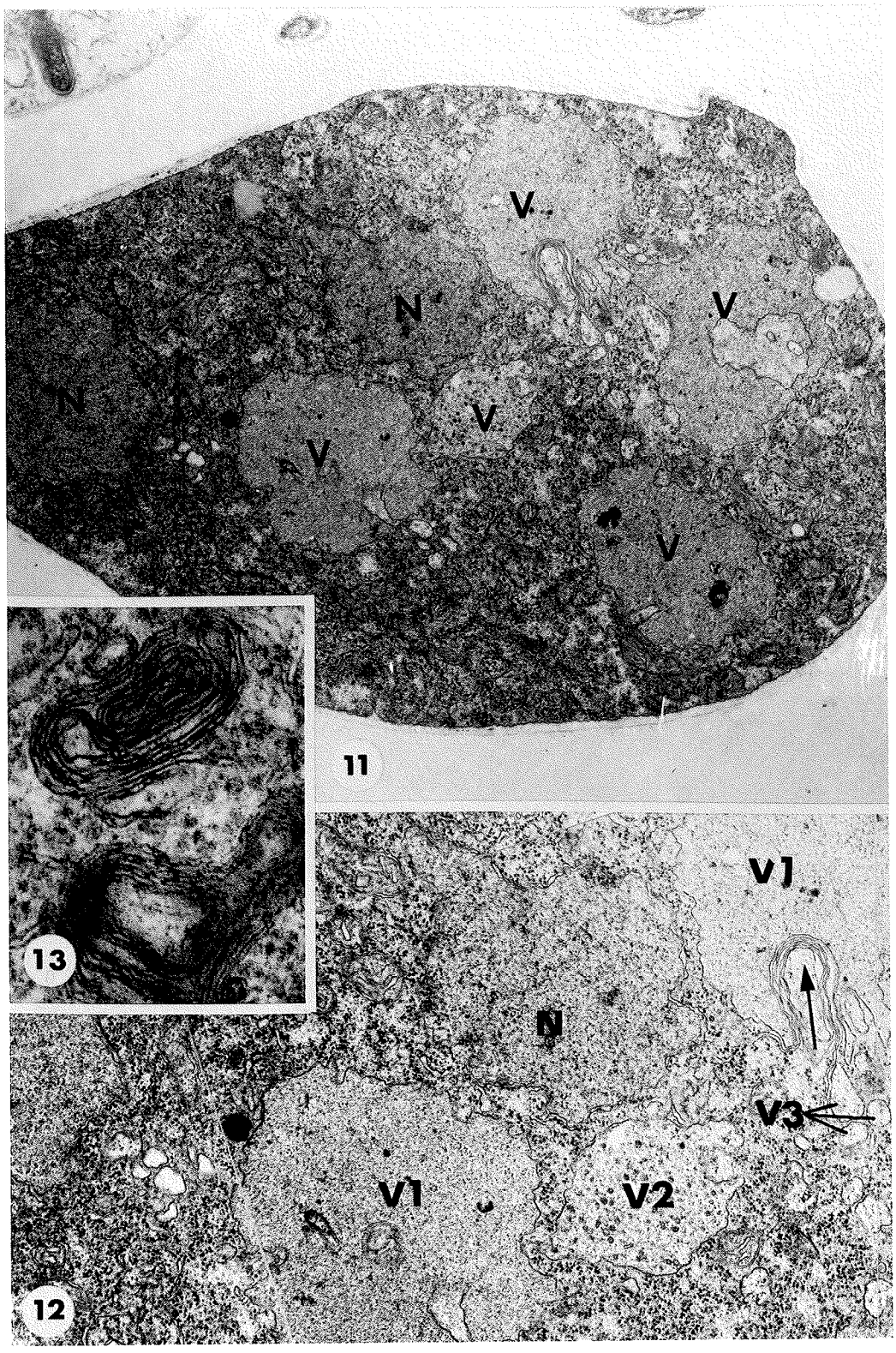
In a review of the biochemistry and function of vacuoles, Matile (1978) pointed out that the wide variety of functions carried out by vacuoles, one of which relates to the presence of hydrolases in the vacuoles of certain plant species; hydrolases were normally characteristic of lysosomal organelles. He also noted that vacuoles isolated from Saccharomyces cervisiae Meyen ex Hansen have been found to contain proteinases and carboxypeptidases as well as ribonucleases, invertases and other enzymes. Vacuoles also serve a storage function, and it is believed that these functions are mutually exclusive (Griffin 1981). Storage products would include nitrogen in the form of amino acids, particularly arginine and glutamine; these appear to accumulate preferentially (Matile 1978).

It is apparent from Figures 11 and 12 that the protoplasm of the young sporangium was relatively unorganized, with organelles randomly scattered through the cytoplasm. However the cytoplasm was rich in ribosomes evenly distributed throughout the sporangium, and smooth endoplasmic reticulum was prevalent; some of the latter appeared to be coiled in the enlarging zoosporangia and was generally associated with vesiculated regions of the cytoplasm (Fig. 12, arrow; Fig. 13).

In the enlarging zoosporangium distinct regions of smooth, tubular endoplasmic reticulum associated with cisternae that contain dark staining granules measuring 45 nm in diameter were apparent (Figs. 14, 15). This tubular endoplasmic reticulum had a diameter of 45-65 nm and appeared similar to the "macrotubules", sensu Lessie and Lovett (1968) in the enlarging zoosporangia of B.emersonii. Barstow and Lovett (1975) and Barstow (1979) have reported on the association of such tubular endoplasmic reticulum with the granule containing cisternae of B.emersonii and C.anguillulae, respectively. The latter author also reported the occurrence of rough endoplasmic cisternae containing the same 45 nm granules in Allomyces macrogynus; however, these lacked associated smooth tubular endoplasmic reticulum. Barstow also indicated that the 45 nm granules were believed to be the progenitors of gamma bodies. Evidence indicating how the tubular endoplasmic reticulum was formed was not obtained during this study, perhaps suggesting very rapid formation of such tubules. The tubules also seemed to disappear very quickly and regions were observed in the cytoplasm of mature sporangia where only a few tubules are seen associated with the granule containing cisternae (Fig. 16, open arrow). Barstow and Lovett (1972, 1975) presented

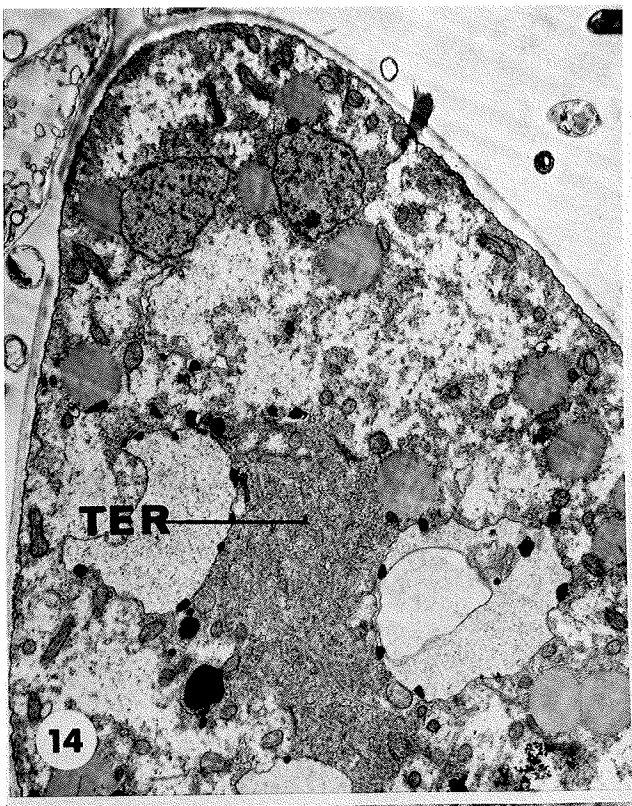
LEGEND

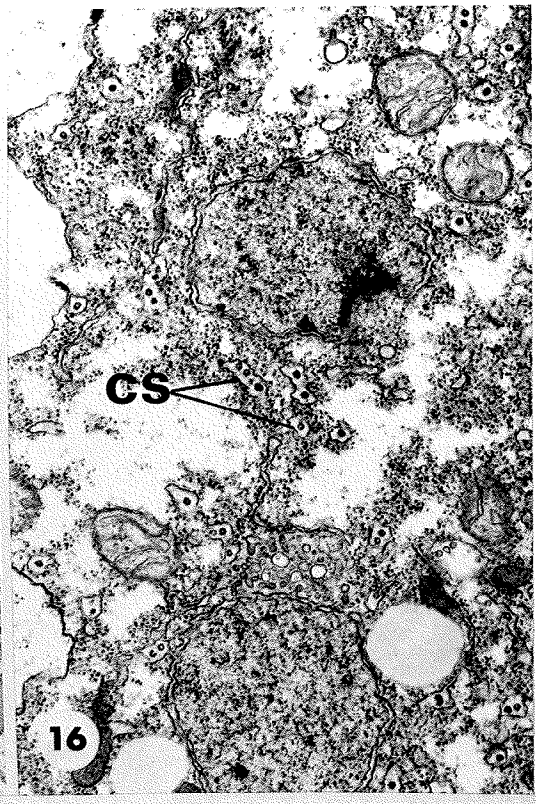
Figures 11-13.	TEM micrographs of young developing zoosporangium.
Figure 11.	Overall view of young developing ZS, x16,000.
Figure 12.	Enlargement of vesiculated region in Figure 11. At least 2 types of vacuoles (V1, V2) are apparent as well as vesicles (V3), A region of smooth coiled ER is closely associated with a V, x26,000.
Figure 13.	Region of coiled smooth ER observed in cytoplasm of young ZS, x86,000.

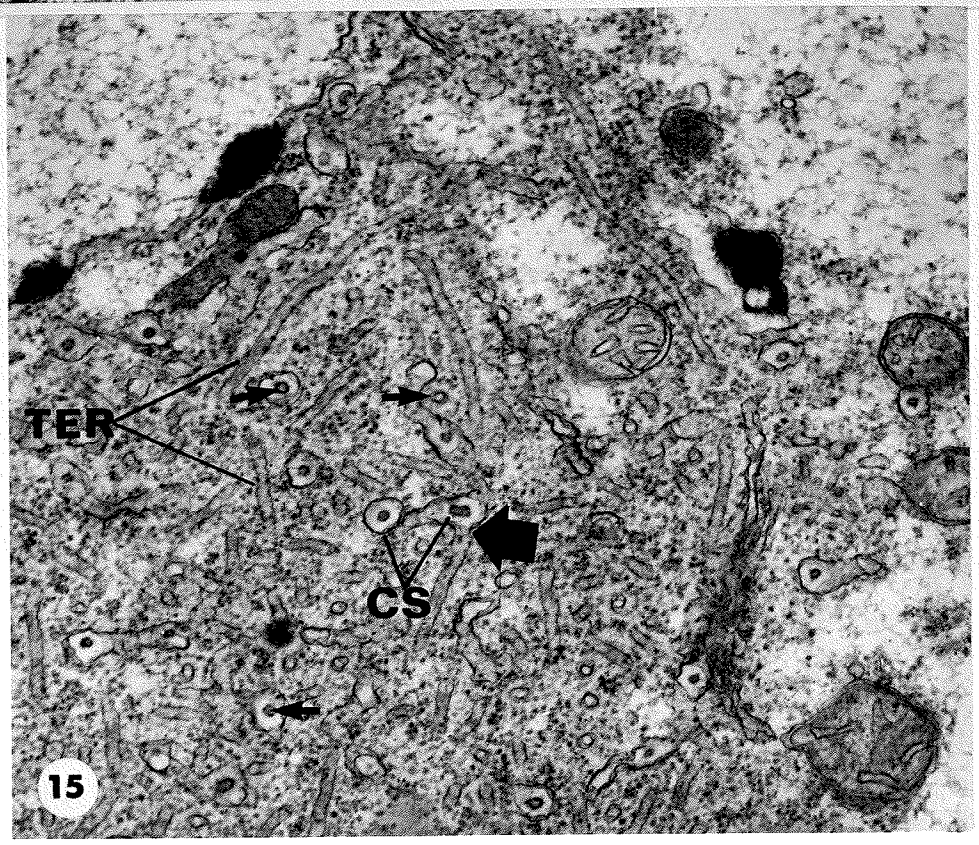

V
N
V
N
V
V
V
11
13
V1
N
V3
V1
V2
12

LEGEND

Figures 14-16.	Smooth tubular ER and associated cisternae containing dark staining granules from developing zoosporangium.
Figure 14.	Centrally located region of TER in young ZS, x7,000.
Figure 15.	Enlargement of region of TER in Fig. 14. Note the association of CS with the TER (solid arrow). The CS contains dark staining granules with an electron lucent centre (small arrow), x38,300.
Figure 16.	A section from a more mature ZS. Note the occurrence of granule-containing CS and the absence of TER, x19,000.


TER
14


CS
16


TER
CS
15

evidence suggesting such macrotubules were produced individually, and then aggregated into bundles of up to thirty tubules in the sporangia of B.emersonii. They also claimed that such bundles became associated with the filled cisternae. Lessie and Lovett (1968) also noted the occurrence of similar bundles in the log phase sporangium of B.emersonii but did not discuss either their formation or function.

The apparent instability of these macrotubules as evinced by their transient state was reminiscent of microtubules. A class of microtubules referred to as macrotubules does exist (Dustin 1978). The macrotubules are distinguished from the former on the basis of a larger diameter (31-52 nm) and the arrangement of the tubulin sub-units. However, it will be necessary to determine the composition of the "macrotubules" in C.anguillulae before any conclusions can be drawn.

Other tubular arrangements were observed in the developing zoo-sporangia of C.anguillulae which were only found in the rapidly growing young sporangia. Such tubules generally occurred in clusters (Figs. 17, 18) and had a centre distance of approximately 45 nm. Each of the tubules was generally in direct contact with, and surrounded by, six other tubules (Fig. 17). While the three dimensional arrangement of their structure was not determined, Figure 17 suggests that the structure had a highly convoluted configuration, possibly with a core of vesicles (Fig. 17). These structures also appeared to be either directly or closely associated with the cell wall (Figs. 17, 19, 20), and were often located in regions of growth such as in the developing basal rhizoids (Figs. 19, 20). It is worth repeating that these structures

LEGEND

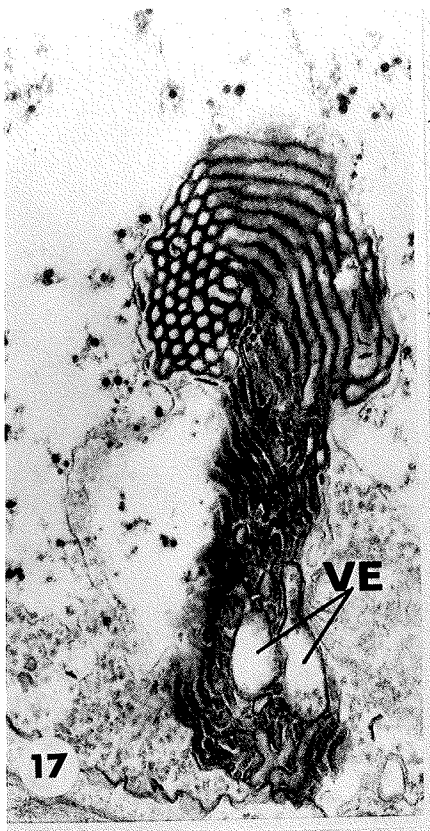
Figure 17-20. TEM micrographs of tubular bundles in the late stages of development in the zoosporangia.

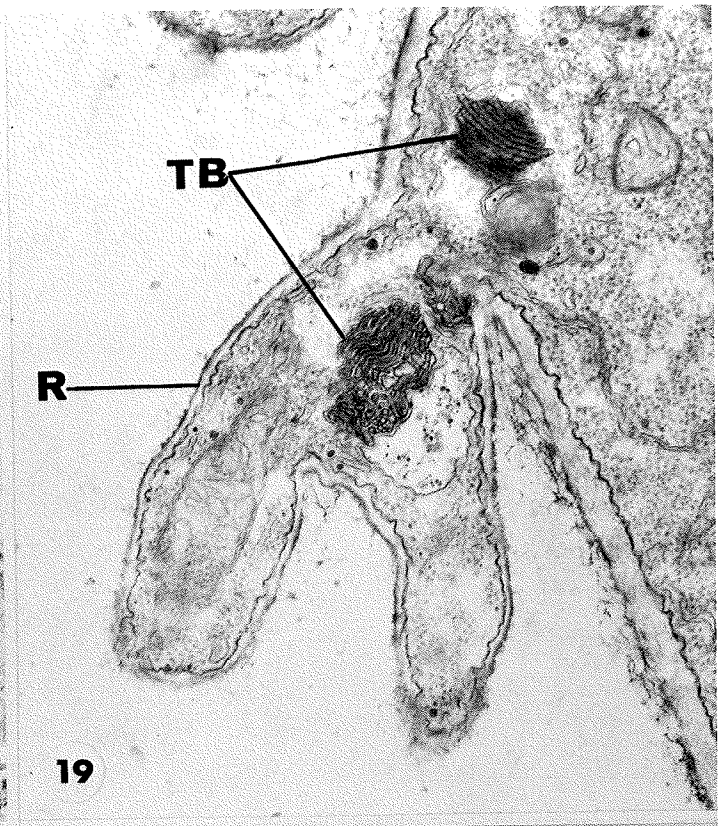
Figure 17. TB apparently in contact with the plasmalemma. Note the orderly appearance of tubules, which are in direct contact with one another and occurrence of VE in matrix of TB, x56,250.

Figure 18. Oblique section of TB confirming the orderly arrangement of tubules and the occurrence of VE, x80,000.

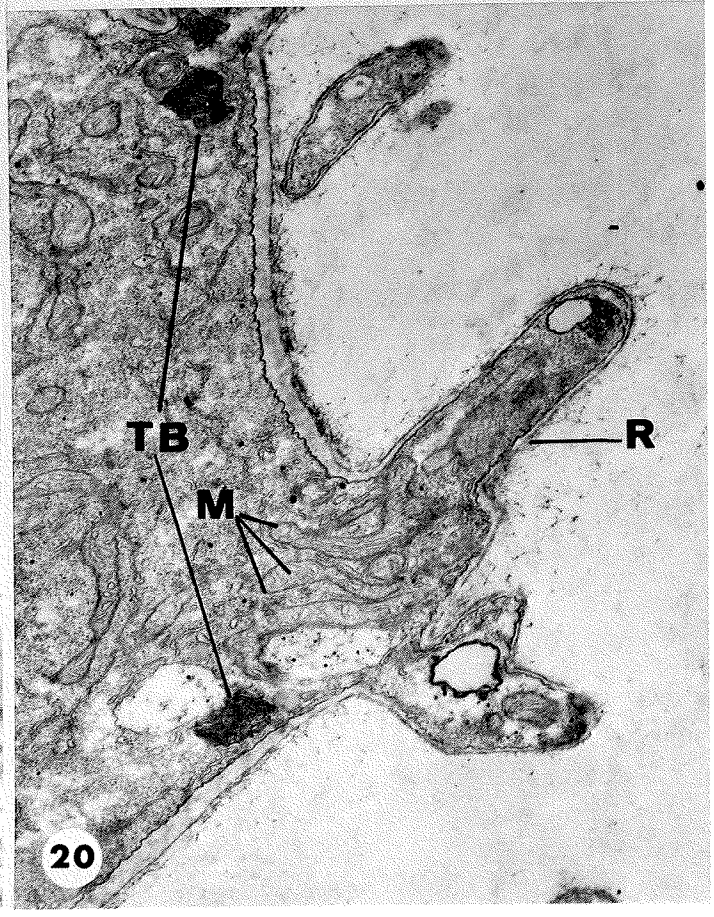
Figure 19. Association of TB with developing R, x36,500.

Figure 20. TB and elongate M associated with developing R. Note the proximity of TB with the cell wall, x19,000.


VE
17


TB
R
19


VE
18


TB
R
M
20

were not observed either in the mature zoosporangia or in the very young developing sporangia but appeared to be specific to the later stages of development in the enlarging zoosporangia.

There were only a few reports in the literature of bodies that seem to bear any resemblance to these tubular configurations. One such report concerned the occurrence of mesosomes in the bacterium Bacillus subtilis as noted by Van Iterson and Leene (1964). Mesosomes are membranous organelles located at the periphery of the cytoplasm and positioned along developing cross walls in the bacterium. The aforementioned authors believed that mesosomes may be involved in either the genesis of new flagella, or in the formation of new walls. Chambers et al. (1967) also reported on the occurrence of a tubular structure in the zoospore of Nowakowskiella profusa Karling, consisting of a series of cylindrical tubular elements, each bounded at one end and on the sides by a clearly defined electron-dense margin; also each of the tubules was in contact with six neighbouring ones with a centre to centre distance of ca 45 nm. This structure, however, did not have the same irregular elongated, twisted configuration as the tubular bundle observed in C.anguillulae, and instead had a compact honeycomb appearance.

Whole mounts of the fungus showed that the swellings that develop, on the hyphae, that will mature into zoosporangia, were somewhat elongate and possessed well-developed rhizoids early in their development. Each zoosporangium was separated by a hyphal isthmus throughout which, initially at least, the protoplasm was continuous with the zoosporangium. Early in the development of the zoosporangium a septum was

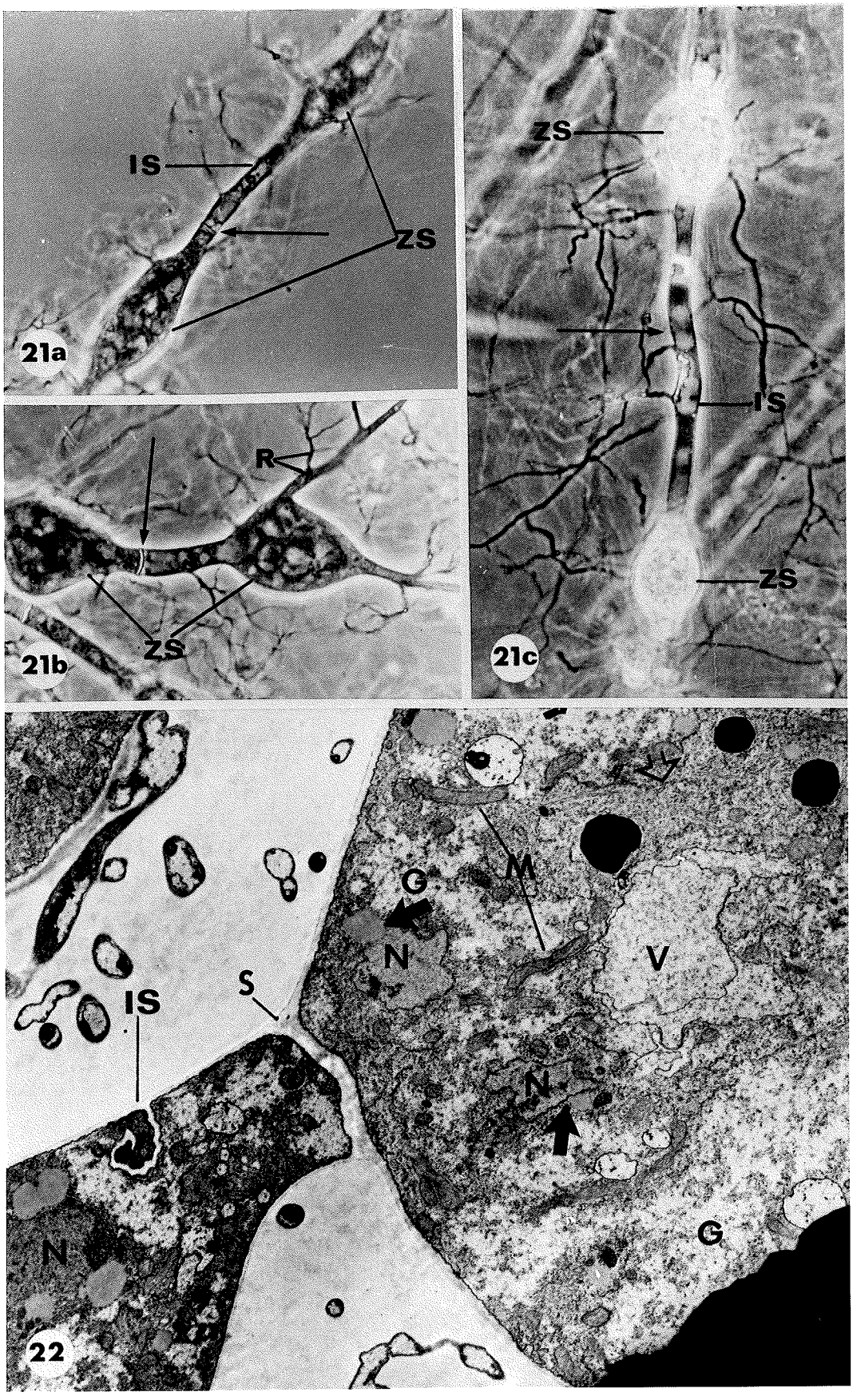
produced in the isthmus a short distance from the more mature developing zoosporangium (Fig. 21a, b, arrow). Shortly after this septum forms, a second cross wall developed at the end of the isthmus distal to the first septum but, while this was forming, there was a migration of the protoplasm out of the isthmus leaving a vacuolate hyphal strand between the two septa (Fig. 21c, arrow). Sometimes three cross walls were produced per isthmus, with the number of septa appearing to be dependent on the length of the isthmus.

It is difficult to determine whether cross wall formation is correlated with a particular stage of development. However, Couch (1945) reported that the hyphal swellings which develop into zoosporangia enlarged before and after the production of septa in the isthmi. However, he also noted that septa were never observed in the growing region of the hyphae.

Figure 22 illustrates the fine structure of the enlarging sporangium and the septate isthmus. It is apparent that the protoplasm was continuous with the distal sporangium. The developing zoosporangium at this stage was multinucleate and all nuclei appeared to be associated with the periphery of the cytoplasm (Figs. 22, 23). The more central regions of the sporangia appeared to contain vacuoles and vesicles of various sizes and what appeared to be extensive regions of glycogen deposits. Elongate mitochondria, as well as lipid bodies with closely associated microbodies were evident (Fig. 23, small arrow). The lipids appeared to take on a more organized appearance, being more closely positioned to the nucleus (Figs. 22, 23, arrows) in a manner similar to that noted in the young zoosporangium of B.emersonii (Lessie and Lovett 1968),

LEGEND

Figure 21a-c.	Phase contrast photomicrographs of septate isthmus separating young zoosporangia.
Figure 21a and 21b.	The first S (arrows) in the IS occurs closest to the more mature ZS, x640.
Figure 21c.	As the ZS mature, the IS separating them becomes highly vacuolate (arrow), x640.
Figure 22.	TEM micrograph of later stages of the log phase zoosporangia after production of septum in the isthmus.
Figure 22.	S is separating ZS from IS, note the association of L and N (solid arrows), elongate M and a region of irregular ER (open arrow) as well as large deposits of G, x8,000.


IS
ZS
21a
R
ZS
21b
ZS
IS
ZS
21c
G
M
N
V
S
IS
N
N
G
N
22

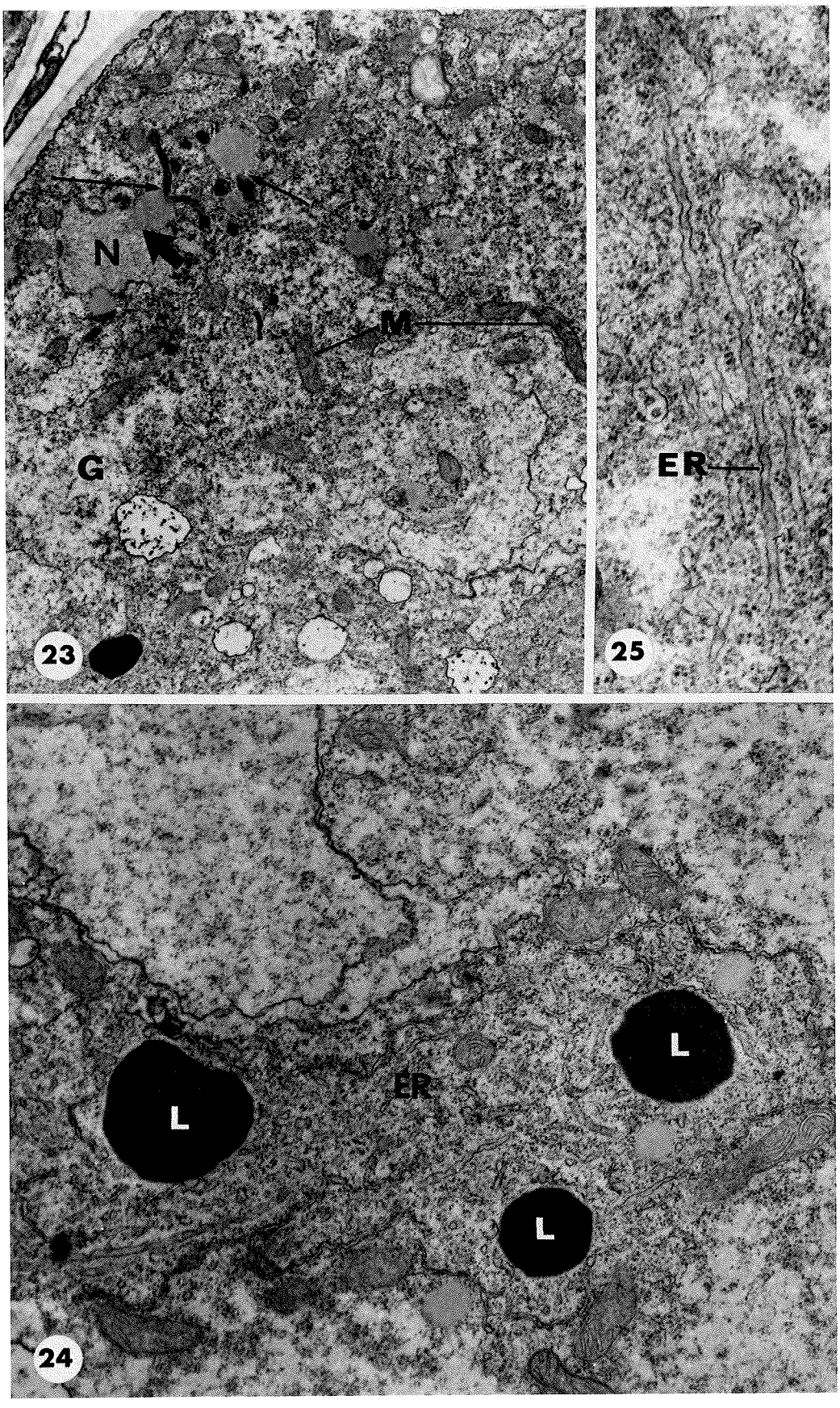
Coelomyces indicus (Madelin and Beckett 1972) and a Phlyctochytrium sp. (McNitt 1974). The sporangium also contained distinct regions of irregularly oriented smooth endoplasmic reticulum (Figs. 22, open arrow; 24), and these regions also appeared to have few ribosomes associated with them. The function of these regions of smooth endoplasmic reticulum was not clear. However, as it is commonly accepted that smooth endoplasmic reticulum in animal cells is responsible for lipid synthesis, it is possible that it performed the same function in C.anguillulae; particularly as the number of lipid bodies increased markedly as the zoosporangium matured. The appearance of dark staining lipid bodies surrounded by the zones of smooth endoplasmic reticulum was also noted (Fig. 24). These regions resembled the endoplasmic reticulum found in farina glands of plants thought to be involved in the production of terpenoid substances (Gunning and Steer 1975) except that those in C.anguillulae appeared to be concentrated in this ribosome-poor region.

Regions of the cytoplasm containing smooth endoplasmic reticulum with a more laminate appearance were also apparent (Fig. 25). In contrast to the other form of smooth endoplasmic reticulum (Fig. 24), these laminate regions appeared to be rich in ribosomes. However, the ribosomes were free in the cytoplasm and not directly associated with the endoplasmic reticulum. This condition was typical of cells not exporting protein, but only producing it for intracellular use (Gunning and Steer 1975).

It was interesting to note that the endoplasmic reticulum was found in discrete regions of the precleavage sporangium, whereas in less developed stages the endoplasmic reticulum seemed to wind through the

LEGEND

Figure 23.	TEM micrograph displaying cytoplasmic detail in a zoosporangium prior to the final nuclear division.
Figure 23.	ZS shows evidence of L closely associated with N (solid arrow) and a number of MB, oval and elongate, in close proximity to L (small arrows). G appears to be the prevalent storage product at this stage, x10,000.
Figures 24 and 25.	TEM micrographs of ER.
Figure 24.	Enlargement of region of smooth irregular ER as depicted in Figure 22. Note the lack of ribosomes and the presence of L in this region, x19,000.
Figure 25.	Laminated arrangement of E.R., typical of ZS, x40,000.


N
M
G
23
ER
25
L
ER
L
L
24

cytoplasm in a much less organized fashion. Heath (1976) reported that most of the endoplasmic reticulum of the zoosporangic fungi occurred in fenestrated sheets that were frequently connected to the nuclear envelope. He also reported that ribosomes adorned the surface of the endoplasmic reticulum except when adjacent to Golgi bodies. Whether an analagous arrangement exists in the Chytridiomycetes such as C.anguillulae, which also lack a typical Golgi, depends on whether the vesiculated zone of ribosome exclusion is equivalent to the Golgi apparatus. It was also not clear from this study as to whether the zones of smooth endoplasmic reticulum were always associated with these vesiculated regions in C.anguillulae. Bracker (1968) in his study of sporangial development in Gilbertella persicaria (Eddy) Hesseltine, indicated that there was a "change in configuration of endoplasmic reticulum from continuous sheets to fenestrated and branching forms, so that a single section intercepts the membranes as discontinuous profiles". This is a more than adequate description of the changes that occurred in the precleavage sporangium of C.anguillulae.

Concomitant with the expansion of the swellings into mature zoosporangia, there appeared to be an increase in the number, size, and complexity of the lomasomes. Lomasomes are accumulations of membranes between the plasmalemma and the cell wall. They may be derived from the passage of vesicles or multivesicular bodies through the plasmalemma, or by the proliferation or budding off of vesicular structures from the plasmalemma. Plasmalemmasome is a term often used to refer to those membranous configurations that are external to the plasmalemma but are located in a pocket thereof which projects into the cytoplasm. Lomasomes

and plasmalemmasomes have only been reported in regions of active wall synthesis (Heath and Greenwood 1970).

Marchant and Robarts (1968) discussed the occurrence of paramural bodies (a term referring to both plasmalemmasomes and lomasomes) in higher and lower fungi. They believed that paramural bodies function in cell wall synthesis since such structures are always associated with actively developing cell walls. However, Heath and Greenwood (1970) concluded that plasmalemmasomes occur whenever more plasma membrane is produced than is needed to line the cell wall; this appears to be the prevailing belief. Heath and Greenwood also suggested that lomasomes are produced whenever plasmalemmasomes become sequestered in the developing wall.

A variety of lomasomes and plasmalemmasomes were found in the later developmental stages of maturing sporangia of C.anguillulae, although the tubular bodies observed in the germling (Fig. 2d) may be a form of plasmalemmasome. Figures 26-28 illustrate typical examples of the plasmalemmasomes observed in the latter stages of the developing and mature zoosporangia, while Figure 29 is typical of the lomasomes observed. Some of these appeared to be composed of single and possibly double unit membrane in a coiled configuration (Fig. 27). Other forms appeared to be more vesicular (Fig. 26) or tubular (Fig. 28). While there was certainly a similarity between the tubular plasmalemmasomes (Fig. 28) and the tubular bodies seen earlier (Figs. 17-20), the plasmalemmasome had a much less organized appearance with loosely packed tubules that were not directly associated with each other.

The use of ruthenium red tends to provide better definition to the

LEGEND

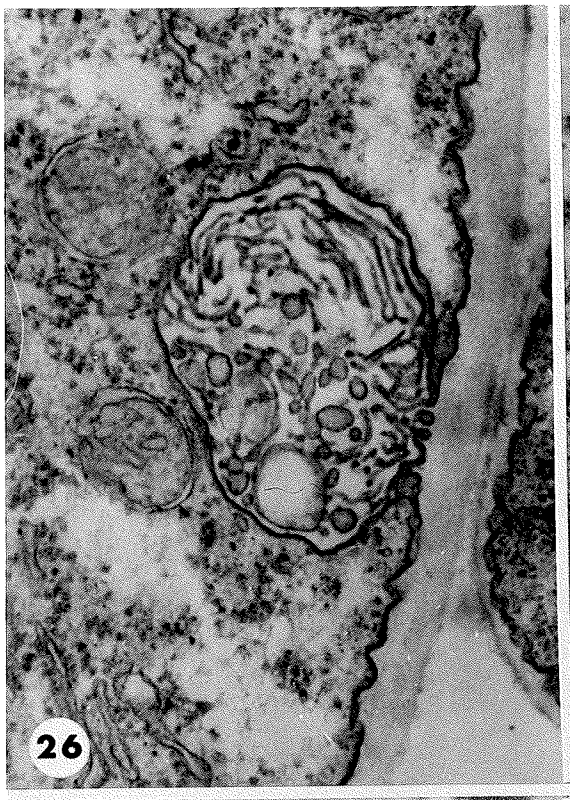
Figures 26-29. TEM micrographs of plasmalemmasomes and lomasomes in the developing zoosporangium.

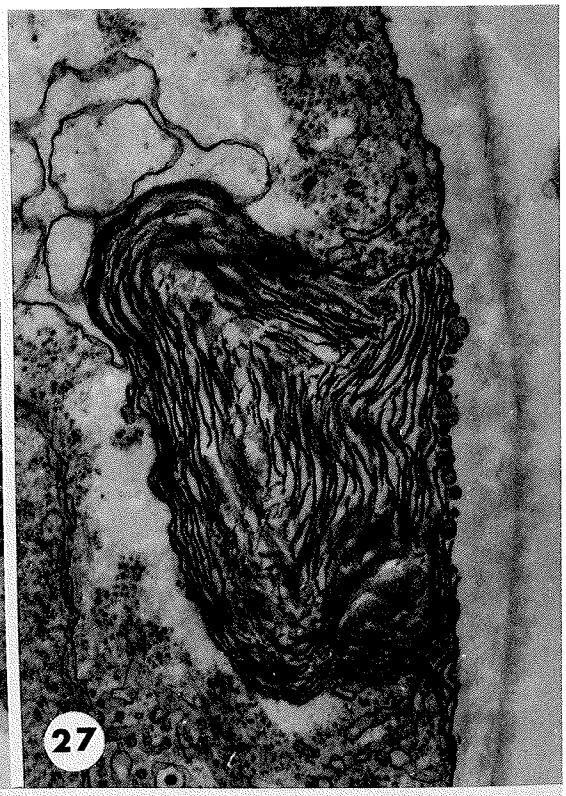
Figure 26. Vesiculate appearing plasmalemmasome, x59,500.

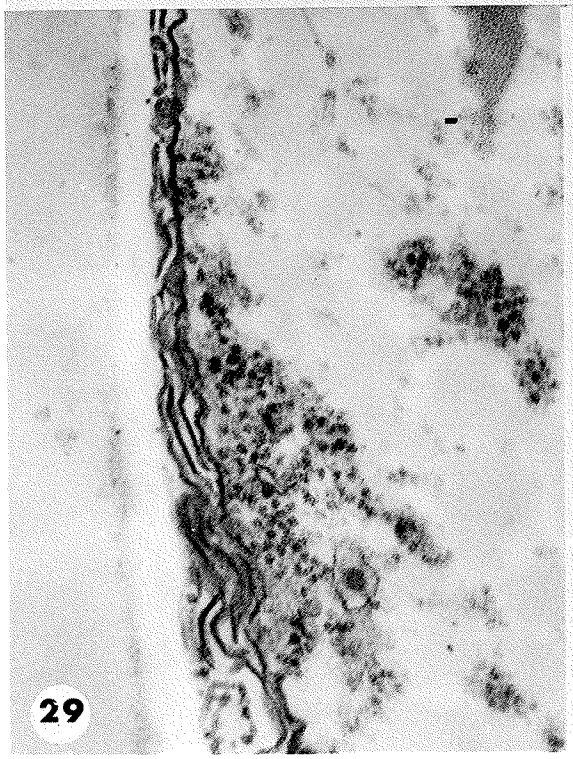
Figure 27. Plasmalemmasome with a laminated configuration due to the occurrence of layers of double membrane, x36,000.

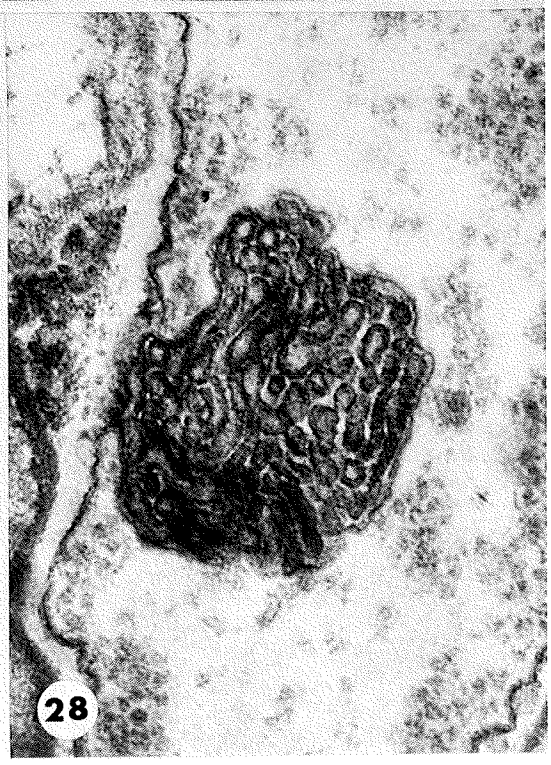
Figure 28. Plasmalemmasome with a pronounced tubular appearance, x107,500.

Figure 29. Illustration of a lomasome. Note that the double membrane composing the lomasome is exterior to the plasmalemma and is not invaginated into the cytoplasm, x86,000.


26


27


29


28

plasmalemmasomes; Figures 26 and 27 provide an illustration of this fact. Figure 27 is a section from a zoosporangium that was fixed using ruthenium red in the osmium tetroxide postfixative, while Figure 26 is a section taken from a sample in which ruthenium red was not used. The thickened dark staining walls surrounding the membrane of the plasmalemmasomes was not observed when ruthenium red was not used. Considering the nature of the stain this may be indicative of a polysaccharide or mucopolysaccharide coating (Luft 1971).

With the enlargement of the zoosporangia, a well-developed rhizoidal system was observed. Rhizoids were established early in the life cycle of C.anguillulae, with the germ tube functioning as a rhizoid as it comes into contact with the substrate, as was apparent with B.emersonii (Lessie and Lovett 1968) and a Phlyctochytrium sp. (Porter and Smiley 1980).

Rhizoids generally function to anchor the thallus to the substrate and also provide a means of increasing surface area for the uptake of nutrients. The rhizoids of C.anguillulae generally arose singly from the thallus but often became branched, and tended to be fairly uniform in diameter (1.2-1.5 um diam.). Typically, rhizoids are anucleate, though they do contain a number of other cytoplasmic organelles. Occasionally, elongate nuclei were observed in rhizoids originating from the zoosporangium (Fig. 30, solid arrow); the fate of such nuclei is uncertain.

The mitochondria associated with the rhizoids appeared abnormally long (Fig. 30, small arrow) and this was typical of the situation found in some other zoosporic fungi (Lessie and Lovett 1968). Elongate mitochondria were also present in the zoosporangial cytoplasm adjacent to

the developing rhizoids (Fig. 20). These mitochondria also appeared to be oriented parallel to the long axis of the rhizoids, although such orientation seemed to be typical only of developing rhizoids and not those that were nearly mature.

Microtubules were also present in the rhizoids, and like the mitochondria appeared to be oriented parallel to the cell wall of the rhizoid. This is similar to the findings of Barstow and Lovett (1974b), who also reported that rhizoidal microtubules were so oriented and often extended into the growing tip of B.emersonii. These authors also reported that the microtubules present in the rhizoids ran parallel to the mitochondria and a similar arrangement was observed in C.anguillulae (Fig. 31). Microtubules have also been reported as being responsible for the migration of mitochondria (Heath and Heath 1978) as well as nuclei (Raudaskoski 1972). Similarly Taylor and Fuller (1980) felt that rhizoidal microtubules might be responsible for the transport of nutrients and organelles from the rhizoid into the sporangia of Chytridium confervae. They believed that since the rhizoids were the only portion of the thallus in intimate contact with the substrate, such a transport mechanism would be valuable.

As the zoosporangium reaches maturity cross walls develop at the junction of the zoosporangium and the rhizoids. The formation of such septa involved two processes which appeared to occur simultaneously: 1) protoplasm migrated from the rhizoids into the sporangium and 2) the septum developed.

The actual protoplasmic migration out of the rhizoids is difficult to document. However, Lessie and Lovett (1968) observed an increase in

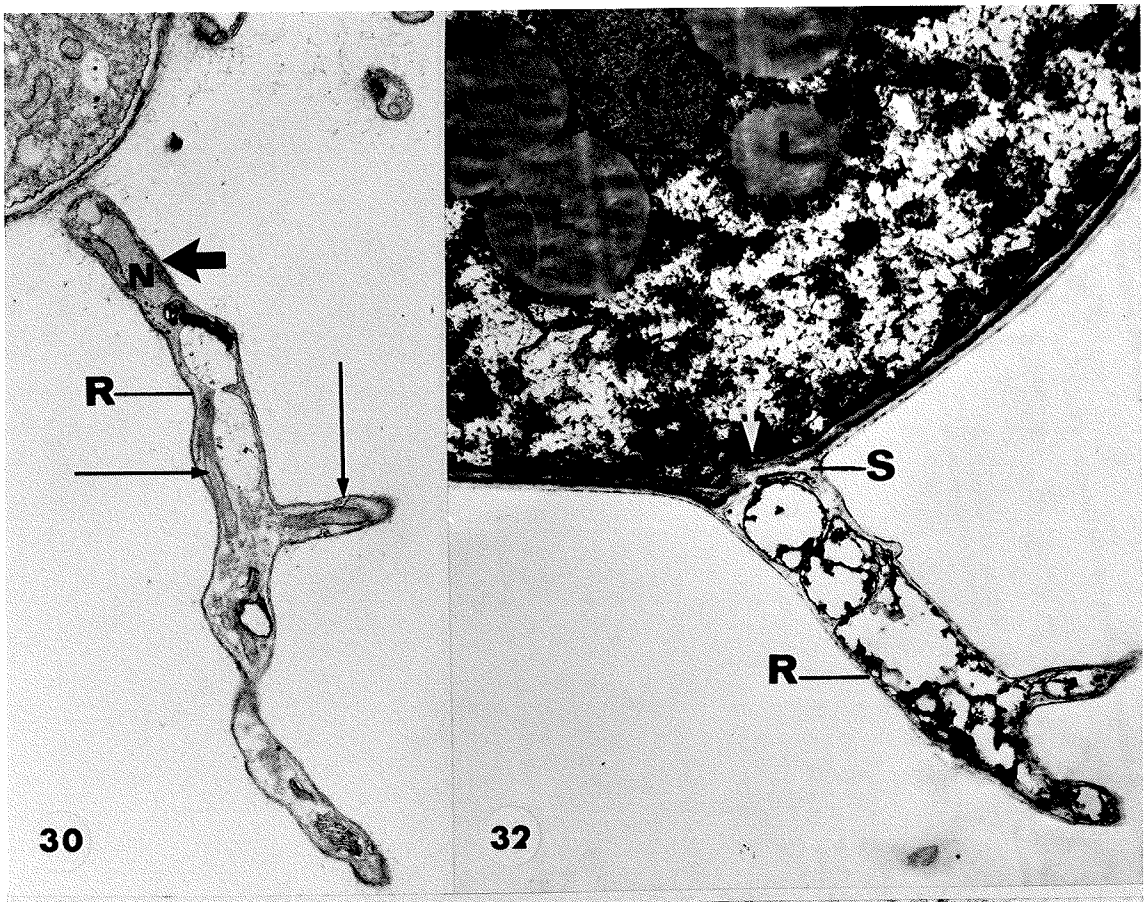
the density of ribosomes and other cytoplasmic material at the region of rhizoidal attachment during cross wall formation in B.emersonii. The rhizoidal septum in C.anguillulae developed inwardly, in a progressive manner, from the cell wall (Fig. 33, arrow) while there was still protoplasmic continuity between the sporangium and the rhizoid. After the septum was mature, the rhizoid was extensively vacuolate (Fig. 32) and the septum appeared to be an extension of the cell wall, and the plasmalemma in the zoosporangium appeared to have developed in association with the septum (Fig. 32, arrow).

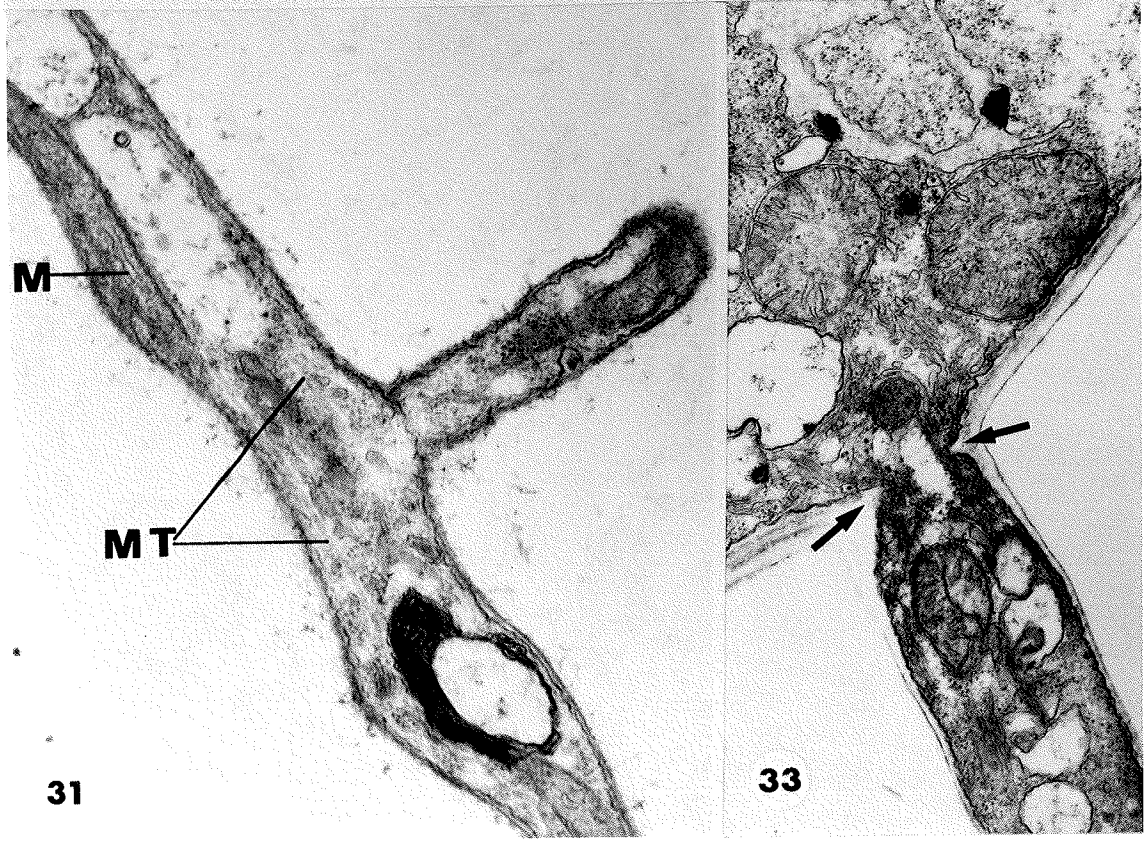
The true nature of the process of septal formation was not clarified by this study; however, other authors have noted organelles in association with the septum that may be responsible for its formation. Organelles found in association with the septa include multivesicular bodies noted in Allomyces macrogynus, and microbodies and vesicles in Rhizopus sexualis (Smith) Callen (Hawker and Beckett 1971).

At the time septa developed in C.anguillulae, cytoplasmic differentiation seemed to be occurring in the zoosporangium, and it appeared that the process of septum formation in the rhizoids was triggered by the initiation of zoospore production. Sparrow (1960) recognized this change in relationship between the sporangium and rhizoids during early stages of reproduction in the zoosporic fungi. As Sparrow (1960) stated "when extension of the rhizoids ceases the residue of material within them is soon drained into the future reproductive body, which is then delimited by septa from the now empty vegetative systems". This production of cross walls prior to zoosporogenesis is also in evidence in other fungi such as Saprolegnia ferax (Gay and Greenwood 1966) and appears

LEGEND

Figures 30-33.	TEM micrographs of zoosporangial rhizoids.
Figure 30.	Longitudinal section of an R, showing evidence of N migrating from ZS (solid arrow), also the occurrence of elongate M oriented parallel to the cell wall (small arrows), x13,500.
Figure 31.	Enlargement of a portion of Fig. 30, depicting MT oriented parallel to the cell wall and M, x36,000.
Figure 32.	Vacuolate R separated from mature ZS by an imperforate S. Note that the ZS plasmalemma is continuous across the S (white arrow), x11,500.
Figure 33.	Longitudinal section through the developing R septum, which is produced by an inward growth of the cell wall (arrows), x22,500.


N
R
30
S
R
32


M
M T
31
33

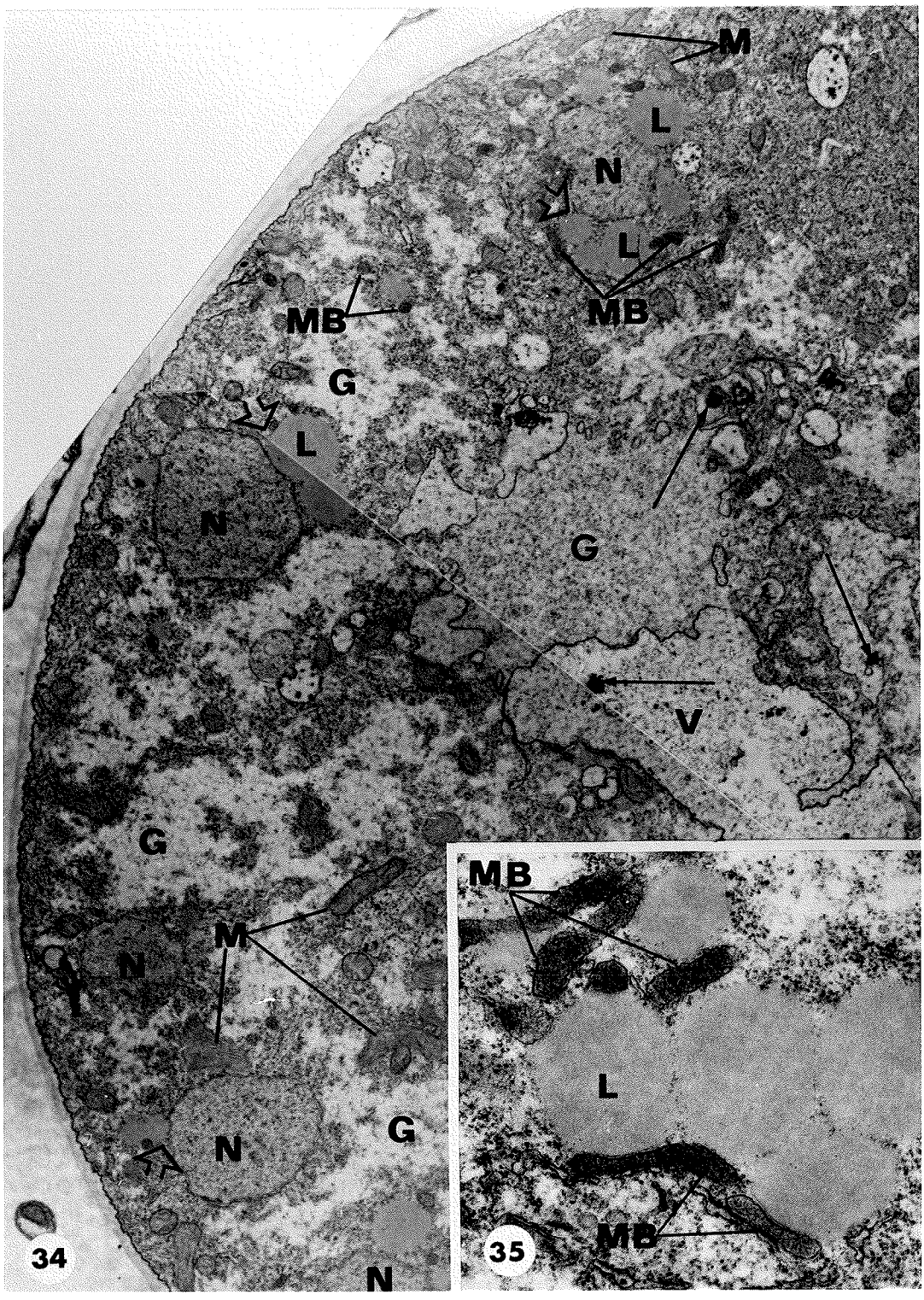
to be a general phenomenon in the lower fungi.

Under optimum conditions the zoosporangium reached its full size within forty eight hours in agar culture, prior to formation of the discharge tube the zoosporangia were pyriform to spherical in shape, the size being related to the degree of crowding; however, a maximum diameter of ca 175 um is often reached. The sporangium had a double layered wall, 150-190 nm in thickness. The discharge tube and accompanying papilla, however, were not produced until nuclear division had ceased.

Figure 34 illustrates the fine structure of a zoosporangium prior to the final series of nuclear divisions. The nuclei were located at the periphery of the cytoplasm and appeared to be associated with large lipid bodies (open arrows). Microbodies were also closely associated with the lipid bodies and a large number of oval and sausage-shaped mitochondria were present in the cytoplasm, although these did not appear to be specifically associated with the nuclei. Figure 34 may well illustrate the stage of development at which the microbody lipid complex was becoming reassociated (Fig. 35); there is no indication that this reassociation occurred in a synchronous manner, nor were mitochondria involved in the complex. It was not possible, without three dimensional reconstruction, to determine whether the microbodies associated with the lipids (Fig. 35) were individual microbodies, or merely portions of a larger single, lobed, microbody, as described by Powell (1979). However, the microbodies at this point appeared somewhat longer than those seen in younger sporangia, at least giving the impression of elongation. If this was the case then this elongation could be the

LEGEND

Figures 34 and 35.	Detail of a zoosporangium prior to the final nuclear division.
Figure 34.	ZS with a number of N at the periphery of the cytoplasm, N with associated L (open arrows), MB present only in association with L. Large deposits of G are observed as are a number of large M. Small membrane-bound dark staining bodies (small arrows) are present, x11,000.
Figure 35.	Association of elongate MB with a number of L, indicative of the re-establishment of the microbody lipid complex, x32,500.


M
L
N
L
MB
MB
G
L
N
G
V
G
M
N
G
N
N
34
MB
L
MB
35

prelude to the formation of the symphomicrobody.

In C.anguillulae, zoosporangia contained regions of heavy ribosome concentration which appeared to be interspersed with extensive deposits of glycogen (Fig. 34). This is in contrast to the situation found in the sporangium of Allomyces macrogynus (Barron and Hill 1974) where in the precleavage sporangia there was an even distribution of ribosomes. The situation in C.anguillulae was somewhat similar to that in Phlyctochytrium arcticum (Chong and Barr 1973) and Olpidium brassicae (Wor.) Dang. (Temmink and Campbell 1968); in both these species glycogen deposits were located in the cytoplasm of the zoosporangia, but these tended to disappear as cleavage occurred.

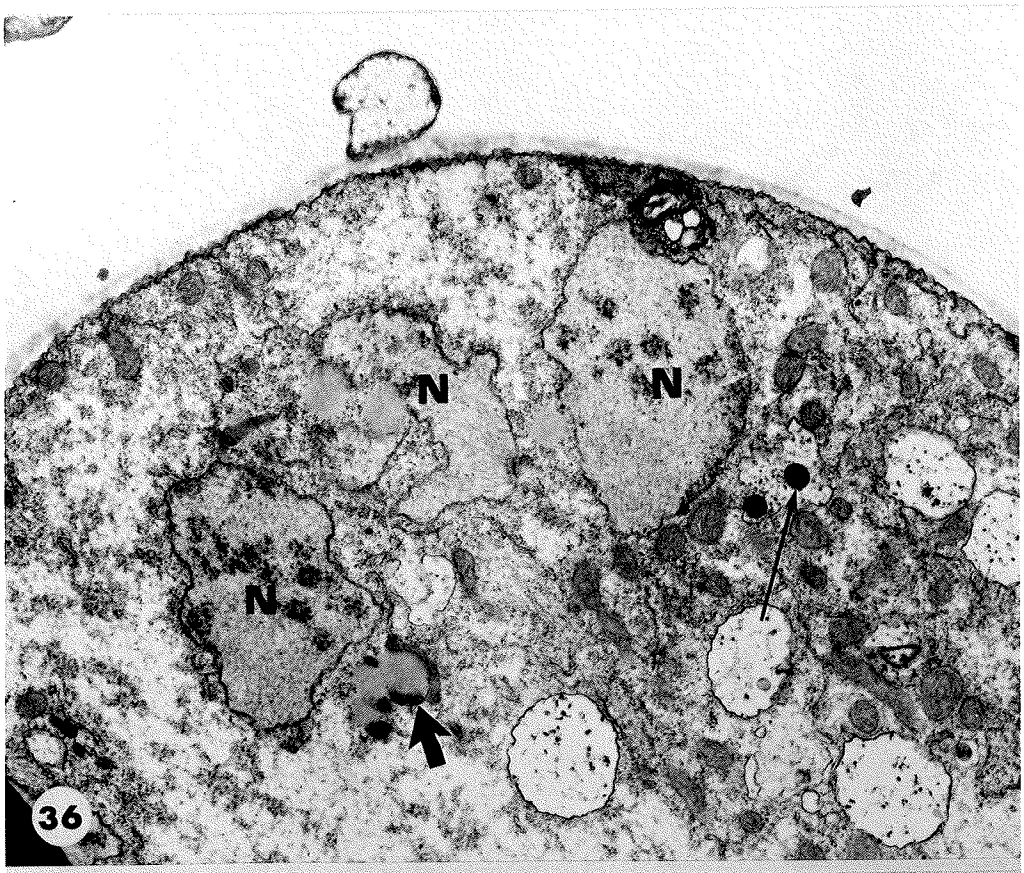
The clumping of the ribosomes which was noted may represent the initial stages of nuclear cap formation. At this point the zoosporangium also contained a number of vacuoles of varying sizes. Many of these contained small dense staining bodies (Fig. 34, 36, arrows) similar to those first observed in the germling and discussed by Gay and Greenwood (1966). A large vacuole was conspicuous in the central region of the zoosporangium (Fig. 34). Lessie and Lovett (1968) noted that the number and size of the vacuoles varied from sporangium to sporangium in the developing thalli of B.emersonii and this should also be expected of C.anguillulae. The occurrence of such large vacuoles also indicated that the sporangium had not yet reached maturity; large vacuoles were not conspicuous in older sporangia.

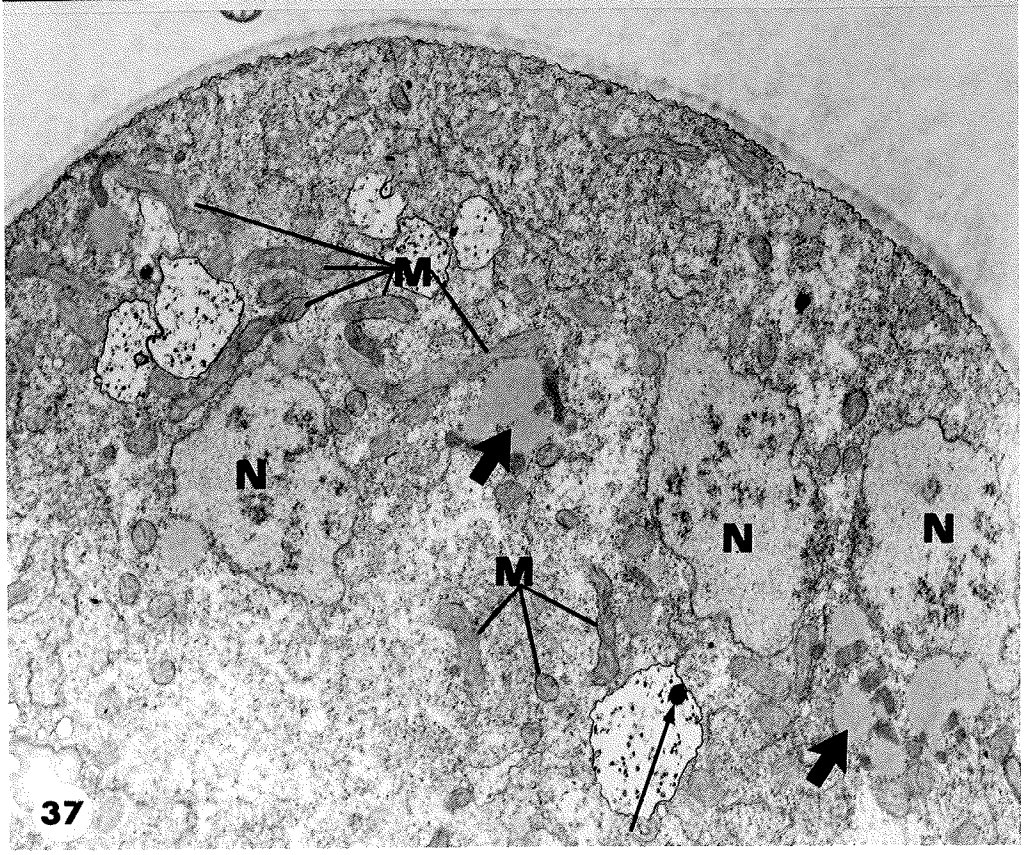
Nuclear division was very evident at this stage, after the final mitotic division the sporangia will enter the late precleavage stage; a term proposed by Madelin and Beckett (1972). These authors divided

LEGEND

Figures 36 and 37. TEM micrographs depicting synchronous nuclear division in the zoosporangium.

Figures 36 and 37. M remain associated with L during nuclear division (solid arrows). Note also the occurrence of dark staining membrane-bound bodies (small arrows, Fig. 36) and a number of elongate M in the cytoplasm (Fig. 37), x10,500.


N
N
N
36


M
N
M
N
N
37

the later stages of the life cycle of Coelomyces indicus, a member of the Blastocladiales, into four stages: "1) early precleavage - nuclei in stages of division; vacuoles present; 2) late precleavage - nuclei no longer dividing, lipid droplets in clusters associated with electron-dense sacs, small vacuoles present; 3) early postcleavage - sporangial protoplast cleft into immature planonts with ribosomes dispersed throughout the cytoplasm; 4) late postcleavage - ribosomes in planonts chiefly in membrane-bounded nuclear caps. Figures 36 and 37 illustrate the synchronous division of the nuclei of the early precleavage zoo-sporangium, here in early anaphase. It appears that the microbody-lipid association remained throughout nuclear division (Figs. 36, 37, solid arrows), and a large number of mitochondria appeared to be in close proximity to the dividing nuclei. In some sections the occurrence of more elongate mitochondria was noted (Fig. 37) and these may be analagous to the large mitochondria found in zoospores by Chong and Barr (1974).

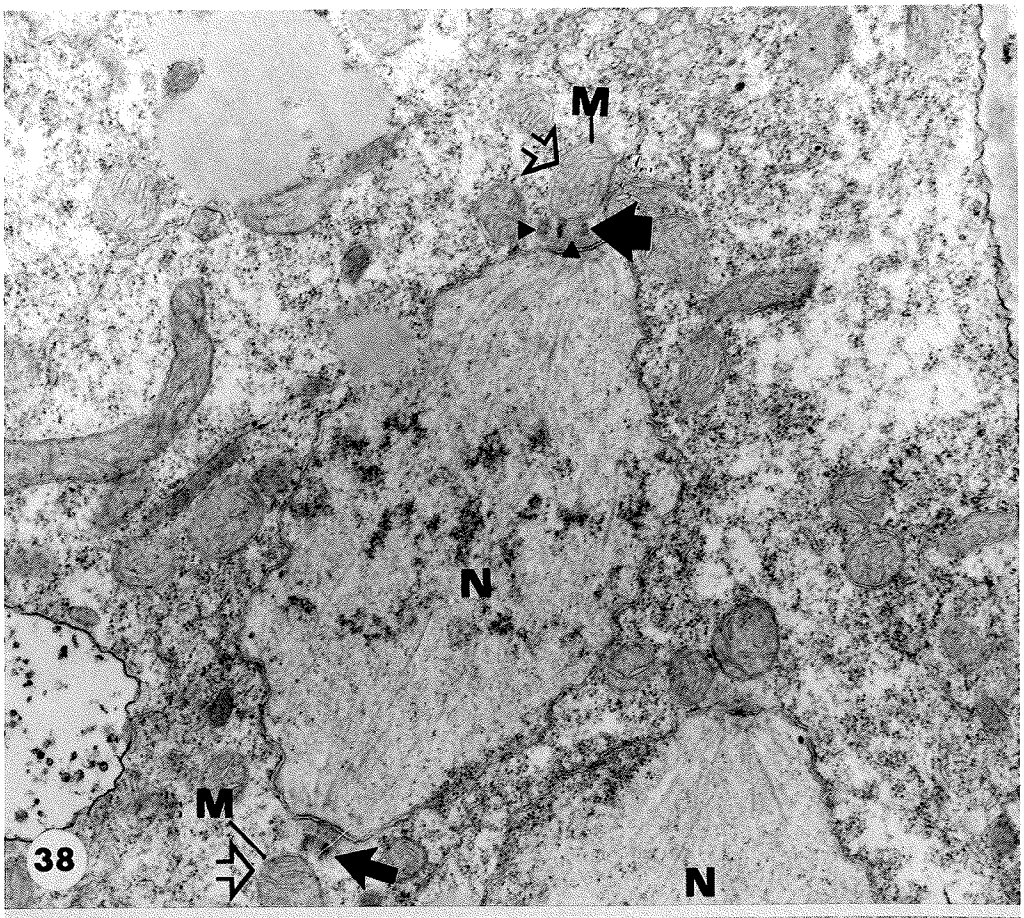
Cultures of C.anguillulae grown on PYG agar underwent only mitotic divisions. These divisions occurred within an intact membrane; this being "a unifying feature of all Phycomycetous mitoses" (Heath 1976). The centrioles were paired at each pole and oriented at right angles to each other (Fig. 38, solid arrows). It is unclear as to whether replication of centrioles occurred at the poles or during migration of the centrioles to the poles. Ichida and Fuller (1968) indicated that the centrioles divide sometime between the time when they become polarized and metaphase. In this study paired centrioles were never observed prior to the production of spindles (Fig. 39, solid arrows). The centrioles were exterior to the nuclear membrane and while never

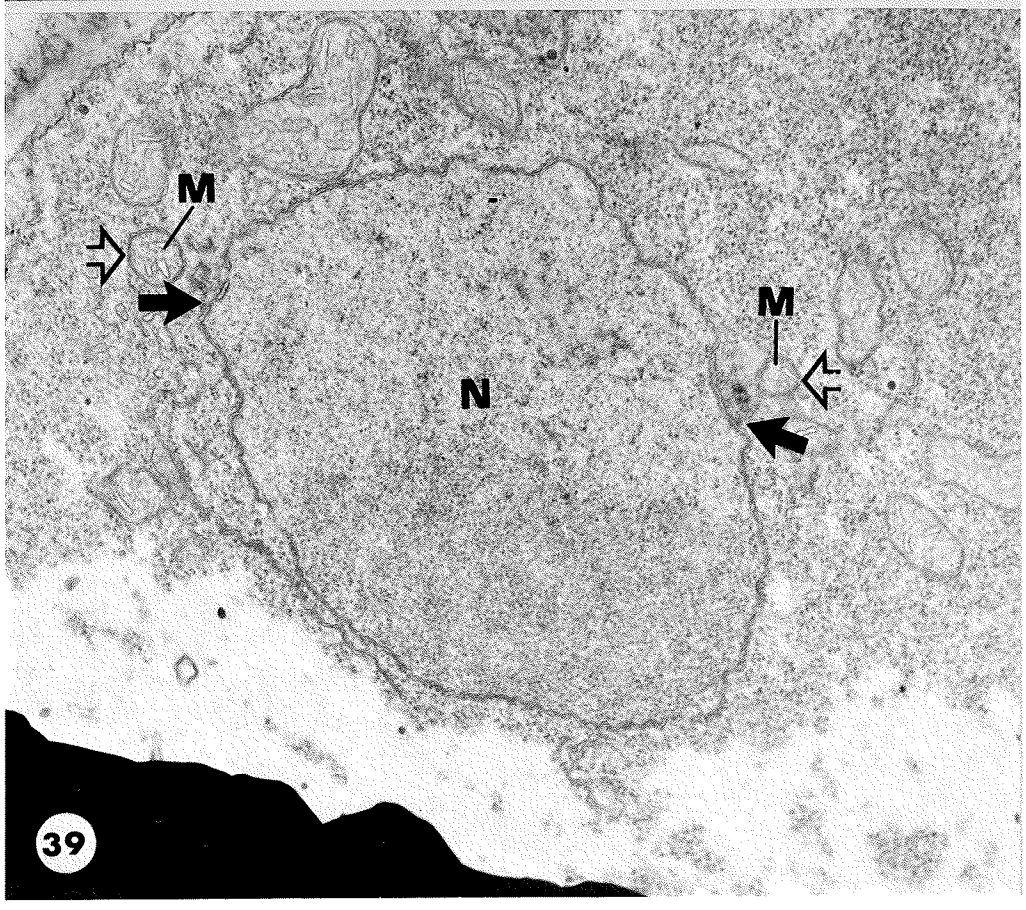
LEGEND

Figures 38 and 39. TEM micrographs depicting stages of mitotic division.

Figure 38. Dividing N (early anaphase) displays polarized centrioles (solid arrows), the centrioles being doubled (arrowheads) and oriented at right angles to one another. Note the presence of M capping the centrioles (open arrows), x18,000.

Figure 39. N (early prophase) showing evidence of M caps (open arrows). Paired centrioles are not observed prior to spindle formation (solid arrow), x29,000.


M
N
M
N
38


M
M
N
39

appearing directly attached were always associated with a pocket in the nuclear membrane. This association within a differential pocket of the nuclear membrane is typical for all species of the Chytridiomycetes which have been studied (Heath 1976) as well as some Oomycetes (Heath and Greenwood 1968).

The centrioles were about 250 nm long and 160 nm wide, measurements similar to those reported by Ichida and Fuller (1968) for their isolate of C.anguillulae. The spindle fibres appeared to originate just within the nuclear membrane (Fig. 38; Fig. 40, solid arrow) and seemed to terminate in the chromatin regions (Fig. 40, arrowheads). It appeared that kinetochores were absent in the dividing nucleus; however, true kinetochores have only been conclusively demonstrated in a few fungi (Aist and Williams 1972; Heath 1974). Plates positioned at the end of the spindle fibres which appear to function as kinetochores have been observed in an Entophlyctis sp. (Powell 1975) and a Phlyctochytrium sp. (McNitt 1974). However, there was no evidence of any such structure in C.anguillulae; this, of course, may be the result of inadequate preservation during fixation.

There also appeared to be a close relationship between the centrioles and the mitochondria which appeared to form a cap directly over the large centrioles (Figs. 38, 39, open arrows). This association has been reported in a Saprolegnia sp. (Heath and Greenwood 1968), B.emersonii (Lessie and Lovett 1968), and C.anguillulae (Ichida and Fuller 1968). Powell (1975) observed a single mitochondrion constantly associated with the centriole pair at each end of the interphase nucleus and after migration to opposite poles, each of the centrioles was capped by a

mitochondrion.

While the morphology of the mitotic apparatus of C.anguillulae is similar to that of most of the reported Chytridiomycetes, it appears that the fungus differs from other zoosporic fungi in the process of nuclear division (Ichida and Fuller 1968, Powell 1975). Whereas in most of the Chytridiomycetes chromosomal movement is initiated by a shortening of the chromosomal microtubules, in C.anguillulae the chromosomes appear to be separated as a result of nuclear elongation and separation of the poles, not a shortening of the microtubules (Ichida and Fuller 1968).

During nuclear division, the nucleolus of the fungus was undetectable and, in this respect, C.anguillulae is similar to other members of the lower fungi, with the exception of the Saprolegniales.

According to Madelin and Beckett (1972), the final series of nuclear divisions marks the end of the early prophase, and the zoosporangia that have undergone nuclear division enter the late precleavage stage. A large number of nuclei were observed in the sporangia at this stage in close proximity to one another (Fig. 41). Large deposits of glycogen were evident as well as a large number of lipid bodies. However, it was not possible to determine whether there was an actual increase in any of the other organelles, except the nuclei, in comparison with the early precleavage zoosporangium. In the initial phase of zoospore production the nuclei were still observed at the periphery of the zoosporangium and these were surrounded by lipid bodies (Fig. 42). This arrangement appeared to represent the "lipid crown" described by Blondel and Turian (1960) in the gametangia of an Allomyces sp. Paired centrioles positioned

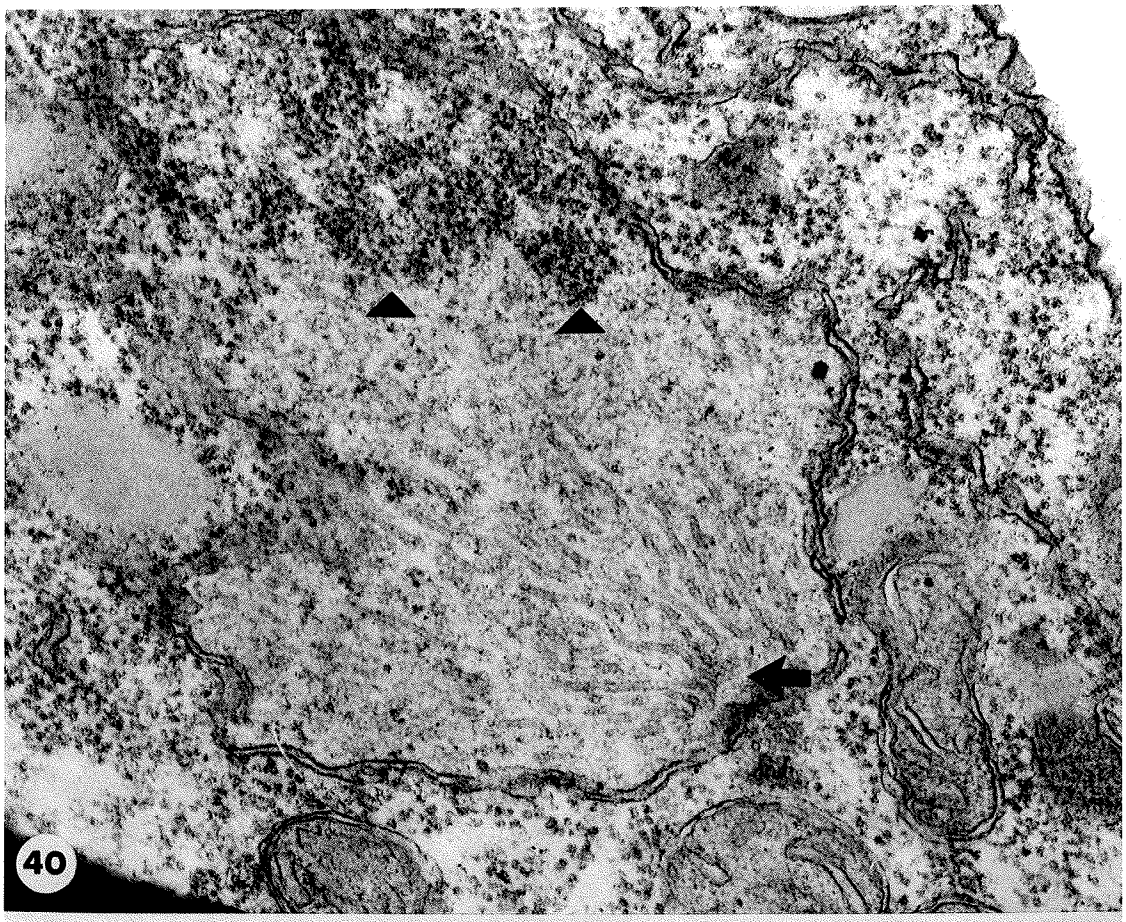
LEGEND

Figure 40. TEM micrographs of a dividing nucleus.

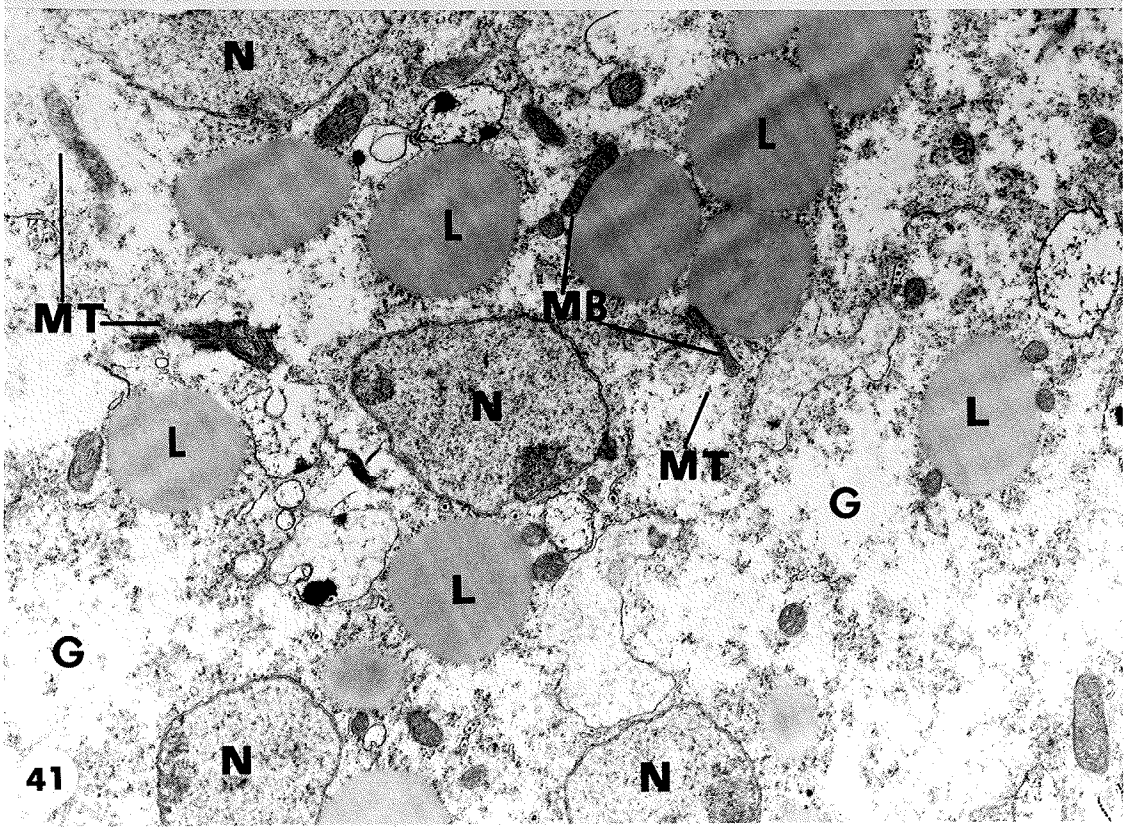
Figure 40. Longitudinal section shows point of origin of the spindle fibres within the nuclear membrane (solid arrow). These fibres appear to terminate in the chromatin regions without evidence of kinetochores or similar structures (arrow heads), x59,000.

Figure 41. TEM micrograph of cytoplasm of mature zoosporangium.

Figure 41. A number of N are present in close proximity to one another, with a number of L and their associated MB. Also note the occurrence of G deposits and cytoplasmic MT, x12,000.



40



41

at right angles to each other, were observed during this stage (Fig. 42, arrows). It is interesting to note the proximity of the nucleolus to the centrioles, at this stage. The nucleolus was at the opposite end of the nucleus; when flagellum production occurred the nucleolus was adjacent to the elongating centriole (Fig. 43).

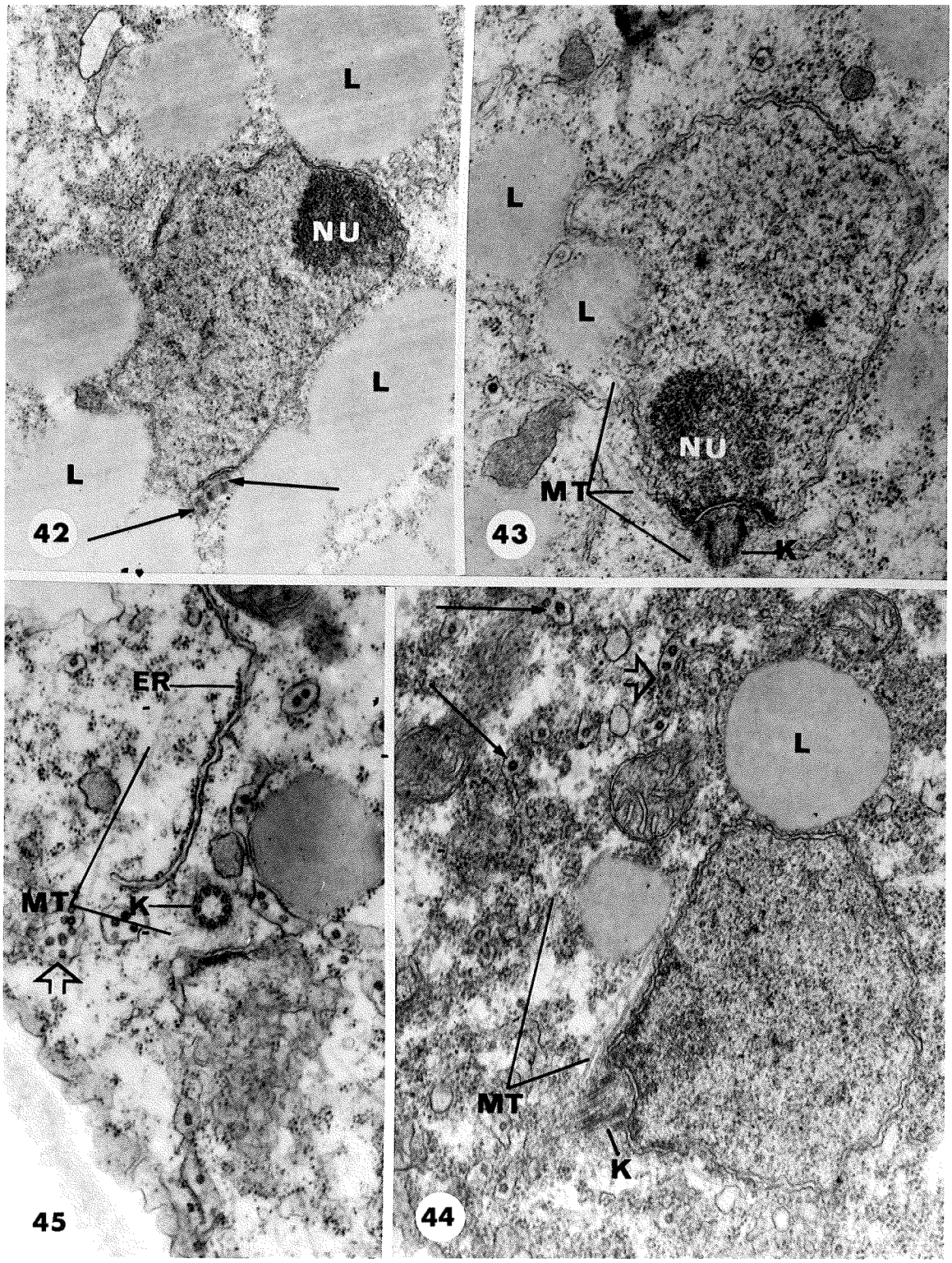
Subsequent to the lipid crown stage, a proliferation of microtubules was also evident flaring out for some distance from the kinetosome (Figs. 43, 44) into the surrounding cytoplasm (Fig. 41). According to Olson et al. (1981) these microtubules are produced by both the functional and non-functional centrioles; however, while this may be the case with C.anguillulae, microtubules were only observed emerging from the functional centriole. The term functional refers to those centrioles which differentiate into flagella. A similar situation has been reported in the Saprolegniales by several workers (Heath and Greenwood 1971; Hoch and Mitchell 1972; Armbruster 1982). Heath (1976) discussed the occurrence of such microtubules in the Saprolegniales but noted that the same situation had not been reported in the uniflagellate zoosporic fungi; Olson et al. (1981) were the first to report the occurrence of centriolar microtubules in the zoosporangia of the Blastocladiales. Heath and Greenwood (1971) suggested that cytoplasmic microtubules possibly were responsible for the control of cytoplasmic cleavage during zoospore production. They believed that the cytoplasmic regions delimited by the microtubules were stable, whereas the cytoplasm outside these regions was weak and therefore cleavage would occur outside of the microtubule delimited zones. Olson et al. presented evidence that suggested that a similar situation existed in the Chytridiomycetes, concluding that

"the cytoplasmic microtubules served to delineate the boundaries of the zoospore initial and in some way establishes cytoplasmic domains through which the flagellum, and or cleavage furrows traverse". Barr (1978) had also noted cytoplasmic microtubules surrounding the nucleus and the nuclear cap of the zoospore of an Allomyces sp. However, as these microtubules form during the last stages of zoospore assembly, Barr believed that the skeletal microtubules differed from those appearing during cytoplasmic cleavage in the Saprolegniales. Microtubules are not peculiar to the early stages of zoosporogenesis and have been observed in the cytoplasm of earlier stages of the life cycle of C.anguillulae, as noted earlier in this study. However, microtubules appeared to occur more frequently at this stage, and seemed to originate at the centriole (Figs. 43, 44). Therefore it was likely that the cytoplasmic microtubules observed in the zoosporangium served in the control of cytoplasmic cleavage.

The 45 nm dark staining granules contained within endoplasmic reticulum cisternae were still present in the late precleavage and early post cleavage zoosporangia (Figs. 44, 50, arrows). These cisternae appeared much as they did in the enlarging sporangia but with the development of the kinetosome from the centriole, the frequency of cisternae which contained more than three granules appeared to increase perhaps due to the fusion of cisternae (Figs. 44, 45, open arrowheads). According to Barstow (1979) these larger cisternae are usually prevalent during early cleavage. He also noted the fusion of these smaller granules to form the gamma particles does not occur until cleavage is complete.

LEGEND

Figures 42-45.	Early stages of zoospore production.
Figure 42.	Lipid crown stage of zoospore initial. Note the paired centrioles (arrows) and polarized position of NU, x16,000.
Figure 43.	Section showing the elongation of a centriole into a K. Note that the NU is now adjacent to K and also the presence of MT arising from K, x36,500.
Figure 44.	MT apparently flaring out from the K. Also note the occurrence of granule-containing CS (small arrows) some containing 6 or more granules (open arrow), x36,500.
Figure 45.	Cross section of a K showing typical arrangement of nine doublet tubules. Note the associated ER and large CS containing a number of dark staining granules (open arrow), x37,000.


L
NU
L
L
42
L
L
NU
MT
K
43
ER
MT
K
45
L
MT
K
44

According to Olson et al. (1981) the next event following the production of cytoplasmic microtubules is the mobilization of membrane-bound electron-dense inclusions in the cytoplasm. In the Blastocladiales these electron-dense bodies were thought to be responsible for the production of primary cleavage vesicles. However, in this study the only electron dense bodies associated with vesicles were those similar to that shown in Fig. 46 (arrows), and these occurred infrequently at this stage of development and certainly unlike those reported by Olson et al. (1981) or Heath (1976).

Flagellar development in C.anguillulae was initiated prior to cleavage of the cytoplasm. The centriole which was composed of a series of nine triplet tubules elongates into a kinetosome as reported by Olson et al. (1978). The kinetosome, which is merely a name applied to an elongated centriole, elongates and typically develops a terminal plate (Fig. 46, solid arrow) of osmiophilic material termed a basal plate (Renaud and Swift 1964), which upon spore maturation migrated to the surface of the spore where the flagellum emerged (Heath 1976). The flagellum proper develops from the basal plate and was composed of a series of nine doublet tubules; the third member of the triplet terminated at the basal plate (Fig. 48). The elongating flagellum appeared to be engaged by a number of small vesicles (Fig. 47, small arrows) which appeared to fuse and form a large single vesicle (Fig. 48, 50, solid arrow) referred to as the primary flagellum vesicle (Sorokin 1962). The primary vesicle formed a double membrane sheath around the growing flagellum (Fig. 49, arrow). According to Renaud and Swift (1964), in Allomyces arbusculus, the inner membrane of the flagellar vesicle forms the outer sheath while

LEGEND

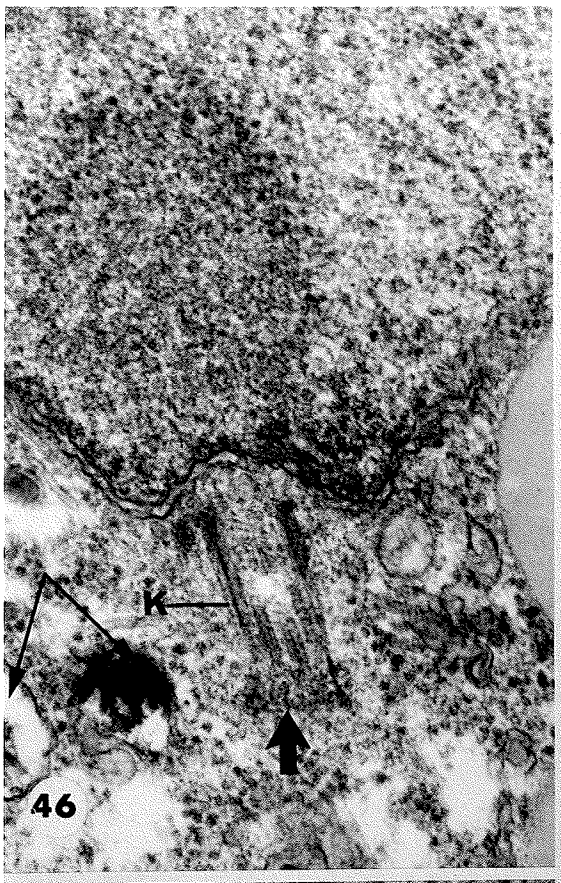
Figures 46-49. TEM micrographs of flagellum formation in C.anguillulae.

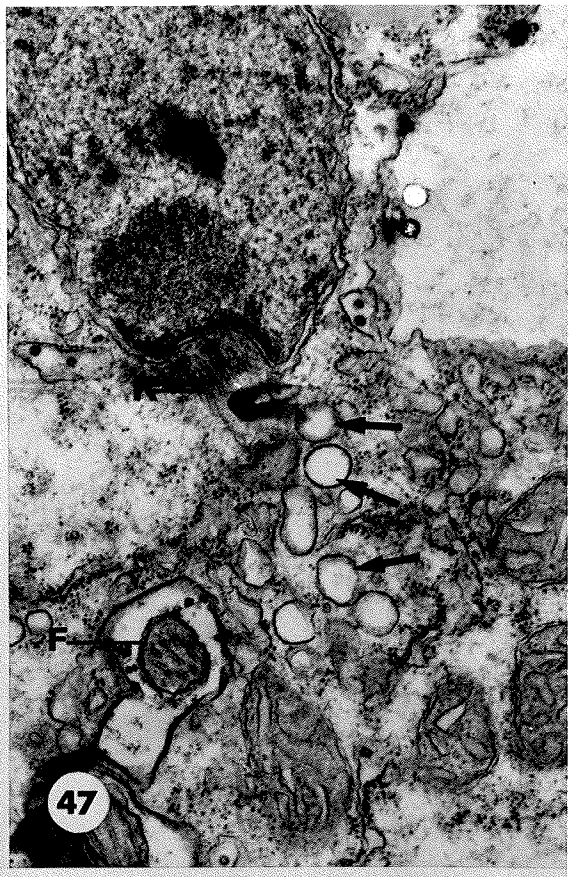
Figure 46. Section through K showing termination at a dark staining terminal plate (solid arrow). Note the occurrence of membrane-bound ribosomes, x77,500.

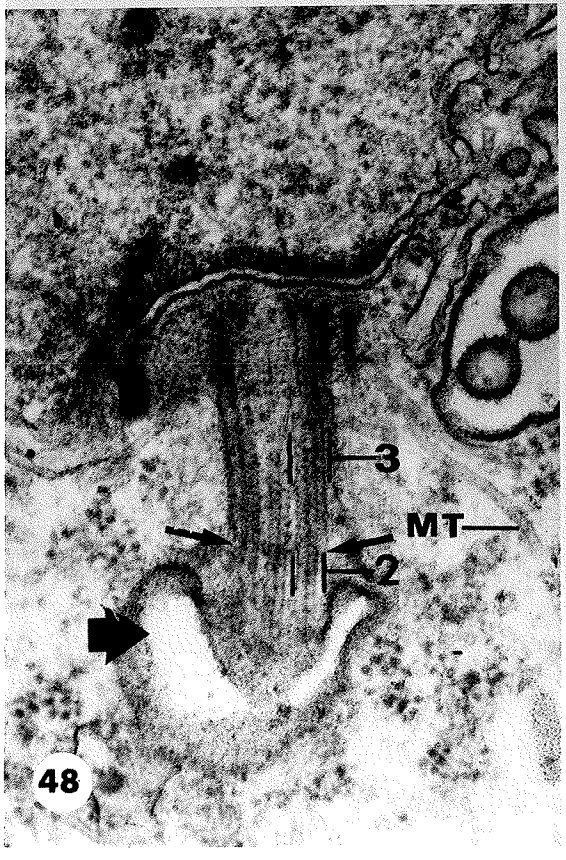
Figure 47. Initial production of primary flagellar vesicles by progressive fusion of small VE (solid arrows). Note the cross section of a F, showing the typical 9 + 2 arrangement of MT, x33,500.

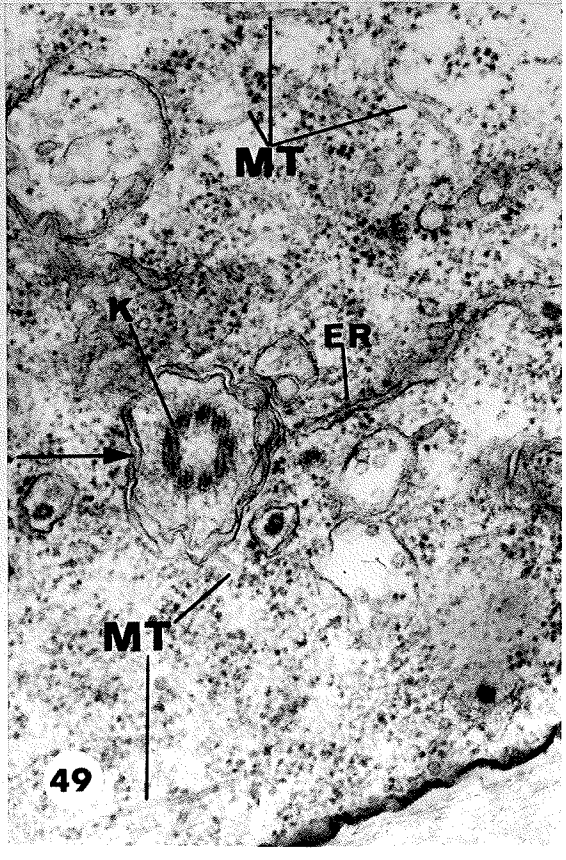
Figure 48. Elongation of the K into the primary flagellar vesicles (solid arrow). Triplet arrangement of MT terminates at the terminal plate (small arrows) to become a doublet. MT also in evidence in association with the K, x80,000.

Figure 49. Cross section through the primary flagellar vesicles showing the double membrane sheath (arrow) surrounding the elongating K. Also note the association of ER with the sheath, and the concentration of MT in the surrounding cytoplasm, x52,000.


K
46


K
F
47


3
MT
2
48


MT
K
ER
MT
49

the outer membrane would be produced by the cleavage vesicles that surround the zoospore initial as suggested by Barron and Hill (1974).

The origin of these small vesicles is uncertain. Heath and Greenwood (1971) and Chong and Barr (1973) speculated that the Golgi body was involved in the synthesis of these vesicles. Renaud and Swift (1964) indicated that the plasma membrane was the source of the flagellum membrane. They suggested that as the kinetosomes near the cell membrane, they induce the formation of vesicles, which then surround and contribute to the development of the elongating flagellum. However Lessie and Lovett (1968) and Olson et al. (1981) argued against this, indicating that the flagellar vesicles apparently appeared throughout the cytoplasm simultaneously. However, the origin of the vesicles is still unclear and as Heath (1976) stated, "until autoradiographic investigations have been undertaken...speculation is not very valuable".

The flagellum continued to grow into the enlarging primary flagellar vesicle (Figs. 50, 51); the latter apparently elongating by continuous fusion of additional small flagella vesicles (Renaud and Swift 1964; Lessie and Lovett 1968; Barron and Hill 1974). Many of the flagella develop in close proximity to each other and fusion of the primary flagellar vesicles was evident (Fig. 50, open arrow).

After the flagellum had been produced, cleavage of the sporangial cytoplasm took place. Concomitant with this the ribosomes appeared to aggregate in the cytoplasm and large mitochondria began to become associated with the regions occupied by the zoospore initials (Fig. 52, solid arrows). For the first time gamma bodies were sited in the cytoplasm (Fig. 52, small arrows; 53), as well as granule-containing cisternae

LEGEND

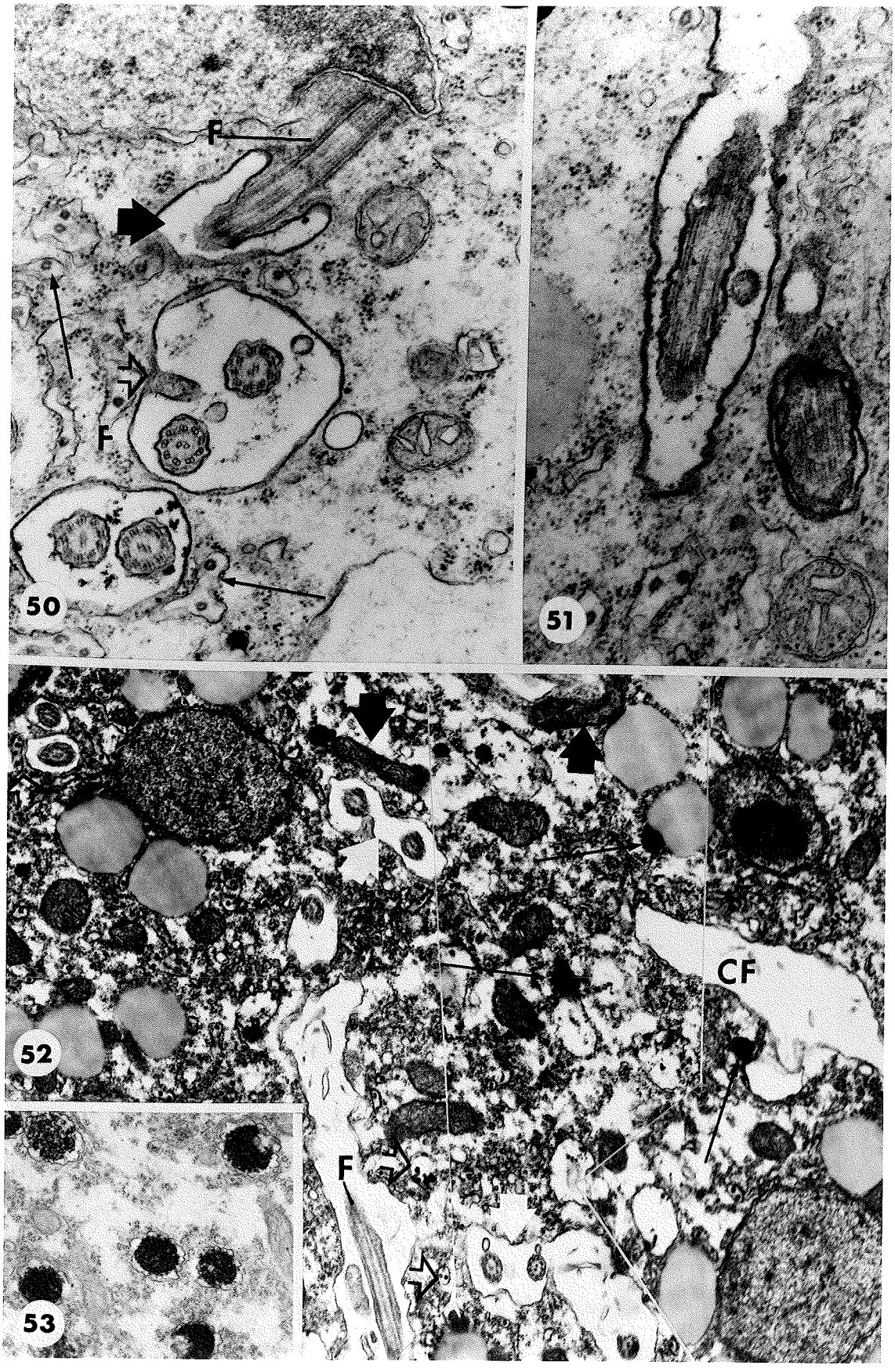
Figures 50-53. TEM micrographs of stages of zoospore production.

Figure 50. Section through the cytoplasm of ZS showing elongation of F into the primary cleavage vesicle (solid arrow) and the commonly observed fusion of these vesicles after F production (open arrow). Granule-containing CS are still present (small arrows) but gamma bodies are not yet evident, x19,000.

Figure 51. Longitudinal section through a primary flagellar vesicle, x50,500.

Figure 52. Initial stages of cytoplasmic cleavage with appearance of CF and fusion of primary flagellar vesicles (white arrow) along with the occurrence of large M in the region of the Z initial (solid arrows). The appearance of gamma bodies is noted (small arrows) in addition to the persistence of granule-containing CS (open arrows), x17,000.

Figure 53. Illustration of mature gamma bodies, x17,000.


F
F
50
51
52
CF
F
53

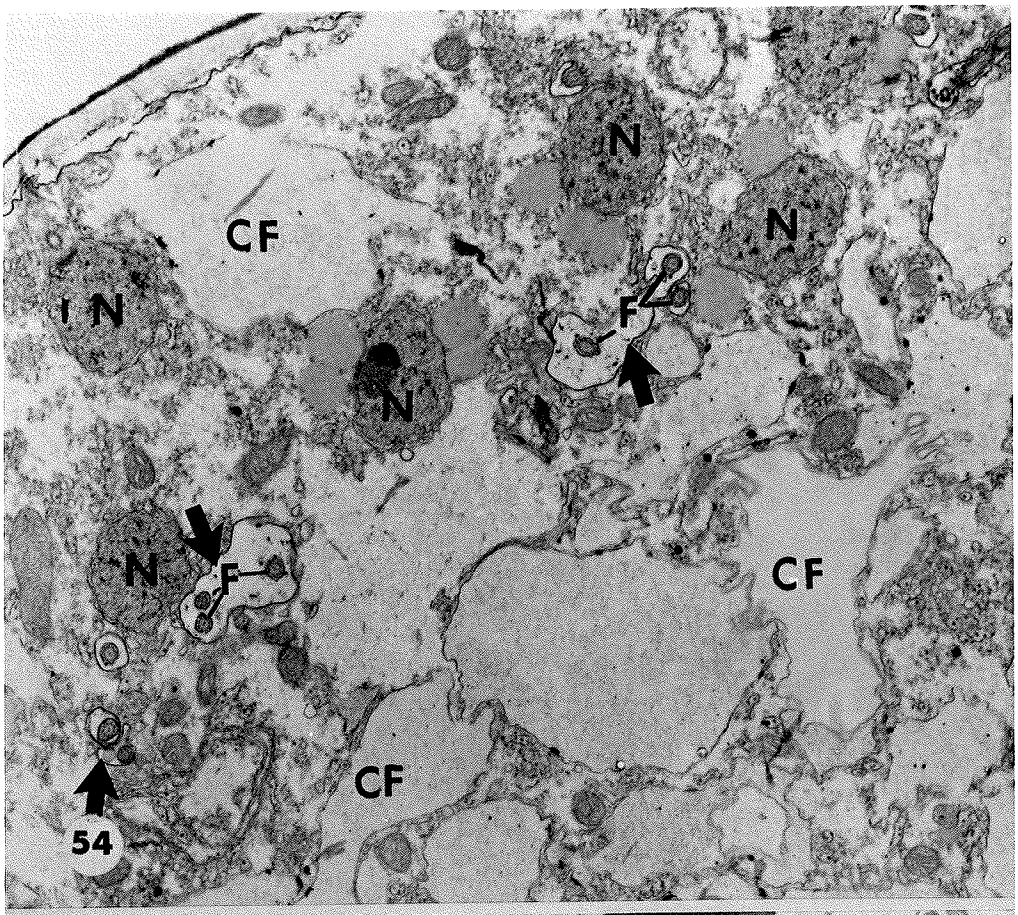
(Fig. 52, open arrows). This implies that gamma body production was not synchronous. In most uniflagellate fungi "cytoplasmic cleavage is brought about by the alignment and subsequent fusion of a reticulum of small vesicles" (Olson et al. 1981). It was evident that in C.anguillulae the cytoplasm cleavage was brought about, at least partially, by the fusion of primary cleavage vesicles. Other workers have reported that cleavage of the cytoplasm occurs through the fusion of cleavage vesicles, primary flagellum vesicles, cell membrane and, in some cases, a central vacuole (Renaud and Swift 1964; Lessie and Lovett 1968; Heath 1976; Williams and Webster 1970). The cleavage itself occurs very quickly and large furrows between the zoospore initials were evident just prior to the final cleavage (Fig. 54), after which the individual zoospores were produced (Fig. 55).

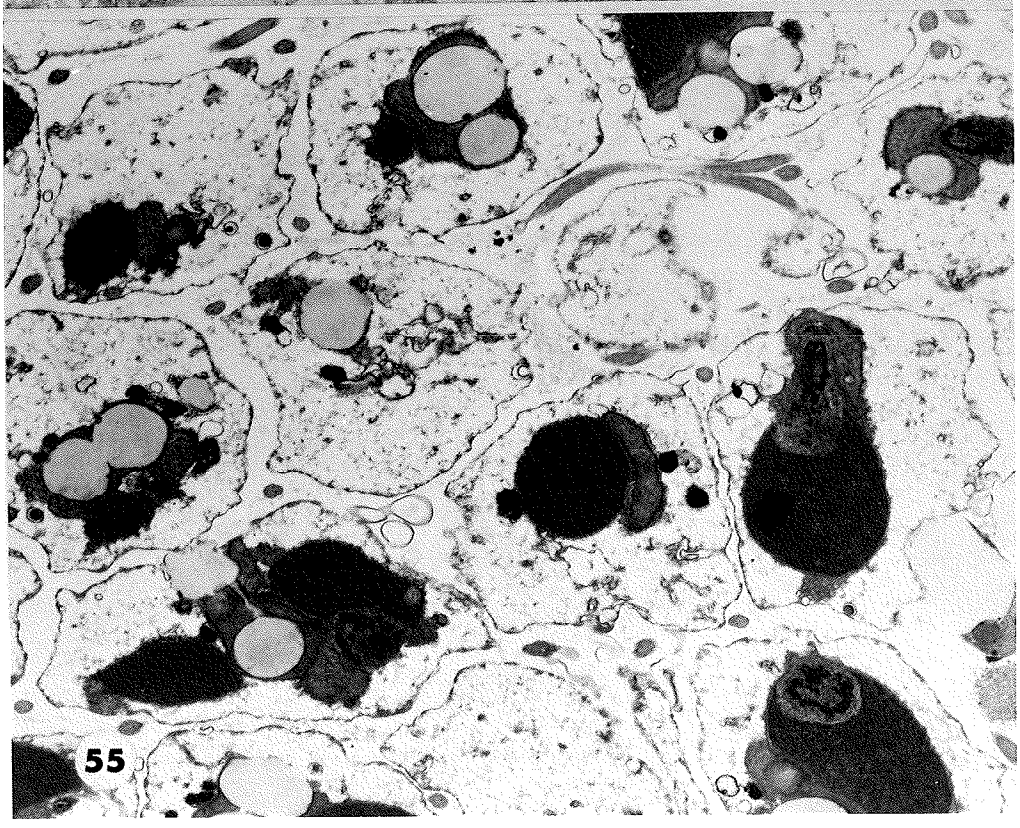
Cleavage of the cytoplasm does not always follow this same pattern. In Harpochytrium hedinii the appearance of cleavage furrows marks the beginning of cytoplasmic differentiation and development of the flagella does not begin until cleavage is complete (Travland and Whisler 1971). This is also the case in Phlyctochytrium spp. (Chong and Barr 1973; McNitt 1974), a member of the Chytridiales.

The biflagellate zoosporic fungi have a somewhat different method of cleavage. In Saprolegnia spp. a central vacuole is formed at the initiation of cleavage. This vacuole expands between the uninucleate blocks of cytoplasm, thus cutting out the zoospore initials and finally fusing with the plasmalemma (Gay and Greenwood 1966; Gay et al. 1971). In Phytophthora parasitica cleavage is brought about by the orderly arrangement of vesicles equidistant from neighbouring nuclei and along the wall of the sporangium. In addition to this a large central vacuole is produced and the ultimate

LEGEND

Figures 54 and 55.	TEM micrographs of the final stages of zoospore production.
Figure 54.	Numerous large CF are apparent in addition to fusing primary flagellar vesicles (solid arrows); both serve to divide the cytoplasm into uninucleate portions, x10,500.
Figure 55.	Section through a mature ZS, showing the closely packed zoospores, x11,500.


N
CF
N
I N
F
N
N
F
CF
CF
54


55

fusion of these small vesicles with each other and with the central vacuole produces the uninucleate zoospores (Hohl and Hamamoto 1967).

The final organization of the zoospore was not observed in this study and it was not very clear from the literature what could happen. It appears that at some point subsequent to the cleavage of the cytoplasm the single basal mitochondrion that was associated with each zoospore initial became appressed to the nucleus and was bordered by the microbody lipid complex. The nuclear cap was also produced by an aggregation of the cytoplasmic ribosomes into the zoospore initial, and eventually formed a cap over the nucleus. The cap is surrounded by a membrane produced by the fusion of vesicles that possibly arise from electron dense bodies (Olson et al. 1981) or pinched off from cisternae (Barron and Hill 1974). It is not clear which of these methods, if either, functions in C.anguillulae; but the presence of some endoplasmic reticulum in the late cleavage zoosporangia (Figs. 45, 49) suggested that the latter method is the more probable.

The life cycle of C.anguillulae, like all of the lower fungi, is of very short duration and when first studied appeared quite simple. However, it is obvious that the biology of these organisms is not completely understood and certainly the changes that the thallus undergoes appear to be very complicated when observed at the ultrastructural level; thus a truly simple life cycle may be a misconception. It is apparent from this study that much more work needs to be undertaken before this group of fungi is truly well understood.

SUMMARY

1) The life cycle of C.anguillulae can possibly be divided into four developmental stages on the basis of gross morphology:
   i) The zoosporic stage: The free swimming zoospore swam for a period of time then came to rest on the substrate.
   ii) Encystment and germination: The zoospore encysted and after a brief period produced an elongate germ tube.
   iii) Prosporangial stage: The migration of protoplasm out of the zoospore into the germ tube led to the production of a swelling on the latter, which enlarged to become the prosporangium. The prosporangium, initially uninucleate, underwent a series of nuclear divisions and produced hyphal elements into which its protoplasmic contents migrated.
   iv) Development of zoosporangia: Swellings developed on the hyphae wherever nuclei were situated; these swellings later enlarged to become zoosporangia.
2) The ultrastructural investigations revealed that a number of changes occurred in the composition of the protoplasm during the development of the thallus:
   i) Glycogen was noted to occur in the prosporangium and appeared to increase with maturity of the thallus. A vesiculated zone of ribosome exclusion was observed; this zone had been reported to be the functional equivalent of the Golgi apparatus by other workers. The prosporangium was also observed to become more vacuolate prior to nuclear migration into the hyphal elements.

ii) The young zoosporangium contained distinct regions of smooth endoplasmic reticulum, some of which occurred in a coiled configuration. A region of smooth tubular endoplasmic reticulum associated with cisternae was also observed. The cisternae contained dark staining granules and have been reported to be the progenitors of the gamma bodies.

Aggregations of thick-walled tubules apparently associated with the cell wall were observed in the enlarging zoosporangium, often in regions of active growth, such as the site of rhizoid development. Rhizoids growing from the enlarging zoosporangia display abnormally long mitochondria and microtubules both oriented parallel to the cell wall. Prior to zoospore production a septum was formed that separated the zoosporangium and rhizoids simultaneous to the migration of the rhizoidal protoplasm into the zoosporangium.

iii) As the zoosporangium reached maturity the number of nuclei increased at the periphery of the sporangium, and became associated with large lipid bodies. The reassociation of large microbodies with lipids was also observed as well as the occurrence of larger mitochondria.

iv) Nuclear division in the zoosporangium was observed to be synchronous and was characterized by the retainment of the nuclear envelope, and the absence of a nucleolus. Pairing of the centrioles was observed at opposite poles of the dividing nucleus; each of the long centrioles being capped

by an oval mitochondrion.

v) The initiation of zoosporogenesis was signalled by the lipid crown stage and was followed by the elongation of the centriole into a kinetosome. The kinetosome developed into a flagellum by elongating into a primary flagellar vesicle, the latter being produced by the fusion of smaller vesicles.

vi) Cleavage of the zoosporangial cytoplasm was brought about by the fusion of developing cleavage furrows with fusing primary flagellar vesicles. The cytoplasm was separated into uninucleate portions that became organized into zoospores. The process of cleavage was possibly controlled by cytoplasmic microtubules that originated from the kinetosome, and may have acted to stabilize portions of the cytoplasm.

BIBLIOGRAPHY

Aist, J.R. and P.H. Williams. 1972. Ultrastructure and time course of mitosis in the fungus Fusarium oxysporum. J.Cell Biol. 55: 368-389.

Armbruster, B.L. 1982. Sporogenesis in three genera of the Saprolengiaceae. II. Primary spore initial to secondary spore initial stage. Mycologia 74: 975-999.

Barr, D.J.S. 1970. Two varieties of Rhizophydium sphaerocarpum (Chytridiales). Can.J.Bot. 48: 1067-1071.

Barr, D.J.S. 1973. Six Rhizophydium species (Chytridiales) in culture. Can.J.Bot. 51: 967-975.

Barr, D.J.S. 1975. Morphology and zoospore discharge in single, epibiotic Chytridiales. Can.J.Bot. 53: 164-178.

Barr, D.J.S. 1978. Taxonomy and phylogeny of Chytrids. Biosystems 10: 153-165.

Barron, J.L. and E.P. Hill. 1974. Ultrastructure of zoosporogenesis in Allomyces macrogynus. J.Gen.Microbiol. 80: 319-327.

Barstow, W.E. 1979. Ultrastructure of the formation of particles during zoosporogenesis in Allomyces macrogynus and Catenaria anguillulae. Exp.Mycol. 3: 28-41.

Barstow, W.E. and J.S. Lovett. 1974a. An Atlas of Fungal Ultrastructure. Beckett, A., T.B.Heath and D.J.McLaughlin, eds. Longman Group Ltd., London.

Barstow, W.E. and J.S. Lovett. 1974b. Apical vesicles and microtubules in rhizoids of Blastocladiella emersonii: Effects of Actinomycin D and Cycloheximide on development during germination. Protoplasma 82: 103-117.

Barstow, W.E. and J.S. Lovett. 1975. Formation of gamma particles during zoosporogenesis in Blastocladiella emersonii. Mycologia 67: 518-529.

Bimpong, C.E. and C.J. Hickman. 1975. Ultrastructural and cytochemical studies of zoospores, cysts and germinating cysts of Phytophthora palmivora. Can.J.Bot. 53: 1310-1327.

Bland, C.E. and H.V. Amerson. 1973. Electron microscopy of zoosporogenesis in the marine phycomycetes Lagenidium collinectes Couch. Arch.Mikrobiol. 94: 47-64.

Blondel, B. and G. Turian. 1960. Relation between basophilia and fine structure of cytoplasm in the fungus Allomyces macrogynus. Em.J. Biophys.Biochem.Cytol. 7: 127-134.

Boolis, M.G. and R. Mankau. 1965. Parasitism and predation of soil microorganisms. In Ecology of Soil-Borne Plant Pathogens. Edited by K.F.Baker and W.C.Snyder. Univ.of California Press, Berkeley.

Bracker, C.E. 1967. Ultrastructure of fungi. Ann.Rev.Phytopathology 5: 343-374.

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

Braun, A. 1856. Uber Chytridium, eine Gattung einzeliger Schmarotzergewachse auf Algen und Infusurien. Abh.Berlin Akad. 1855: 21-83.

Brenner, S. 1974. The genetics of Caenorhabditus elegans. Genetics 77: 71-94.

Burnett, J.H. 1976. Fundamentals of Mycology. Edward Arnold, London.

Butler, J.B. and J.J.C. Buckley. 1927. Catenaria anguillulae as a parasite of the ova of Fasciola hepatica. Sci.Proc.Royal Dublin Soc. 18: 497-512.

Butler, J.B. and A. Humphries. 1932. On the cultivation in artificial media of Catenaria anguillulae, a chytridiacean parasite of the ova of the liver fluke, Fasciola hepatica. Sci.Proc.Royal Dublin Soc. 20: 301-324.

Cantino, E.C. and Horenstein, E.A. 1956. Gamma and the cytoplasmic control of differentiation in Blastocladiella. Mycologia 48: 443-446.

Chambers, T.C. and L.G. Willougby. 1964. The fine structure of Rhizophylictus rosea, a soil phycomycete. J.Roy.Microscop.Soc. 83: 355-364.

Chambers, T.C., K. Markus and L.G. Willoughby. 1967. The fine structure of the mature zoosporangium of Nowakowskiella profusa. J.Gen. Microbiol. 46: 135-141.

Chapman, J.A. and R. Vujicic. 1965. The fine structure of sporangia of Phytophthora erythrospetica Pethyb. J.Gen.Microbiol. 41: 275-281.

Chong, J. and D.J.S. Barr. 1973. Zoospore development and fine structures in Phlyctochytrium arcticum (Chytridiales). Can.J.Bot. 51(7): 1411-1420.

Chong, J. and D.J.S. Barr. 1974. Ultrastructure of the zoospores of Entophlyctis confervae-glomeratae, Rhizophydium patellarium and Catenaria anguillulae. Can.J.Bot. 52(6): 1197-1204.

Couch, J.N. 1945. Observations on the genus Catenaria. Mycologia 37: 163-193.

Dangeard, M. 1885. Note sur le Catenaria anguillulae Sorok. et Chytridium zootocum. A. Br.Bull.Soc.Normandie IIIe 9: 126-135.

Doward, D.W. and M.J. Powell. 1982. Cross-linking bridges associated with the microbody-lipid globule complex in Chytriomyces aureus and Chytriomyces hyalinus. Protoplasma 112: 181-188.

Dustin, P. 1978. Microtubules. Springer-Verlag, New York.

Emerson, R. 1941. An experimental study of the life cycles and taxonomy of Allomyces. Lloydia 4: 77-144.

Fischer, A. 1892. Phycomycetes. Rabenhorst's kryptogamenflora, 1, IV, Abt. kummer, Leipzig.

Fitzpatrick, H.M. 1930. The Lower Fungi. Phycomycetes. McGraw-Hill, New York.

Frederick, S.E., P.J. Gruber and E.J. Newcomb. 1975. Plant microbodies. Protoplasm 84: 1-29.

Gay, J.L. and Greenwood, A.D. 1966. Structural aspects of zoospore production in Saprolegnia ferax with particular reference to the cell abd vacuolar membranes. In The Fungus Spore, Colston Papers. M.F. Madelin, ed., pp. 95-110. Butterworth's, London.

Gay, J.L., A.D. Greenwood and I.B. Heath. 1971. The formation and behavior of vacuoles (vesicles) during oosphere development and zoospore germination in Saprolegnia. J.Gen.Microbiol. 65: 233-241.

Goodey, J.B. 1957. Laboratory methods for work with plant and soil nematodes. Technical Bulletin No.2, Ministry of Agriculture and Fisheries and Food. Her Majesty's Stationery Office, pp.1-47.

Griffin, D.H. 1981. Fungal Physiology. Wiley and Sons, New York.

Grove, S.N., C.E. Bracker and D.J. Morre. 1970. An ultrastructural basis for hyphal tip growth in Pythium ultimum. Am.J.Bot. 57: 245-266.

Gull, K. 1978. Form and function of septa in filamentous fungi. In The Filamentous Fungi. III. Developmental Mycology. Edited by J.E.Smith and D.R.Berry. J.Wiley and Sons, New York.

Gunning, B. and M.W. Steer. 1975. Ultrastructure and the Biology of Plant Cells. Edward Arnold, London.

Hawker, L.E. 1965. Fine structure of fungi as revealed by electron microscopy. Biol.Rev. 40: 52-92.

Hawker, L.E. amd A. Becket. 1971. Fine structure and development of Rhizopus sexualis (Smith) Callen. Phil.Trans.Roy.Soc.Lond. 263: 71.

Heath, I.B. 1974. Mitosis in the fungus Thraustotheca clavata. J.Cell Biol. 60: 204-220.

Heath, I.B. 1976. Ultrastructure of freshwater phycomycetes. In Recent Advances in Aquatic Mycology. Edited by G.Jones. Elek Science, London.

Heath, I.B. and A.D. Greenwood. 1968. Electron microscopic observations of dividing somatic nuclei in Saprolegnia. J.Gen.Microbiol. 53: 287-289.

Heath, I.B. and A.D. Greenwood. 1970a. The structure and formation of lomasomes. J.Gen.Microbiol. 62: 129-137.

Heath, I.B. and A.D. Greenwood. 1970b. Centriole replication and nuclear division in Saprolegnia. J.Gen.Microbiol. 62: 139-148.

Heath, I.B. and A.D. Greenwood. 1971. Ultrastructural observations on the kinetosomes and Golgi bodies during the asexual life cycle of Saprolegnia. Z.Zellf.Mikroskop.Anat., Abt. 112: 371-389.

Heath, I.B. and M.C. Heath. 1978. Microtubules and organelle movements in the rust fungus Uromyces phaseoli var.vignae. European J.Cell Biol. 16: 393-411.

Hemmes, D.E. and H.R. Hohl. 1971. Ultrastructural aspects of encystment and cyst-germination in Phytophthora parasitica. J.Cell Sci. 9: 175-191.

Hepler, P.K. and B.A. Palevitz. 1974. Microtubules and microfilaments. Ann.Rev.Plant Physiol. 25: 309-362.

Hess, W.M. 1966. Fixation and staining of fungus hyphae and host plant root tissue for electron microscopy. Stain Technol. 41: 27-35.

Hillegas, A.B. 1940. The cytology of Endochytrium operculum (de Wildeman) Karling in relation to its development and organization. Bull. Torrey Bot.Club 67: 1-32.

Hoch, H.C. and J.E. Mitchell. 1972. The ultrastructure of Aphanomyces euteiches during asexual spore formation. Phytopathology 62: 149-160.

Hohl, H.R. and S.T. Hamamoto. 1967. Ultrastructural changes during zoospore formation in Phytophthora parasitica. Amer.J.Bot. 54: 1131-1139.

Ichida, A. and M.S. Fuller. 1968. Ultrastructure of mitosis in the aquatic fungus Catenaria anguillulae. Mycologia 60: 141-155.

Karling, J.S. 1937. The structure, development, identity and relationship of Endochytrium. Amer.J.Bot. 24: 352-364.

Kazama, F. 1972. Development and morphology of a chytrid isolated from Bryopsis plumosa. Can.J.Bot. 50: 499-505.

Lessie, P.E. and J.S. Lovett. 1968. Ultrastructural changes during sporangium formation and zoospore differentiation in Blastocladiella emersonii. Amer.J.Bot. 55: 220-236.

Lu, B.C. and N.B. Raju. 1970. Meiosis in Coprinus. II Chromasome and lampbrush diplotene stage of meiotic prophase. Chromasoma 29: 305-316.

Luft, J.H. 1971. Ruthenium Red and Violet. I. Chemistry, purification, methods of use for electron microscopy and mechanism of action. Anat.Rec. 171: 347-368.

Lunney, C.Z. and C.E. Bland. 1976. Ultrastructural observations of mature and encysting zoospores of Pythium proliferum de bary. Protoplasma 90: 119-137.

MacLeod, H. and P.A. Horgen. 1979. Germination of the asexual spores of the aquatic fungus Achyla bisexualis. Experimental Mycology 3: 70-82.

Madelin, M.F. and A. Beckett. 1972. The production of planonts by thin-walled sporangia of the fungus Coelomyces indicus, a parasite of mosquitoes. J.Gen.Microbiol. 72: 185-200.

Manier, J.F. 1977. Cycle, ultrastructure d'une Catenaria (phycomyces, Blastocladiales) parasite de Crustaces Cyclopoides. Annales de Parasitologie 52: 363-376.

Marchant, R. and A.W. Robarts. 1968. Membrane systems associated with the plasmalemma of plant cells. Ann.Bot. 32: 457-71.

Matile, P. 1978. Biochemistry and function of vacuoles. Ann.Rev. Plant Physiol. 29: 193-213.

Maxwell, D.R., V.N.Armentrout and L.B. Graves, Jr. 1977. Microbodies in plant pathogenic fungi. Ann.Rev.Phytopath. 15: 119-34.

Maxwell, M.D., G. Hanssler, V.N. Armentrout, G.M. Murray and H.C. Hoch. 1975. Microbodies and glyoxylate-cycle enzyme activities in filamentous fungi. Planta 124: 109-123.

McLaughlin, D.J. 1973. Ultrastructure of sterigma growth and basidiospore formation in Coprinus and Boletus. Can.J.Bot. 51: 145-150.

McNitt, R. 1974. Zoosporogenesis in Phlyctochytrium irregulare. Cytobiologie 9: 290-306.

Mills, G.L. and E.C. Cantino. 1979. Trimodal formation of microbodies and associated biochemical and cytochemical changes during development in Blastocladiella emersonii. Exp.Mycol. 3: 53-69.

Morrison, P.J. 1977. Gametangial development in Allomyces macrogynus. II. Evidence against mitochondrial involvement in sexual differentiation. Arch.Microbiol. 113: 173-179.

Myers, R.B. and E.C. Cantino. 1974. The gamma particle. A study of cell-organelle interactions in the development of the water mold Blastocladiella emersonii. In Monographs in Developmental Biology (A.Wolsky, ed.), Vol.8. Korger, Basel.

Olson, L.W. and R. Reichle. 1978. Meiosis and diploidization in the aquatic phycomycete Catenaria anguillulae. Trans.Br.Mycol.Soc. 70: 425-437.

Olson, L.W., L. Lange and R. Reichle. 1978. The zoospore and meiospore of the aquatic phycomycete Catenaria anguillulae. Protoplasma 97: 53-71.

Olson, L.W., V.M. Eden and L. Lange. 1981. Zoosporogenesis model systems - problems - possible approaches. pp.43-70. In The Fungal Spore: Morphogenetic Controls. Edited by G.Turian and H.R.Hohl. Academic Press, New York.

Porter, D. and Smiley, R. 1980. Development of the sporangium and discharge apparatus in a marine chytrid, Phlyctochytrium sp. Botanica Marina 23: 99-115.

Powell, M.J. 1974. Fine structure of plasmodesmata in a chytrid. Mycologia 66: 606-614.

Powell, M.J. 1975. Ultrastructural changes in nuclear membranes and organelle associations during mitosis of the aquatic fungus Entophlyctis sp. Can.J.Bot. 53: 627-646.

Powell, M.J. 1976. Ultrastructure and isolation of glyoxysomes (microbodies) in zoospores of the fungus Entophlyctis sp. Protoplasma 89: 1-27.

Powell, M.J. 1978. Phylogenetic implications of the microbody-lipid globule complex in zoosporic fungi. Biosystems 10: 167-180.

Powell, M.J. 1979. The structure of microbodies and their association with other organelles of Entophlyctis variabilis. Protoplasma 98: 177-198.

Powell, M.J. and W.J. Koch. 1977. Morphological variation in a new species of Entophlyctis. I. The species concept. Can.J.Bot. 55: 1668-1685.

Raghu Kumar, S. 1982. Fine structure of the Thraustochytrid Ulkenia amaeboidea. I. Vegetative thallus and formation of the amoeboid stage. Can.J.Bot. 60: 1092-1102.

Raudaskowski, M. 1972. Occurrence of microtubules in the hyphae of Schizophyllum commune during intercellular nuclear migration. Arch.Microbiol. 86: 91-100.

Renaud, F.L. and H. Swift. 1964. The development of basal bodies and flagella in Allomyces arbusculus. J.Cell Biol. 23: 339-354.

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

Roane, M.K. and R.A. Paterson. 1974. Some aspects of morphology and development in the Chytridiales. Mycologia 66: 147-164.

Robertson, J.A. 1972. Phototaxis in a new Allomyces. Arch.Mikrobiol. 85: 259-266.

Sayre, R.M. and L.S. Keeley. 1969. Factors influencing Catenaria anguillulae infections in a free-living and a plant pasasite nematode. Nematologica 15: 492-502.

Schroter, J. 1897. Chytridiineae. Engler and Prantl, Die Naturlichen Pflanzenfamilien, L,1. pp.64-87. W.Englemann, Leipzig.

Sorokin, S. 1962. Centrioleses and the formation of rudimentary cilia by fibroblasts and smooth muscle cells. J.Cell Biol. 15: 363.

Sorokine, N. 1876. Note sur les vegetaux parasites des Anguillulae. Ann.Sci.Nat. VI. 4: 62-71.

Sparrow, F.K. 1943. Aquatic Phycomycetes. Ann Arbor.

Sparrow, F.K. 1960. Aquatic Phycomycetes. 2nd ed.rev. The University of Michigan Press, Ann Arbor.

Spurr, A.R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J.Ultrastruct.Res. 26: 31-45.

Stirling, A.M. and E.G. Platzer. 1978. Catenaria anguillulae in the mermithid nematode Romanomermis culicivorax. J.Invert.Path. 32: 348-354.

Suberkropp, K.F. and E.C. Cantino. 1972. Environmental control of motility and encystment in Blastocladiella emersonii zoospores at high population densities. Trans.Br.mycol.Soc. 59: 463-475.

Sykes, E.E. and D. Porter. 1980. Infection and development of the obligate parasite Catenaria allomycis on Allomyces arbuscule. Mycologia 72: 288-300.

Taylor, J.W. and M.S. Fuller. 1980. Microtubules, organelle movement, and cross-wall formation at the sporangial-rhizoidal interface in the fungus, Chytridium confervae. Protoplasma 104: 201-221.

Temmink, J.H.M. and R.N. Campbell. 1968. The ultrastructure of Olpidium brassicae. I. Formation of sporangia. Can.J.Bot. 46: 951-956.

Travland, L.B. and H.C. Whisler. 1971. Ultrastructure of Harpochytrium hedinii. Mycologia 63: 767-789.

Truesdell, L.C. and E.C. Cantino. 1971. The induction and early events of germination in the zoospore of Blastocladiella emersonii. In Current Topics in Developmental Biology, Vol.6, 1-44 (A.A.Moscona and A.Monroy, eds.). New York, Academic Press.

Van Iterson, W. and W. Leene. 1964. A cytochemical localization of reductive sites in a gram positive bacterium. J.Cell Biol. 20: 361-375.

Von Minden 1911. Chytridiineae. Kryptogamenflora der Mark Brandenburg. I. 5: 390-419. Leipzig.

Whiffen, Alma J. 1944. A discussion of taxonomic criteria in the Chytridiales. Farlowia 1: 583-597.

Williams, W.T. and P.H. Webster. Electron microscopy of the sporangium of Phytophthora capsici. Can.J.Bot. 48: 221-227.