ABSTRACT

Henry Van Amerson. THE STRUCTURE AND DEVELOPMENT OF LAGENIDIUM CALLINECTES COUCH. (Under the direction of Dr. Charles E. Bland) Department of Biology, August, 1972

Co-ordinated light and electron microscope observations were made, tentatively establishing the life cycle of Lagenidium callinectes. Each stage of this life cycle was studied in detail. The zoospores are typical for an oomycete, oval and biflagellate. Spore encystment involves flagellar retraction or detachment and the formation of a cyst wall from fibrous and wall vesicles. Structures resembling polysynaptonemal complexes are present in the nucleus of encysting spores and are indicative of meiosis at this stage. Germination of the encysted spores is generally monopolar with the cytoplasm moving into the germ tube leaving the cyst empty. Wall formation in the germ tube and hyphae is accomplished by small wall vesicles. Spore development in L. callinectes occurs in a gelatinous cleavage vesicle formed at the tip of an unbranched hypha. Cytoplasm discharges from this hypha into the vesicle where a process of vacuolar fusion results in the cleavage of the sporogenic cytoplasm into zoospores. The spores are released when the vesicle ruptures.

THE STRUCTURE AND DEVELOPMENT OF LAGENIDIUM CALLINECTES COUCH

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DEDICATION

This thesis is dedicated to my wife, Ellen, who has endured so much during its preparation.

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ABBREVIATIONS

A - axoneme

AT - anterior tinsel flagellum

B - bead

BB - basal body

BP - basal plate

C - cyst

Ce - centriole

CM - central mass

CR - connecting rootlets

CV - central vacuole

D - dictyosome

DB - dense body

EB - enlarged base

ER - endoplasmic reticulum

EW - elongate wall vesicle

F - flagellum

FC - flagellar cavity

FV - fibrous vesicle

Fil - filamentous vesicle

Fl V - flagellar vesicle

GCV - gelatinous cleavage vesicle

GT - germ tube

GV - granular vesicle

L - lipid

LE - lateral element

LG - longitudinal groove

Lo - lomasome

LR - long rootlet

M - mitochondria

MA - matrix area

Mb - membrane

ME - medial element

MT - microtubules

NB - nuclear beak

No - nucleolus

P - polysome

PC - polycomplex

PM - plasma membrane

PW - posterior whiplash flagellum

R - rootlets

SLW - single layered wall

SR - short rootlet

SW - spherical wall vesicles

T - tinsel

Th - thread

TLW - three layered wall

TP - tinsel packet

V - vacuole

VF - vacuole fusion

VT - vesicular tip

INTRODUCTION

Lagenidium callinectes Couch, class Oomycetes, family Lagenidiaceae, was first observed and collected in 1941 growing on eggs of the blue crab, *Callinectes sapidus* Rathbun (Couch, 1942). Specimens collected in 1942 and sent to J. N. Couch for identification were described by him as a marine species of *Lagenidium* (Couch, 1942). Couch showed parasitism of the crab ova to occur via zoospores which attacked the eggs and germinated on them by means of a single germ tube. Destruction of infected eggs followed with the eggs becoming completely filled with hyphae. Couch showed that during spore formation "stout" hyphae within the crab egg penetrated the egg wall and served as discharge tubes. A gelatinous vesicle formed at the end of these discharge tubes, and cytoplasm within them flowed into the vesicle. Following this, cleavage of the spores occurred with their subsequent discharge from the vesicle. Couch described these biflagellate spores as being posteriorly rounded, anteriorly pointed, and measuring 9.6 by 12.6 um.

Shortly after Couch's work, Sandoz, Rogers, and Newcombe (1944) reported the occurrence of *L. callinectes* on eggs of *C. sapidus* collected in tidewater Virginia. These investigators showed that infected eggs either failed to hatch or hatched abnormally. Also, crabs collected from various tidewater regions showed wide variation in the degree of infection.

Rogers-Talbert (1948) made a thorough survey of the distribution and effect of L. callinectes on crab populations in Chesapeake Bay, Virginia. This worker found L. callinectes to be a peripheral parasite on the egg mass (sponge) of the blue crab. It was shown that the fungus rarely penetrated more that three mm below the surface of the sponge. Infection occurred in more than fifty percent of the crabs sampled; however, the author felt that the peripheral nature of this parasite limited its danger to the crab population. Laboratory experiments with L. callinectes showed that infection and transmission potential was very great. Further experiments revealed normal development of the fungus over a meso-euryhaline range, 5-30 ppt. Only in fresh water was the development described as abnormal.

Johnson and Bonner (1960) reported the occurrence of L. callinectes on ova of the barnacle, Chelonibia patula Ranzani. They, as did Couch (1942), gave descriptions of hyphal structure, sporangium and vesicle formation, and planont structure. Johnson and Bonner found that large masses of profusely branching extramatrical hyphae were present when L. callinectes developed on barnacle ova. This differed from Couch's description of the fungus in crab ova, where extramatrical hyphae did not exist except for sporangial discharge tubes. Minor differences were also reported in sporangium and vesicle formation. Planont structure was described by Johnson and Bonner as identical to that reported by Couch. Johnson and Bonner succeeded in infecting crab ova with spores from L. callinectes that had been collected from barnacle ova. This fungus when grown on crab ova, appeared morphologically identical to that described by Couch in 1942. This cross innoculation with slight alteration of form in

different hosts was seen as evidence that the fungus observed by Johnson and Bonner was the same species as described by Couch (1942).

Fuller, Fowles, and McLaughlin (1964) demonstrated that L. callinectes was a facultative parasite rather that obligate by bringing the organism into agar culture. Isolates were obtained from various algae such as Chordaria, Cladophora, Ceramium and Ectocarpus. However, the authors did not consider L. callinectes parasitic on these algae, but merely a surface dweller. In addition to isolation and pure culture studies, these authors again described the process of spore formation. They found that "walled off" hyphae served as discharge tubes, with the cytoplasm in these hyphae being discharged into a vesicle which formed at the hyphal tip. This of course agreed with the description of previous authors. Cleavage was reported to begin about one minute after the protoplasm entered the vesicle with flagella becoming visible after about ten minutes. Release of the biflagellate zoospores occurred about 30 minutes after cleavage began. Fuller et al. concluded their consideration of L. callinectes with a brief description of the zoospore and zoospore germination.

Recent work by Bland and Amerson (1972) has provided further information about sporogenesis, spore structure and spore germination in L. callinectes. The results of this study are given in the results section of this thesis.

It is obvious from the above papers that much information has been accumulated on the nature and general morphology of L. callinectes. There still, however, exists a need for a fine strucutre study of this

organism. Questions remain concerning the process of sporogenesis, the deposition of the cell wall, even the life cycle of the organism, and many other facets that can only be answered at the ultrastructural level.

MATERIALS AND METHODS

The strain of L. callinectes used in this study was isolated during the summer of 1971 from ova of blue crabs collected from the Newport Estuary, North Carolina. Isolation and pure culture of this fungus were accomplished using the media described by Fuller et al (1964). One million units of penicillin "G" and one gram of streptomycin sulfate were dissolved in 2 cc. of sterile distilled water and added to one liter of medium while warm. This dosage was approximately twice that used by Fuller et al. Infected ova were washed in sterile sea water and plated on the antibiotic medium. Twenty-four to forty-eight hours later hyphal tips were transferred to new plates. Generally a pure culture was obtained on this second plate.

As suitable growth on the medium described by Fuller et al. was not obtained subsequent to isolation, various nutrient sources were tried. Of the various media, the best hyphal growth was obtained in broth prepared by mixing 5.5 g. of Difco PYG in 1,000 ml. of sea water. 1.5% agar plates of this same PYG medium were used also in maintaining the fungus. All media and other materials, except antibiotics, involved in the isolation and culture of this organism were autoclaved for 15 minutes at 15 psi and 121 degrees centigrade.

Propagation of L. callinectes was accomplished through the transfer of spores rather than hyphae. This transfer method was employed to prevent the fungus from becoming purely vegetative, as often happens

through hyphal transfer alone. An innoculum of zoospores was easily obtained by placing the hyphae from a 5-7 day broth or agar culture in sterile sea water. For this, hyphae were washed in 3 changes of sterile sea water, 100 ml. each, at 30 minute intervals with the hyphae remaining in the third wash. Approximately 12-15 hours after the first wash, spore production began and continued over a 48 hour period. At any time during this two day period, 2-3 ml. of the spore innoculum were transferred to a new broth solution or agar plate. The new broth cultures showed prolific growth within three days. Newly innoculated plates were completely covered by many small patches of hyphae within a week.

Phase contrast microscopy was employed in most of the light microscope observations as these observations were generally made with living material. Some observations, however, were made with fixed material. This material, generally spores, was fixed for three minutes in the fumes of 2% osmic acid. All photomicrographs were made with a Zeiss WL research microscope equipped with phase contrast optics and a Nikon AFM Microflex camera. Kodak Panatomic X film was used for all light micrographs.

For electron microscopy numerous fixation procedures were employed; however, only one method consistently gave suitable results. This procedure was a double fixation using glutaraldehyde and osmium tetroxide. First, the material was fixed for 10-15 minutes in 3% glutaraldehyde mixed in sea water. No buffer was necessary as the sea water acted as a dilute salts buffer. This fixation was followed by a 12-24 hour wash

in three changes of sea water. Following the final wash, material was post-fixed for 10-15 minutes in 2% osmium tetroxide in sea water. This post-fixation was followed by two 15 minute washes in sea water. Dehydration in a graded ethanol series was followed by propylene oxide dehydration, infiltration and embedding in Araldite 6005. The entire infiltration process was conducted with accelerator in the resin.

Material for the study of spore structure and spore encystment was fixed as above, however, centrifugation at 1470 x g for two minutes was necessary with each solution change. To prevent flagellar loss the spores were fixed before the initial centrifugation. Fixation was accomplished by mixing 100 ml. of 6% glutaraldehyde in sea water with a 100 ml. spore solution. Whole mounts of spores were prepared according to the techniques of Sharp et al. (1952) and shadowed with chromium.

Sections were cut with a Du Pont diamond knife on a Reichert OmU2 Ultramicrotome. Sections, 600-900 Å, were double stained with 1% uranyl acetate and Reynolds lead citrate (Reynolds, 1963). Staining periods varied from 4-15 minutes for each stain depending on the thickness of the specimen.

All electron microscope observations were made with a Hitachi HS-8 electron microscope operating at an accelerating voltage of 50 kv. Electron micorgraphs were taken on Kodalith LR film.

RESULTS

Thus far, no definite life cycle has been established for any member of the Lagenidiaceae (Alexopoulos, 1966). Several life histories have been proposed, but none have been confirmed (Alexopoulos, 1966). Cook (1935) presented a life cycle for Lagenidium rabenhorstii Zopf, which has been generally accepted as being representative of the family; however, even this cycle has not been confirmed.

Based on information obtained during the present study, a proposed life history for L. callinectes has been formulated. Although supported by information obtained in this work, it should be stressed that this life cycle is still only tentative.

Life History

The infective agent of L. callinectes is the spore and thus represents a good point at which to open a discussion of the life history. In the proposed cycle, free swimming diploid spores settle on the host surface and encyst. Presumably meiosis occurs in the encysted spores just prior to germination; thus, the emerging hyphae are haploid. These haploid hyphae may undergo asexual reproduction producing haploid zoospores, or they may function in sexual reproduction. In addition, resistant structures termed resting bodies have been observed; however, it is not known if these are sexually or asexually formed. In the sexual process gametangia, so far unseen in L. callinectes, are formed on the haploid hyphae, and these function in the production of a diploid zygote. Diploid hyphae arise from the

zygote with such hyphae giving rise to diploid zoospores. Release of these diploid zoospores returns us to our starting point. A diagram of the proposed life cycle of L. callinectes is given in Figure 1.

The Zoospore

The zoospore of L. callinectes as viewed with the light microscope immediately after emergence from the cleavage vesicle, generally measures 9.1 x 12.5 um, is irregularly shaped, and biflagellate (Fig. 2 & 3). Shortly after emergence, the spore assumes an oval shape (Fig. 3). Numerous refractive bodies, presumably lipid, can be observed in the spore. The oppositely directed flagella, anterior tinsel and posterior whiplash, measure 15-25 um and may occasionally show flagellar beads (Fig. 4). The flagella arise from a longitudinal groove that runs along one side of the spore (Fig. 5). The path of the actively swimming spore is helical.

Electron microscopy reveals the zoospore surface to be bound by only a plasma membrane (Figs. 6-7). Various vesicles and vacuoles lying slightly under this membrane cause the surface irregularity of the spore (Figs. 6-7). The flagella, as seen with the electron microscope, arise from a raised central area of the diagonal, longitudinal groove (Fig. 7). The axoneme exhibits the typical 9+2 arrangement of microtubules (Fig. 8). The flagellar membrane and matrix cytoplasm form a sheath that encases the axoneme. The anterior flagellum is covered with mastigonemes that measure 1.4 um by 15-20 nm. These mastigonemes consist of a microtubule shaft and an enlarged base that

narrows at the point of attachment (Fig. 81a). The anterior tinsel and posterior whiplash flagella are each anchored by a basal body which together have an interior angle of approximately 120° (Figs. 7, 9). The structure of these basal bodies is typical as they are composed of 9 triplets of microtubules. A basal plate is present at the junction between the flagellum and basal body (Figs. 7, 9, 10a, 10b). Two types of anchoring rootlets are attached to the basal bodies. One rootlet is short and projects towards the spore center (Fig. 10b), whereas the other rootlet is quite long and runs along the spore surface (Fig. 10a, 10b). In cross section, the basal bodies resemble pinwheels with projecting rootlets (Figs. 11, 18). A third rootlet is sometimes seen connecting the two basal bodies (Figs. 7, 9). Microtubules radiate outward from the basal bodies and continue around the elongate, beaked portion of the nucleus in the free spore (Figs. 11, 12). These nuclei are elongate structures with strongly beaked or pointed tips (Figs. 11, 12, 13). Occasionally more than one beak may be present (Fig. 14). The overall dimensions of the nucleus are 3.1-4 um by 1.7-2.3 um. Each nucleus usually contains a centrally located nucleolus which is round to slightly elongate (Figs. 13, 14). One to several dictyosomes may be seen close to the nucleus (Fig. 13). Large membrane bound vesicles, .5-.75 um in diameter, are often grouped near the spore surface (Fig. 15). These vesicles, subsequently referred to as fibrous vesicles, have a coarsely granular to fibrous texture which gives them a mesh or net-like

appearance (Fig. 15). A second vesicle type, granular, is present also in the zoospore. These membrane bound vesicles have a finely granular texture and vary in size from .5-1.5 um (Fig. 15). It is often difficult to distinguish the granular vesicles from lipids as the staining qualities of the two are similar. The function of both the granular and fibrous vesicles is not evident in the zoospores and will thus be reviewed in later developmental stages. Numerous vacuoles of variable size are present also in the free spore (Fig. 15).

Other cell components such as mitochondria, endoplasmic reticulum, ribosomes and lipid appear much the same in all stages of the life cycle and will therefore be described fully for only the spore. In longitudinal section, the mitochondria are elongate, sausage-shaped structures with filiform cristae (Figs. 6, 16). In cross section, the mitochondria are round (Figs. 17, 18, 19). Normally, mitochondria measure 1.2-1.5 by .5-.6 um; although some 2 um or longer were observed. Mitochondria in the free spore are quite numerous with 7-15 of them being visible in a single thin section. The endoplasmic reticulum is of the granular type (Fig. 18), and is quite sparse in the zoospore. In addition to those ribosomes attached to the endoplasmic reticulum, the cytoplasm is virtually littered with free ribosomes (Figs. 15, 18) and polysomes (Figs. 15, 18, 28). Lipid droplets in L. callinectes are typically very electron dense and highly variable in size and shape, and are usually greater than 1 um in diameter (Fig. 19).

Spore Encystment

Spore encystment in L. callinectes is a process by which an unwalled, biflagellate spore is transformed into a round, nonflagellate, walled cyst that measures 9-10 um in diameter. Several events in the encystment process are worthy of consideration.

One of the initial steps in encystment is retraction or detachment of the flagella. This process occurs a few seconds after the free spore settles on a substrate. Both flagella are often, although not always, retracted simultaneously. Several methods of flagellar retraction have been observed for both flagella. The methods are as follows: vesicular retraction, straight-in retraction, vesicular detachment and simple casting-off of flagella (Koch, 1968). Of these methods, those of vesicular retraction and detachment are the ones most commonly encountered in L. callinectes. Vesicular retraction as described by Koch (1968) proceeds as follows: Coiling of the axoneme within an inflated area of the flagellar sheath initiates retraction (Figs. 20, 21, 22). Coiling continues until the flagellar vesicle fuses with the spore and the axoneme enters the spore body (Fig. 23). Thin sections of encysting spores of L. callinectes reveal retracted flagella looped or coiled within the spore (Fig. 24). Vesicular detachment, the second method commonly encountered, was described by Ho and Hickman (1967). Their description of the detachment process is quite similar to Koch's description (1968) of retraction. The major difference in the two processes is of course the detachment of the coiled flagella rather than their absorption by the spore. During both retraction and

detachment, vigorous lashing of the flagella was observed and some flagella even continued such movements for a few seconds after detachment.

Following flagellar retraction, or detachment, the oval shape of the free spore is lost as the spore rounds up (Fig. 25). Internally, the nucleus of the spore assumes a spherical configuration (Figs. 24, 26, 28); however, beaked nuclei persist occasionally in encysting spores (Fig. 27). Whorls of endoplasmic reticulum around the nucleus are also evident during encystment (Fig. 28). Finally, the secretion of a wall around the encysting spore involves the fibrous vesicles described earlier to occur in the free spore (Fig. 15). In encysting spores, these vesicles are numerous and clumped near the spore surface (Fig. 29). Secretion of the granular to finely fibrous contents of the vesicles results in formation of the wall (Figs. 30, 31b) by fusion of these vesicles with the plasma membrane of the spore. Vesicles may also fuse with each other at the spore surface resulting in the release of much wall material in a given area (Fig. 30, 31b). The wall secretion process lays down a thin, outer wall which subsequently thickens to .25-.5 um as encystment and wall deposition continue. Special wall vesicles (Fig. 31a) are believed to function in this thickening of the cyst wall. These vesicles will be described fully in the consideration of the germinating spore.

Often present in the nucleus of encysting spores is a nuclear inclusion which closely resembles a compound synaptonemal complex (Figs. 32, 33, 35, 36). These structures, termed polycomplexes, have

been observed on many occasions in encysted spores, but never have they been found in other stages. The presence of the polycomplex in the encysted spore implies that meiosis occurs only at this stage in the life cycle. Usually only one polycomplex is seen per nucleus; however, on occasion two may be seen (Fig. 34). The polycomplex appears to be composed of several synaptonemal complexes in which the lateral elements have fused. The overall size of the polycomplex is variable, generally between 1000 and 4000 Å in width (Figs. 34, 35). Each polycomplex is composed of three subunits: dense lateral elements, a light matrix and a narrow dense band in the matrix (Fig. 36). The lateral elements are dark-staining, rod-like structures which are 250-300 Å wide. In the polycomplex these lateral elements are stacked with a space 400-500 Å wide separating each of them. Centrally located, in the space between each pair of lateral elements, is a narrow dense region, the medial element, approximately 100 Å thick. This medial element is separated from each lateral element by a light staining matrix 150-200 Å thick.

Spore Germination

In sea water, germination begins within one hour after spore encystment. Although bipolar germination has been observed (Fig. 37), normally the process is monopolar with a single germ tube approximately 2-4 um thick developing from each cyst (Figs. 38, 41, 42, 43). As the germ tube emerges from the cyst, cytoplasm fills both the cyst and the short tube (Fig. 41). Next, cytoplasm can be seen filling the tube with the cyst becoming partially empty (Fig. 42). Finally, the cyst

appears completely devoid of internal structures with the cytoplasm having moved into the tip of the long, slender germ tube (Fig. 43). Eventually this tube undergoes profuse branching and an increase in width, thus forming the hyphae.

Electron microscopy of early germination reveals that the emerging germ tube ruptures the cyst wall causing the outer wall layer to fold back (Fig. 44). The inner layer of the cyst wall appears to be continuous with the wall of the young germ tube (Fig. 44). Also, in early germination many vacuoles, some containing dense bodies similar to lipid, are present in the cytoplasm of the cyst and young germ tube. Most of the vacuoles are small and have undergone little fusion with other vacuoles (Fig. 45). As development continues and the contents of the cyst continues moving into the tube, the vacuoles within the cyst can be seen to fuse with one another (Fig. 46). Eventually most of the vacuoles will coalesce to form one or two very large vacuoles which occupy almost the entire cyst (Fig. 47). A thin layer of cytoplasm is left just inside the cyst wall (Fig. 47). The exact mechanism governing the movement of cytoplasm from the cyst into the tube is not known; however, it appears that the process of vacuolar fusion may in some way account for this movement.

New cell components and structural changes in existing components occur in both the germinating cyst and in the germ tube. The new components and structural changes most evident during germination are related to vesicular activity. In the germinating spore four vesicle types are present and all are directly or indirectly related to wall synthesis. The vesicle types present are: granular, filamentous, fibrous and wall.

Granular vesicles, described earlier in the spore, are numerous in germinating spores and hyphae while sparse in other stages. The role that granular vesicles play in wall formation will be reviewed later in this section.

Filamentous vesicles, like granular vesicles, are most numerous in germinating spores and hyphae. The filamentous vesicles are approximately the same size as the granular vesicles and are also membrane bound (Figs. 44, 48). Filamentous vesicles contain long fibers or filaments approximately 100 Å thick (Figs. 44, 48). Cross sections of these filaments appear as dots only slightly larger than ribosomes (Fig. 48). Often the orientation of filaments within a vesicle is such that both their length and width may be observed at once (Fig. 48).

Fibrous vesicles seen earlier in free and encysting spores are also present in germinating spores. Although numerous, they appear to have little direct role in wall deposition during germination, as these large vesicles can no longer be seen fused with the plasma membrane. Some of the fibrous vesicles in germinating spores break apart and release their contents into nearby vacuoles (Fig. 49a, 49b).

The granular, filamentous, and fibrous vesicles all appear to function in secretion of the cyst wall in encysting spores. In this process, it is the sequential change of one vesicle type to another that eventually produces the cyst wall material. First in this sequence of vesicles are the granular vesicles. The fine granular contents of these vesicles condense and give rise to the filamentous vesicles (Figs. 50, 51a). In turn these filamentous vesicles break down to form the fibrous vesicles (Figs. 49b, 50, 51a, 51b) which are directly responsible for laying down the cyst wall (Figs. 30, 31b). This process of vesicular

activity occurs throughout the life cycle of L. callinectes in a manner that insures an abundance of fibrous vesicles in encysting spores. It is probably for this reason that those stages immediately following encystment contain many granular and filamentous vesicles, while those immediately preceeding encystment contain mostly fibrous vesicles.

Present also in germinating spores, especially in the germ tube, are numerous small wall vesicles. These wall vesicles have highly variable shapes and dimensions. Most, however, are rounded (Fig. 52a) with a few being elongate (Fig. 52b). The contents of these wall vesicles are coarsely granular and quite similar in appearance to the material found in the fibrous vesicles. These vesicles line the inner wall of the germinating spore just below the plasma membrane (Fig. 53). A narrow region at the germ tube tip, while being devoid of other organelles, is filled with these wall vesicles (Figs. 53, 54). As well as functioning in cyst wall thickening (Fig. 31a) the wall vesicles are directly responsible for the wall deposition and maintenance that occurs in the germ tube and in the elongating hyphae. Evidence supporting such a function can be seen in figure 53 in which several vesicles may be seen fusing with the plasma membrane, thus allowing their contents to be added to the wall.

The nucleus also undergoes significant change in the germinating spore. As the nucleus migrates from the cyst into the germ tube, it looses its round shape and becomes elongate (Fig. 47). Nucleoli are rarely seen in the germ tube nucleus.

On crab eggs, the infection process begins at the point of germination, when a spore settles on the egg surface and sends a single germ tube through the egg wall (Fig. 39). The contents of the cyst move into the tube with the empty cyst persisting (Fig. 40).

Hyphae

Once branching of the germ tube has occurred, one can consider germination to be completed and that subsequent development will involve the hyphae. The hyphae of L. callinectes are sparsely septate, coarsely granular with many refractive inclusions, and measure 14-23 um in diameter (Fig. 55). The extramatrical hyphae are easily seen on crab eggs with the aid of a light microscope (Figs. 56, 58). When infected eggs are transferred to PYG agar, growth into the agar is easily observable within 12-24 hours. Each point of innoculation forms a colony 5-10 mm in diameter (Fig. 57). The hyphae initially grow out from eggs as long unbranched filaments (Fig. 58). These hyphae however soon undergo branching and the older segment becomes catenulate (Fig. 59).

Intramatrical hyphae may be seen within the crab eggs (Fig. 60). These hyphae often contain thick walled resting bodies (Figs. 61a, b, c). The resting bodies develop within expanded areas of the hyphae, are approximately 25-32 um in diameter, and are extremely variable in appearance. Some resting bodies appear to contain granular material only (Fig. 61a) whereas others appear to contain mostly large refractive droplets (Fig. 61b). Some contain both droplets and granules (Fig. 61c). Germination of these resting bodies has not been observed.

Electron microscopy of young hyphae shows that the cytoplasm contains numerous small vacuoles, (Fig. 62), as does the cytoplasm in germ tubes. However, as the hyphae mature, the vacuoles undergo fusion, as in the spore cyst, eventually forming one or two large vacuoles which occupy much of the space within the hyphae (Fig. 63).

Few apparent differences exist between hyphal and germ tube structure; however, some minor differences can be seen in vesicular activity. Granular vesicles and filamentous vesicles are more numerous in hyphae than in germ tubes (Figs. 63, 64a, 64b). However, fewer wall vesicles are present in hyphae than in germ tubes.

The elongate, slender nucleus found in germinating material is rarely present in hyphae. Here, the nuclei are again becoming rounded (Fig. 63). Also, the hyphae are multinucleate (Fig. 63) as opposed to the germ tube which is initially uninucleate.

Electron microscope observation of mature L. callinectes hyphae reveals the presence of a heavy cell wall, .25-.5 um thick (Fig. 65a). This wall appears granular to finely fibrous and may consist of a single layer (Fig. 65a), or three layers (Fig. 65b). Those walls consisting of three layers have a dark, dense, central layer between two lighter layers (Fig. 65b). Quite possibly the age of the wall or fixation conditions may affect its appearance.

Sporogenesis

The production of spores, sporogenesis, may be easily induced in L. callinectes by placing hyphae from a 5-7 day old broth culture into sterile sea water. Spore production begins within 12-15 hours and may

last for up to 48 hours. The sporogenic process consists of three stages: discharge of the cytoplasm from the discharge tube into a vesicle, cleavage of the spores within the vesicle, and release of the spores.

With the light microscope, those hyphae which will function as discharge tubes may be recognized by the dense accumulation of cytoplasm within them and by the hyaline appearance of their tips (Fig. 66). In addition, these hyphae are generally unbranched and septum delineated. As discharge begins, cytoplasm flows towards the hyphal tip and expansion of this tip forms an amorphous, gelatinous vesicle which encases the cytoplasm (Figs. 67, 68, 69, 70a). The cytoplasm does not move through the discharge tube as one unit, but rather in small distinct units which are connected by a thread (Figs 69, 70b-c). As a unit of cytoplasm enters the vesicle, it remains attached to the succeeding unit by this thread, and apparently pulls this unit into the vesicle (Figs. 70d-e). Upon entering the vesicle each unit fuses with the cytoplasm already present, eventually forming a rough, spherical mass (Figs. 70f-g).

Light microscopy shows that cleavage of the cytoplasm may begin before all of the sporogenic units have migrated into the vesicle (Fig. 71). In this situation, a thread still persists between the cleaving units and those units still in the sporangium (Fig. 71). Once all of the cytoplasm in the sporangium enters the vesicle, the thread snaps (Fig. 70i). Cleavage is generally completed 7-10 minutes after spore-like units first become visible within the vesicle (Fig. 70h). Following complete cleavage, the spores swim rapidly within the vesicle,

with discharge of the spores occurring 3-10 minutes later (Fig. 70k). After discharge, the vesicle persists around the tip of the empty discharge tube (Fig. 701).

Electron microscopy of the connecting thread shows it to be simply a continuation of the cytoplasm (Fig. 72). Membranes running lengthwise in the thread are believed to be the most important connecting agents (Fig. 73). Also, electron microscope observation of the discharge units shows that flagella may be present on these units prior to their migration into the cleavage vesicle (Fig. 74).

Electron microscope observations of cleavage show that the cleavage process may be divided into four phases: early, mid, late and final. In the sporogenic cytoplasm of all of these stages granular and fibrous vesicles are still present; however, filamentous vesicles are no longer seen (Figs. 75, 78, 79, 80). Small, wall-secreting vesicles seen in hyphae and germinating material also appear to be absent. In the early stage of spore cleavage, many small vacuoles, some of which contain dense bodies, are present in the sporogenic cytoplasm with only a few vacuoles having fused. Nuclei, both round and beaked, are present in early and mid cleavage with the basal bodies usually located near a beaked nuclear tip (Fig. 78). Flagella in the early phase are enclosed within cavities or spaces in the sporogenic cytoplasm (Fig. 76). Small, rod-shaped, membrane bound vesicles which contain microtubules are also commonly visible during early cleavage (Figs. 77a, 77b). These vesicles measure 1.4 x .2 um with the enclosed microtubules being about .15 nm in diameter. They

have been seen in zoospores, cysts, and germinating spores, but are more abundant during cleavage. These vesicles are believed to function in flimmer development in later stages.

In the second or mid cleavage stage, cleavage furrows or vacuoles become recognizable (Fig. 78). These furrows result from the fusion of many of the smaller vacuoles. Although no definite patterns have been found, vacuolar fusion seems to occur such that most of the remaining cytoplasm becomes peripheral rather than centric. In this phase, cleavage has progressed to the point that uninucleate cytoplasmic masses are becoming recognizable (Fig. 78). The disposition of the nuclei in these masses is such that the beaked portions, associated with the basal bodies and flagella, are near an exposed surface. Generally this results in a nuclear projection towards the gelatinous retaining vesicle (Fig. 78).

The late cleavage phase shows continued vacuolar fusion resulting in enlargement of the furrows. Distinct cytoplasmic units are now clearly recognizable (Fig. 79). These units are almost separated and are joined only by narrow cytoplasmic bridges (Fig. 79).

The final stage is one in which the spores are completely separated and swimming freely within the gelatinous vesicle (Fig. 80). During this stage, the mastigonemes become visible on the anterior flagellum (Figs. 81a, b). These tinsels, or mastigonemes, appear to develop from the microtubules found in the rod-shaped vesicles, flimmer packets, visible during the earlier stages (Figs 77a, b). Although the exact mechanism of flimmer development is not known,

it is believed that the tinsel packets move through the cytoplasm and aggregate around the anterior flagellum (Fig. 82). The occurrence of these tinsels only on free flagella further suggests that the actual attachment is extracellular. The tinsels appear to attach directly to axoneme of the flagellum (Fig. 81a).

DISCUSSION

Vesicular Activity

Germ Tube and Hyphal Wall Formation:

Numerous workers have recently suggested that the addition of wall material to the cell wall in fungal hyphae is accomplished by vesicles that fuse with the plasma membrane and release their contents to the overlying wall region (Hemmes and Hohl, 1971; Heath, Gay and Greenwood, 1971; Grove and Bracker, 1970). Rizvi and Robertson (1965) and Marchant and Smith (1968) established the hyphal apex as the main site for the "addition of material to fungal walls." Such a point of addition is in agreement with recent works which show hyphal apices almost completely filled with vesicles thought to function in wall deposition (Grove and Bracker, 1970; Heath, Gay and Greenwood, 1971). In L. callinectes, wall deposition and maintenance in hyphae and germ tubes appears also to involve small vesicles almost identical to those described by other workers (Grove and Bracker, 1970; Heath, Gay and Greenwood, 1971). The apical concentration of the vesicles found in L. callinectes corresponds closely to the results of these authors. Questions have arisen as to the origin of wall vesicles in the oomycetes. Some workers feel that the vesicles are of golgi origin (Grove and Bracker, 1970), while others (Heath, Gay and Greenwood, 1971) suggest that the origin may be elsewhere in the endomembrane system. In L. callinectes evidence can be seen for both golgi origin (figure 53) and for origin in the endoplasmic reticulum (figure 52b).

Cyst Wall Formation:

Figures 30 and 31b indicate that deposition of the outer portion of the cyst wall in L. callinectes occurs via the fibrous vesicles which fuse with the plasma membrane and release their contents to the outside. Grove (1970) described a similar process in Pythium aphanidermatum (Edson) Fitzpatrick. Although the role of fibrous vesicles in subsequent cyst wall development in L. callinectes is not known, figure 31a shows that wall vesicles apparently play a role in this thickening. Such a two step process is similar to that described by Hemmes and Hohl (1971) for Phytophthora parasitica Dast. In P. parasitica, it was shown that the initial cyst wall formed from flattened vesicles and fibrillar vacuoles and that maturation of the wall involved dictyosome derived vesicles.

Although the encystment process in L. callinectes involves the formation of a thin cyst wall by the fibrous vesicles with subsequent thickening of this wall, it should not be overlooked that much vesicular activity preceeds these final steps. The results of the present study suggest that fibrous vesicles are formed as a result of the breakdown of filamentous vesicles, and filamentous vesicles are themselves formed from granular vesicles. Thus the vesicular acitivity involved in encystment proceeds from granular vesicles to filamentous vesicles to fibrous vesicles. The presence of numerous fibrous vesicles in encysting spores coupled with the scarcity of granular vesicles and filamentous vesicles at this stage provides some evidence supporting this concept. Further evidence may be found in the reappearance of numerous granular and filamentous vesicles in germinating spores and hyphae, the stages immediately following encystment.

To the knowledge of the author, no similar process of vesicular interaction has been reported in other fungi. However, the occurrence of individual vesicles similar to those involved in the encystment process in L. callinectes has been reported by various workers. The fibrillar vacuoles reported by Hemmes and Hohl (1971) in P. parasitica are similar in both size and texture to the granular vesicles in L. callinectes. Hohl and Hamamoto (1967) reported fiber containing vesicles in P. parasitica similar to the filamentous vesicles found in L. callinectes. Reichle (1969) described vesicles in P. parasitica var. nicotania B. de Haan that appear to be very similar to the fibrous vesicles of L. callinectes in both size and content. Reichle further noted that these vesicles were usually found close to the plasma membrane.

Polycomplex

Moses (1956) first described the appearance of chromosomal cores in crayfish spermatocytes. In a later study, Moses (1958) termed these cores synaptonemal complexes. He described these structures as linear complexes consisting of three parallel strands. The outermost strands, lateral elements, were thick, dense structures with the innermost strand, central element, being thinner and less dense. These synaptonemal complexes were shown to be part of the bivalent chromosome thus relating them to synapsis. Since that time, the occurrence of compound synaptonemal-like structures has been reported (Roth, 1966; Jaworska and Lima-De-Faria, 1969; and Moens and Rapport, 1971). Those described by Roth, and Jaworska and Lima-De-Faria are quite similar

to the polycomplexes found in encysting spores of L. callinectes. Roth found polycomplexes to be numerous in the oocytes of the mosquito Aedes aegypti L. during meiotic prophase. In addition, a few polycomplexes were found in accompanying nurse cells. Roth proposed that the polycomplex was composed of numerous synaptonemal complexes that had released their chromatin material and fused laterally. This fusion occurred in such a manner as to form a sheet of joined synaptonemal complexes. Roth further pointed out that such a process requires that the lateral elements which fuse be only half as thick as those in a single synaptonemal complex. He proposed that removal of half of the lateral component with the chromatin accounts for the fact that the fused lateral elements of the polycomplex have the same 40 nm width as those in a single synaptonemal complex in A. aegypti. The bipartite nature of the lateral elements in polycomplexes of L. callinectes as seen in figure 36 supports the work of Roth (1966). The overall size of the polycomplexes observed by Roth (1966) were generally larger than those seen in L. callinectes; however, differences in chromosome number and the angle of section could lead one to expect different overall sizes. A single complex, measured from the outer edge of one lateral component ot the outer edge of another lateral component, from the Aedes polycomplex was about 160nm thick while a similar complex in L. callinectes was 100 nm thick. Most of this difference is seen in the thickness of the medial element.

Jaworska and Lima-De-Faria (1969) found polycomplexes almost identical to those described by Roth to be present in the oocytes of

Acheta domesticus (house cricket) during interphase and prophase of meiosis. In Acheta, the polycomplexes were associated with a heterochromatic DNA body. Lima-De-Faria, Brinsteil and Jaworska (1969) showed that gene amplification resulting in the production of ribosomal cistrons occured in this heterochromatic body in Acheta. Jaworska and Lima-De-Faria (1969) believed the polycomplexes to be in some way related to this gene amplification; however, no exact relation was given. Despite the finding of Jaworska and Lima-De-Faria no generalization as to the function of polycomplexes in L. callinectes can be made as heterochromatic bodies, as seen by these workers, apparently are not present in L. callinectes.

Although proposals have been made for alternative sites, it is generally believed that the synaptonemal complex exists between paired chromosomal homologs during meiotic prophase (Moses, 1958; Roth, 1966; and Comings and Okada, 1971). Thus, the finding of synaptonemal complexes can be considered as being indicative of meiosis. In L. callinectes single synaptonemal complexes have not been found; however, polycomplexes are present. This author, as well as others (Roth, 1966; Jaworska and Lima-De-Faria, 1969) believes that the polycomplexes are in fact multiple synaptonemal complexes. Thus the polycomplexes may be considered as valid indicators of meiosis. It should be pointed out that the polycomplexes observed by Roth and Jaworska and Lima-De-Faria were observed in cells undergoing meiosis. Since in L. callinectes the polycomplexes are observed in encysting spores, this author feels justified in assuming that meiosis occurs at this point. This is indicated in the tentative life cycle presented in figure 1.

Cytoplasmic Discharge and Cleavage

Cytoplasmic discharge in L. callinectes is accomplished by the sequential movement of discrete cytoplasmic units out of the sporangium or discharge tube and into a gelatinous cleavage vesicle. Bland and Amerson (1972) pointed out that this discharge method is similar to that described by Couch (1924) for Aphanomyces and Leptolegnia. In some species of the organisms described by Couch (1924) and in L. callinectes the discharge units are connected by threads. Couch (1924) reported these threads in Leptolegnia to correspond to cilia; however, electron microscopy of the thread in L. callinectes revealed no special structure, with the connecting thread being simply a continuation of the cytoplasm.

Light microscopy of the sporogenic cytoplasm revealed that cleavage in L. callinectes may begin within the vesicles before all of the cytoplasmic units have entered. Such a process often resulted in incomplete cleavage and release of some multi-flagellate masses of cytoplasm. An examination of cleavage with the electron microscope revealed that the sporogenic cytoplasm was divided into uninucleate spores as a result of vacuolar fusion involving both clear vacuoles and vacuoles containing dense bodies. The dense bodies in some of the vacuoles appeared similar to lipid bodies, and may be simply small particles of lipid that have been engulfed. Gay, Greenwood and Heath (1971) showed that similar dense bodies in Saprolegnia contained a high percentage of the polar lipid phosphatidal choline.

Cleavage in L. callinectes is not unlike that described by Gay and Greenwood (1966) for the zoospores of Saprolegnia ferax

(Gruithuisen) Thuret. In this organism cleavage of the cytoplasm was accomplished by the fusion of large vesicles, thought to develop from vesicles containing dense bodies, which eventually formed cleavage furrows and a central vacuole. The cleavage furrows radiated out from this central vacuole toward the plasma membrane dividing the cytoplasm into individual spores which were located peripherally. A similar process was described by Hohl and Hamamoto (1967) for sporangial cleavage in Phytophthora parasitica. In this organism, cleavage was accomplished by the fusion of cleavage vesicles which lined up between neighboring nuclei. Fusion of these cleavage vesicles with a central vacuole or vesicle and peripheral vesicles near the sporangial wall delimited uninucleate spores.

While considering cleavage in L. callinectes, some attention should be given to the rod-shaped microtubule packets first seen in early cleavage. These microtubules have the same 15-20 nm diameter as the flagellar mastigonemes and the packets may be seen clustered around certain flagella during cleavage. It is thus believed that these microtubule packets later become the flagellar mastigonemes. Similar processes involving similar or identical packets of microtubules have been reported in many other fungi and algae (Bouck, 1969; Deason, 1971; Heath, Greenwood and Griffiths, 1970; Leedale, Leadbeater and Massalski, 1970; and Fuller and Reichle, 1965). With the exception of Fuller and Reichle who drew no conclusion, all of the other authors have concluded that flagellar mastigonemes are formed from packets of microtubules. The origin of the tubules in L. callinectes is presently unknown, however the works of Bouck (1969); Heath, Greenwood and Griffiths (1970); Deason (1971); and Leedale, Leadbeater and

Massalski (1970) all indicate the nuclear envelope and endoplasmic reticulum as the points of origin for the microtubule packets in the organisms they observed. The packets seen in L. callinectes may have a similar origin. It is believed that attachment of the microtubule (flimmer) packets to the flagella in L. callinectes occurs extracellularly and directly to the axoneme. Bouck (1969) proposed an extracellular attachment process in which the packets were released from the cell and attached at certain sites on the flagellar sheath. The work of Heath, Greenwood and Griffiths (1970) also supports attachment of the mastigonemes to the sheath. However, Deason (1970) presents evidence for a method of intracellular attachment directly to the axoneme. L. callinectes semmingly has characteristics of both methods described and thus does not completely fit any mechanism thus far proposed. Quite possibly the methods of attachment could vary with the species studied.

Zoospore Structure

Couch (1942) described the zoospores of L. callinectes as being anteriorly pointed, posteriorly rounded and possessing a diagonal groove from which the cilia arise. These spores measure 9.6 x 12.6 um. This description is in close agreement with the findings of Johnson and Bonner (1960) and with the present work. In the present work, however, some additional characteristics not mentioned by Couch or Johnson and Bonner were found. First, the free swimming zoospores of L. callinectes occasionally possessed flagellar beads similar to those described by Ho, Zachariah and Hickman (1967) in Phytophthora megaspermae var. sojae Hildebrand. Secondly, vesicular retraction of

the flagella as described by Koch (1968) was commonly observed in encysting spores. Also, the zoospores of L. callinectes were observed in this study to round-up during encystment. This finding differs from observations made by Couch (1942) in which he described encystment as occurring without rounding-up of the zoospore. The nucleus of the free swimming spore of L. callinectes was found in all cases to have at least one elongate beak. This beak projected toward the nearby basal bodies. Microtubules radiating from these bodies could be seen quite close to the nuclear envelope. Heath and Greenwood (1970) have reported similar nuclear beaks in Saprolegnia and have suggested that the microtubules close to the nuclear envelope could be responsible for the beaked shape of the nucleus. Each flagellum in L. callinectes is anchored by a single basal body with the basal bodies being so positioned as to form a 120° angle. The structure of the basal bodies appears quite typical. The structure of the rootlets, anchoring structures of the basal bodies, appears to be very similar to that described by Reichle (1969) for the zoospores of Phytophthora parasitica. In L. callinectes, as in Phytophthora, each basal body was found to have one short rootlet and a long rootlet which ran along the spore surface slightly under the plasma membrane as well as a rootlet connecting the basal bodies.

Spore Germination

L. callinectes spores in sea water were shown to be capable of germination within one hour of encystment. Germination normally is monopolar with almost all of the encysted spores germinating. This finding differs somewhat from the finding of Couch (1942) as he

reported failure of the zoospores to germinate unless encystment occurred on crab eggs.

Light microscope observations reveal that as germination continues the cytoplasm flows out of the cyst and into the germ tube, eventually moving to the tube tip and leaving part of the germ tube empty. The process responsible for emptying the cyst will be discussed later. The failure of L. callinectes to manufacture enough cytoplasm to fill the germ tube is presumably due to the low nutrient level in the sea water.

Electron microscopy of the zoospore reveals that considerable activity and change occurs during germination. Granular and filamentous vesicles are numerous in germinating spores as opposed to their scarcity or absence in free or encysting spores. In germinating spores these vesicles seemingly are actively undergoing conversion processes. Fibrous vesicles are also numerous, and some of these may be seen emptying into vacuoles (Figures 49a and b). As the young germ tube emerges from the spore cyst, the outer layer of the cyst wall is ruptured and folded back. The inner layer of the cyst wall apparently is continuous with the wall of the newly forming tube. This is consistent with the recent findings of McCully and Bracker (1972) in germinating bud cells of heterobasidiomycetous yeasts. In L. callinectes elongation of the germ tube continues with the cytoplasm moving from the cyst into the tube. A process of vacuolar fusion, involving both clear vacuoles and vacuoles containing dense bodies, is thought to be responsible for the formation of a few larger vacuoles from the smaller ones. It is believed that as this fusion occurs the cyst cytoplasm is actually pushed into the tube by the enlarging vacuoles.

Although accounts of similar processes have not been found, Gay, Greenwood and Heath (1971) do show that the fusion of small dense-bodied vesicles in Saprolegnia ferax results in the formation of large vacuoles in germinating spores. Also, Hawker and Abbott (1963) revealed that in germinating Rhizopus sporangiospores the cytoplasm flowed into the germ tube with vacuoles developing in the most distal regions of the cyst.

In germinating spores nuclear shape varies from round to elongate. Generally the elongate structure is seen as the nucleus migrates into the tube. Although mitosis was not observed in the germ tube, presumably nuclear division does occur during germination or shortly thereafter as the very young hyphae were shown to be multinucleate. The extreme tip of the germ tube was found to be composed of small wall vesicles having an organization very similar to that described by Grove and Bracker (1970) for Pythium aphanidermatum and for other oomycetes.

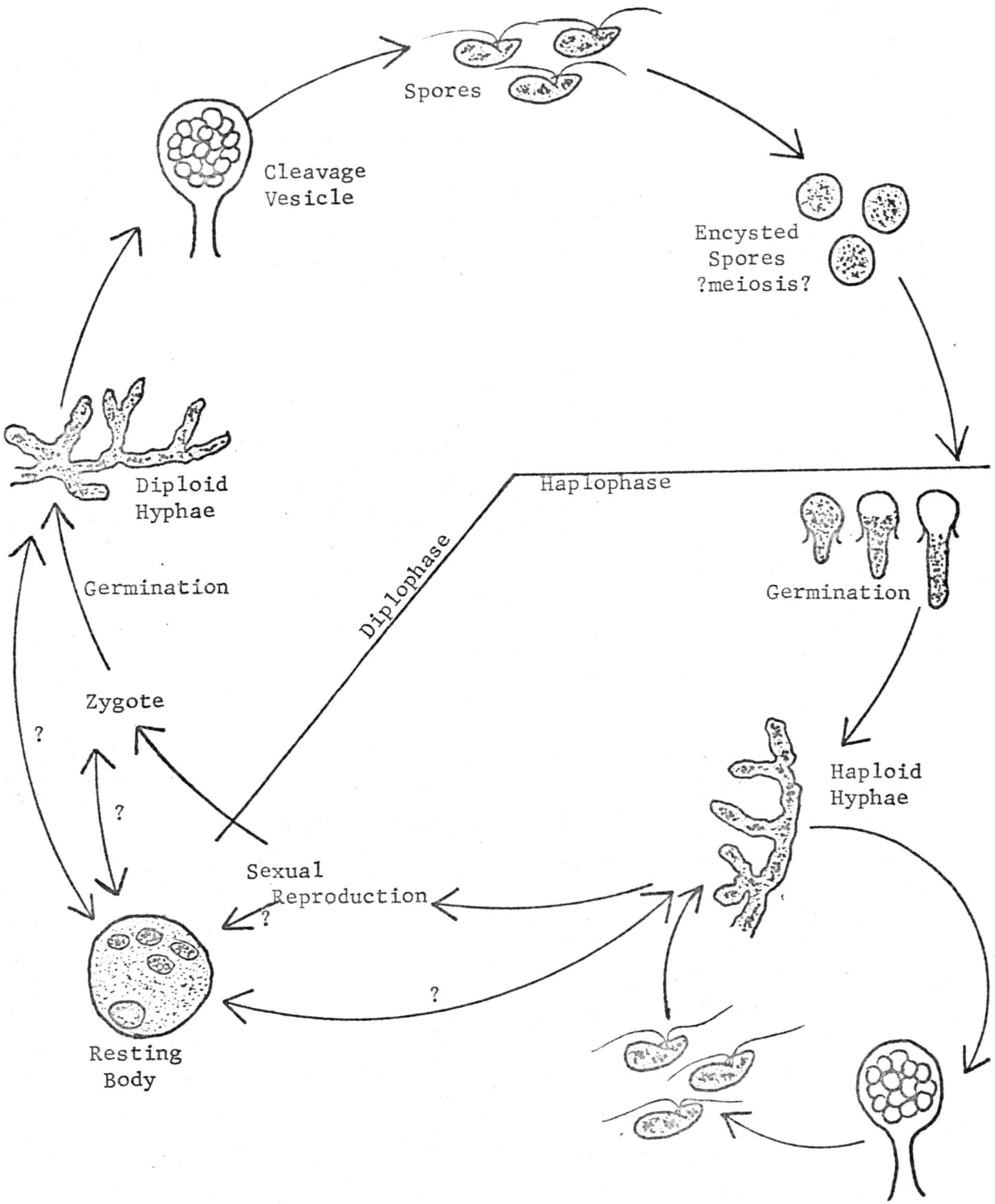


Figure 1. Proposed life history diagram for Lagenidium callinectes. Note the point of meiosis, the presence of haploid and diploid hyphae, and the uncertain position occupied by resting bodies.

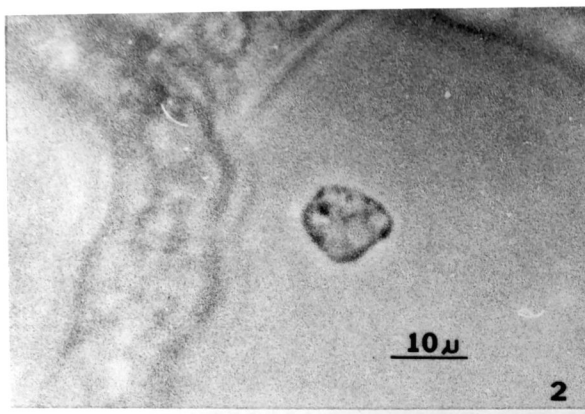
Figure 2. Light micrograph of Lagenidium callinectes spore showing highly irregular shape immediately after emergence from the cleavage vesicle. 960 X.

Figure 3. Oval shaped zoospore showing extended flagella, anterior tinsel and posterior whiplash. 1,300 X.

Figure 4. Zoospore showing the presence of flagellar beads. 1,560 X.

Figure 5. Zoospore showing longitudinal groove along one side of the spore. 2,400 X.

Figure 6. Electron micrograph showing irregular zoospore surface and underlying vesicles and vacuoles. 23,375 X.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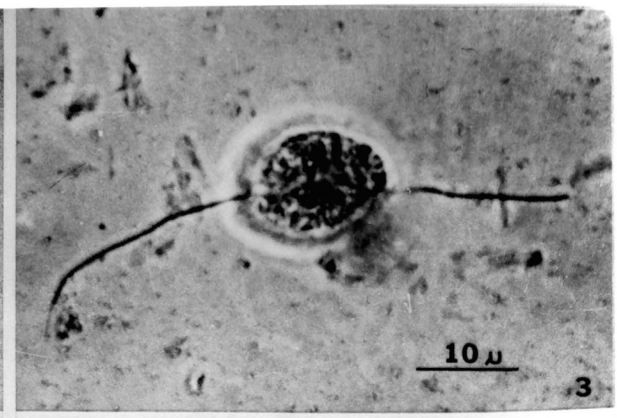
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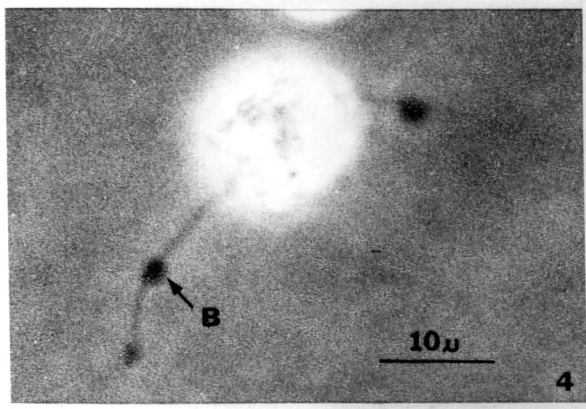
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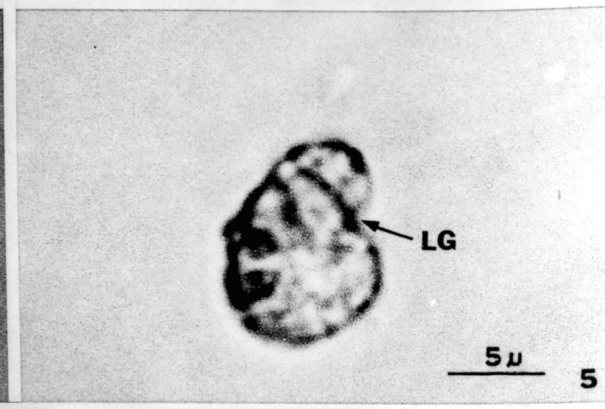
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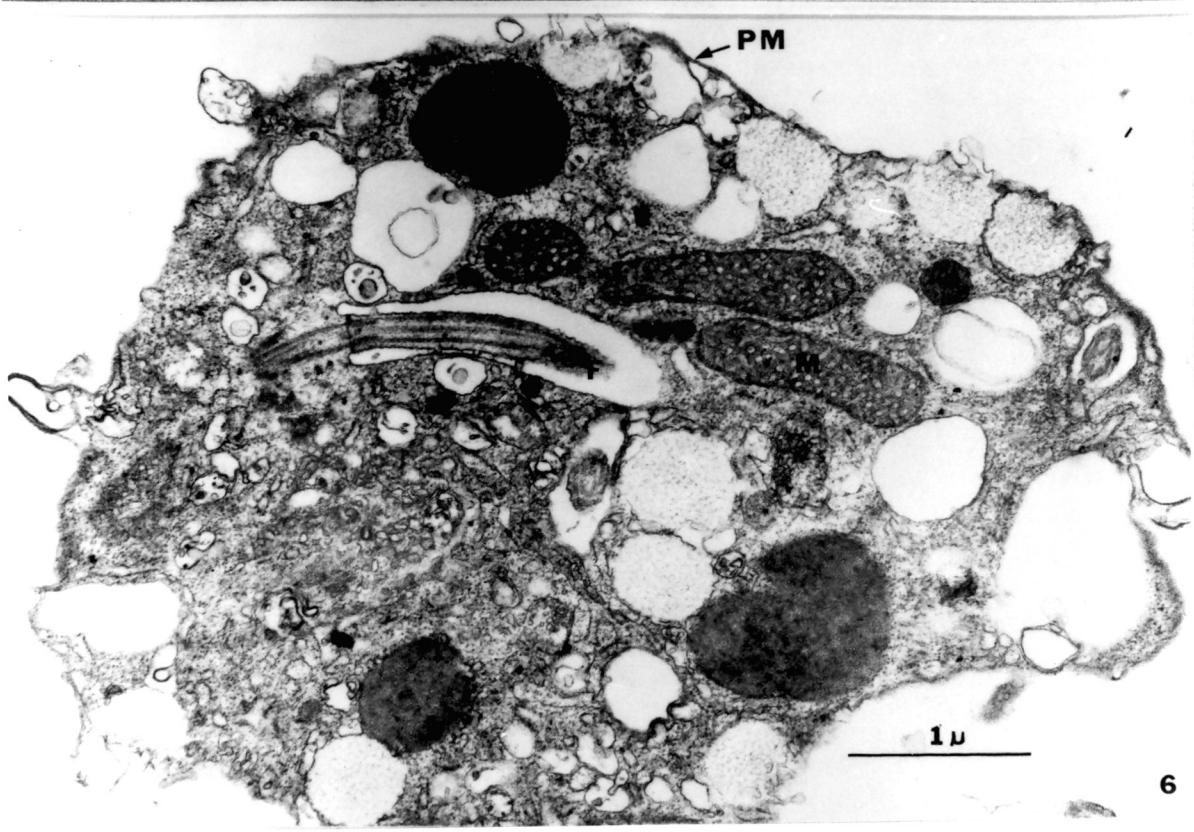
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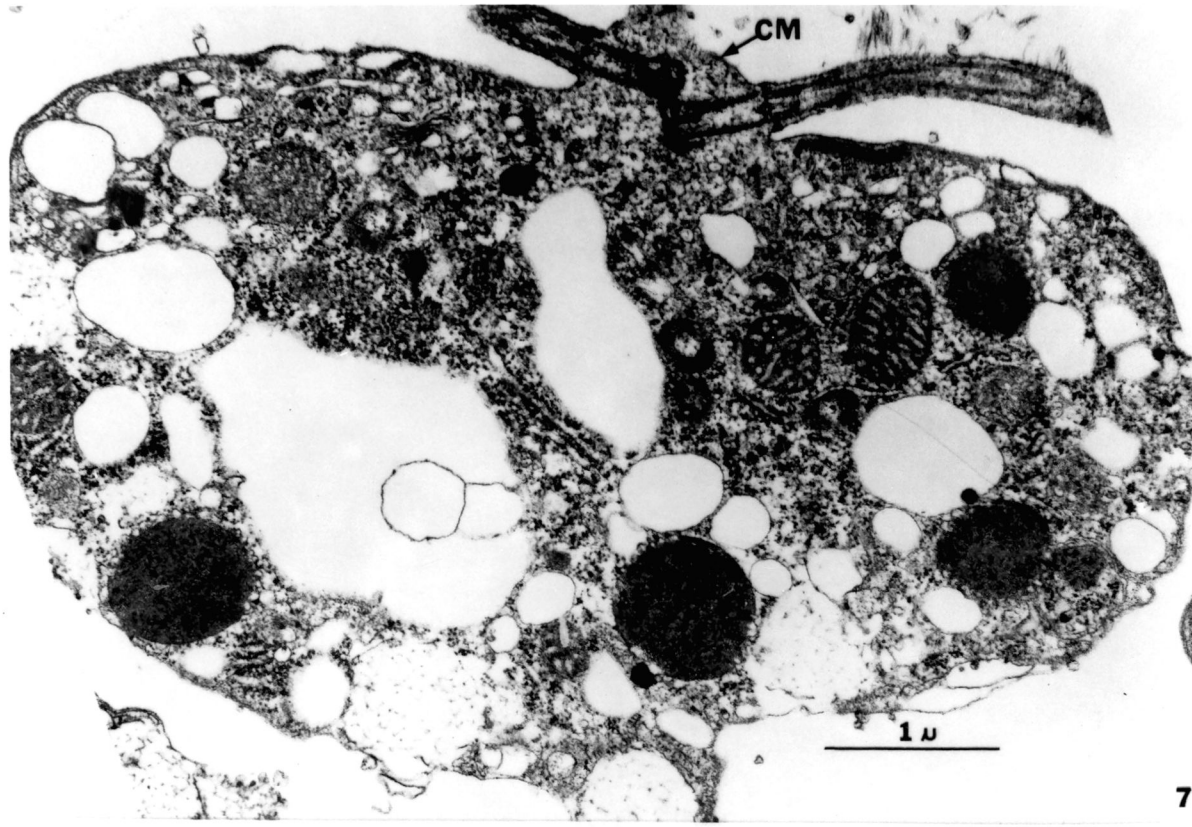
Figure 7. Cross section of zoospore showing shallow longitudinal groove with flagella arising from a raised area in the groove. 22,310 X.

Figure 8. Cross section of a flagellum. Note 9+2 arrangement. 213,125 X.

Figure 9. Longitudinal section of basal bodies, tinsel flagellum and whiplash flagellum. Note basal plate (solid arrow) and rootlet connecting basal bodies (arrowhead). 36,750 X.



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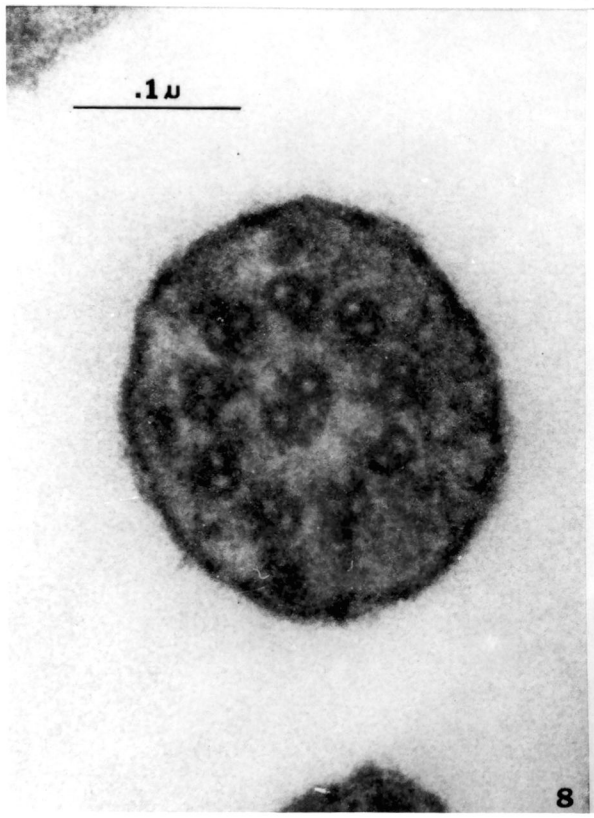

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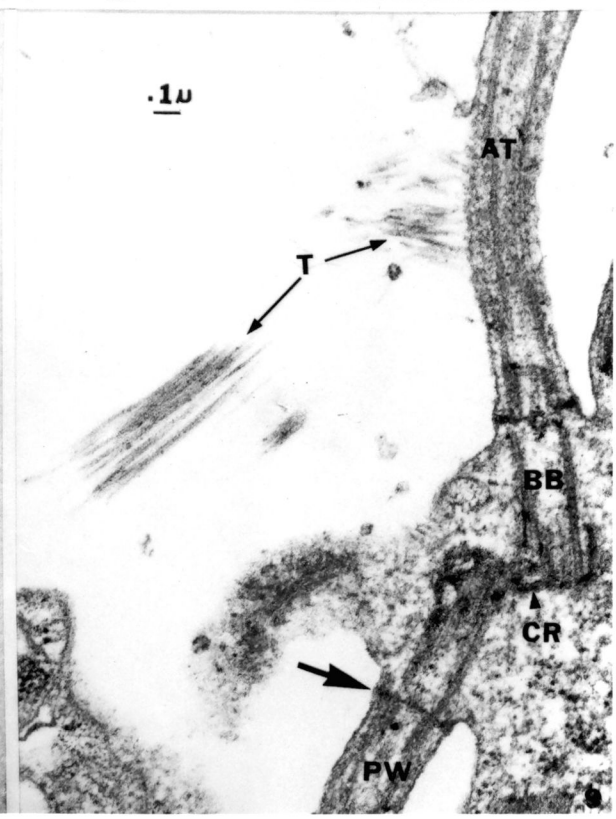

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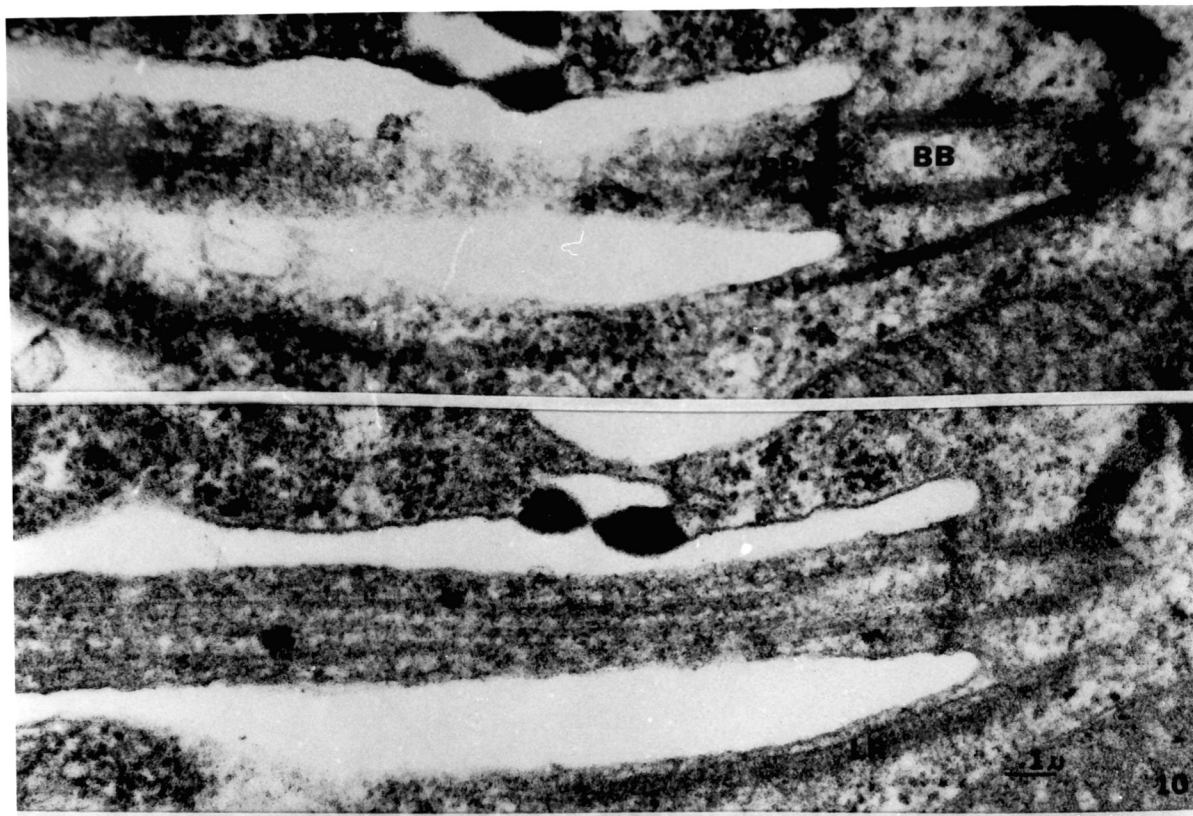
Figure 10a & b. Serial longitudinal sections through a flagellum and basal body. Note short rootlet and the long rootlet near the spore surface. 63,000 X.

Figure 11. Cross section of basal body showing projecting rootlets. Also note beaked nucleus. 47,250 X.

Figure 12. Electron micrograph showing a beaked nucleus and a glancing section of a basal body with microtubules radiating outward. 47,250 X.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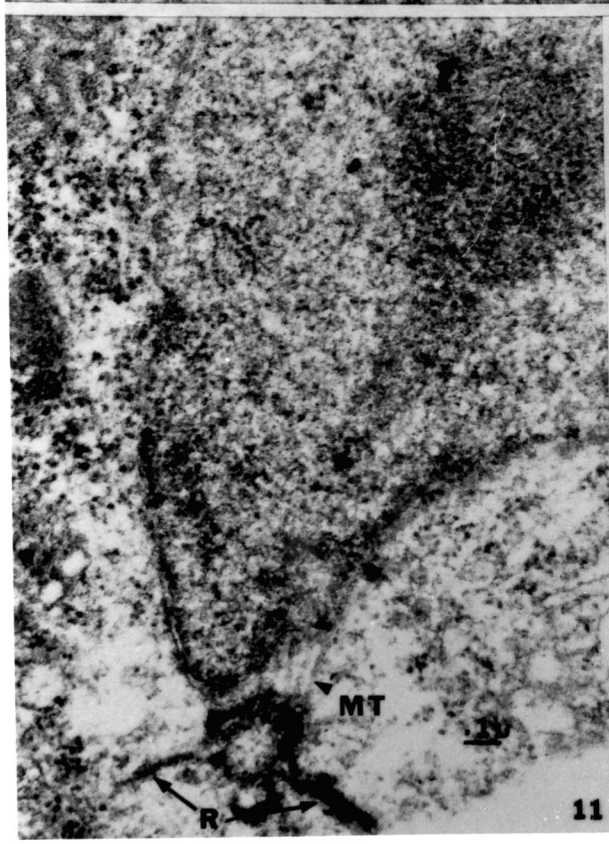
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Figure 13. Strongly beaked nucleus with dictyosome nearby. 34,000 X.

Figure 14. Double beaked nucleus. Arrows indicate beaks. 36,000 X.

Figure 15. Section through a zoospore showing fibrous vesicles near the spore surface. Also note the large, dark staining granular vesicles. 21,600 X.

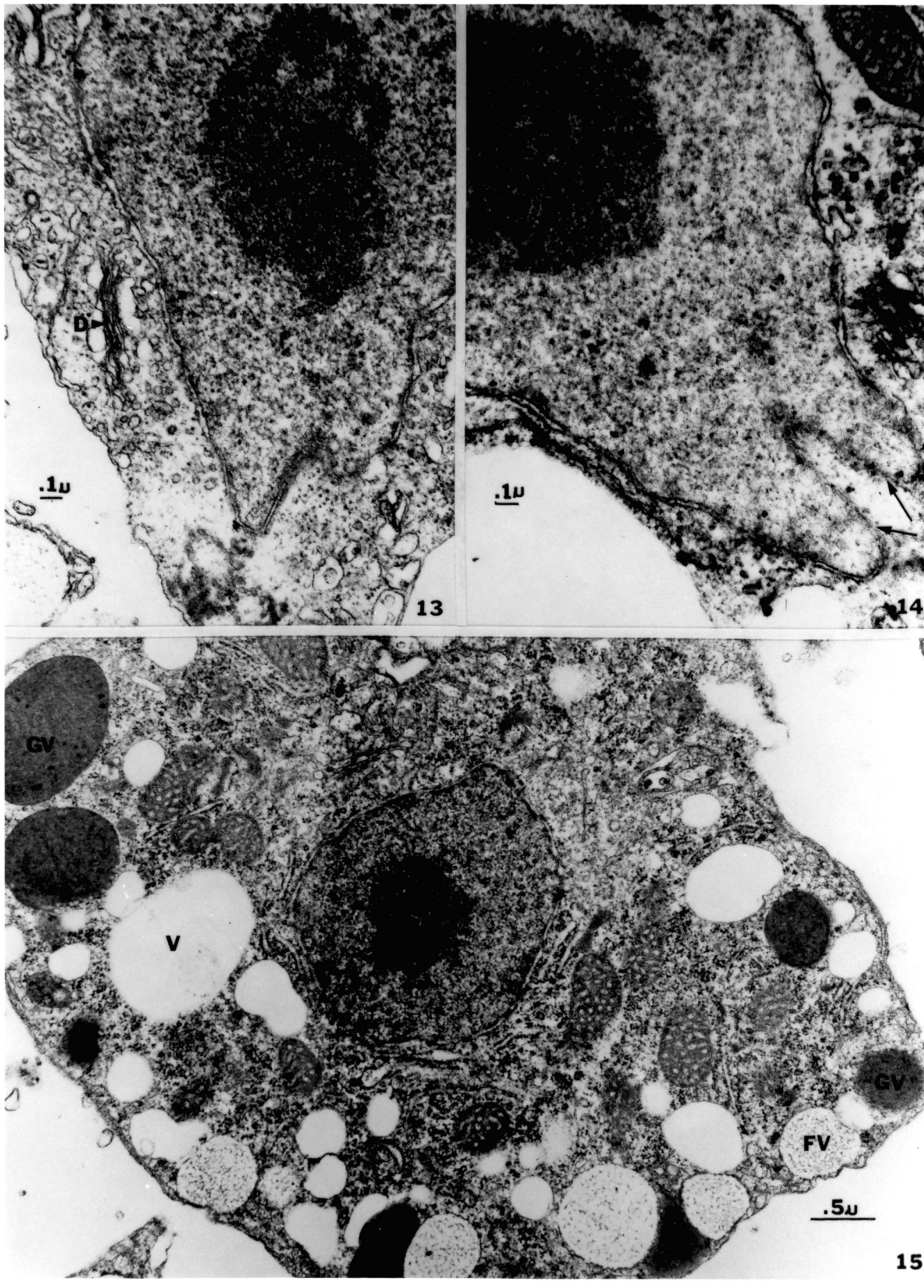
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Figure 16. Electron micrograph showing mitochondria in longitudinal section. 17,875 X.

Figure 17. Mitochondrial cross sections. 17,875 X.

Figure 18. Electron micrograph showing endoplasmic reticulum heavily studded with ribosomes. Polysomes are also present. 40,500 X.

Figure 19. Section through a zoospore showing very dense lipid droplets. 22,000 X.

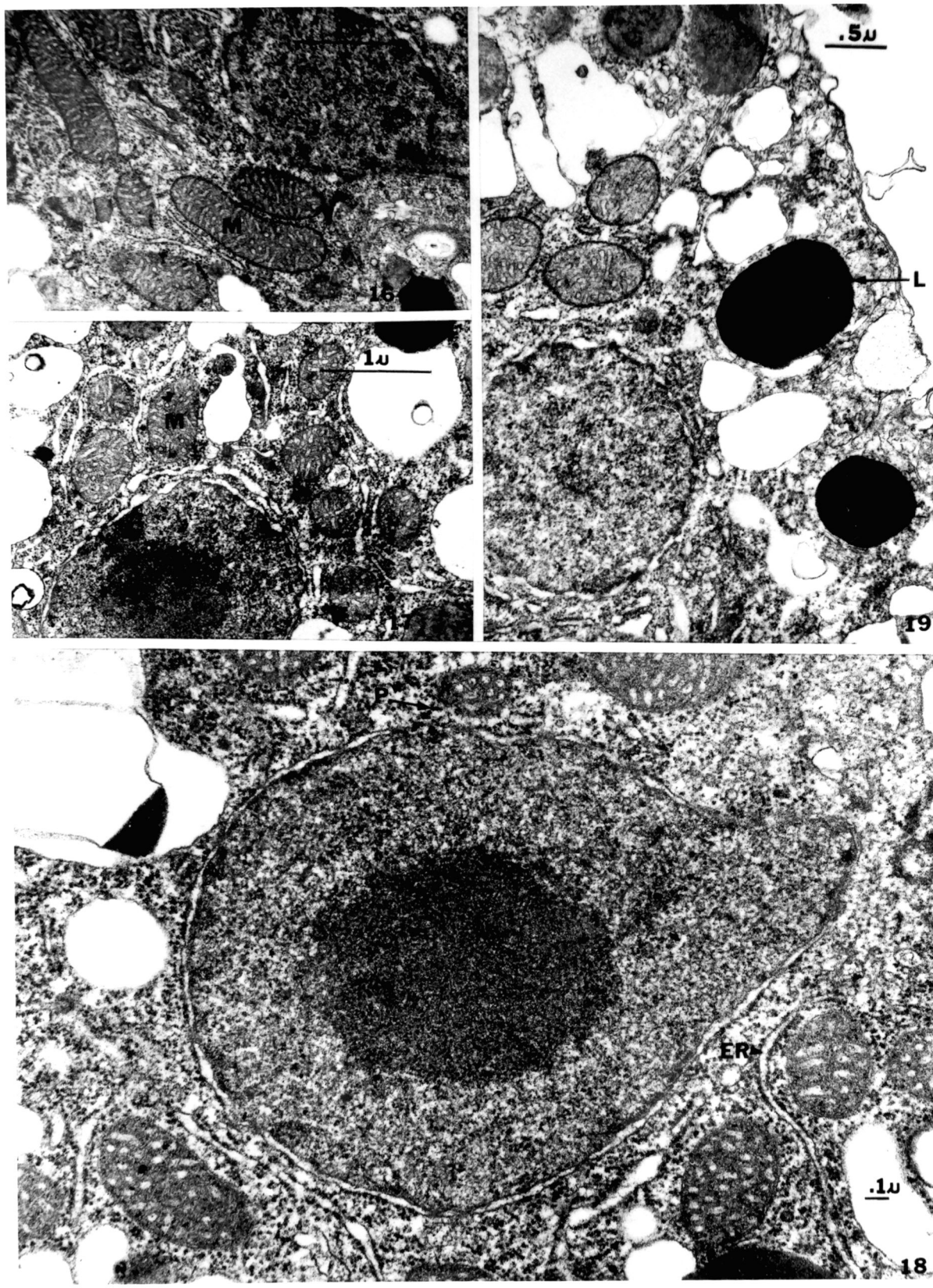
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Figure 20. Whole mount of zoospore showing retracting posterior, whiplash flagellum. Note axoneme coiling in expanded flagellar sheath. 4,725 X.

Figure 21. Anterior tinsel flagellum coiling within flagellar sheath. Note mastigonemes (tinsels). 4,990 X.

Figure 22. Spore retracting both flagella. Note axoneme in flagellar vesicle (arrow). 1,600 X.

Figure 23. Light micrograph showing the flagellar vesicles close to the spore body. 1,500 X.

Figure 24. Electron micrograph of spore with retracted flagellum inside. 24,750 X.

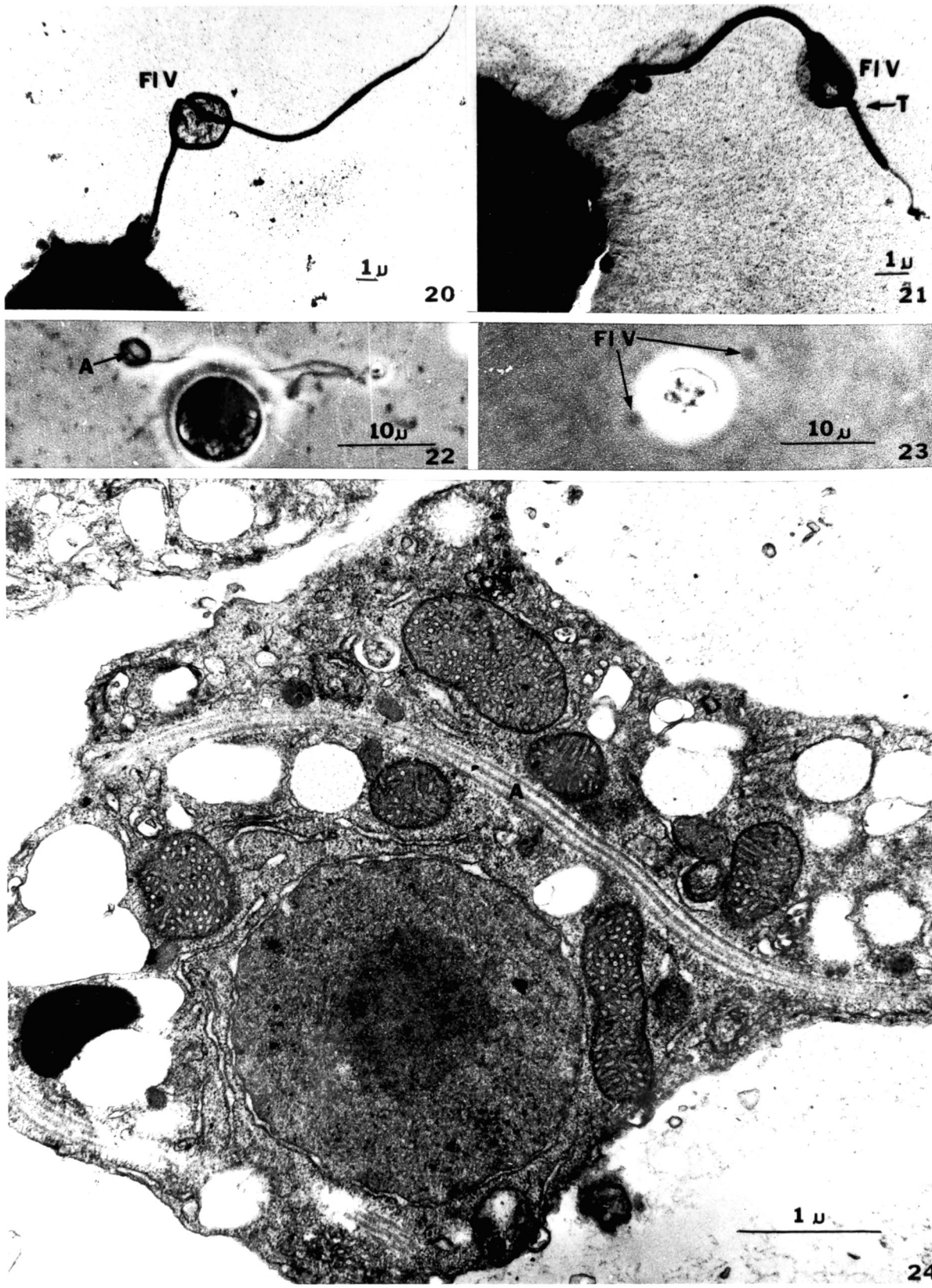
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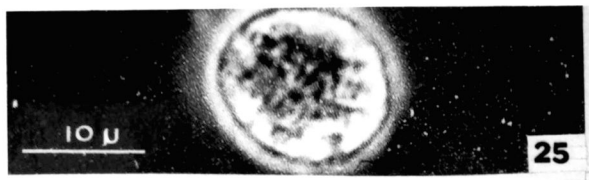
Figure 25. Light micrograph showing encysting spore that has become round. 1,500 X.

Figure 26. Spherical nucleus in an encysting spore. 26,400 X.

Figure 27. Encysting spore that still has a beaked nucleus. Note basal body at the tip of the beak. 40,500 X.

Figure 28. Spherical nucleus in an encysting spore surrounded by whorls of granular endoplasmic reticulum. 29,250 X.

Figure 29. Low magnification micrograph of an encysting spore showing fibrous vesicles near the spore surface. 11,900 X.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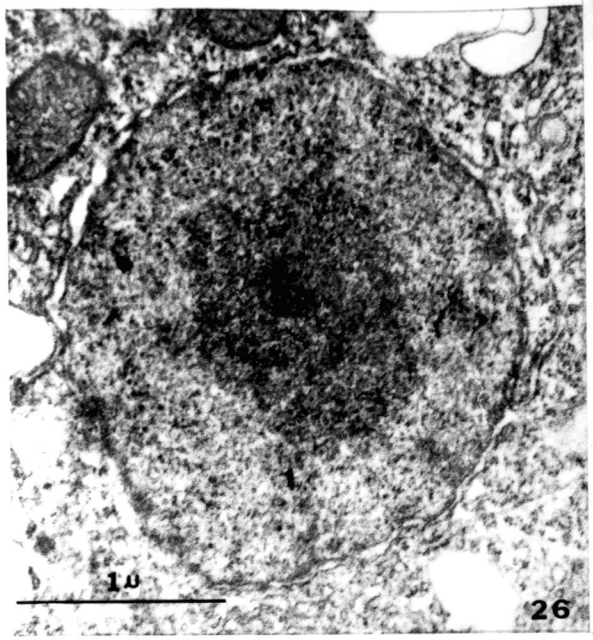
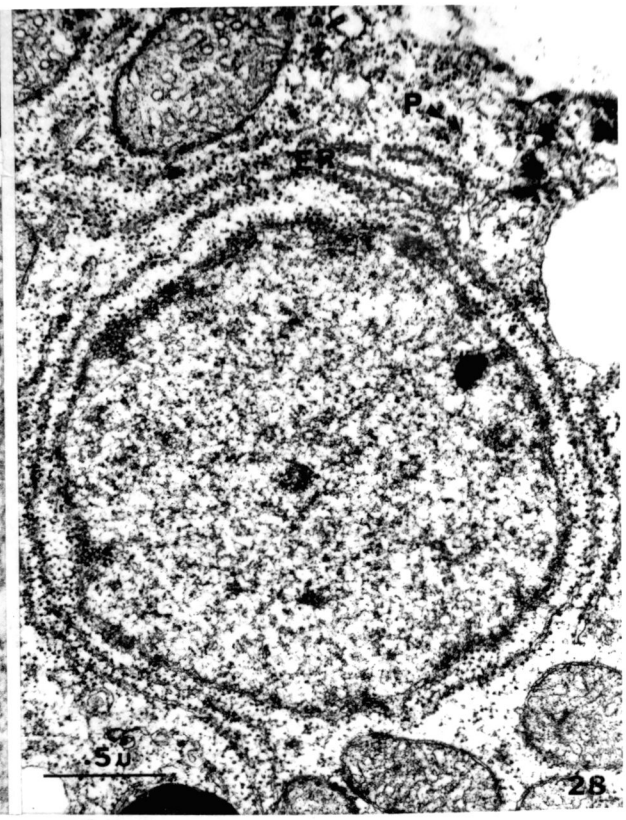
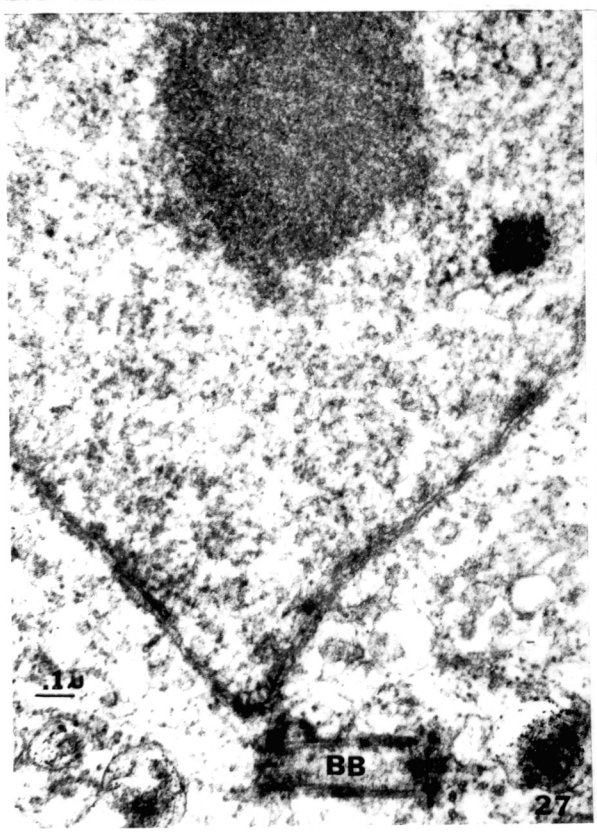

1µ
26


P
ER
5µ
28


1µ
BB
27

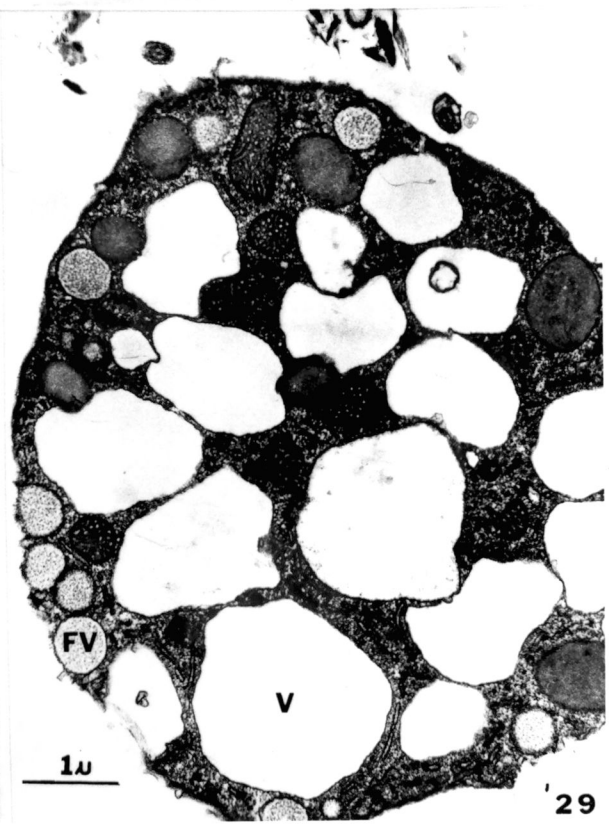
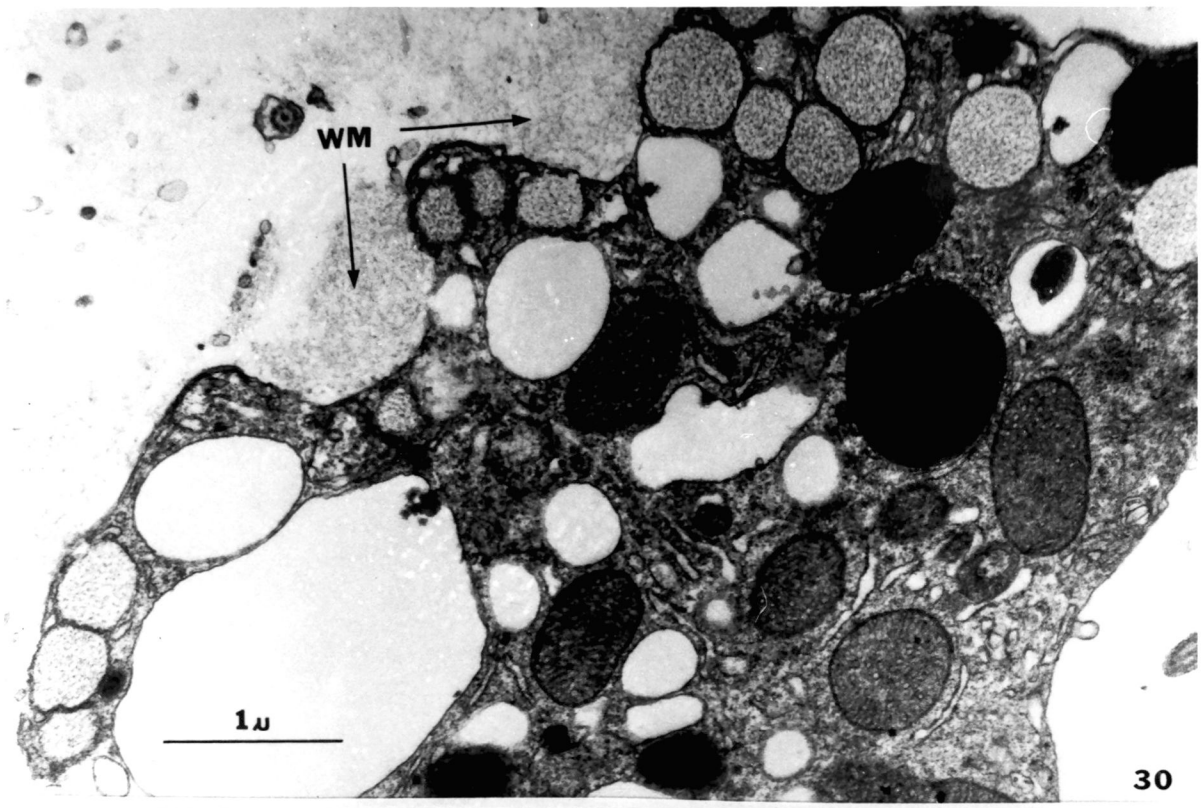

FV
V
1µ
29

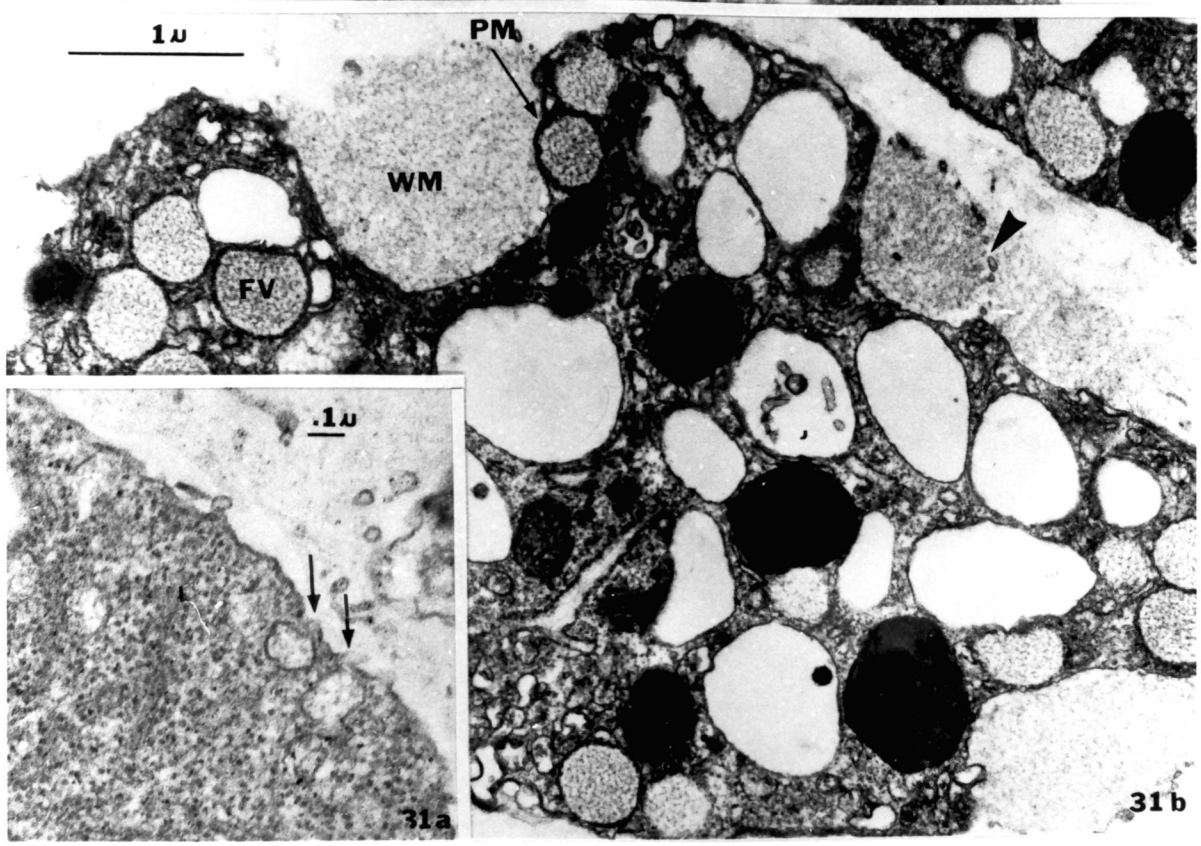
Figure 30. Encysting spore. Note clumped fibrous vesicles. Arrows indicate two places where fibrous vesicles are releasing cyst wall material. 23,375 X.

Figure 31a. Encysting spore in which wall vesicles appear to be adding material to the maturing cyst wall. Note the irregularity of the plasma membrane. Arrows show points of addition. 54,000 X.

Figure 31b. Encysting spore. Arrowhead indicates point at which fibrous vesicles are fusing. 22,000 X.



30



31a

31b

Figure 32. Nucleus containing polycomplex with five lateral elements visible. 33,750 X.

Figure 33. Nucleus containing two polycomplexes with three lateral elements each. 33,750 X.

Figure 34. 1,000 Å wide complex. 63,000 X.

Figure 35. 4,000 Å wide complex. 63,000 X.

Figure 36. Polycomplex with four lateral elements. Solid arrowhead indicates the double nature of one of these elements. Arrow points to dense medial element. 45,000 X.

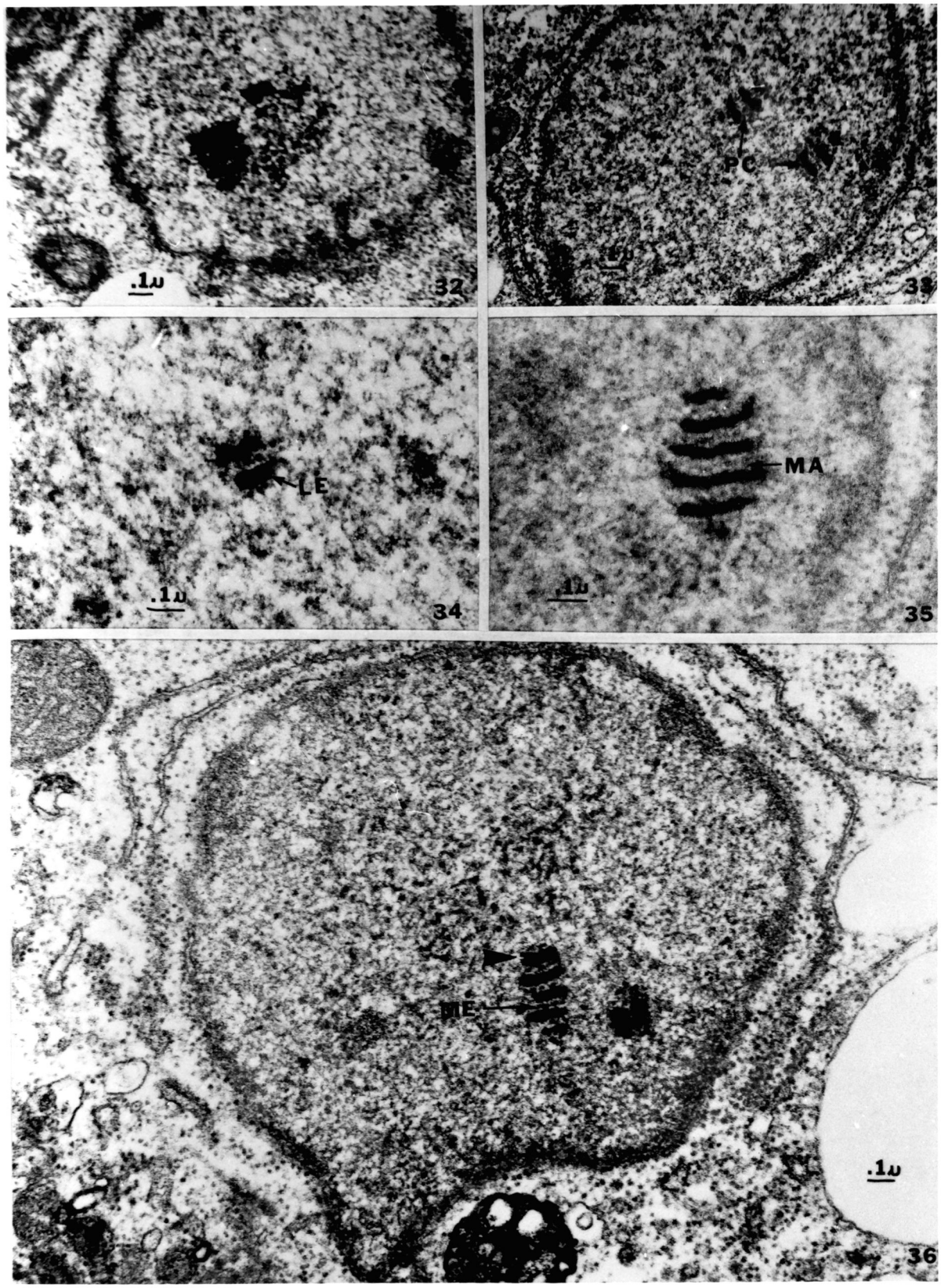

.1μ
32
PC
33
LE
.1μ
34
MA
1μ
35
ME
.1μ
36

Figure 37. Electron micrograph showing bipolar germination of spore. Note that elongate nucleus appears to be pointed toward the tubes. 7,100 X.

Figure 38. Germinating spore in seawater. 620 X.

Figure 39. Young germinating spore on crab egg. 500 X.

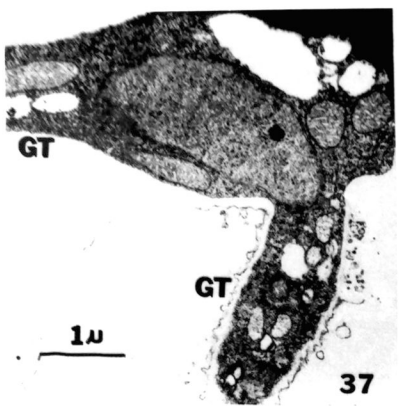
Figure 40. Germinating spore on crab egg. Note the empty cyst remaining on the surface of the egg. 500 X.

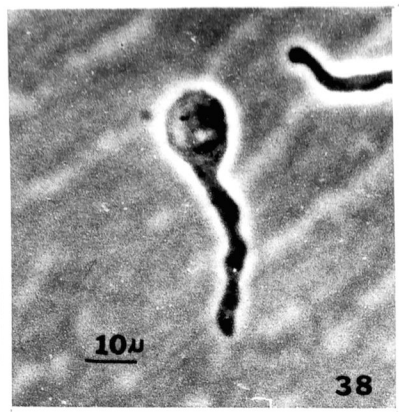
Figure 41. Young germinating spore in seawater. 620 X.

Figure 42. Germinating spore in which cytoplasm fills the tube with a small amount of cytoplasm remaining visible in the cyst (arrow). 620 X.

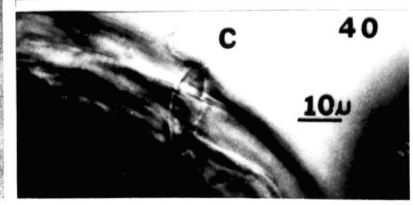
Figure 43. Old germinating spore in which the cytoplasm is moving toward the tube tip. 620 X.

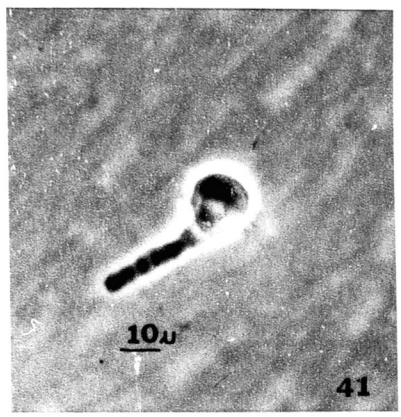
Figure 44. Electron micrograph of a germinating spore with the germ tube rupturing the outer cyst wall. Note that the inner cyst wall is continuous with the tube wall. 18,000 X.

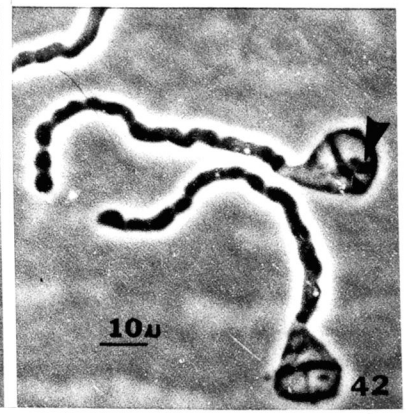

GT
GT
1μ
37

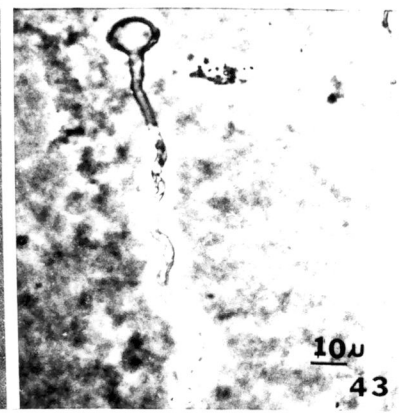

10μ
38


10μ
39
GT


C
40
10μ


10μ
41


10μ
42


10μ
43

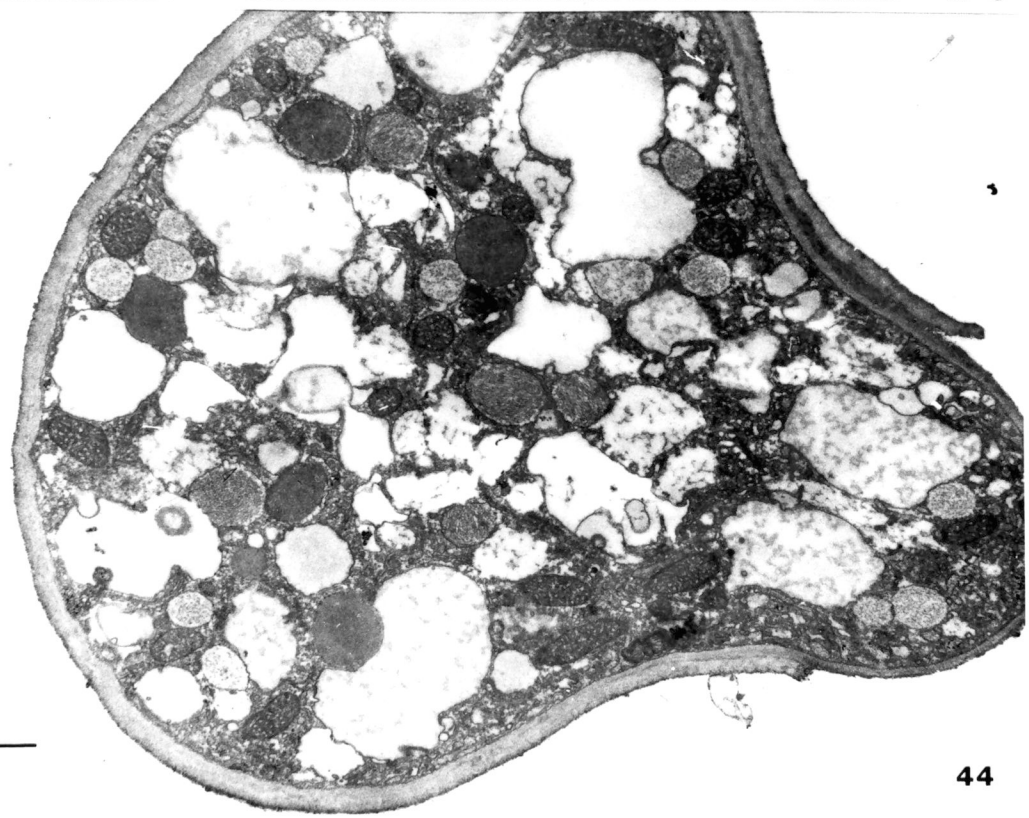
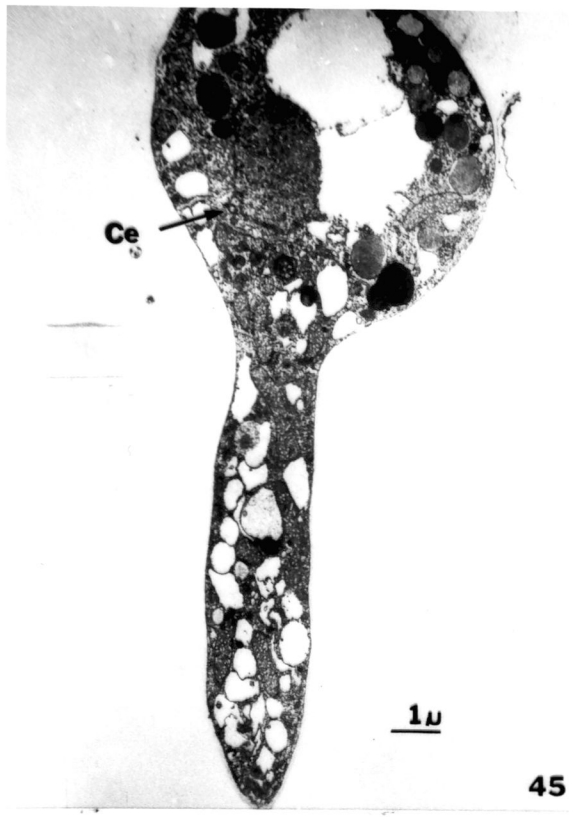

1μ
44

Figure 45. Young germinating spore showing numerous small vacuoles. Note that the centriole still has projecting rootlets. 6,000 X.

Figure 46. Germinating spore in which some of the vacuoles have fused forming two larger vacuoles. Note vacuoles having dense bodies. 10,500 X.

Figure 47. Germinating spore in which a single large vacuole occupies most of the cyst area. 10,560 X.



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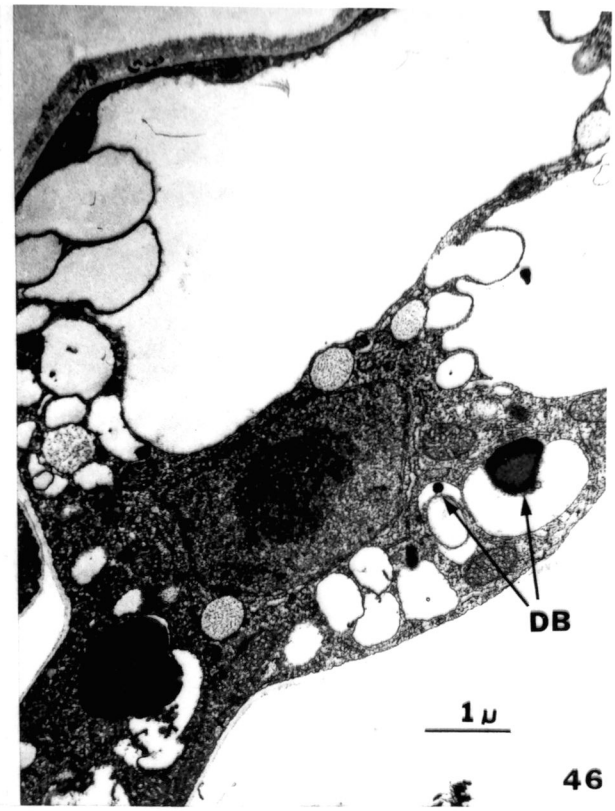
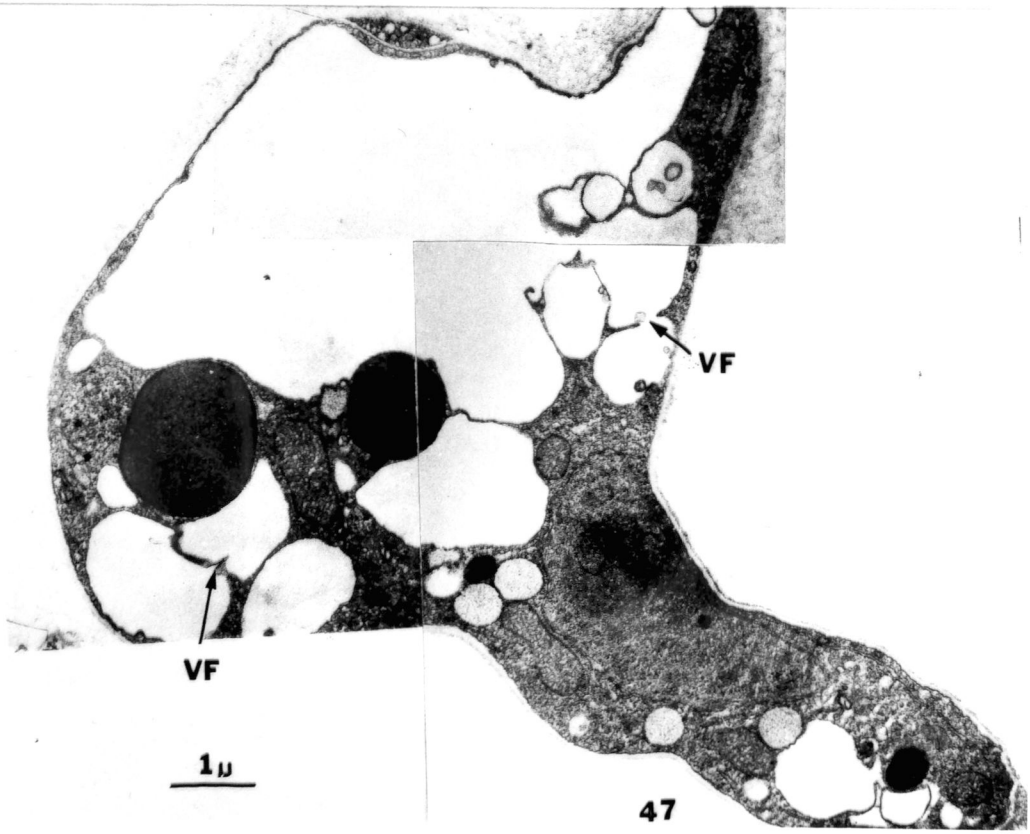


46

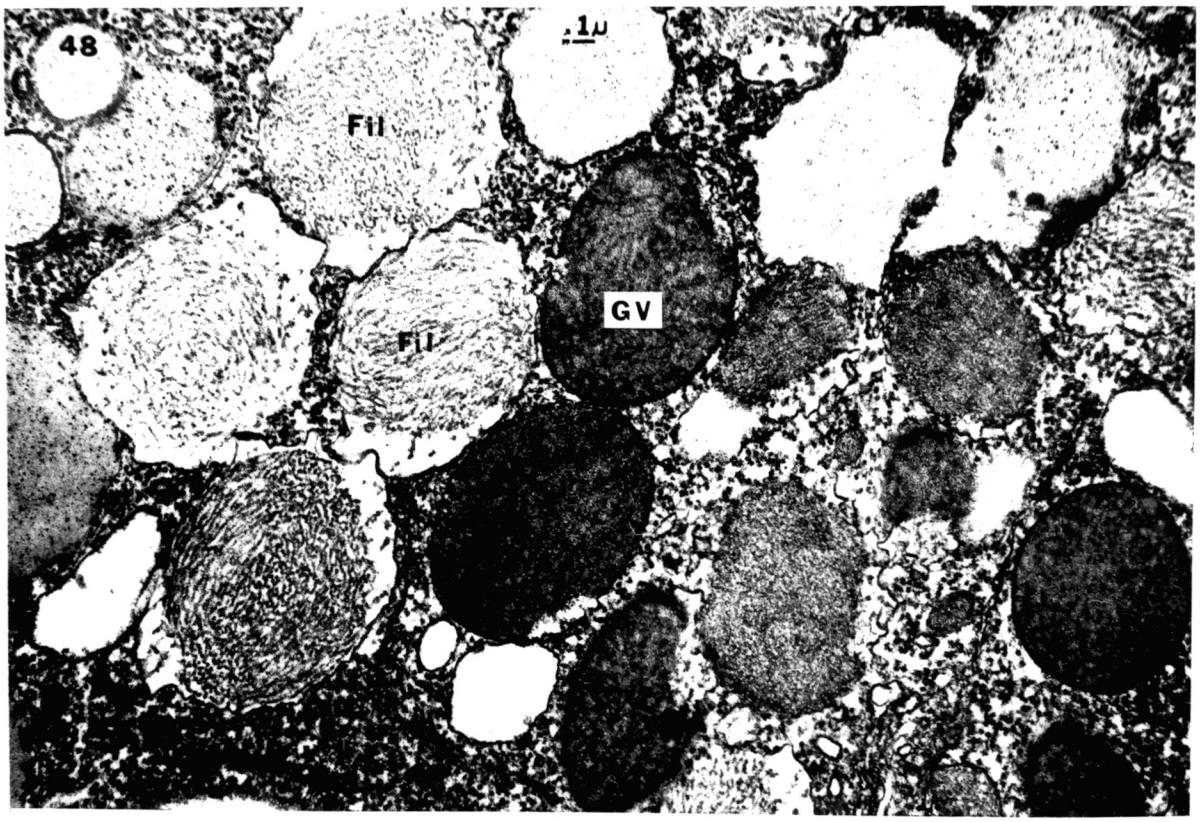


47

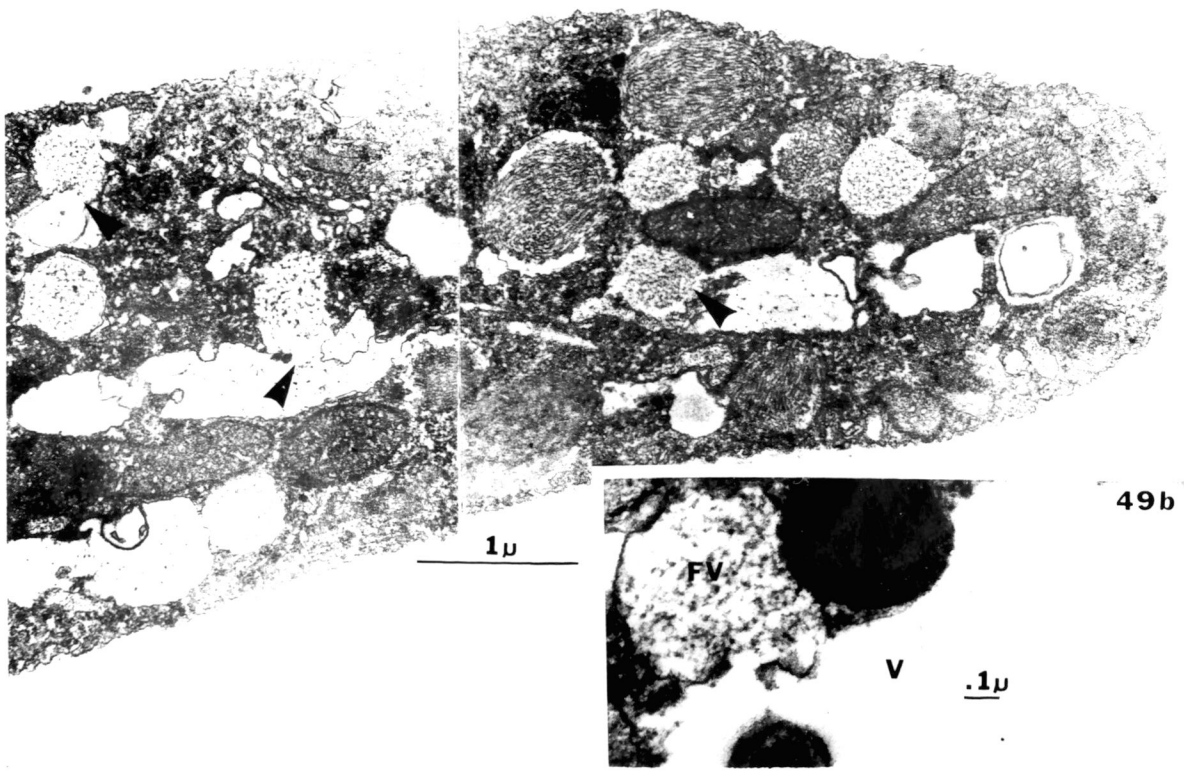
Figure 48. Vesicles within a germ tube. 40,500 X.

Figure 49a. Germ tube showing fibrous vesicles emptying into vacuoles. Arrowheads denote points of breakdown. 21,600 X.

Figure 49b. High magnification micrograph of fibrous vesicle emptying into a vacuole. 40,500 X.



49a

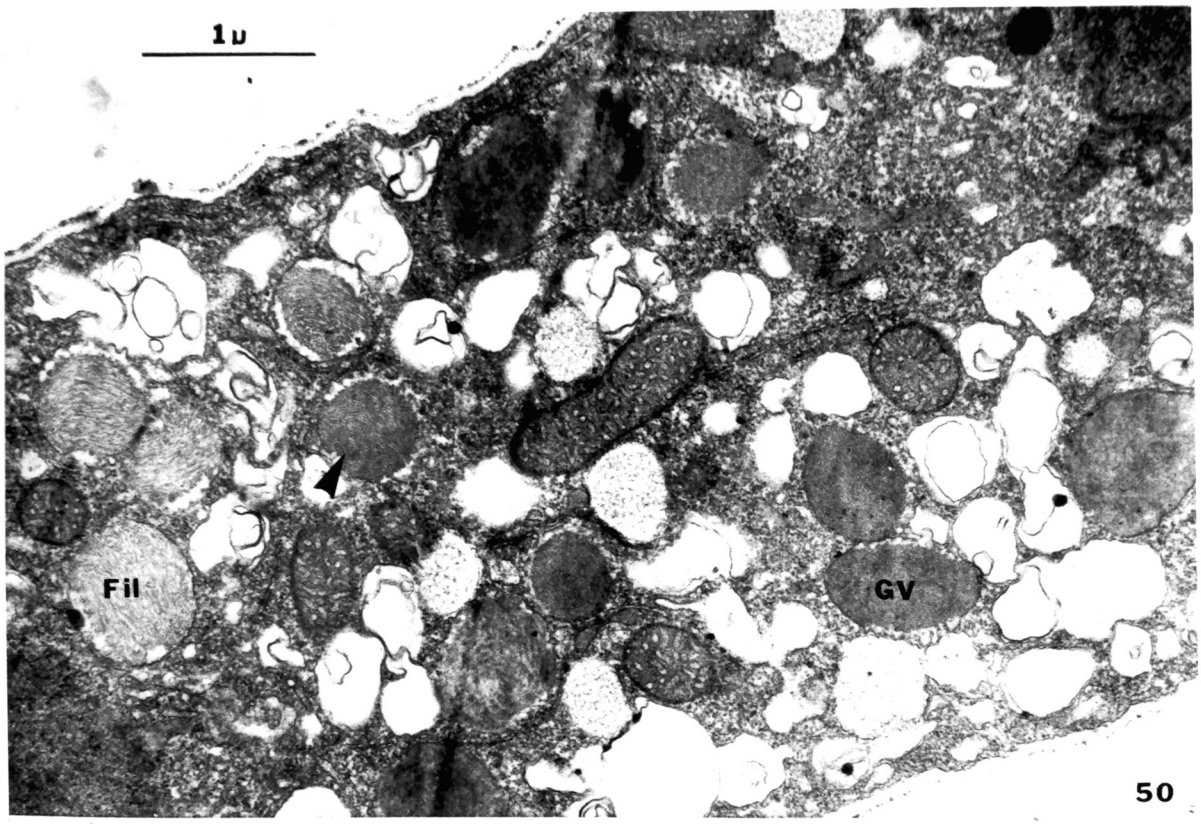


49b

Figure 50. Germ tube section showing granular vesicles, and granular vesicles changing into filamentous vesicles. Arrowhead indicates changing granular vesicle. Note the many filamentous vesicles. 22,000 X.

Figure 51a. Section through spore cyst showing the conversion process of the various vesicles. Arrowhead indicates granular vesicle changing to filamentous vesicle; hollow arrowhead indicates filamentous vesicle that has begun to breakdown into a fibrous vesicle. 38,500 X.

Figure 51b. Granular vesicle changing to filamentous vesicle. 38,500 X.



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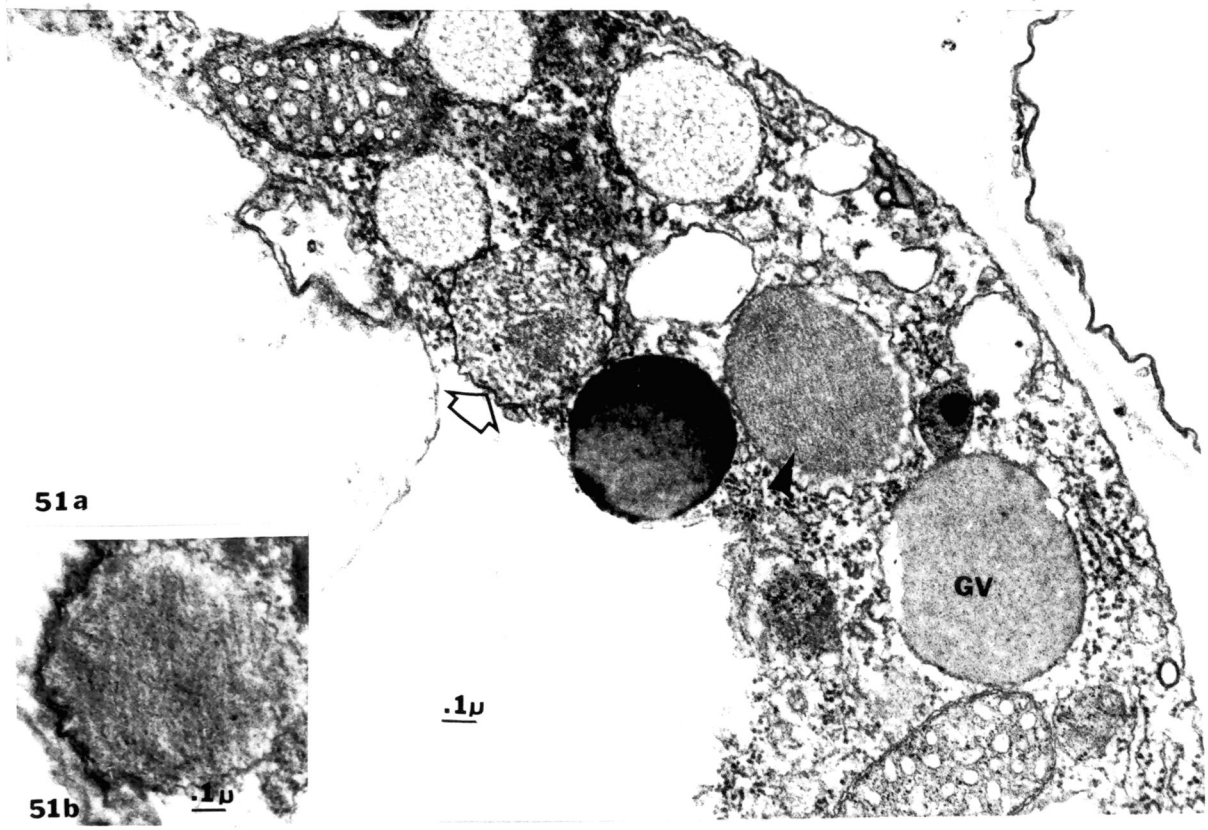


51a

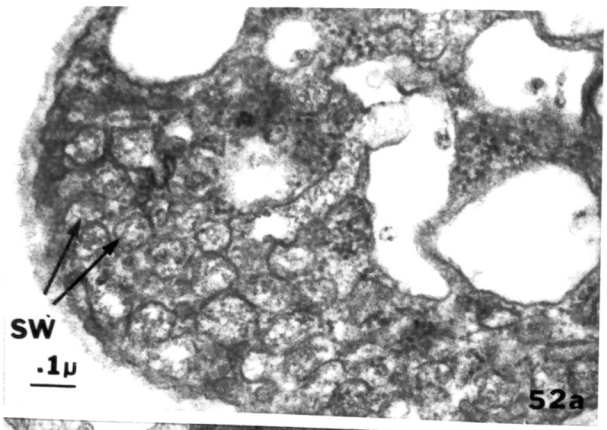
51b

Figure 52a. Spherical wall vesicles in germ tube. 47,250 X.

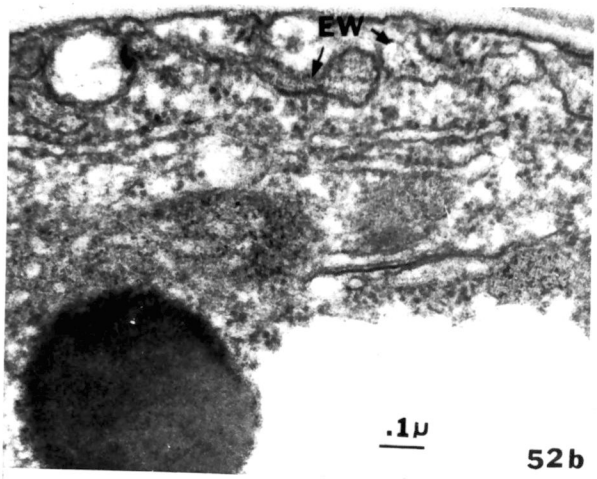
Figure 52b. Elongate wall vesicle forming from endoplasmic reticulum. 54,000 X.

Figure 53. Longitudinal section of a germ tube showing wall vesicles just below the plasma membrane. The tube tip is filled with wall vesicles. Note small vesicles near dictyosome (arrows). 38,000 X.

Figure 54. Longitudinal section of a germ tube. 40,500 X.



52a



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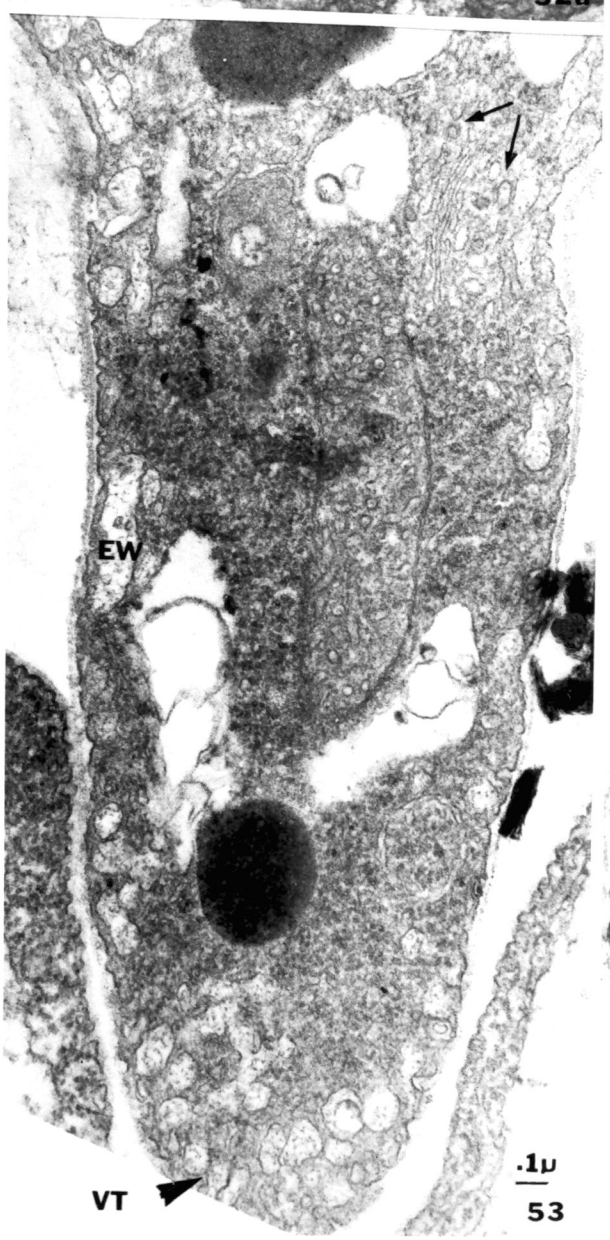

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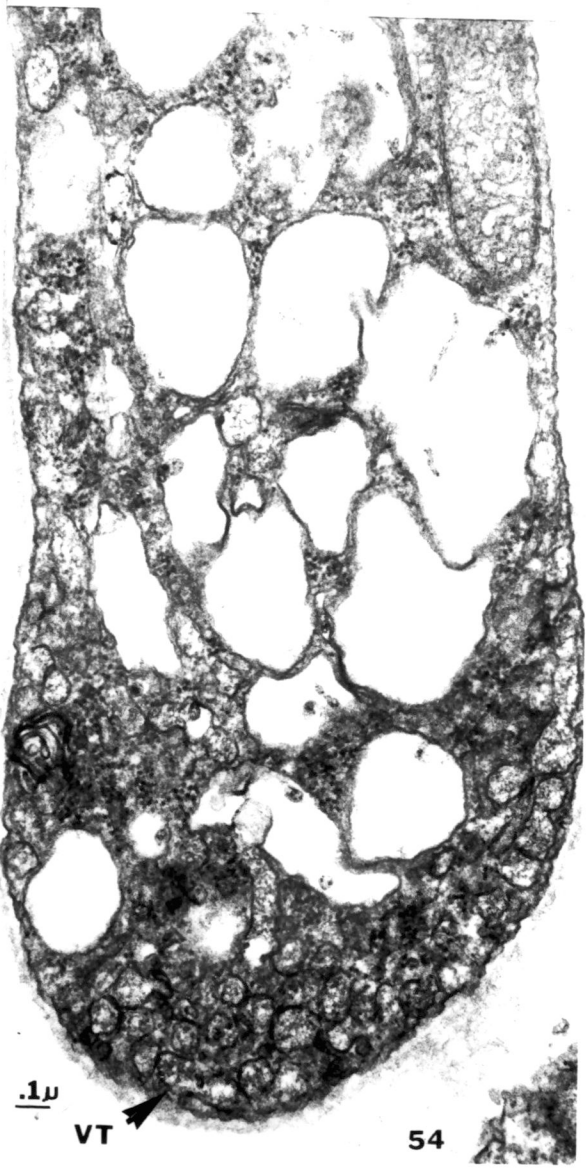


54

Figure 55. Hyphae in broth culture. 185 X.

Figure 56. Hyphae growing from crab egg. 75 X.

Figure 57. Old hyphal clumps on agar plate. Actual size.

Figure 58. Young unbranched hyphae emerging from crab egg. 75 X.

Figure 59. Old catenulate hyphae. 540 X.

Figure 60. Hyphae within a crab egg. 115 X.

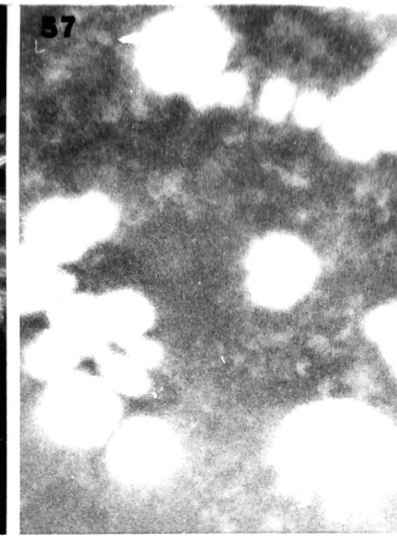
Figure 61a. Granular textured resting bodies. 940 X.

Figure 61b. Resting body with large droplets. 1,250 X.

Figure 61c. Resting body showing both granules and droplets. 1,250 X.


55
10 μ


56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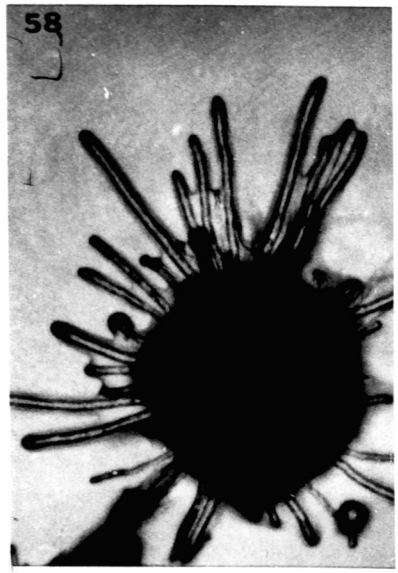
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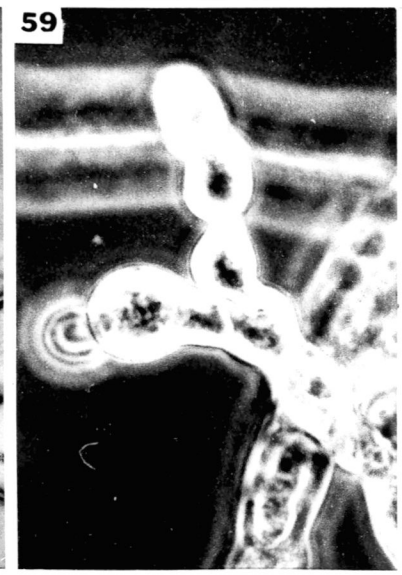
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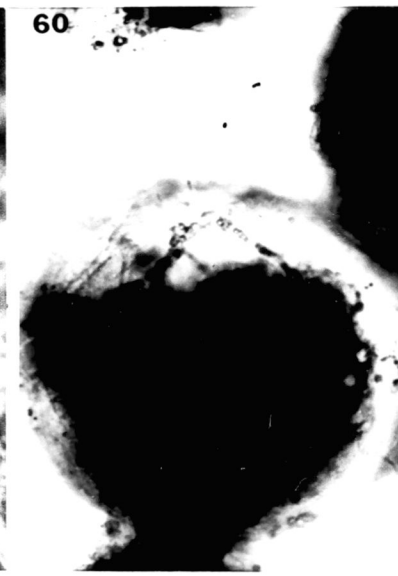
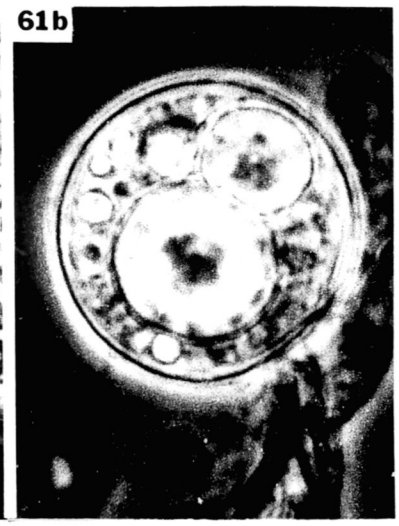

60


61a


61b

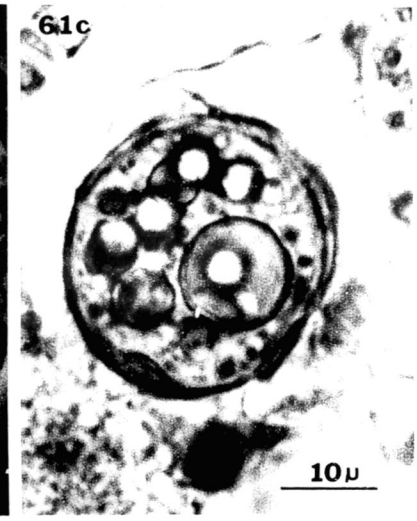
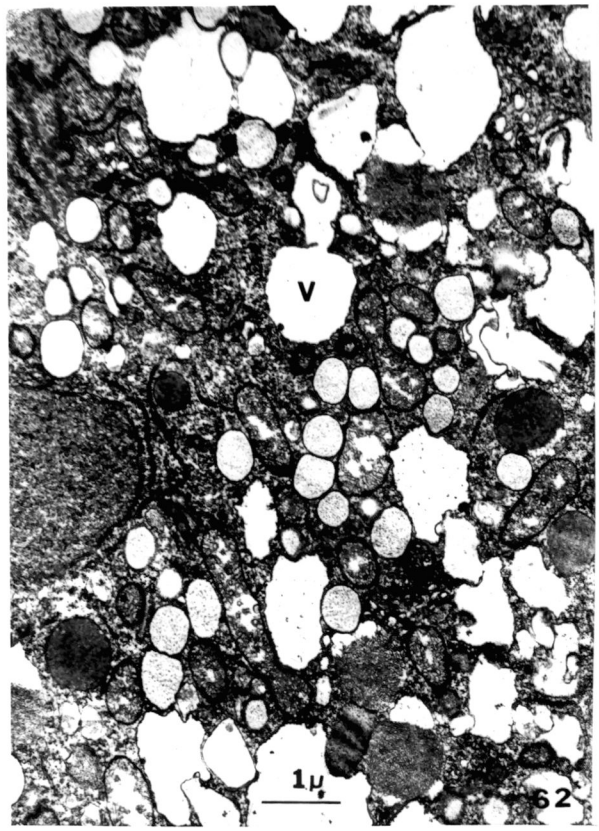

61c
10 μ

Figure 62. Electron micrograph of young hypha showing many small vacuoles. 10,200 X.

Figure 63. Electron micrograph of maturing hypha showing large central vacuole. 9,120 X.

Figure 64a & b. Granular and filamentous vesicles in hyphae. 29,250 X.



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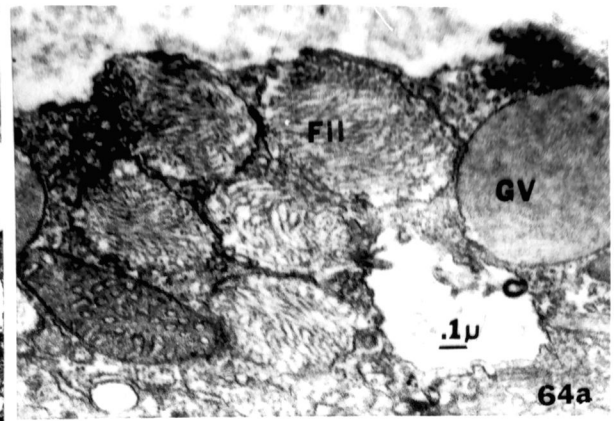
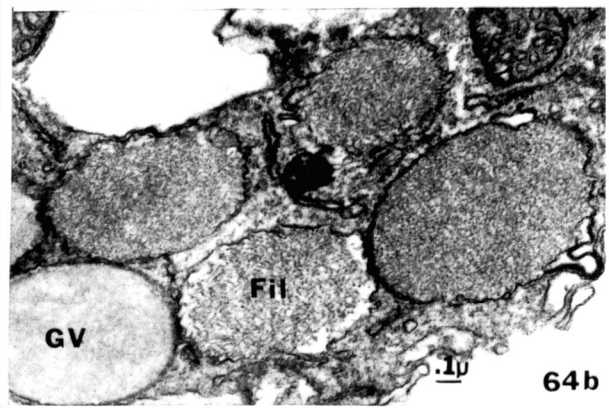
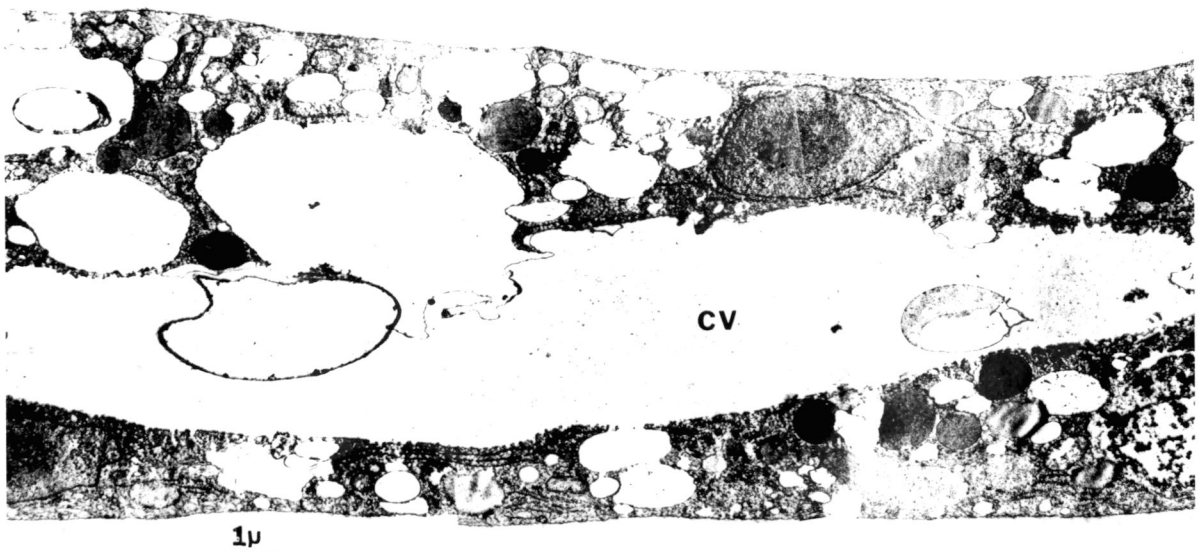


64a



64b



63

Figure 65a. Hyphal section showing single layered wall. Note lomasome. 36,000 X.

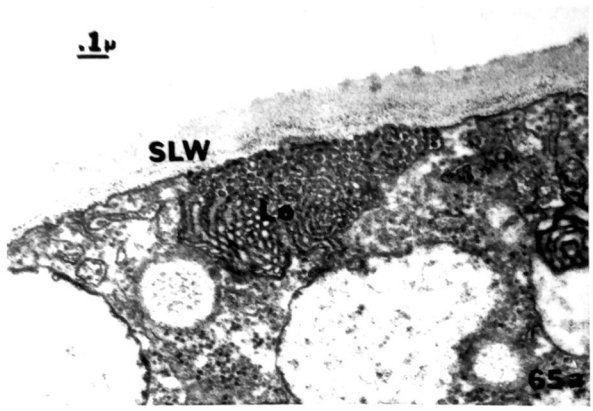
Figure 65b. Hyphal section showing three-layered wall. 45,000 X.

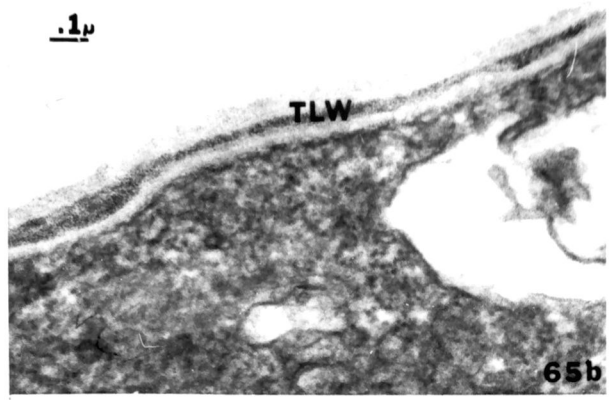
Figure 66. Hyphal tip destined to form cleavage vesicle. Note hyaline appearance of tip at arrowhead. 1,000 X.

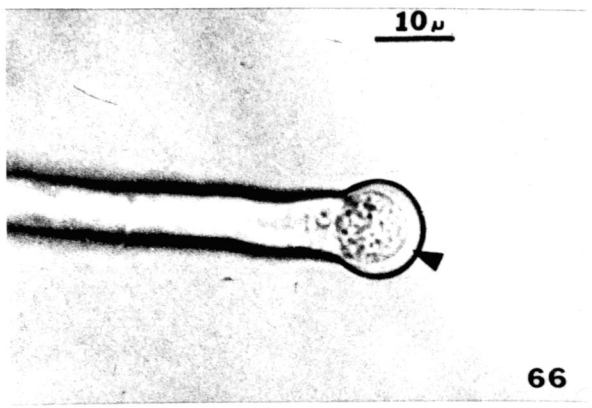
Figure 67. Cleavage vesicle forming at hyphal tip. 800 X.

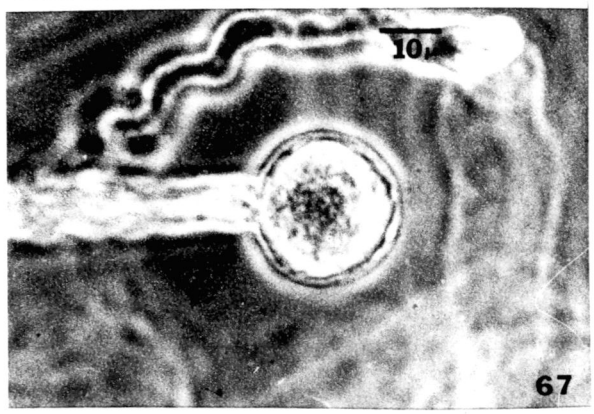
Figure 68. Enlarging cleavage vesicle at hyphal tip. 800 X.

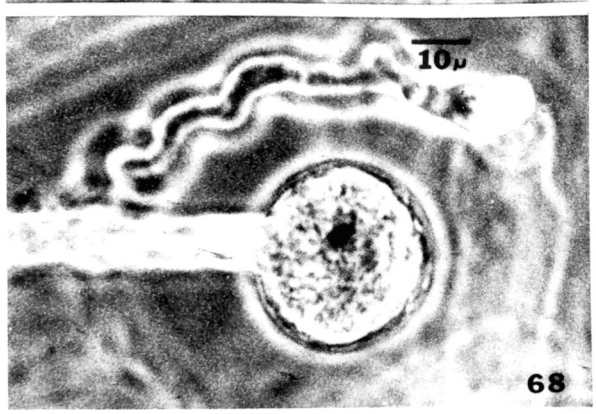
Figure 69. Cytoplasmic flow into gelatinous vesicle. Arrow indicates the direction of flow. A small thread (arrowhead) can be seen connecting the cytoplasmic units flowing into the vesicle. 900 X.

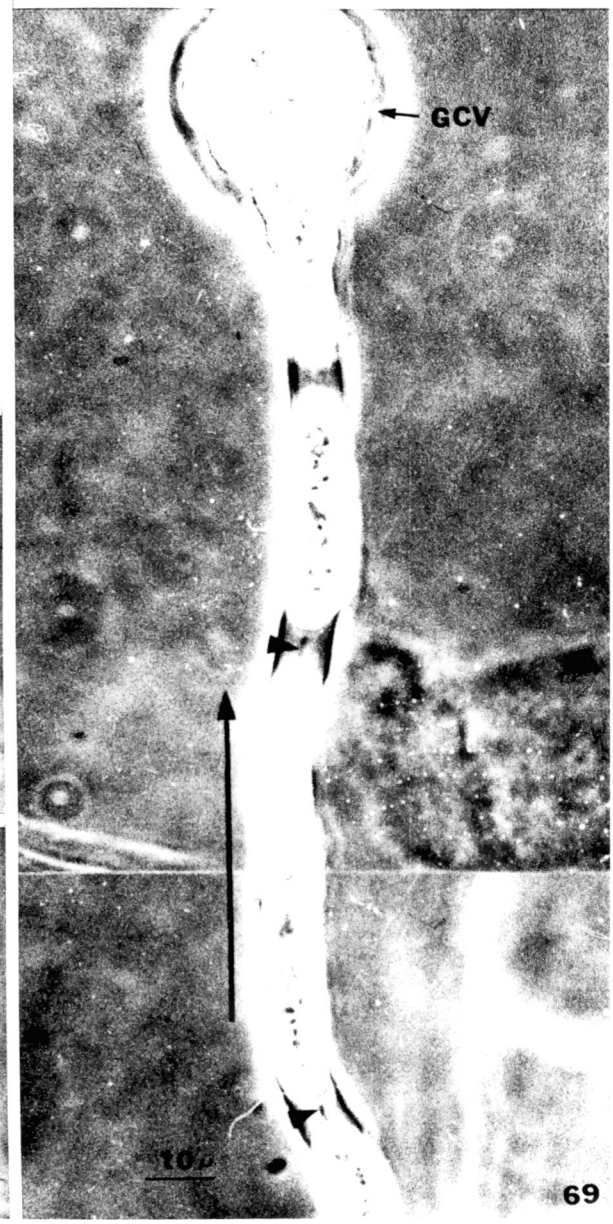

.1μ
SLW
Lo
65a


.1μ
TLW
65b


10μ
66


10μ
67


10μ
68


GCV
10μ
69

Figures 70a-f. Discharge of sporogenic cytoplasm into gelatinous cleavage vesicle. 500 X.

Figures 70g-j. Spore cleavage. 500 X.

Figure 70k. Spore discharge. 265 X.

Figure 701. Vesicle collapsed around hyphal tip. 500 X.
(Total time of this sequence - 43 minutes)

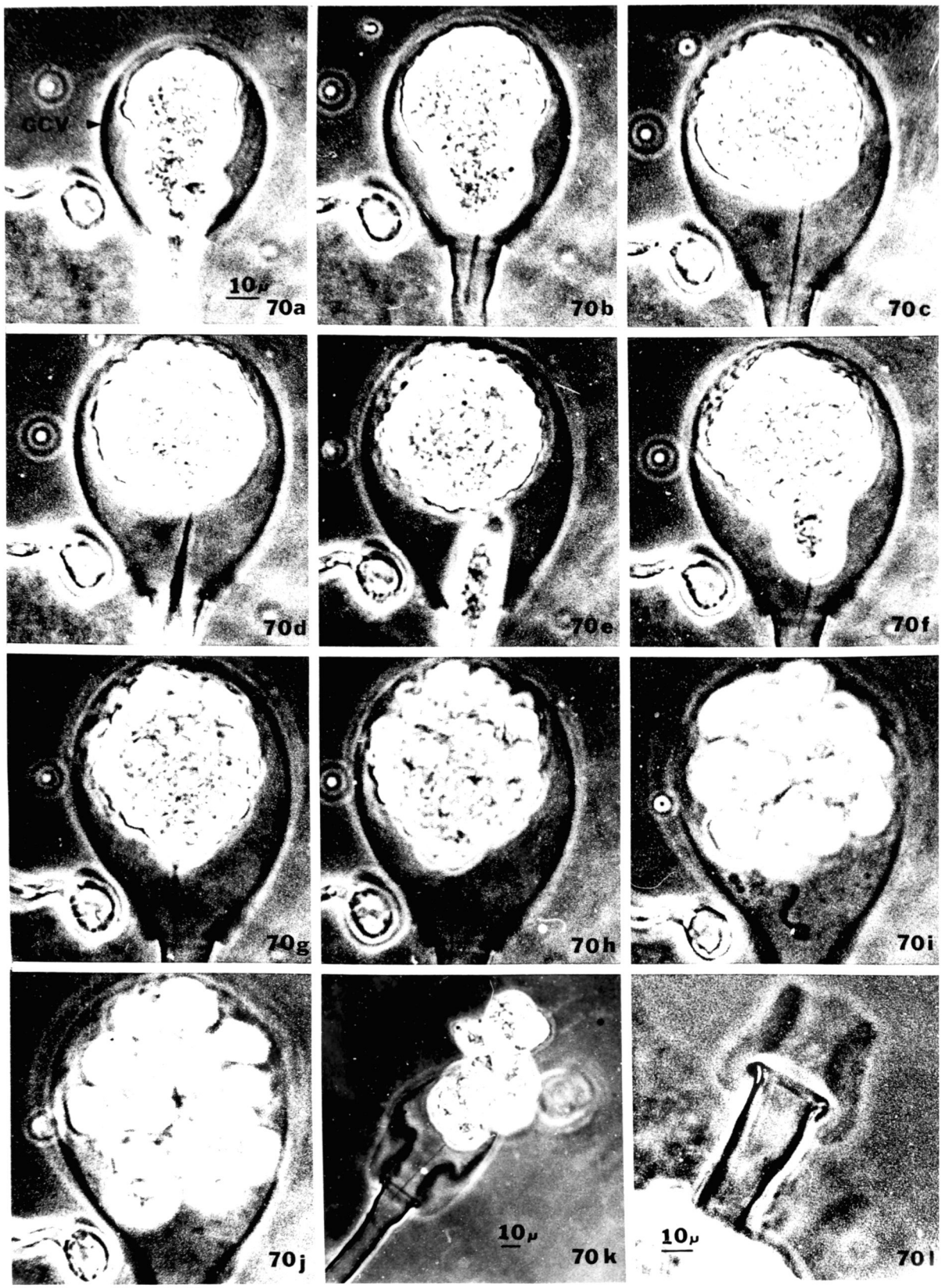
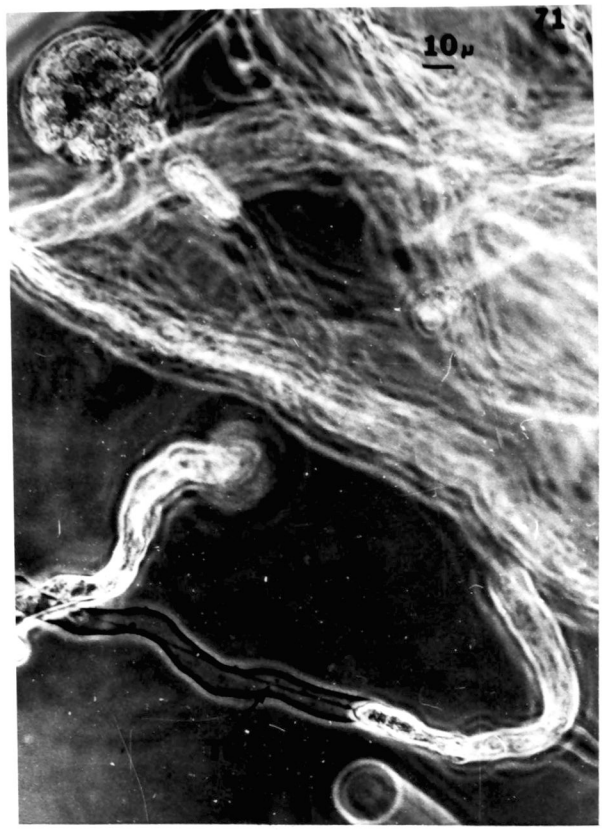

GCV
10μ
70a
70b
70c
70d
70e
70f
70g
70h
70i
70j
10μ
70k
10μ
70l

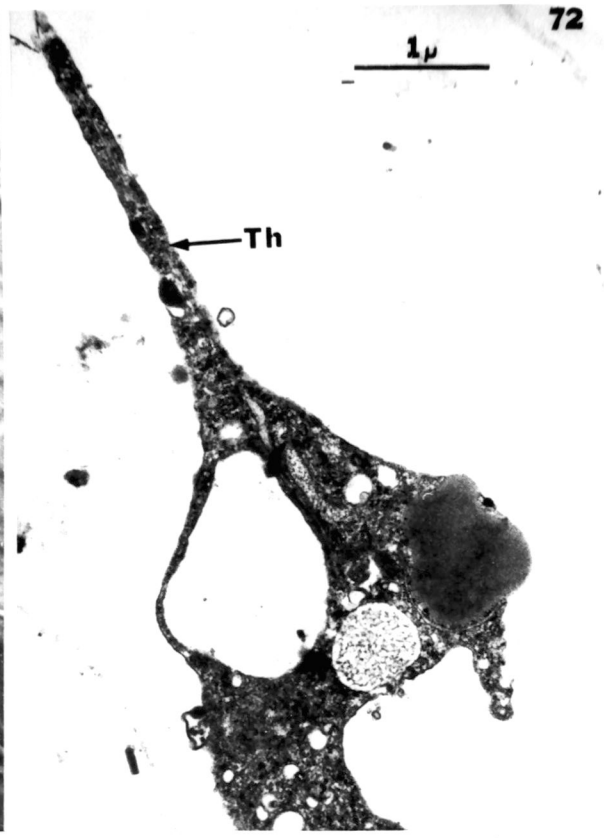
Figure 71. Movement of cytoplasm into cleavage vesicle in which cleavage has already begun. Note thread extending back into the discharge hypha (arrow). 360 X.

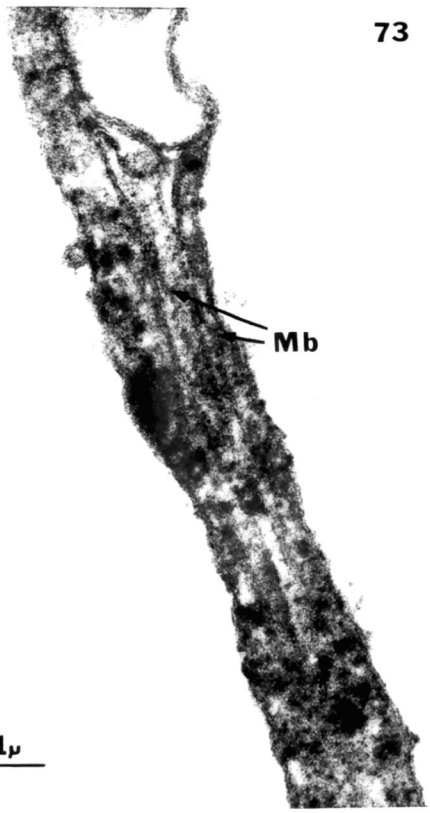
Figure 72. Electron micrograph of thread showing it to be simply a continuation of the cytoplasm. 16,800 X.

Figure 73. High magnification micrograph of thread showing membranes. 106,875 X.

Figure 74. Cytoplasmic unit in discharge hypha. Note that the basal body and flagellum are already present. 27,000 X.


71
10μ


72
1μ
Th


73
Mb
.1μ

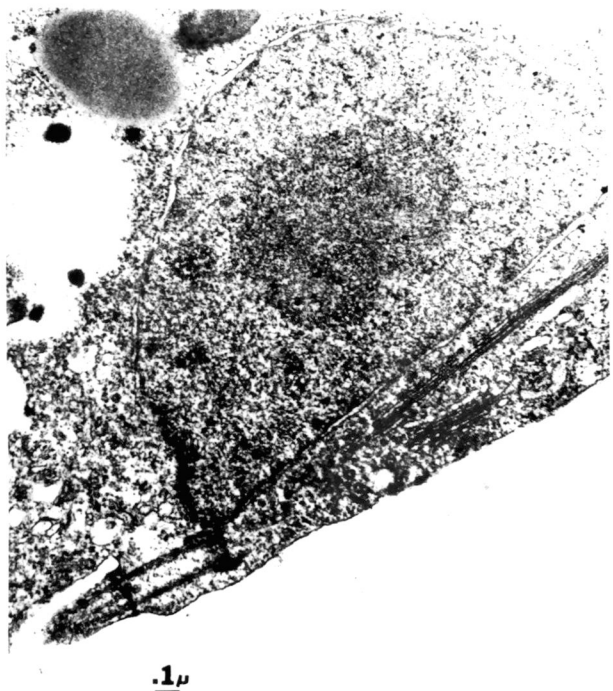
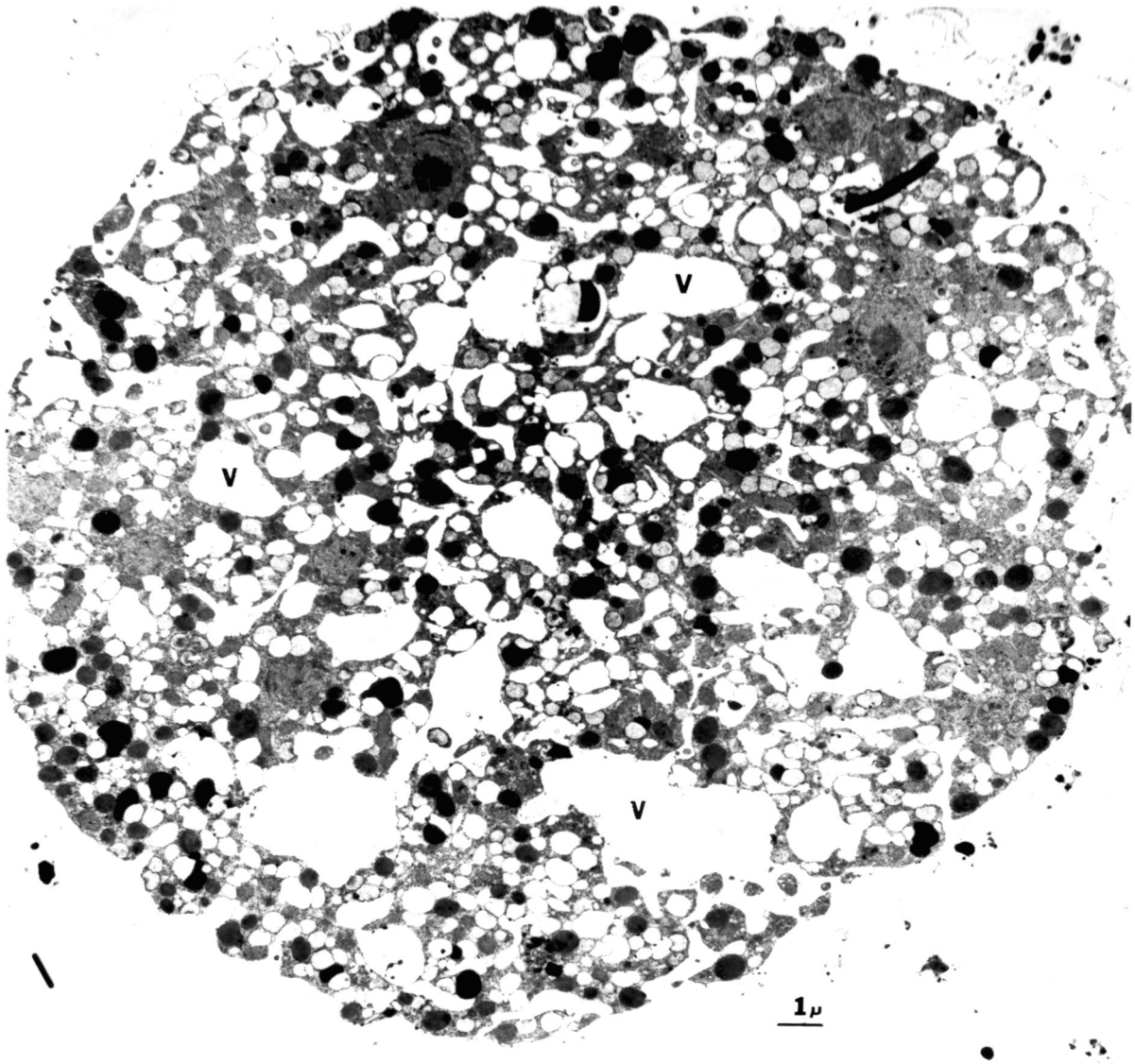

74
.1μ

Figure 75. Low magnification electron micrograph of a young cleavage vesicle. 6,300 X.

Figure 76. Flagella present in vesicle within cavities. 27,600 X.



75

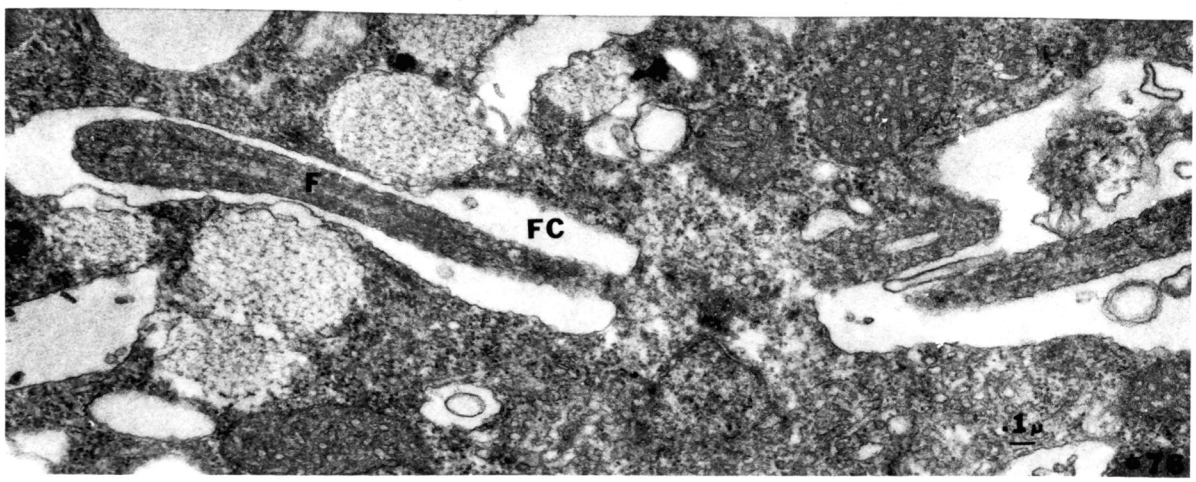
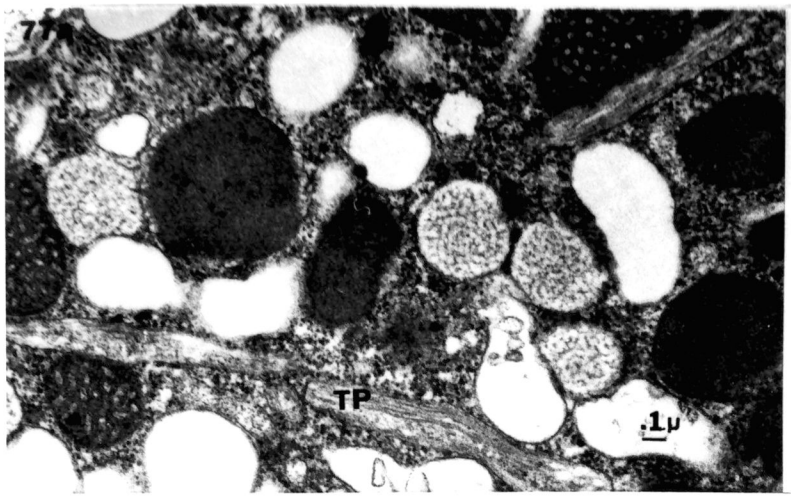
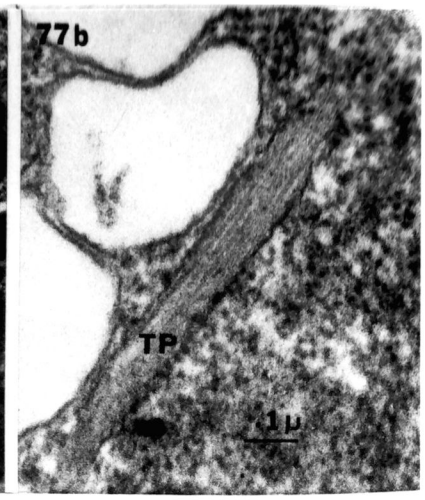


Figure 77a. Rod-shaped flimmer packets containing microtubules. 26,400 X.

Figure 77b. High magnification micrograph of flimmer packet. 63,000 X.

Figure 78. Low magnification micrograph of a mid cleavage vesicle. Note vacuoles starting to fuse. 6,300 X.





78

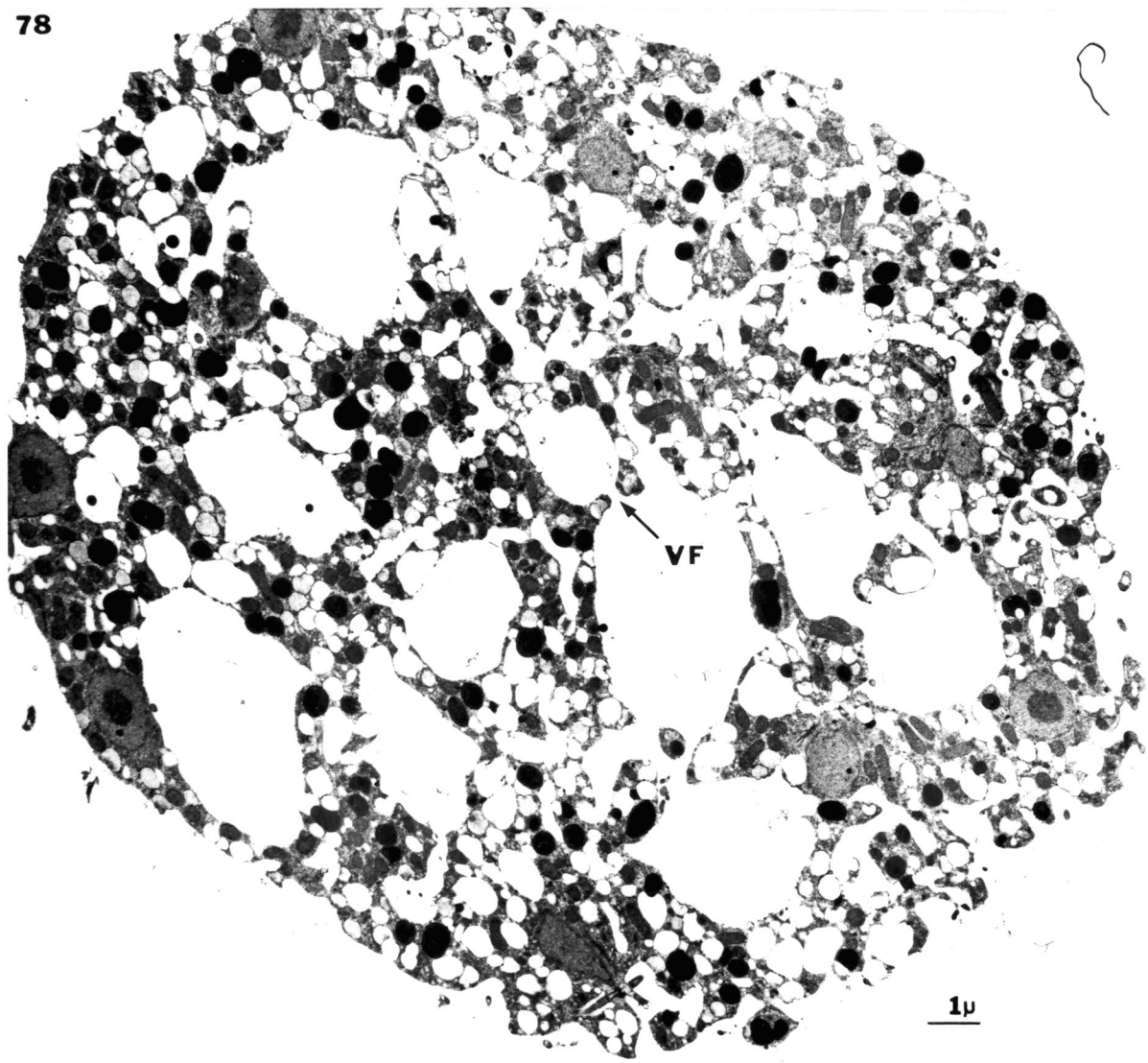


Figure 79. Micrograph of late cleavage vesicle. Arrows indicate narrow connecting cytoplasmic bridges. 6,300 X.

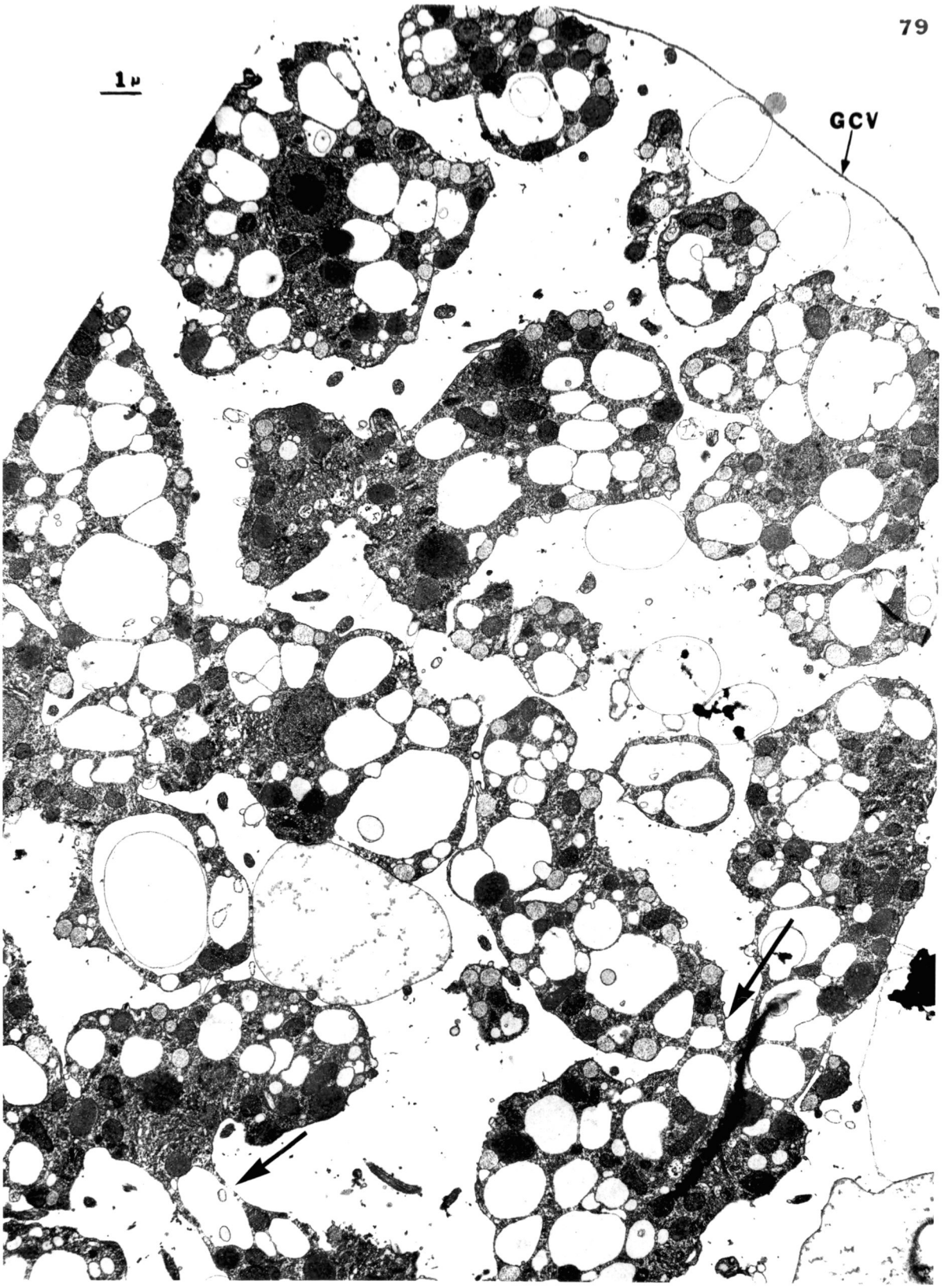

1µ
GCV

Figure 80. Electron micrograph of final cleavage stage with free spores in cleavage vesicle. 4,460 X.

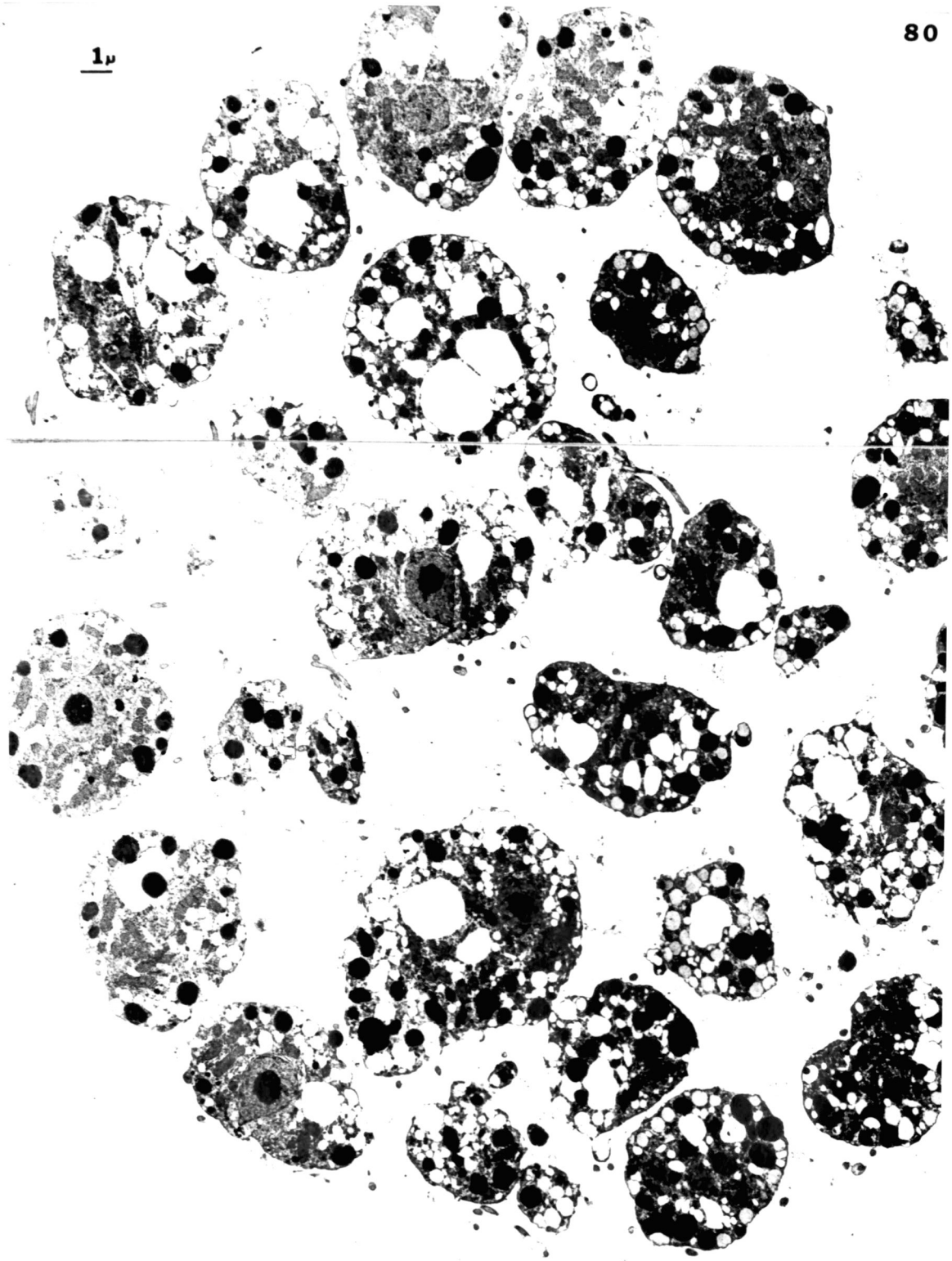
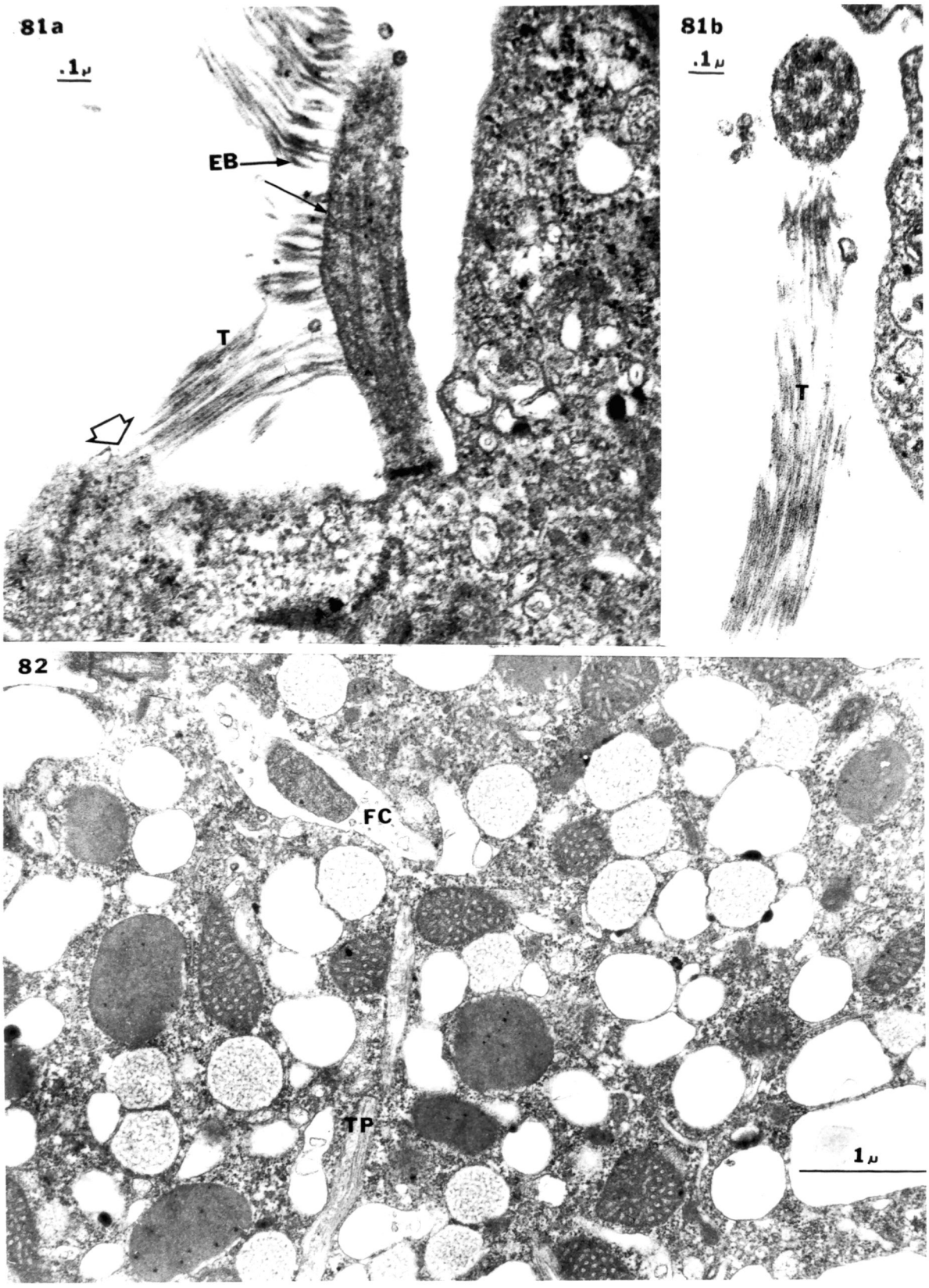

1μ

Figure 81a. Longitudinal section of tinsel flagellum. Note that tinsels are connected to the cytoplasm at hollow arrowhead. Note that tinsels seemingly attach to axoneme at arrow. 49,500 X.

Figure 81b. Cross section of tinsel flagellum. 54,000 X.

Figure 82. Flimmer packets aligned near flagellum. A glancing section of the flagellum may be seen within cavity. 21,600 X.


81a
.1μ
EB
T
81b
.1μ
T
82
FC
TP
1μ

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