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City University of New York PH.D. 1979

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ABNORMAL DEVELOPMENT OF *THRAUSTOCHYTRIUM AUREUM*, A BIFLAGELLATE MARINE PHYCOMYCETE

by

AVROM POLLAK

A dissertation submitted to the Graduate Faculty in Biology
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy, The City University of New York.

1979

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This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

ABNORMAL DEVELOPMENT OF THRAUSTOCHYTRIUM AUREUM,

A BIFLAGELLATE MARINE PHYCOMYCETE

by

AVROM POLLAK

Advisors: Professors Frederick L. Schuster and Solomon Goldstein

The effects of environmental manipulations on the morphology and development of Thraustochytrium aureum, a marine fungus, has been studied by light and electron microscopy. Under anaerobic conditions sporangia display unique patterns of cellular differentiation not evident in normal aerobic cultures, the most striking of which is the presence of large masses of endoplasmic reticulum-derived membranous tubules. These tubules are observed after 48 hrs of anaerobic growth and closely resemble tubuloreticular structures found in some virus-infected animal cells. Continued exposure to anaerobic conditions results in the formation of numerous, hexagonal-shaped, 150 nm, viruslike particles, in approximately 15% of the cells. The VLPs possess an RNA core and are surrounded by a triple layered shell 20-25 nm thick. There is no indication that the nucleus has any role in the development of these particles. VLPs were not observed in cells subjected to extremes of pH, salinity, and temperature. Possible relationships between the membranous tubules and the VLPs are considered, and comparisons are made between the VLPs found in T. aureum and those found in other eucaryotic microorganisms. It is suggested that T. aureum harbors a latent virus

whose recrudesence is dependent upon external environmental factors. These observations have ecological implication since marine fungi of this type are often found in polluted coastal waters.

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INTRODUCTION

Marine phycomycetes, because of their ubiquitous nature, have recently become the subject of numerous physiological, ecological, ultrastructural, and taxonomical studies. To date relatively little is known regarding their relationships with other marine microorganisms or their mode of response to changing environmental conditions. In a recent review on zoosporic marine fungi, Goldstein (1973) attributed the general paucity of information concerning these organisms to their near anonymity and slow growth rates relative to the burgeoning numbers of other marine microorganisms. However, new isolation procedures and the realization that thraustochytriaceous fungi possess unique physiological and ultrastructural traits, have served to focus attention on these formerly obscure fungi.

It is not the purpose of this review to give a detailed account of the various research efforts involving thraustochytrids. However, a summary of the major areas of interest is presented for a proper understanding of the experimental studies to follow in the response of *Thraustochytrium aureum* to environmental manipulations and the formation in this fungus of viruslike particles.

Taxonomy

Thraustochytriaceous fungi were first described by Sparrow (1936) in a report that verified the existence of saprophytic lower fungi in marine habitats. Sparrow (1943 and emended 1960) established the

Thraustochytriaceae as a family in the Saprolegniales (Oomycetes) to accomodate monocentric, eucarpic, marine phycomycetes which produce biflagellate zoospores. The genus Thraustochytrium accounted for those species in which zoospores are liberated upon the bursting or dissolution of the sporangial wall. Other genera included in the Thraustochytriaceae are Japonochytrium Kobayashi and Ookubo 1953, Schizochytrium Goldstein and Belsky, 1964, and Aplanochytrium Bahnweg and Sparrow, 1972. Sexual reproduction has not been observed in any of these genera. Thus, a relatively simple life cycle complete upon the production of asexual zoospores makes these forms useful tools in marine physiological and ecological investigations.

The ectoplasmic nets or slime tracks of the labyrinthulas bear a remarkable similarity to the rhizoids of many Thraustochytriaceae. Both lack cell walls and do not contain any cytoplasmic organelles. In addition, both the ectoplasmic nets and the rhizoids appear to be intimately associated with a sagenogenetosome, a term coined by Perkins (1972) to describe a structure with net-forming abilities. The sagenogenetosome, or sagenogen as abbreviated by Olive (1975), is truly a unique organelle. It is thought that it controls transport of cellular components between the membranous channels within the cell and the extracellular net elements. Similar observations by Porter (1972, 1974) have led him to conclude that the rhizoids of the Thraustochytriaceae are very unlike any structures in the Saprolegniales and that they more closely resemble the network

associated with cells of *Labyrinthula*.

The behavior of the centrioles during mitotic division and the fine structure of the mitotic apparatus in *Thraustochytrium* sp (Kazama, 1974) are considerably different from other Saprolegniales studied (Heath, 1974; Whisler and Traviand, 1973). In this respect the thraustochytrids differ significantly from the labyrinthulids where the vegetative interphase centrioles are absent (Perkins, 1970; Porter 1972).

The study of the structure, method of formation, and chemical composition of the cell wall in thraustochytrids is another experimental approach employed towards defining the taxonomic affinities of the group. Biochemical composition of cell wall material is a reliable indicator in considering fungal phylogeny (Bartniki-Garcia, 1970). Results by Darley et al. (1973) reveal distinct differences in *Schizochytrium aggregatum* and *Thraustochytrium* sp from other Saprolegniales. The cell walls of these two thraustochytrids consist of laminated structures which upon sonic disintegration yield thin flexible scales. Developing wall scales were found within cisternae of the Golgi apparatus, and examination of freeze-etched preparations showed plate-like indentations on both inner and outer surfaces of the plasmalemma. Similar scales have been observed in *Althornia couchii* (Jones and Alderman, 1970), another marine biflagellate thought to be a thraustochytrid.

Most workers familiar with these chytrid-like organisms agree

that the biflagellate nature of the zoospore is not sufficient reason to include them among the Saprolegniales. Evidence that these fungi are indeed taxonomically misplaced comes from a number of ultrastructural and biochemical studies (Breen and Goldstein, 1975; Darley and Fuller, 1970; Darley et al., 1973; Kazama, 1974; Perkins, 1969, 1972; Porter, 1974).

The labyrinthulids, another taxonomic headache, share certain features with the thraustochytrids, encouraging Olive (1975) to include each group as a family in a newly erected order Labyrinthulida. At the second International Symposium of Marine Mycology held in 1972, the assembled mycologists agreed that any link between *Thraustochytrium* and the order Saprolegniales is artificial, and therefore recommended rearrangement of this taxon between the protozoa and fungi (Gaertner et al., 1974).

Physiology and Ecology

Goldstein (1973) in a recent review of zoosporic marine fungi suggested that these marine coccoid fungi may be most useful for probing salient features relating marine microorganisms to their environment. The increasing frequency of isolation from polluted littoral waters ensures these primitive forms a significant role in ecological studies.

Adair and Vishniac (1958) demonstrated that *Thraustochytrium globosum* plays an integral part in the oceans' thiamine and B_{12}

cycles. Goldstein and Belsky (1963) showed that at least three other thraustochytrids, including T. aureum, are dependent upon their environment for B_{12}, and are the only fungi reported to require this growth factor. Recently Breen and Goldstein (1975) demonstrated that strain S-3, an unidentified Phycomycete used for assaying thiamine and cobalamine levels in sea water (Vishniac 1961) is a bonafide member of the genus Thraustochytrium.

Determination of optimal culture conditions for marine phycomycetes has been the subject of a number of investigations. In general, the organisms will thrive over a broad range of environmental conditions, a fact not surprising, since members of the Thraustochytriaceae are prevalent in all major oceans and their tributaries (Bahnweg and Sparrow, 1972).

Bremer (1974), in an exhaustive study investigated temperature and salinity optima and light effects on 19 different isolates of thraustochytrids. All of the isolates were classified as euryhaline, although Goldstein and Belsky (1964) had previously regarded some of these same isolates stenohaline. Similar discrepancies can be found with regard to ideal temperature, and are probably due to differences in strains and media composition.

An interesting observation illustrating the usefulness of these organisms in studying the physiological ecology of a marine habitat has been made with T. roseum and species of Dermocystidium. Siegenthaler, et al. 1967) and Belsky, et al. (1970) have demonstrated that phosphate uptake can be attributed specifically to Na^+ concentration,

and that the role of NaCl extends beyond its osmotic functions. Thus the obligately marine nature of thraustochytrids is related to a need for Na^+ which cannot be replaced by other osmotically active substances.

For additional information see the substantial review by Goldstein (1973) on the physiology and ecology of marine Phycomycetes.

Ultrastructure

Ultrastructural studies of thraustochytriaceous fungi show that these organisms exhibit features common to most protists and higher organisms. Mitochondria with tubular cristae, endoplasmic reticulum typical of eucaryotes, Golgi apparatus, centrioles, and other organelles have been observed in all species examined. A detailed account of the ultrastructure of thraustochytrids and labyrinthulids was presented by Olive (1975). The several unusual morphological characteristics shared by these and other related marine forms have been the prime cause for speculating on possible closer phylogentic relationships between species of *Thraustochytrium*, *Schizochytrium*, *Dermocystidium*, and *Labyrinthula*. To date, most of the ultrastructural studies had been initiated as an attempt to resolve, using morphology, the complicated taxonomic relationships within the group. In *Thraustochytrium* sp. zoospores, Kazama (1972a) reported electron opaque structures within the lumen formed by kinetosome fibrils. Similar granules have been found in other thraustochytrids and labyrinthulids, but in no other fungi (Perkins and Amon, 1969;

Porter, 1974).

Biochemical analysis reveals galactose (mostly in L-isomer form) to be the principle sugar liberated upon hydrolysis of thraustochytrid cell walls, whereas the walls of Saprolegniales consist of glucans and cellulose as do all other members of the class Oomycetes. Another significant finding was the large protein fraction unique to the cell walls of thraustochytrids.

Viruslike Particles

A report demonstrating the uniqueness of thraustochytriaceous fungi, and of great interest to experimental mycologists, was the finding by Kazama and Schornstein (1972, 1973) of a herpes-type virus infection in one of their *Thraustochytrium* isolates. This was the first report of a herpes-type virus infecting a non-vertebrate host. Of particular interest was the observation that simple manipulation of culture conditions could lead to a "permissive" state with viruses in various developmental stages readily apparent. Preparations in which zoospores were nutritionally stressed by immobilization in thioglycollate agar 11 hours prior to fixation revealed abundant viruslike particles (VLPs). The claim that these particles belong to the family Herpesviridae is based on detailed ultrastructural observations.

The VLPs originate in the nucleus and become enveloped by two nuclear unit membranes during passage into the cytoplasm. In the cytoplasm the particles lose their membranous envelopes and develop

a fibrillar coat. Subsequent invasion of cytoplasmic organelles or Golgi-related vesicles appears to be the final maturation step before release of particles from the cell. These sequence of events, as well as size and symmetry of the particles, closely resemble other known herpes virus infections. Evidence that the dense particle cores are composed of DNA comes from experiments showing core digestion in thin sections treated with DNAse but not with RNAse.

When culture conditions favored the rapid completion of the fungal life cycle hardly any of the VLPs were visible. Attempts to generate single zoospore cultures free of VLPs were unsuccessful. Based on these results, Kazama and Schornstein suggested that their Thraustochytrium isolate is "virogenic", where cells maintain a potential to produce VLPs when stressed or stimulated in an appropriate fashion. Such a hypothesis is compatible with numerous reports describing VLPs in many different fungal groups. Fungal viruses are reviewed by Bozarth (1972), Hollings and Stone (1971), Wood (1973), Lemke and Nash (1974), Lemke (1976), Saksena and Lemke (1978), and Hollings (1978).

The assumption that most fungal viruses are latent and represent a benign symbiotic relationship is based primarily on the fact that such viruses are rarely responsible for any impairment of fungal growth. Functional properties such as infectivity have rarely been demonstrated. Most of the reports are based exclusively on electron microscope observations and do not conclusively prove that the particles observed are actually viruses. Koch's postulates

of infection, transmission, cure, and reinfection have generally not been taken into account because of technical difficulties inherent in the experimental material. Those fungal VLPs which have been characterized by physicochemical means are nucleoprotein in nature with a double stranded RNA, and possess an icosahedral symmetry.

The extent that a viral nature can be attributed to these particles is a controversial subject and is the very reason why the ambiguous term "viruslike" is so prevalent in the literature. Lemke argues in his review (1974) that, although labeling these particles viruses may be presumptive, little is to be gained by the continued use of "viruslike". Most investigators prefer to be more prudent and persist in describing observed particles as "viruslike", unless infection by cell free filtrates has been demonstrated. "Mycoviruses", a term designated by Bozarth (1972) in the first review of fungal viruses appears to be the best alternative. This term would be valid for fungal VLPs regardless of whether or not infectivity has been demonstrated.

For some of the more extensively studied mycoviruses, there is evidence that definite, persistent, heritable morphological traits are associated with the presence of VLPs. In the cultivated mushroom, Agaricus bisporus, Hollings (1963) showed that Die-back disease, in which rapid mycelial degeneration takes place, is caused by a virus. Hollings actually isolated the first fungal viruses from such

diseased mushrooms.

Banks et al. in a series of papers (1968, 1969, 1970) demonstrated morphological variations in virus infected colonies of Penicillium stoloniferum. Colonies of the infected strain had a noticeable irregular margin and were a different green color from the uninfected strain. Furthermore, double stranded RNA, extracted from isolated particles in this and several other infected Penicillium strains, was capable of inducing interferon activity in animals. Stimulation of interferon production by viral double stranded RNA, as well as the ability to eliminate viral particles by differential heat treatment, is part of the justification Banks and coworkers use in designating these particles viruses, although demonstration of infectivity was not possible.

Also included among the mycovirus-infected Pencillium sp are those which produce plaques on agar surfaces similiar to those produced by bacteriophages. Borré et al. (1971) noted marked morphological modifications in strains of P. citrinum and P. variabile subcultured from the center of lytic areas containing numerous VLPs.

The above cited examples where mycoviruses are associated with disease states or lytic phenomenon are not truly representative of the majority of published reports concerned with fungal viruses. Most reports are based on electron microscope findings and simply reveal the presence of particles or crystalline aggregates, thought to represent latent viruses. For the Phycomycetes, in other than the herpes-type particles reported by Kazama and Schornstein, there have been only a few reports of substance describing VLPs.

Khandjian et al. (1974, 1975) observed VLPs in the Bali I

strain of the fresh water chytridomycete Allomyces arbuscula. These particles were extracted, partially purified and characterized as RNA viruses. In mature hyphae the spherical or polyhedral 40 nm particles appear mostly in the cytoplasm in random aggregates during different stages of gametophytic and sporophytic generations. In mature gametes, probably as a consequence of their RNA content, the particles are preferentially localized in the mass of ribosomes making up the nuclear cap (Roos et al., 1976). Such localization in the gametes suggests a possible mechanism for the transmission of the virions throughout the life cycle. Although the VLPs were consistently found in many cultures fixed at different time intervals, Roos and coworkers found no evidence of ary pathogenicity. Apparently this putative virus is avirulent since no physiological or morphological disorder was evident either at macroscopic or microscopic levels.

Another phycomycete with VLPs is the oyster pathogen Labyrinthomyxa marina, a marine protist of uncertain taxonomic position. The cells glide through mucoid tracks as do members of the Labyrinthuales (protozoa), while morphologically (and on the basis of planont differentiation) they more closely resemble the holocarpic Saprolegniales (Perkins and Menzel, 1967). Actually the original description of this organism by Mackin et al. (1950) was under the name Dermocystidium marinum, a thraustochytrid. The close similarities of the labyrinthulids and thraustochytrids, and the consensus that both groups have been taxonomically misplaced has

already been discussed. An electron microscope study by Perkins (1969) designed to compare the ultrastructure of L. marina with other phycomycetes led to the unexpected discovery of VLPs in the nucleus and cytoplasm of all cellular stages of L. marina isolated from Virginia oysters. Similar studies on pathogens isolated from Florida oysters showed no evidence of VLPs. The observed particles were approximately 50 nm in diameter and by the use of photographic rotation techniques, were shown to be icosahedrons with a five or sixfold symmetry.

Schnepf et al. (1970) reported VLPs in thalli of Aphelidium sp, another aquatic phycomycete of uncertain taxonomic position, which parasitizes the green alga Scenedesmus armatus. The polyhedral particles are about 200 nm in diameter and appear in the remains of Aphelidium protoplasts which have disintegrated within the algal host. The majority of the cells do not become infected and complete a normal developmental cycle where they either form resting spores or divide to produce zoospores. Those cells which fail to develop completely do not divide or produce a cell wall within the host. Their cellular organelles become disorganized in conjuction with the formation of a large spherical conspicuous body at the periphery of which VLPs are formed. The body, referred to by Schnepf and coworkers as a "virogenous body" is at first approximately 2 µm in diameter and gradually decreases in size as the number of particles increases.

Thraustochytrium aureum, the organism used in this research, also

has the capability of producing VLPs. When cultured under anaerobic conditions hexagonal particles approximately 150 nm in diameter can be observed in the cytoplasm of many developing sporangia (Pollak, 1975). In a sense the induction of VLPs in *T. aureum* under anaerobic conditions is analogous to Kazama's and Schornstein's findings (1972, 1973). In their *Thraustochytrium* isolate the cells are "virogenic" with production of VLPs preceded by some yet unknown stimulus.

Unlike *Aphelidium* and *L. marina*, members of the genus *Thraustochytrium* are not parasitic. They are saphrophytes and can be isolated from a wide variety of natural substrates including alga of many different genera (Booth and Miller, 1968; Fuller et al., 1964). Data describing ecological relationships between thraustochytrids and marine algae are almost nonexistent, this is because phycomycetes, although ubiquitous, have relatively slow growth rates compared to other marine microorganisms. Only through special baiting and enrichment procedures and use of antibiotic - containing media has detection of these evanescent forms become practical.

Recent reports of large polyhedral VLPs in green, red, and brown algae (see reviews by Lemke, 1976; and Andrews, 1976) resembling the *Aphelidium* and *Thraustochytrium* particles should be seriously evaluated when considering ecological relationships

between marine algae and phycomycetes. In most instances VLPs in algae have five- or six-sided polygonal profiles indicative of icosahedra and are generally associated with varying degrees of cellular disruption. As in the case of most mycovirus-infected fungi, actual proof that these are true viruses is lacking since reports are based solely on descriptive morphology.

One exception has been reported for the green alga Chara corallina (Gibbs et al., 1975) where long rod shaped particles with RNA cores were isolated, purified and shown to be serologically related to two known tobacco mosaic viruses. Through microinjection techniques, transmission of virus into healthy, previously uninfected cells was possible providing unequivocal proof that the particles are true viruses.

In several instances investigators have noted VLPs in only a singe culture or in experimental material intended for other purposes where cells were either manipulated or treated in some novel fashion. Only upon ultrastructural examination was there any indication of a possible virus infection. Chapman and Lang (1973) employed a synchronizing light regime in a study designed to reveal stages in karyokinesis of the red alga Porphyridium purpureum. During the course of their study, the authors observed unusual inclusions, termed concentrosomes, and small circular or polygonal particles approximately 40 nm in diameter, morphologically similar to some of the mycoviruses infecting higher fungi.

Markey (1974) found VLPs within sporangia of the brown

alga *Pylaiella littoralis* from a single preparation that had been left standing 24 hours prior to fixation. The hexagonal particles present in cells showing evidence of nuclear breakdown were 130-170 nm in diameter and resembled viruses of the iridescent group in size and shape. Infected sporangia also contained cytoplasmic modifications, the most striking of which were unusual membranous components enclosing electron-dense material. Although there is no direct evidence that allowing the algal plants to stand 24 hours had direct influence on VLP formation, the possibility that this procedure did in fact act as a stimulus inducing their formation cannot be ignored. Thus *Pylaiella littoralis* may be another example of a "virogenic" system where latent viruses complete their developmental sequences only under some undefined abnormal environmental condition.

VLPs in the green alga *Cylindrocapsa* perhaps best represent an instance of cells possessing a latent virus where mature particles are not produced unless the cells are subjected to environmental manipulations. Hoffman and Stanker (1976) found that when zoospore release in *Cylindrocapsa* was triggered by exposing algal filaments to a 40°C heat-shock treatment for six hours, the germlings derived from these zoospores contain large icosahedral particles up to 230 nm in diameter. Germlings from the same *Cylindrocapsa* strain not produced by the heat-shock method never gave rise to VLPs. Furthermore, repeated subculturings of surviving germlings from samples of heat-shocked material developed into healthy, actively growing filaments. However, there was one series of experiments

interpreted by Hoffman and Stanker to be inconsistent with a latent virus infection. In these experiments individual germlings from infected populations were brought into culture and repeatedly transferred every two weeks. Eight months after the original isolation, heat-shock treatment was ineffective in producing infected germlings. Although such results suggest an ordinary virus transmission and infection for Cylindrocapsa germlings, Hoffman and Stanker do not rule out a latent virus hypothesis because of the unusual heat-shock conditions required for the occurrence of VLPs.

Although reports of viruses in eucaryotic microorganisms have become increasingly common in recent years, there is still a paucity of information on the effect these VLPs have on the morphology and metabolism of their hosts. The ecological significance of these putative viruses should be considered regardless of whether or not VLPs are eventually proven to be viruses within the concepts of animal and plant virology. In the case of phytopathogenic fungi, there are many findings (reviewed by Lemke and Nash, 1974) showing them to contain a high incidence of VLPs, yet little is known whether the pathogenicity of the virus, if any, is directed against the fungus or the parasitized host plant.

A good illustration of the ecological significance of mycoviruses is found in the study by Bozarth et al. (1972) of the presence of VLPs in the Southern corn blight fungus, Helminthosporium maydis. Bozarth and coworkers screened twelve strains of the fungus from

geographically isolated areas for virus and detached three different VLPs from strains capable of causing severe disease symptoms in corn. The observation that strains in which VLPs were absent are only mildly pathogenic, seemingly adds a new dimension to plant pathology studies involving host-parasite relationships.

Many of the eucaryotic microorganisms that are known to harbor latent viruses have close associations with other aquatic or terrestrial organisms. This has led to much speculation suggesting that such VLP-containing microbes serve as vectors for virus diseases in other plants and animals. In polluted marine habitats, Goldstein (1973) noted the significance of the herpes-type virus found in Thraustochytrium sp (Kazama and Schornstein, 1972), the VLPs in the thraustochytrid-labyrinthulid L. marina, and a herpes-type virus infection in oysters living at elevated temperatures reported by Farley et al. (1972). The oyster herpes-type virus infection also provided the basis for speculation by Pearson and Norris (1974) that marine algae may serve as vectors of viruses for marine animals. Their speculation was the result of their having found intranuclear VLPs, similar in morphology to the oyster particles, in Platymonas sp, a marine green alga widely distributed along the west coast of North America.

In a discussion of the large polygonal-shaped VLPs in eucaryotic algae, Hoffman and Stanker (1976) also suggested the possibility that algae serve as virus reservoirs for different forms of aquatic life. Such speculation seems quite plausible in light of published reports describing virus infections in aquatic animals by viruses similar in

morphology to those found in many algae.

This study employing primarily ultrastructural techniques is an attempt to investigate the effects of changing environmental conditions on the development of the thraustochytrid, T. aureum. The ability of this organism and other closely related forms to display a wide variety of morphological alterations dependent upon growth condition is well documented at the light microscope level. The majority of ultrastructural studies to date are primarily concerned with developing a better understanding of the phylogenetic relationships existent among the aquatic phycomycetes. Little information is available detailing morphological and developmental variations of a single species or the pathological responses to stress induced by environmental manipulations. The significance of such developmental studies is enhanced by the recent surge in environmental research of marine and estuarine ecosystems.

Furthermore, the fact that viruslike particle formation can be induced in this organism, makes T. aureum an ideal candidate for investigating precisely and to what extent environmental manipulations are a factor in mycovirus proliferation. Induction of mycovirus production in apparently healthy cultures by simple manipulation of culture conditions also provides the opportunity to assess at the ultrastructural level the effects these putative viruses have on host cell development, a subject having received scant prior attention Thus the aims of this study are: First, determine the degree of morphological plasticity and developmental alterations at the ultrastructural level;

and second with regard to the viruslike particles, trace their mode of formation, define the conditions under which they can be induced, and consider any pathological effects on the host cell.

On a broader scale it is felt that experimentation with these poorly understood forms can add significantly to an appreciation of complex ecosystems such as polluted marine littoral waters in which these organisms are particularly prevalent.

MATERIALS AND METHODS

Organism

Cultures of Thraustochytrium aureum Goldstein (1963b) were provided by Dr. S. Goldstein. This strain was isolated and brought into axenic culture from littoral waters bounding Woods Hole, Massachusetts. Other unidentified Thraustochytrium spp were isolated from polluted costal waters in the New York area.

Media and Culture Methods

T. aureum is obligately marine and was routinely cultivated at 23^{0}C in a glucose-glutamate marine medium (see Table 1), a modification of the ASP6 medium of Provasoli et al. (1957). The organism was transferred every two weeks into 16 X 150 mm, screw-cap tubes containing 7.5 ml of media. Adequate aeration of the cells was ensured by the addition of 0.15% agar to the media, which tends to keep the cells at the surface.

Inocula for the various experiments were prepared by adding 1 ml from a tube with dense growth into a 50 ml Erlenemeyer flask containing 24 ml of media. After incubation in a shaker at 23°C for 4-5 days, 2.5 ml of the logarithmic vegetative phase cells were added to each of the experimental growth vessels.

In several instances the inoculum consisted entirely of a population of motile zoospores. These were prepared by adding a 2.5 ml cell suspension to Petri dishes containing medium solidified with 1.7% agar. After 24 hrs., the excess liquid was drained, so that the plate surfaces dried and the sporangia became firmly anchored

in the agar. The plates were then stored in a refrigerator for up to 1 month. Discharge of zoospores from sporangia was triggered by adding 10 ml of sterile distilled water to the dry plates. Approximately 5-6 hours later the water overlay contained a relatively pure, dense population of zoospores, which could be pipetted directly from the surface of the plate. The zoospores were counted with a Petroff-Hauser bacteria counter.

For light microscope observations, cells were cultured in sea water baited with Pseudotsuga taxifolia pollen. This method was employed to minimize morphological variations common with an enriched medium.

Anaerobic growth was achieved by culturing the organism in a BBL anaerobe jar with a hydrogen-carbon dioxide Gas Pak, or in a desiccator from which oxygen was continuously removed with a constant flow of nitrogen gas. Anaerobiosis was monitored with a Gas Pak methylene blue anaerobic indicator.

In one series of experiments total removal of oxygen was not attempted, and no hydrogen or nitrogen gas was added Cells were simply placed in a desiccator with a burning candle. Sealing the desiccator extinguished the candle, thereby providing a high CO_2 environment for the cells.

In addition to anaerobic growth, cells were subjected to stress conditions with extremes of temperature, salinity, and pH. (see Table 2). Unless stated otherwise, growth chambers were opened,

cells harvested, and prepared for electron microscopy at 3, 5, and 7 days after inoculation.

Treatment with Halogenated Pyrimidine Derivatives

One ml of inoculum was added to 24 ml of fresh media containing 20, 100, 200, or 300 µg of 5 - bromo - 2 deoxyuridine (BUdR), 5 iodo - 2 - deoxyuridine (IdU), and 5 - fluorodeoxyuridine. (Sigma Chemical Co.). The cells were harvested for electron microscopy after five days in an incubator shaker at 23°C.

Incubation of Cells with Labeled Precursors

One ml zoospore suspensions were added to sterile 60 x 15 mm tissue culture dishes (Falcon Plastics) containing 4 ml of liquid media. The dishes were left standing until all the zoospores encysted. When the encysted zoospores had settled at the bottom of the dishes, uridine-5-^{3}H (New England Nuclear, sp.act. 26.2 Ci/m mole), or thymidine methyl - ^{3}H (NEN, sp. act. 20.0 Ci/m mole) was added to separate dishes at concentrations of 40 µ Ci/ml. After 20 hours the dishes were drained and the cells washed 3 times with 25% sea water; care being taken not to dislodge any of the cells. Five ml of fresh medium was added and the dishes incubated in an anaerobe jar for 2-5 days at 23°C.

Electron Microscopy

Cell suspensions were prefixed (1 part fixative to 5 parts

medium) and then centrifuged lightly to form a pellet. The fixative employed was 2% glutaraldehyde, buffered with veronal-acetate or collidine (pH 7.2). The possibility that tonicity factors were responsible for some of the observations was considered. Several samples were prepared for electron microscopy where either the buffer was made up in 2.4% (w/v)NaCl, or 8% glutaraldehyde was added directly to fresh medium to a final concentration of 2%, and the pH adjusted to 7.2. Fixation time was generally 2 hours. Cells were postfixed with veronal-acetate-buffered OsO_4 for 20 minutes.

After fixation the cells were washed in 0.01% CaCl and then stained in an aqueous solution of saturated uranyl acetate for 1 hour. Following dehydration through graded ethanols and propylene oxide, the cells were embedded in Maraglas epoxy resin (Spurlock et al. 1963). Thin sections were cut on a Porter-Blum MT-2B ultramicrotome using a diamond knife. Sections were mounted on formvar-filmed, carbon-stabilized grids stained with Pb citrate (Reynolds 1963), and examined in an RCA EMU-3G electron microscope operating at 100 kV, or a Zeiss EM 9S-2 at 60 kV.

Electron Microscopy Autoradiography

Labeled cells were prepared for electron microscopy in the usual manner. Thin sections were mounted on carbon stabilized filmed grids and coated with Ilford L4 emulsion by means of a

wire loop following the technic of Ehret et al. (1964). Emulsion-coated grids were stored in light tight boxes at room temperature for 3-4 weeks. Silver grains were developed with Kodak Microdol-X, immediately followed by Pb citrate staining to enhance contrast.

Acid Phosphatase

Localization of acid phosphatase activity using a modified Gomori technique (Barka and Anderson 1962) was performed on both anaerobically grown cells and cells grown aerobically at 33°C. Five day-old cultures were fixed in collidine- buffered glutaraldehyde and then incubated at 37°C in the reaction mixture for 24 hours. Cells were prepared for electron microscopy in the usual manner except that staining with uranyl acetate and Pb citrate was omitted so that the lead phosphate reaction product would be more readily distinguished.

Table 1.

NaCl	2.4 g
$MgSO_4 \cdot 7H_2O$	0.8 g
KCl	0.07 g
$CaCl_2$	15 mg
"Tris"	0.2 g
NaH glutamate	0.2 g
Glucose	0.2 g
Na^+ Glycerophosphate	20 mg
Vitamin B_{12}	1.0 µg
Vitamin mix 8[1]	0.1 ml
Trace Metals	
Na_2EDTA	5.0 mg
Fe (as $FeSO_4 \cdot 7H_2O$)	0.05 mg
Zn (as $ZnSO_4 \cdot 7H_2O$)	0.02 mg
Mn (as $MnSO_4 \cdot H_2O$)	0.01 mg
Co (as $CoSO_4 \cdot 7H_2O$)	2.0 µg
Cu (as $CuSO_4 \cdot 5H_2O$)	0.2 µg
B (as H_3BO_3)	2.0 µg
Mo (as $NaMoO_4 \cdot 2H_2O$)	2.0 µg

Glass distilled H_2O to 100 ml

pH 7.4 after autoclaving

[1] 1 ml of vit. mix 8 contains: Thiamine HCl, 0.2 mg; nicotinic acid, 0.1 mg; Biotin 0.5µg.

Table 2.

Environmental Parameter	Optimal[1]	Upper Extreme	Lower Extreme
Temp. (C)	23°	33°	15°
Salt Conc. % NaCl[2]	2.4	4	1.0
pH	7.0-7.6	8.4	5.5

[1] Based on observations made by Goldstein (1963)

[2] KCl concentrations remained constant at 0.07%

RESULTS

Life Cycle and Morphology

Descriptions of the life cycle and external morphology of Thraustochytrium aureum are based on the repeated examinations of sea water pollen cultures at room temperature. The use of hanging drop preparations greatly facilitates prolonged observations of single cultures during the entire course of development. These light microscope observations are almost identical to those reported by Goldstein (1963b).

Freshly discharged zoospores are smaller and more elongated than those formed on nutrient media. They are somewhat ellipsoidal, have a large refractile globule, measure 3.7 µm (range 3.2-4.0 µm) in length and 2.4 (range 2.0-2.7 µm) in width. They possess two laterally attached flagella, one posteriorly directed whiplash flagellum and a considerably longer anteriorly directed tinsel flagellum measuring approximately 15 µm as observed by phase contrast microscopy. Zoospores swim rapidly for 1-3 hours with sudden and frequent changes of direction before becoming stationary and withdrawing their flagella. The spores encyst on the surface of pollen grains and form germ tubes capable of digesting the resistant outer wall of the pollen grain. Inside the grain the germ tube becomes branched and forms a network of rhizoids which supply nutrients to the developing sporangium on the surface of the pollen grain. As the sporangium continues to increase in size, the wall thickens, numerous

mitotic divisions occur, and eventually the sporangial contents are cleaved into individual zoospores.

When fully mature the large number of zoospores begin to swim freely within the sporangium causing a rupture of the sporangial wall from which the zoospores haphazardly escape. Often a basal cell or rudiment separates from the rest of the sporangial cytoplasmic remains and by internal proliferation produces a secondary sporangium together with a new basal rudiment. At room temperatures *T. aureum* completes its life cycle in 26-32 hours.

Ultrastructure

Ultrastructural examination of aerobic logarithmic phase cultures reveals that *T. aureum* possesses many of the distinguishing characteristics of the Thraustochytriaceae (Alderman et al., 1974). More specifically it shares many ultrastructural details with the extensively studied 70-2 *Thraustochytrium* isolate described by Kazama (1972a, 1972b, 1973, 1974a, 1974b, 1975) and the *Japonochytrium* described by Harrison and Jones (1974). In addition several ultrastructural features were observed that have not been previously described for any other thraustochytrid.

Longitudinal sections through the flagellar apparatus and associated structures of *T. aureum* zoospores reveal the presence of electron-dense structures within the kinetosome lumen (fig 1). These structures appear to be identical to the kinetosome granule and the complex terminal plate-basal disc region described by Kazama (1972a). The kinetosome-flagellum

complexes of both anterior and posterior flagella have an identical ultrastructure and are difficult to distinguish from one another without the benefit of serial sections. Mastigonemes, known to be present on the anteriorly directed tinsel flagellum, apparently fall off while the material is being prepared for electron microscopy.

The morphology and development of mitochondria show greater complexity than other thraustochytrids previously studied. Figures 2 and 3 suggest that in the zoospore stage there is a single large mitochondrion per cell. The mitochondria, which have tubular cristae, are sometimes branched, and encircle the nucleus. In later developmental stages the mitochondria fragment into numerous segments which are distributed throughout the cytoplasm of the developing sporangium (fig 4). The concentric double membranes enclosing cristae-free areas within mitochondria that were reported by Goldstein et al. (1964) are rarely observed in young cultures but are a prominent feature of aged cells (fig 29).

The spherical, centric nucleus (fig 3, 4 and 9) is surrounded by a well developed nuclear envelope with pores and contains a prominent nucleolus. Electron dense chromatin is distributed throughout the nucleoplasm. Adjacent to the nucleus is a single active Golgi apparatus (fig 4 and 9) which proceeds to divide during successive nuclear divisions. Vesicles derived from the Golgi are directly associated with the nuclear membrane. Lying close to the nucleus within a nuclear indentation is a pair of centrioles (fig 9 and 25) situated at an angle of 120-140° relative to each other. The centrioles contain an electron

dense core morphologically similar to the granules found within the kinetosome.

Enclosed within the cytoplasmic matrix are ribosome free areas (Goldstein et al. 1964), smooth endoplasmic reticulum which may be continuous with both nuclear and mitochondrial outer membranes, small and large cytolysomes (Kazama, 1973), and multi-vesicular bodies.

The gradual increase in the thickness of the cell wall results from the deposition of thin, circular, amorphous scales on the surface of the developing sporangium (fig 8). Large numbers of these Golgi-derived scales (Darley et al., 1973) overlap in a complex structural pattern forming a multilamellated wall over the convoluted plasma membrane (fig 8).

Towards one pole the sporangial cell wall forms an elaborate apophysis-like structure (fig 4) consisting of intertwining ectoplasmic net elements. Since these structures appear to be more prevalent in liquid cultures than in cells grown on solid substrates, they do not seem to be involved in the absorbption of nutrients or as a mechanism for movement and attachment.

A single sagenogenetosome is present and forms soon after zoospore encystment. Figure 3 shows the presence of a sagenogenetosome in a cell prior to any nuclear divisions or mitochondrial fragmentation. The sagenogenetosome (figs 5-7) is an electron dense organelle composed of a series of endoplasmic reticulum channels converging just beneath the plasmalemma. Presumably the sagenogenetosome is the focal point from which cell wall elements are transported to the surface of the developing sporangium.

Directly beneath the plasmalemma are unidentified structures which superficially resemble in size and shape the gamma particle described by Cantino and Mack (1969) for the freshwater chytrid Blastocladiella emersonii. The dense horseshoe-shaped structures (figs 4 and 8) are approximately 450 nm in diameter and are observed only in sporangia that have not yet undergone cleavage into individual zoospores. Figure 8 is a grazing section through the cell wall and adjacent cytoplasm of a developing sporangium. The various configurations shown in figure 8 and in other sections not presented here, suggest an open ended cylinder composed of inner and outer unit membranes separated by electron dense material. There is no indication that these bodies are oriented in any specific manner. Unlike the gamma particles these structures are not completely surrounded by a continuous unit membrane.

Anaerobic Cultures

The developmental pattern and morphological details outlined above are strikingly different from those observed in cells grown under anaerobic conditions. Representative micrographs taken of anaerobic cultures fixed and processed for electron microscopy 2-5 days after inoculation are shown in figures 10-27. Freshly discharged zoospores placed in anaerobe jars encysted, increased in size, and underwent mitotic divisions, but were unable to complete the entire developmental cycle by forming mature sporangia with motile zoospores.

At two days both nuclear and cytoplasmic effects were clearly visible in roughly 50% of the cells examined. Nuclear membranes of these cells were often swollen and filled with an amorphous substance (fig 10). It is unlikely that this represents an osmotic artifact since changing to buffers isoosmotic with sea water yielded similar results. In addition, the mitochondria, which are generally indicative of the quality of fixation, appear unswollen. The distribution of chromatin is altered (fig 12) by showing a large degree of margination. Golgi bodies, a prominent feature of aerobically grown cultures, are conspicuously absent. In the cytoplasm there are numerous inclusions (fig 10-15) which consist of membranous components and crystalline arrays which are sometimes lost during the processing for electron microscopy. The crystalline structures seen in figures 13-15 probably remained intact because the plane of section is perpendicular to the long axis. A higher magnification through one of these crystalline arrays (fig 15) shows the crisscrossing of electron dense rods to form a lattice-like arrangement with a center-to-center spacing of 21 nm. The structures enclosed within the large empty spaces in figures 10 and 12 resemble the polyphosphate granules observed by Wool and Held (1976) in encysted zoospores of Rozella allomycis. The notion that these morphologic alterations are simply degenerative changes taking place in dying cells is unlikely, since electron microscope autoradiographs (fig 22) of anaerobically cultured cells pulsed with ^{3}H thymidine indicate that they are actively synthesizing DNA.

The most striking morphological manifestation of anaerobic growth is the accumulation of large masses of endoplasmic reticulum-derived tubules observed in approximately 5% of such cells. These unique membranous structures are evident in two day anaerobic cultures (fig 10 and 11) and by 3-4 days enlarge to form discrete complexes displaying regular repeating patterns. Ultrastructural examination of these complexes (figs 16-19) show them to be composed of a series of undulating tubules approximately 23 nm in diameter. Depending on the plane of section the tubules are revealed as either circles, loops, or a combination of these configurations embedded within a granular cytoplasmic matrix. The inside diameter of the circles measures from 80-115 nm and the mean distance between adjacent circles is 70 nm. The walls of the tubules are composed of two electron-dense, double membranes 8-12 nm apart. The cytoplasm enclosed within the walls is identical in density and granularity to the cytoplasmic matrix outside the walls. In figure 18, the walls of the tubules appear continuous with the rough ER at the periphery of the membranous complex. In several instances elaborate ER complexes were observed to be directly associated with the crystalline aggregates described above. Figure 13 shows such an association and suggests a possible role for these specialized regions of ER.

A search of the literature for earlier reports of ER-derived tubules with similar symmetrical configurations revealed that they have indeed been previously described, primarily in clinical reports, in a variety of tissues of different species. There is much uncertainty regarding

their biogenesis and biochemical nature; however, from the many different conditions in which they have been observed it is clear that they share many features in common. Although many authors have referred to these structures under various descriptive terms, they are now commonly labeled tubuloreticular structures (TRS). The significance of TRS and their possible role in anaerobically grown cells of T. aureum will be discussed later.

From the variety of profiles and from semiserial sections a three-dimensional graphic reconstruction was prepared (fig 42) representing one of several possibilities that would account for the circles, loops and net-like configurations observed. In this model the ER undergoes a transformation where apposing membranes converge to form membranous sheets lined on each side with a double unit membrane. Several of these sheets become stacked in a plate-like fashion. The entire stack then becomes compressed and corrugated with uniform ridges and grooves in each sheet. A second compression of the stack at a 90° angle to the first results in the corrugated appearance illustrated. Thus, the undulating tubules observed are cross sections through undulating plate-like formations of specialized ER.

Viruslike Particles

When cultures of T. aureum were maintained under anaerobic conditions from 4-5 days, progressive degeneration of the nuclei and surrounding cytoplasm was observed in roughly 15% of the cells. In many cells,

except for the cell wall, there was a complete lack of any organized structure. This near lysis of the cells was associated with the appearance of numerous hexagonal-shaped viruslike particles (VLPs) 150 nm in diameter, distributed throughout the cell (fig 20). The VLPs are contained within the still intact striated cell wall and embedded in a "viral stroma" composed of ribosomes, vacuoles, fibrous material, and lipid droplets. VLPs or cells with the same degenerative pattern were never observed in cultures grown aerobically. Polyhedral 150 nm VLPs found occasionally in 3 day anaerobe cultures (fig 14) probably represent the earliest stages in VLP formation.

Figure 21 shows the VLPs at a higher magnification. They have an opaque fibrillar core surrounded by a triple layered shell 20-25 nm thick. The shell itself is constructed of two unit membranes separated by an electron lucent zone. Often the VLPs appear as empty shells (fig 20). Such empty particles are thought to represent immature virions and are common in early stages of assembly of infectious particles.

The six-sided appearance of the VLPs in section and their envelopment with a distinct membrane suggest that they are icosahedra, morphologically resembling the iridescent group of insect viruses reviewed by Bellett (1968) and Tinsley and Harrap (1978). Partial evidence that the cores of these putative virions are composed of RNA can be seen in figures 23 and 24. Autoradiographs of VLPs show grains when cells are pulsed with ^{3}H-uridine. However, no grains in the vicinity of the particles were evident when cells were similarly pulsed with ^{3}H-thymidine.

It is of interest to note that the measurements and morphology of the VLP outer shell are almost identical to those of the tubular structures prevalent in 3-4 day old cultures. Furthermore, the inner core of the VLPs measures approximately 100 nm, which is about equal to the inner diameter of the circles formed by the undulating tubules. Although there is no direct evidence linking the tubular structures with the production of VLPs, the fact that they approximate each other in size and morphology, and that both VLPs and tubules appear only in environmentally stressed cultures, is cause for suspecting an ontogenetic relationship. Such speculation is consistent with reports of the presence of tubuloreticular structures in some known virus infections of mammalian cells (Grimley and Schaff, 1976). Alternatively, it is equally possible that the presence of VLPs and tubular structures in anaerobically grown cells is symptomatic of a stressed environment and that no developmental relationship exists.

Attempts to increase the percentage of cells producing VLPs by lengthening the incubation periods in anaerobic environments were unsuccessful. Maintenance of anaerobic conditons for up to 14 days did not significantly increase the number of VLP producing cells or the number of cells displaying cytoplasmic deterioration associated with VLP production.

In order to determine whether or not the effects of anaerobiosis were reversible on those cells which showed no evidence of degeneration, cells from the 14 day anaerobic culture were reincubated aerobically an

additional 48 hrs in the presence of 40 µCi/ml of ^{3}H-uridine. Light microscope examination revealed a fresh growth spurt as evidenced by the presence of numerous zoospores. Figure 25 is an electron microscope autoradiograph of a fully mature sporangium with morphologically normal zoospores. The distribution of grains throughout the sporangium in the individual zoospores indicates that these are newly formed cells.

The results so far presented are compatible with a latent virus hypothesis in which virogenic cells form viral particles in response to specific stimuli. This would explain how cultures inoculated with apparently healthy cells become infected despite the fact that extracellular VLPs were never observed. Such a hypothesis would also account for the transmission of the presumptive virions in anaerobic cultures, since the particles are undetectable in zoospores, and it is unlikely that the particles can penetrate the multilamellar wall of a developing sporangium. Assuming that T. aureum does harbor a latent virus, the viral genome is not necessarily present in the genome of each individual cell. Thus, those cells unaffected by exposure to anaerobic environment were never "virogenic" in the first place.

Several other interesting morphological alterations have been observed in anaerobically cultured cells (fig 26-30). However, because of their infrequent appearance it is difficult to state with certainty that they are a characteristic feature of such cultures. A second class of cytoplasmic VLPs 45-50 nm in diameter can be seen in figures 26

and 27. These particles bear no morphological resemblance to the polyhedral 150 nm particles that are present in the cells of this same population in far greater numbers. These smaller VLPs are evenly spaced and interconnected by fine strands radiating outwards from their single layered coats. In figure 27 both the small VLPs and undulating tubules of ER can be seen in the same section.

In several instances annulate lamellae were observed (fig 28). These distinctive cytoplasmic organelles are continuous with endoplasmic reticulum cisternae and are composed of stacks of double membranes interrupted at periodic intervals by pores. Generally, annulate lamellae are found in proliferating or differentiating cells of neoplastic or embryonic tissues (Kessel, 1965; Wischnitzer, 1970). Thus, the formation of these structures in cells of _T. aureum_ displaying signs of deterioration is highly unusual.

Other Environmental Manipulations

The production of VLPs and associated morphological modifications described above were experimentally induced under anaerobic conditions. However, no evidence was presented showing that this altered course of development is a direct consequence of an anaerobic environment and not a general pathological response to a state of stress. For this reason cultures were subjected to other environmental stresses including extremes of temperature, salinity and pH (see Table 2). In addition

candle-jars were employed as a means of providing cultures with high levels of CO_2. The jars were opened from 20-30 minutes daily and before being resealed a burning candle was placed inside. This procedure provided a relatively high CO_2 concentration without removing the oxygen dissolved in the medium. In this system methylene blue anaerobic indicators did not lose their color. Finally, in order to determine whether aged cells manifested any of the unusual morphological alterations, senescent cultures were allowed to stagnate in their own spent medium for 3-4 weeks before being fixed and processed for electron microscopy.

Ultrastructural examination of senescent cells failed to reveal the presence of any specialized endoplasmic reticulum or signs of VLPs. The effects of aging were mostly evident in the dense mitochondria with central membranous inclusions (fig 29), myelin bodies (fig 30), and occasionally in cells undergoing degeneration and fragmentation of cytoplasmic organelles (fig 32). Apart from these age-related signs of deterioration, complexes of intensely stained microtubules were observed in several instances (fig 31). These complexes are composed of short, 16-18 nm in diameter, electron-dense microtubules arranged parallel to one another. The aggregates are twisted and turned to form an orderly assemblage of linked microtubules. In contrast, the microtubules seen in figure 29 are long, have few twists, are not closely packed, have an electron-lucent core, and are much wider, measuring 45-50 nm in diameter.

Goldstein (1963b) has shown that T. aureum is obligately marine and requires 2.0% NaCl for optimal growth, but will survive and grow, although poorly, at NaCl concentrations ranging from 1-4%. Ultrastructural examination of cells cultivated at both 1 and 4 % were normal and did not display any morphological anomalies. Similar manipulation of pH had no marked effect other than reducing yield. This is of particular interest since measurement of pH after 4 days under anaerobic conditions showed a drop from an initial pH of 7.4 to 5.7, presumably due to an accumulation of fungal metabolites. Thus the lowered pH of the culture medium is not a factor in inducing the altered developmental sequences observed in anaerobically grown cells.

Response to High Temperature

Cultivation of T. aureum at 34°C was effective in inducing some of the morphological changes observed in anaerobic cultures. Figure 33 shows tubuloreticular structures present after 4 days of incubation at these elevated temperatures. However, the percentage of cells possessing these membranous elaborations is much lower than in the anaerobically grown cultures. The large, 150 nm, hexagonal particles found in anaerobe cultures were not present even after 7 days of incubation at 34°C.

The raised temperature at which these cells were incubated was responsible for the formation of large cytoplasmic inclusions. These inclusions (figs 34 and 35) were present in the majority of cells examined and contained electron-dense particles 35-55 nm in diameter surrounded by membrane fragments. Also present but lying free in the cytoplasm are the

45-50 nm VLPs (fig 36) observed infrequently in the anaerobic cultures (fig 26 and 27). The large, unusual, ribosome-free cytoplasmic inclusions presented in figures 34 and 35 resemble in some respects the cytolysomes described by Kazama (1973). However, incubation of these cells in modified Gomori's medium, used to detect acid phosphatase activity, showed high levels of this enzyme only in the mitochondria, Golgi apparatus, and endoplasmic reticulum but not within the cytoplasmic inclusions (fig 41).

Dense rods composed of microtubules fused together often appear within these inclusions (fig 35). The conclusion that these rods are made up of microtubules is based on micrographs showing evenly spaced 16 nm microtubules within the inclusions in cells incubated at 34°C in the presence of 500 µg/ml of colchicine (fig 37 and 38).

The function or significance of these inclusions with their dense particles and microtubules, as a response to elevated temperatures, is not known. It is unlikely that they are virogenous bodies since the particles observed are not uniform in size and shape as are the other VLPs found in this organism. The high magnification micrograph presented in figure 38 shows fine fibrillar bridges connecting the individual microtubules one to another, suggesting a possible contractile role for the microtubular bundles.

High CO_2 Levels

The altered developmental sequences induced by exposure to anaerobic

conditions could not be duplicated by high CO_2 levels alone. Zoospores inoculated into candle-jar cultures encysted and formed apparently healthy sporangia within 48 hours. However, cleavage of the sporangial contents into a new generation of zoospores was inhibited. At 4 days many of the sporangia developed large central vacuoles, evident at the light microscope level. Figure 39 is a median section through one of these donut-shaped sporangia. Surrounding the vacuole a normal complement of sporangial organelles can be seen including nuclei, mitochondria, Golgi apparatus, endoplasmic reticulum, ribosomes, and gamma-like bodies. Tubuloreticular structures, VLPs, inclusion bodies and microtubular bundles were not observed.

In normal aerobic cells final cleavage of a sporangium into zoospores seems to take place by a process of cytoplasmic vacuolization. In this process a separate zoospore results from each sporangial fragment becoming completely surrounded by vacuoles. Since vacuolization normally occurs during cleavage, it is conceivable that the high CO_2 concentrations which inhibit the final stages of zoospore formation are somehow linked to the large central vacuoles observed in candle-jar cells.

Effects of Halogenated Pyrimidine Derivatives

Since cultures of T. aureum apparently harbor a latent virus whose formation may be intimately related to tubuloreticular structures, it was decided to cultivate the cells in the presence of halogenated pyrimidine derivatives. These compounds are known to activate latent viruses in a variety of cells (Lowy et al., 1971; Gerber, 1972; Grimley

et al., 1973a; Margalith et al., 1975). Such an experiment seemed particularly appropriate in view of the fact that bromodeoxyuridine and iododeoxyuridine were effective in inducing the formation of tubuloreticular structures in human lymphoid cell lines known to be infected by virus. (Grimley et al. 1973a, b, and c).

In preliminary experiments the thymidine analogs were added to cultures in concentrations ranging from 20-300 μg/ml. 5-iododeoxyuridine and 5-fluorodeoxyuridine had a far greater inhibitory effect on growth of cells than did BUdR. Even at the relatively high concentration of 200 μg/ml, 5 days of BUdR treatment was responsible for only a 30% decrease in cell numbers.

Although VLPs could not be detected in any cultures incubated with these thymidine analogs, approximately 3% of the cells grown in the presence of 200 μg/ml of BUdR developed tubuloreticular structures, Figure 40 is a micrograph of a cell exposed to 200 μg/ml of BUdR, where the specialized form of endoplasmic reticulum can be clearly visualized. Prolongation of the incubation period up to 10 days did not increase the number of cells with tubuloreticular structures or induce the formation of VLPs. It is of interest to note that the tubuloreticular structures observed in these cells are not as compact as are those observed in anaerobically grown cells, and more closely resemble the loosely arranged structures present in cells grown at 34°C (fig 33).

DISCUSSION

As a result of this study it appears that the most important morphogenetic changes in cultures of T. aureum subjected to environmental manipulations are the appearance of a highly specialized form of endoplasmic reticulum called tubuloreticular structures, and the induction of virus-like particles. In addition this study includes a more comprehensive ultrastructural survey unrelated to morphogenetic changes, than was previously available, making it possible to determine the degree in which T. aureum is related to other established taxa.

Tubuloreticular Structures

Distinctive regions of endoplasmic reticulum appearing as a series of either undulating tubules or short branched anastomosing tubules, and resembling the configurations seen in figures 16-19, 33 and 40, have been observed in cells from normal and neoplastic tissues and a wide variety of virus-induced infections either in vivo or in vitro. For review articles on these structures see Uzman et al., 1971; Andres et al., 1972; Schaff et al., 1972; and Grimley and Schaff, 1976. A proliferation of terms by different investigators in describing these ER elements has been responsible for much confusion. Examples include "undulating tubules" found in human cancer cell lines (Chandra, 1968) "crystalline arrays" in experimental herpes encephalitis (Baringer and Griffith, 1969), and "viruslike structures" in Sticker

sarcoma (Lombard et al., 1967) and systemic lupus erythematosus (Andres et al., 1972; Kobayashi and Asboe-Hansen, 1972).

In an effort to reduce confusion, Schaff et al. (1972) proposed that arrays of ER membranes be designated tubuloreticular structures (TRS). They argued that this term precisely describes the appearance of these structures without limiting their different morphological aspects, which may range from loosely interwoven tubules to extremely orderly patterns of paracrystalline conformation. Most authors agree that TRS is an appropriate name and in more recent reports they use TRS or TRI (tubuloreticular inclusions) exclusively (Grimley and Schaff, 1976; Chandra and Stefani, 1976; Hulanicka et al., 1977; and Watanabe et al., 1977). The uniform appearance, size, distribution, and relationship to granular ER is many different systems suggests that they are composed of the same material and represent a discrete morphologic entity. Configurational differences may be due to factors such as species or organ involved stage of disease, fixation, or other variables, which lead to differences in packing density (Baringer and Swoveland, 1972).

The large number of studies depicting TRS in various configurations has led to several different interpretations of the morphogenesis of these structures. In each case the authors agree that conventional ER membranes are somehow modified to form a multilamellar structure. Smith and Dienhardt (1968) suggest that walls of the "cytoplasmic

membrane complexes", which form in tumor cells of the marmoset monkey, arise by fusion of two opposing ER membranes to form a double membrane plate which then becomes further modified by the formation of regularly spaced evaginations or loops. An identical interpretation is provided by Ohman (1974) for the TRS found in retinal components of river lampreys, and by Eakin and Brandenburger (1975) for the ER elaborations present in the photoreceptors of some slugs. Baringer and Swoveland (1972) demonstrated that in cerebral tissue obtained from a case of human herpes simplex encephalitis, the trilaminar unit membrane structure of the ER was preserved in tubule walls, and in certain favorable thin sections it was clearly seen that TRS originate as invaginations of rough ER with individual tubules anastomosing with one another within ER cisternae. Ultrastructural examination by Chandra and Stefani (1976) of a Burkitt lymphoma culture and lymphoblasts cultured from a patient with acute lymphocytic leukemia revealed parallel elements of ER separated by a distance of approximately 24 nm. In such a juxtaposition there was a loss of attached ribonucleoprotein granules in adjacent ER lamellae while the material between them was increased in electron opacity. In some cells several paired lamellae stacked together forming a compound ER which often extended over large portions of the cytoplasm. On the basis of serial sections of such compounds Chandra and Stefani proposed a model on TRS morphogenesis in which undulating tubules emanate from the paired lamellae of a compound

reticulum. The number of undulations of a tubule would depend on the dilation of the ER and the length of the tubules, which in turn probably depend on the physiopathological state of the cell as TRS have been mostly observed in disease conditions.

Cytochemical studies by Schaff et al. (1972, 1973) reveal that TRS are composed principally of phospholipids and acidic glycoproteins. They are easily digested by pronase and pepsin but not by trypsin. Differences in the selective digestion of TRS and normal cytoplasmic ER by proteolytic enzymes show that the two differ chemically, which is interesting since in almost all reported observations of TRS there is a close association between the two and indications are that TRS actually arise from modified ER membranes. In view of the fact that these structures often resemble viruses, Schaff et al. (1972, 1973) attempted to identify a nucleic acid as a component of these structures. However, despite claims by others (Hashimoto, 1970; Helder et al., 1975), that one could demonstrate the presence of RNA by preferential staining or that TRS are susceptible to digestion by RNase, the more comprehensive studies by Schaff et al. failed to confirm the presence of any RNA. The failure to detect nucleic acid by nucleases does not totally rule out the possibility that small amounts in a resistant or protected form still exist. In summary, the available cytochemical evidence indicates that the tubules are constructed of a phospholipid framework coated with acidic proteins with sialic acid residues and is

almost identical in biochemical composition to cell surface membranes and virus envelopes (Grimley and Schaff, 1976).

With respect to the functional significance of TRS, despite a considerable amount of speculation their function remains obscure. The various interpretations fall into two main categories: viral and non-viral. The propenents of a viral theory note the numerous instances in which TRS have been observed in either virus or virus-induced infections (see Baringer, 1971; Schaff et al., 1972; and Grimley and Schaff, 1976 for a compilation of reported observations of TRS in virus infected tissues), as well as the superficial resemblance of TRS membranous components to known viral crystalline aggregates. Thus the appearance of these structures in kidneys affected by lupus erythematosus has generated interest in the possible viral etiology of this disease because the tubular structures resemble nucleoprotein strands liberated from paramyxoviruses (Gyorkey et al., 1969; Hashimoto and Chandler, 1972; Kobayashi and Asboe-Hansen, 1972). Other support for the viral theory comes from studies in which identical structures were propagated in tissue culture medium (Hashimoto and Chandler, 1972), and the induction of TRS in various human lymphoid cell lines with the halogenated pyrimidines, BUdR and IUdR (Grimley et al., 1973a, b, and c; Popoff and Malinin, 1976; Hillman et al., 1977; Hulanicka et al., 1977), substances known to activate latent viruses (Gerber, 1972; Rowe et al., 1972). Most investigators remain unconvinced by such circumstantial evidence and are in accord that TRS do not represent viral material

per se. This belief is based on several factors: the wide range of conditions in which TRS have been detected that have no known viral etiology; infective or cytopathic agents could not be recovered from cells or tissues bearing tubuloreticular inclusions (Feorineo et al., 1970; and Hulanicka et al., 1977); cytochemistry and autoradiography failed to reveal the presence of viral nucleoproteins within the tubules (Schaff et al., 1972, 1973; Cesarini and Prunieras, 1972; Grimley et al. 1973c) and likewise, immunofluorescent studies were unable to correlate the presence of TRS with serum antibody titers against specific virus antigens (Pincus et al., 1970; Goodman et al., 1973; Barry et al, 1976). The morphogenetic studies, referred to previously, in which TRS have been observed to arise directly from ER membranes, also do not support the contention that they are themselves a form of virus. Additional confirmation that individual tubular elements are hollow structures void of nucleocapsids and physically attached to the internal aspect of the ER, as predicted by the Baringer and Swoveland (1972) model, comes from freeze-fracture and high resolution stereography studies by Demsey and Grimley (1975). In their study no substructure was visible in fractured tubules, reinforcing the concept that TRS are of a membrane limited nature.

If TRS are not a form of virus particles then what are they? Included among the various alternative explanations are that they represent specialized areas of ER involved in: the synthesis of immunoglobulins in cells of lymphoid origin (Pothier et al., 1973;

Uzman et al., 1971; Popoff and Malinin, 1976), osmotic regulation and ion transport in the retina of Latimeria chalumnae (Locket, 1973), highly specialized metabolic functions in the dendritic organ of the catfish (van Lennep and Lansing, 1967), and reduction of cell volume during zygote development of cotton plants (Jensen, 1968). A similar explanation was postulated by Jenson et al. (1971) for the role of tubules within an osteosarcoma in which a viral theory was rejected; the TRS are a means of maintaining cell stability and volume. Hurd et al. (1969) found these structures in kidney diseases other than systemic lupus erythematosus, where viruses are not suspected as the causative agents, and speculated that TRS represent cellular material released by damaged cells and phagocytized by kidney endothelial cells. A more plausible explanation which takes into account the diverse biologic sources and pathologic situations in which TRS have been detected is that they represent a cellular response to abnormal conditions (Schaff et al., 1972; Baringer and Swoveland, 1971; Uzman et al., 1971; Grimley and Schaff, 1976; Watanabe et al., 1977). These investigators believe that various forms of cell injury including virus infections are capable of triggering a host cell response. A consequence of this damage is the proliferation of ER membranes. According to Grimley and Schaff (1976) it is conceivable that the many different structures referred to as TRS share little more in common than a physicodynamically efficient macromolecular arrangement.

Careful examination of the membranous configurations observed in approximately 5% of anaerobically grown *T. aureum* (figs 16-19) and less frequently in cells cultivated at elevated temperatures (fig 33) or in the presence of BUdR (fig 40) show that they share many features in common with TRS. The compact nature and the stiff organization of the *T. aureum* tubules are almost identical to compact tubular inclusions (CTI) found in the ER of certain animal cells (see review by Grimley and Schaff, 1976) except that CTI are more compactly arranged so as to present in cross-section a more geometric paracrystalline appearance. Also CTI are invariably associated with a virus disease or one in which a viral etiology is suspected. Such associations were responsible for the erroneous identification of these inclusions as viruses (Breese and Graves, 1966; Koestner et al., 1966; Blinzinger et al., 1969). The term "TRS" is used in this study since it is more general and encompasses CTI and undulating tubules.

It is highly unlikely that these structures in *T. aureum* are fixation artifacts since other cell organelles, particularly the mitochondria, remain relatively intact. Furthermore the post-fixation in osmium tetroxide and staining with uranyl acetate and lead citrate employed in this study is considered ideal for the best possible appreciation of the membranous structure of individual tubular elements (Grimley et al., 1973a). The 23 nm diameter of the tubules in cross-section and the 6 nm thickness of the tubule walls,

approximating the width of a typical endoplasmic reticulum unit membrane, correlates well with the dimensions reported for the TRS in other systems. From figs 17 and 18 it appears that there is a close association and direct continuity with the rough ER. However, as pointed out by Grimley and Schaff (1976), such micrographs are in themselves not conclusive. Since the diameter of a tubule is only one-quarter the thickness of an ultrathin section (approximately 100 nm), the apparent continuity with the ER may be illusive. Presumably freeze-fracture studies would be required in order to demonstrate a direct continuity between the ER and TRS. Depending on plane of section and length of time in which cultures were incubated under anaerobic conditions, the membranous structues appear as short branched anastomosing tubules arranged in tightly packed geometric forms (figs 10, 11, 16, and 17) or in a loose arrangement of loops and circles embedded within the cytoplasmic matrix (figs 13, 18, and 19). Tubular undulations, a characteristic of some TRS, are clearly seen in fig 19 which represents a longitudinal section through several membranous components.

The model presented in fig 42 was constructed to account for the many different configurations observed, owing to variations in the plane of sectioning. In this model, two opposing ER membranes fuse and in the process form a membranous sheet lined on each side with a double-unit membrane. A series of these sheets stacked in register become compressed, which is manifested as a ripple with uniform undulations throughout the stack. A second compression of the stack at a 90° angle to the first results in the corrugated pattern illustrated with evenly spaced grooves and evaginations. The undulations and

corrugations of individual membrane sheets are not necessarily synchronous with one another and may account for profiles in which circles and loops or a combination of these forms appear in a single section. This model is similar to the three-dimensional model prepared by Smith and Dienhardt (1968) to represent "unique cytoplasmic membranes" in Rous sarcoma virus-induced tumors of the marmoset monkey. In their discussion of the mode of formation of these ER-derived structures they noted that a similar structural pattern can be reproduced by compression of a stiff wire mesh first in one axis, resulting in corrugations, and then in an axis at right angles to the first one, resulting in equally spaced surface projections.

The present findings in which TRS are induced in cultures of _T. aureum_ are important in view of the fact that such structures are believed to be an independent ultrastructural entity, and are of considerable pathobiological interest in some disease states. Enhancing the significance of these observations is the simultaneous induction in these same cultures, grown under anaerobic conditions, of virus-like particles, as this is the first non-mammalian system in which TRS appear under the identical conditions required for the induction of what is believed to be a latent virus. Furthermore the convenience and ease of manipulation possible with a microorganism compared to clinical samples or tissue culture material may make _T. aureum_ a practical system for further experimental studies on the constitution and nature of TRS.

Although in this study no experiments were specifically designed to elucidate the functional significance of TRS, speculation, based on what is already known regarding these structures in other systems, is possible. A widely accepted explanation, as previously discussed (see review by Grimley and Schaff, 1976), is that TRS represent an early pathological change to either a latent virus infection, or more generally, a mode of reaction by the endoplasmic reticulum membranes to cell injury or abnormal conditions. This explanation is attractive because it accounts for the appearance of TRS in many different cell types and instances in which no viral presence is suspected. Considering that thraustochytrids are marine fungi with a physiology entirely different from mammalian cells grown either in vivo or in vitro, and the fact that TRS were induced in cultures incubated under anaerobic conditions, elevated temperatures, and in the presence of BUdR, makes it plausible to argue that TRS formation in T. aureum is merely a general cellular response to a variety of stimuli and represents a host cell product rather than a form of virus. This explanation does not rule out the possibility that proliferation of membranous tubules in this marine fungus is a consequence of a latent virus being activated, since anaerobiosis, elevated temperatures, and halogenated pyrimidines may each independently be capable of activating a latent virus which in turn triggers a cellular response manifested in the form of ER proliferation.

As previously discussed most investigators remain skeptical of the possibility that TRS are in themselves a form of virus or virus precursor. However, the induction of TRS in the ERof human lymphoid

cells by exposure to halogenated pyrimidines (Grimley et al., 1973a, b, c; Splinter et al., 1975; Hulanicka et al., 1977; and Hillman et al., 1977), agents known to activate latent viruses, has revitalized the question of a viral origin. Presumably the induction of TRS in cultures of T. aureum cultivated in the presence of 200 µg of BUdR/ml (fig 40) is cause for similar speculation regarding an ontogenetic relationship between the tubular arrays and the VLPs induced under anaerobic conditions (figs 20 and 21). The morphological appearance and size of the membranous configurations is additional cause for such speculation. Circles and loops formed by undulating tubules such as those seen in figs 18 and 19 generally measure about 100 nm in their inner diameter and correspond well with the dimensions of the VLP cores, while the outer shell of the VLPs appear almost identical in structure to the tubules themselves. Despite these intriguing morphological considerations, such speculation is difficult to accept. In anaerobic cultures where TRS and VLPs can be regularly observed after three and five days respectively, no intermediate forms between the two were observed. In fact, it is not possible to state with certainty that VLPs form only in cells with TRS-containing inclusions. Sections such as the one shown in fig 14, in which a VLP is seen in association with a crystalline aggregate, were infrequently observed. The failure to detect intermediate forms between TRS and VLPs may suggest that the two are only indirectly related. An indirect relationship can also explain

the induction of TRS by virus-activating substances such as BUdR, in that TRS are a manifestation of a secondary effect related to the virus infection. This indirect relationship is also in accord with work by Grimley et al. (1973b) in which autoradiographic studies showed no labeling of TRS with tritiated bromodeoxyuridine. From the preceding discussion it seems clear that regardless of the number of stimuli capable of inducing TRS formation, the association in T. aureum with a putative virus, or with actual viral diseases and neoplasia in other systems, means that the formation of tubules is somehow related to alterations in cellular replicative activity.

Relevant to a discussion on the ability to induce tubuloreticular structures in T. aureum by simple environmental manipulations, are several other instances in which a proliferation of cytoplasmic membranes have been observed. In fungi, Zachariah (1970), and Zachariah and Anderson (1973), detailed the morphological appearance and development of "lattice bodies" in apothecial cells of Ascobolus stercorarius. These bodies are described as netted endoplasmic reticulum consisting of 20-45 nm membranous tubules or vesicles arranged in a paracrystalline lattice. A distinguishing characteristic of these lattice bodies is a strong periodic internal arrangement with a periodicity of approximately 40-80 nm. From their published micrograhs it is difficult to determine if lattice bodies are a form of TRS, particularly since an association with cytoplasmic ER was not evident. Zachariah and Anderson do not refer to any published

reports regarding TRS; however, they do compare lattice bodies with other highly differentiated membranous organelles such as those appearing as inclusions in phloem cells and plastids of higher plants. They also do not assign any specific physiological function to these structures, but they do recognize that the striking morphological regularity implies extensive ordered membrane surfaces which may serve a secretory role or a storage and assembly function for a multienzyme system. It is interesting to note that these authors also considered the possibility that lattice bodies may represent an abnormal proliferation of membranes related to viral infection, although unlike the situation in T. aureum, no VLPs were detected.

In electron microscopic studies of coronaviruses isolated from human diploid lung cells, virus particles in various stages of maturation were observed in a condensed tubular network of agranular endoplasmic reticulum (Oshiro et al., 1971; Oshiro, 1973). These cytoplasmic tubules consisted of a densely stained material between smooth membranes approximately 33 nm in diameter and are referred to as reticular membranous tubules. The densely stained material resembled the inner ring of mature coronavirus particles and led Oshiro et al. to conclude that the tubular structures represent a stage in the development and accumulation of viral precursors. Thus, unlike other viral infections discussed previously in which the TRS observed are believed to be only a secondary effect, reticular membranous tubules do seem to be intimately involved in the production of coronavirus particles.

Another association between ER derived membranous bodies and viruses is found in wheat cells infected with wheat spindle streak mosaic virus. Cylindrical inclusions or "pinwheels", a characteristic feature of plants infected with long flexuous viruses (Edwardson, 1966; Edwardson et al., 1968), bear a remarkable resemblance to TRS and develop from ordered plates and tubes of ER which interconnect at regular points to form pinwheels (Hooper and Wiese, 1972; Langenberg and Schroeder, 1973). As a result of these developmental studies on pinwheel formation, it is believed that these membranes play an important role in the actual synthesis of virus particles and represent more than a host response to a viral infection.

In amoeboflagellates Schuster and Rechthand (1975) examined the effects of amphotericin B in three different naegleria strains and observed highly organized configurations of smooth ER in cells exposed to this drug for periods up to 48 hours. The membranous configurations were composed of interconnected tubules continuous with the rough ER. Although the significance of the amphotericin B induced tubules is not known, the possibility that they are yet another form of TRS and represent a cellular response to stress conditions induced by the antibiotic, should be considered.

Viruslike Particles

It is now recognized that mycoviruses exist in many fungal species and in the majority of cases they represent latent or nonsymptomatic infections. Most mycoviruses possess double stranded RNA and it is not uncommon to find several viral forms in a single fungal host. (For

a history, an account of mycoviruses found in the phycomycetes, and references to review articles concerned with mycoviruses, see introductory chapter). Most of the mycoviruses described are either spherical or rod-shaped. The only other mycovirus that morphologically resembles the large 150 nm polyhedral VLPs present in cells of T. aureum (figs 20 and 21) grown under anaerobic conditions is found in Aphelidium sp; a parasite of the green alga Scenedesmus armatus (Schnepf et al., 1970). The VLPs in Aphelidium are approximately 200 nm in diameter with a triple layered structure enveloping a 150 nm core consisting of a mesh of fibrillar strands difficult to visualize individually. The viral nature of the Aphelidium particles, as in the case of the T. aureum VLPs, is presumed from their structure, mode of development, and appearance exclusively in lysing protoplasts.

Except for VLPs observed in the oyster pathogen Labyrinthomyxa marina (Perkins, 1969), a marine protist with possible taxonomic affinities to thraustochytrids, and in a strain of Schitzochytrium aggregatum (Perkins, personal communication), the studies by Kazama and Schornstein (1972, 1973) are the only other reports of mycoviruses among thraustochytrids. From their detailed ultrastructural studies of the replicative cycle of a herpes-type virus in Thraustochytrium sp., it is evident that these particles have few morphological and developmental similarities with the putative virus of T. aureum. The capsids of the herpes-type particles are assembled in the nucleus. During passage into the cytoplasm they are temporarily enveloped by two

nuclear membranes, which are replaced in the cytoplasm by a fibrillar coat. In the final stages of maturation the particles invade cytoplasmic organelles or Golgi-related vesicles before being released from the cells. Treatment of thin-sections with DNAse and RNAse further suggests the herpes-like nature of these particles, since only DNAse is effective in digesting the VLP core. In contrast, the VLPs observed in anaerobic cultures of T. aureum are thought to possess, as do most mycoviruses, an RNA core. This presumption is based on autoradiographs (figs 23 and 24) which suggest labeling of the VLPs with tritiated uridine. Attempts to label the particles with thymidine were unsuccessful. In T. aureum no particles, complete or incomplete, were ever observed in nuclei even in the earliest stages of infection and unlike the herpes-type particles their course of development does not include a sequence in which they bud into other cytoplasmic organelles. However, since nuclear degeneration or lysis is one of the first visible effects attributed to anaerobic growth, early involvement of the nucleus in particle formation cannot be excluded. Another indication that the herpes-type particle represents an entirely different form of mycovirus is that no tubuloreticular structures or other ordered membranous configurations in virus-producing cells were observed.

In the Thraustochytrium sp. examined by Kazama and Schornstein there was no evidence of viral production until cultures containing immobilized zoospores were allowed to sit from 5-20 hours in estuarine

water only without any added nutrients. Sporangia formed from zoospores placed in a nutrient broth showed no recognizable virus particles. The regulation of viral production by controlling culture conditions which were either "permissive" or "nonpermissive" is consistent with a latent virus hypothesis in which apparently healthy cultures can be induced to produce virulent particles capable of causing cell lysis. In this respect, the anaerobically induced mycovirus observed in T. aureum appears to have similar latent properties since VLPs were never observed in nonanaerobically grown cultures. Evidently the ability of these cells to harbor a virus which does not initiate any overt signs of infection depends to some extent on environmental conditions. Similarly Kazama and Schornstein (1973) recognized that the herpes-type virus only remains inapparent when nutrient conditions favor the rapid development of the fungus.

It is possible to speculate that the recrudescenee of mycoviruses in T. aureum under anaerobic conditions is simply due to a slowdown in the rate of fungal growth caused by an inability to utilize available nutrients. However, extremes of pH, salinity, and temperature, (Table 2) tested for their ability to induce mycovirus production, showed no evidence of VLPs associated with cell lysis, although each one of these environmental manipulations cause considerable reductions in the growth rate of T. aureum (Goldstein 1963b). In addition aged cultures, in which cells were left stagnating in their own spent medium for 3-4 weeks, showed no evidence of mycoviruses being activated.

Thus it would appear that activation of what is believed to be a latent virus requires a more specific stimulus than simply interfering with the rate of fungal development. The induction of mycoviruses in cells grown under anaerobic conditions still does not pinpoint any one or combination of factors involved in the onset of recrudescence since anaerobic growth results in numerous metabolic and physiological alterations. Until a great deal more information on cellular control mechanisms in marine fungi becomes available it is unlikely that this question can be satisfactorily answered.

There is at least one other instance in which mycovirus production is dependent upon an external stimulus. Lemke et al. (1973) found that the formation of lytic plaques of mycoviral origin in certain mutant strains of Penicullium chyrsogenum is dependant on a special lactose-based medium. Genetic analysis of progeny derived from crossing a strain which is mycovirus infected but does not produce plaques with a strain that does form plaques suggested that a nuclear allele (s^-) was responsible for plaque formation (Lemke et al., 1976). Segregation patterns for plaque formation of a series of other crosses confirmed the involvement of a nuclear allele in the lysis of fungal material, however in all instances plaque formation was still conditional on the presence of the lactose medium, a nutritional factor.

To a large extent the VLPs observed in T. aureum more closely resemble in morphology some of the eukaryotic algal viruses then they

do other mycoviruses. As in the case of mycoviruses most of the published reports of VLPs in eukaryotic algal cells are single accounts and descriptive in nature. Classical proof of virus infection is lacking and they are generally nonsymptomatic. For recent reviews dealing with algal viruses see Andrews (1976), Lemke (1976), and Sherman and Brown (1978).

Two reports, by Markey (1974) and Hoffman and Stanker (1976), are germane to the situation in T. aureum. In both instances degenerative effects attributed to extranuclear VLPs with a six-sided polygonal profile in sectional veiw were observed in apparently healthy cultures of marine algae. In an isolate of the green alga Cylindrocapsa, Hoffman and Stanker were routinely able to induce VLP formation by subjecting cultures to heat-shock. When surviving Cylindrocapsa germlings from samples of heat-shocked material, known to include numerous VLP-infected cells, were placed in fresh medium, they developed into healthy actively growing filaments in a fashion similar to anaerobic cultures of T. aureum transferred to fresh medium under aerobic conditions. These results led Hoffman and Stanker to consider the possibility that their cultures carried a latent virus infection which can be induced under certain conditions, e.g., heat-shock, to form virulent particles capable of causing cell lysis. The other alga in which there is a likelihood that the cells possess a latent virus is Pylaiella littoralis. Markey (1974) observed VLPs in a single collection which had been left standing 24 hrs in seawater. Presumably the procedure of allowing plants to stand had some influence on the induction of VLPs, since this was the only instance in which they were

detected. In his report Markey does not indicate if the cells were kept in the dark during the 24 hr period, a fact which may have some bearing on whether the cells become nutritionally stressed. As previously discussed, deprivation of nutrients to Thraustochytrium sp zoospores results in the indùction of herpes-type particles (Kazama and Schornstein, 1972, 1973). It is noteworthy that in Pylaiella littoralis membranous elements of the same size as the putative virions seemed to be involved in the development of the VLPs. Although these membranous structures, believed to be a form of modified endoplasmic reticulum, show no periodicity or ordered arrangements as do the prominent tubuloreticular structures in T. aureum, it is possible to speculate that both represent related forms of virogenous bodies.

Transmission of VLPs

Investigators working with fungal viruses are faced with uncertainty in postulating a mechanism for the transmission of these agents, since for the most part even purified mycovirus particles have not yet been proven to be infective. Among the higher fungi it has been convincingly demonstrated that virus transmission occurs by hyphal anastomosis between cells, or by heterokaryosis involving fusion between compatible cell lines (Gandy, 1960; Gandy and Hollings, 1962; Hollings 1962; Hollings and Stone, 1971; Lhoas 1971 a, b; Lemaire et al., 1971; Day and Anagnostakis, 1972; Wood and Bozarth,

1972; Metitiri and Zachariah, 1972; Rawlinson et al., 1973; Koltin and Day, 1976a, b; Lemke et al., 1976). Commercial mushroom growers generally alternate between different mushroom genotypes in order to prevent transmission of mycoviruses.via heterokaryosis from one crop to another (Atkey et al., 1974). Transmission of mycoviruses through spores has been demonstrated in some fungi (see review by Hollings, 1978) and is considered to be epidemiologically important in pathogenic fungi which harbor mycoviruses. For other fungal and algal groups in which VLPs have been observed, no such natural mechanism of transmission is known. Extracellular particles are rarely observed, and even if upon cell lysis they are liberated into the surrounding environment, their ability to penetrate the thick cell walls of algae and fungi remains doubtful. Infection of naked zoospores or damaged sporangia may be one possible mechanism for ordinary virus infection, however, such infections have not been demonstrated. In Allomyces arbuscula, RNA-containing VLPs are preferentially localized in the mass of ribosomes making up the nuclear cap of motile cells (Roos et al., 1976). These authors consider this localization in zoospores as a mechanism for the transmission of viruses through sexual and asexual generations.

Latent Viruses

Since the VLPs in T. aureum appear to have latent characteristics several comments about latent viruses and their relevancy towards viruses of eukaryotic microorganisms will be made. Latency, in the case of the extensively investigated latent herpes simplex viruses

has been defined as that state in which a virus remains present within an individual without initiating any overt infection (Docherty and Chopan, 1974). The most likely explanation as to why even extremely virulent viruses such as the herpes simplex virus enter into a period of quiescence is that conditions in the surrounding environment are no longer conducive to the production of new virions. Roizman (1965) presented two possible mechanisms as to how animal viruses are maintained after having entered the latent state. One possibility known as the static state hypothesis postulates that certain virogenic cells shelter nonreplicating virus particles or its genome between recurrent infections by producing a substance which inhibits virus replication or that the adverse environmental conditions within the cells themselves preclude the multiplication of viruses. The second proposal called the dynamic state hypothesis visualizes a dynamic relationship between cell and virus resulting in a cell-virus equilibrium such that the virus multiplies very slowly. In this second hypothesis latent viruses are not dormant but because of their slow rate of development infections persist at subclinical levels.

Although a great deal of what is known about viral latency in the study of host-pathogen relationships comes from work on animal viruses, a useful analogy may be drawn towards a better understanding of the propagation of non-infectious viruslike particles in a wide variety of species. Stated simply, factors which place a stress on cells or on an organism, such as growth under anaerobic conditions, heat-shock, or

deprivation of required nutrients, result in a physiological imbalance so that potentially virulent particles are produced. These external stimuli may interfere with cellular control mechanisms responsible for the virus remaining inapparent in the case of the dynamic state hypothesis or they may prevent cells from producing a substance which interferes with virus production as per the static state hypothesis. Interestingly, double-stranded RNA of mycoviral origin and intact VLPs from several species of Penicillium and other fungal genera are active inducers of interferon when injected into test animals (see reviews by Bozarth, 1972; Saksena and Lemke, 1978; and Hollings, 1978). However, free viral ds-RNA is more potent in inducing interferon than are virus particles containing an equivalent amount of ds-RNA (Nemes et al., 1969; Buck et al., 1971). Although these differences have not been adequately explained (Saksena and Lemke, 1978), it may be possible that the greater antiviral activity exhibited by free ds-RNA is due to a cellular control mechanism which maintains viral precursors in an integrated state resulting in latent viruses remaining sequestered.

The possession of such endogenous or latent viruses may be far more common than previously believed, as reports in which noninfectious VLPs have been observed cover a large number of species ranging from VLPs in eukaryotic microorganisms to B- and C-type particles in healthy animals. In the phylum Protozoa the EGs strain of Naegleria gruberi is a likely candidate for harboring a latent virus. A series of reports by Schuster (1969), and Schuster and Dunnebacke (1971,

1974a, b, c, 1976, and 1977) describe the development of VLPs in this amoeboflagellate and the conditions under which they are most easily detected. The appearance of particles is triggered by the use of any suitable bacterium as a growth substrate. Elevated temperatures accelerate VLP development, and treatment of the amoeba with the halogenated pyrimidine, BUdR, results in the induction of VLPs even in axenic cultures. Based on these results, Schuster and Dunnebacke postulate that the EGs strain harbors a latent virus whose development is initiated by some modification of growth conditions. In another extensively studied protozoan system, Diamond and coworkers have isolated and identified viral agents from ten strains of Entamoeba histolytica (see review by Diamond and Mattern, 1976). It is interesting, as far as latent viruses are concerned, that apparently all normal and well-established cultures of E. histolytica contain viruses, even though these are not evident when examined by electron microscopy. Furthermore, BUdR was effective in increasing virus production in at least one isolate, and attempts to rid amoebae of their endogenous viruses were all unsuccessful.

The curious phenomenon in which a proportion of a laboratory strain of Drosophila is sensitive to CO_2 may have some relevance to the induction of VLPs in T. aureum. It has been shown (see review by Howatson, 1970) that sensitive flies, unlike CO_2-resistant flies which recover completely after exposure to a CO_2 atmosphere, carry an infectious agent, termed sigma virus, which is responsible for paralysis and eventual death. An

interesting question is whether VLPs in T. aureum have anything in common with sigma virus in the mechanism by which they are induced. Although cultures of T. aureum exposed to relatively high CO_2 concentrations did not show any evidence of VLP formation, the key factor under anaerobic growth conditions may involve CO_2. Conversely, the activation of sigma virus in Drosophila may be due to a state of anaerobiosis resulting from CO_2 exposure.

Lemke, in his review (1976) points out similarities between viruses of eukaryotic microorganisms from taxonomically diverse systems whose activation or increase in numbers is dependent upon external factors. These factors include nutritional factors or elevated growth temperatures for protozoan viruses, requirement of a lactose-based medium for strains of infected Penicillium, heat-shock in Cylindrocapsa, nutritional stress in the Thraustochytrium sp studied by Kazama and Schornstein, and anaerobiosis in T. aureum. All of these, according to Lemke, are consistent with the "phenomenon of latency".

Significance of VLPs

A controversial hypothesis presented by Reanney (1974) on the role played by viruses in nature may add some understanding concerning the widespread occurrence of VLPs in eukaryotic microorganisms. Reanney noting that: 1) most species have associated viruses, 2) viruses are the most numerous genetic objects in the biosphere, 3) virus genes can enter

and leave cell chromosomes, 4) integrated virus genomes are often reiterated, 5) that cytopathic viruses may be atypical, suggested that certain viruses are primarily agents of gene exchange between cells. Thus the number of associated viruses and the extent and speed with which they allow information to be cycled through the total gene pool of a population has a significant effect on the rate and direction of evolution in any given species. Reanney further argues that the large number of observations of noninfectious "passenger" viruses is consistent with the postulate that viruslike particles transduce information without autonomous replicative potential. It follows according to Reanney that questions concerning ecological significance and possible infection cycles posed by many investigators faced with nonsymptomatic VLPs or attempts to demonstrate infectivity with cell-free virus preparations are based mainly on the "mistaken" notion that viruses are chiefly destructive agents responsible for disease. The fact that cytolytic viruses, although relatively few in number, have received so much attention is due to their medical and economic importance to man.

Actually, little of what has been learned about viruses of eukaryotic microorganisms is in conflict with Reanney's hypothesis. Situations in which definite physiological and morphological anomalies have been attributed to viruses may be exceptional, or else laboratory manipulations may, as previously discussed, cause some physiological imbalance resulting in disruption of cell-virus equilibrium. A

case in point may be mycoviruses associated with disease symptoms in Agaricus bisporus (Gandy and Hollings, 1962; Hollings, 1962). Since these are cultivated mushrooms, their diseases may hot be indicative of virus diseases among naturally growing mushrooms. For organisms such as T. aureum in which sexual reproduction is not known to exist, speculation that viruses act as positive agents in various aspects of evolution is particularly attractive since viruses may be the only mechanism which ensures variability of natural populations.

The awareness that marine algae and fungi are subject to viral infections has led to speculation that they act as vectors for viral diseases in marine animals or play a role in phytoplankton blooms (Goldstein, 1973; Pearson and Norris, 1974; Andrews, 1976). Interactions between marine fungi and algae themselves may go beyond nutritional relationships in the light of the fact that the large VLPs in T. aureum morphologically resemble algal VLPs and that thraustochytrids are omnipresent on algal surfaces. Similarly the findings of a herpes-type infection in oysters (Farley et al., 1972) and of the herpes-type VLPs observed by Kazama and Schornstein (1972, 1973) are responsible for speculation that fungi may act as vectors since herpes-type viruses are highly unusual in invertebrates.

Taxonomic Considerations

The systematics and taxonomy of the lower marine fungi depend to a great extent on characteristics such as spore release mechanisms, size of zoospore and sporangium, nature of rhizoids, pigmentation, cell

wall behavior and the presence or absence of a basally proliferating rudiment (Sparrow, 1968). Unlike the spore appendages of other fungi or the frustules of diatoms which are invariable and serve as useful taxonomic criteria in identifying these microorganisms, the characteristics used in the identification of thraustochytriaceous fungi are subject to change depending upon the conditions in which they are grown. As Gaertner (1972) pointed out, this high degree of variability is the most serious difficulty in the accurate classification of the Thraustochytriaceae. For the same reason, difficulties were experienced by Booth and Miller (1968) in a comparative morphological and taxonomical investigation of the genus Thraustochytrium. In their study large numbers of new isolates were cultivated and the above mentioned morphological characters compared to those found in already established taxa. Their results demonstrated a susceptibility to variation with the characters tending to overlap forming a character gradient or cline.

Gaertner (1970, 1972) attributes most of the taxonomic difficulties to culture methods which supply the fungi with a continuous flow of nutrients through diffusion, or the high surface tensions present on the surface of solid media. He argues that these artificial conditions induce abnormalities or suppress crucial diagnostic features. As an alternative he advised the use of sea water pollen cultures which provide the fungus with a limited amount of nutrients of high value yet allow the sporangium to develop in the surrounding water of low nutrient

value. Gaertner (1974) considers pine pollen as a model substrate for the study of thraustochytrids since it is "a very small box" protected from bacteria which cannot penetrate the highly resistant wall of the pollen grain. Motile *Thraustochytrium* spores are chemotactically attracted to the pollen grain by the small amounts of nutrients which escape by diffusion.

Goldstein (1973) agrees with Gaertner and cautions investigators on the pitfalls they are likely to encounter in attempting to accurately diagnose the taxonomic position of new isolates. He maintains that although nutrient media are essential for physiological and ultrastructural studies, they have relatively little taxonomic value. It is interesting to note that *T. aureum* and other thraustochytrids, because of their nondescript characteristics, remained unclassified when first isolated by Vishniac (1955, 1956) and cultivated on a solid agar medium. Only after Goldstein (1963a, b, c, 1964), in a search for more suitable substrates, used pollen grains was he able to unmask individual peculiarities in development and taxonomically diagnose Vishniac's isolates.

In the present study seawater pollen cultures were employed for the light microscope observations on the life cycle and morphology of *T. aureum*. For the ultrastructural studies it was impractical to use pollen cultures. Thus, there is no way to accurately assess the extent in which the artificial culture conditions induced structural irregularities. Alderman et al. (1974), primarily on the basis of ultrastructural studies,

made revisions in the marine biflagellate fungi and introduced them as members of a new order, Thraustochytriales Sparrow. The nutrient media in their study were not defined containing additives such as yeast extract, peptone, and calf serum. Here, too, the difficulties described above in the use of artificial culture conditions warrant some speculation on the validity of their conclusions. Since some of their results are based on scanning electron microscope preparations, it may not prove to be too much of an experimental hardship to employ pollen cultures and still benefit from the resolution afforded by the scanning electron microscope.

Ultrastructure

The fine structure of T. aureum is similar to that of other thraustochytrids reviewed by Alderman et al. (1974). The characteristics of the group, including a distinctive striated cell wall, presence of a sagenogenetosome, and possession of an electron dense granule in the kinetosome are well defined in T. aureum and support the elevation of the Thraustochytriaceae to ordinal rank (Sparrow, 1974). In addition, T. aureum has some unique morphological features some of which are observed in normal cultures, and others which appear only in response to stress. These features are discussed below in greater detail.

Kinetosome Granules

The electron-dense material observed within the kinetosome lumen (fig 1) is added confirmation to Kazama's (1972) view that these granules, not found in other biflagellate Phycomycetes, are an important taxonomic

criterion. It is interesting that although significant differences, such as ribosomal RNA molecular weights, cell wall structure and composition, motility, and centriole behavior during mitosis, exist between thraustochytrids and labyrinthulids (Porter, 1974), electron dense areas have also been observed within the kinetosome lumen of Labyrinthula (Perkins and Amon, 1969). Kazama (1972) points out that the distribution of the granules seems to differ in Labyrinthula; nevertheless the phylogenetic relationship between these two groups remains an unresolved issue. Gaertner (1974), noting the many features which they do have in common, stated, "We do not know if the Labyrinthulae are primitive thraustochytrids or if the thraustochytrids are rare developed Labyrinthulae".

Giant Mitochondria

Large single mitochondria, as observed in the zoospore stage of T. aureum (figs 2 and 3), have not been demonstrated in any other thraustochytrid. Although three-dimensional reconstruction of mitochondrial structure was not attempted in this study, examination of numerous non-serial sections allow comparision with other uniflagellate Phycomycetes in which mitochondrial morphology was studied in great detail (Bromberg, 1974; Lange and Olson, 1976; Barr and Hartman, 1977). The indications are, that because of the highly branched mitochondrial configurations observed (figs 2, 9, and 25), mitochondrial division resembles the process described for Olpidium brassicae (Barr and Hartman, 1977) and Blastocladiella emersonii (Bromber, 1974). The association with the kinetosome, as seen in most uniflagellate Phycomycetes with single mitochondria (Lange and Olson, 1976), was not observed in T. aureum.

From investigations of aquatic fungi virtually nothing is known regarding the functional aspects of mitochondrial fusion and division. However, in other eucaryotic microorganisms. Chlamydomonas reinhardii and Euglena gracilis, where giant mitochondria are temporarily present during the life cycle, Osafune et al. (1972, 1975) demonstrated that the fragmentation of giant mitochondria is accompanied by a marked increase in the oxygen-uptake activity of these cells with a concurrent decrease in activity during mitochondrial fusion. In rats treated with cortisone, a drug known to cause multiple defects inthe respiratory chain, Kimberg and Loeb (1972) showed that fusion of liver mitochondria takes place in response to the drug. They believe that inhibition of respiration acts as a stimulus for mitochondrial fusion. A similar explanation may account for the observation that only in the zoospore stage of T. aureum are giant mitochondria observed. Perhaps after encystment when the endogenous store of nutrients is depleted and a cell wall forms, differences in metabolism and rates of oxygen diffusion act as a stimulus in triggering mitochondrial fragmentation. Such fragmentation of mitochondria in early stages of sporangium development differs considerably from the situation in Chlorella fusca which possesses a single mitochondrion that continues to elongate until its partition among daughter cells at cytokinesis without any fragmentation prior to that time (Atkinson et al., 1974).

Gamma-Like Particles

The unidentified structures shown in figs 4 and 8 bear a superficial morphological resemblance to the gamma particles, extensively studied

by Cantino and coworkers, found in zoospores of the uniflagellate water mold Blastocladiella emersonii. For a historical account as well as the morphology and biochemistry of gamma particles see reviews by Cantino and Myers, 1973; and Myers and Cantino, 1974. The name gamma particle derived from the original belief that the particles controlled a phenotypic variation involving gamma carotene formation. It is now known that the enzyme chitin synthetase resides in the gamma particle and that the particles play an important role in cyst wall formation of B. emersonii zoospores, hence they have been designated a type of encystosome (Cantino and Mills, 1976). Gamma particles have been found to contain both DNA and RNA and may represent an independent genetic system similar to chloroplasts and mitochondria. Calcium is a prominent constituent of the gamma particle as revealed by X-ray microanalysis (Hutchinson et al., 1977) and it is postulated that movements of Ca and K into and out of gamma particles may be responsible for the formation and release of membrane vesicles which migrate from the gamma particles and fuse with the plasma membrane. The main body of these organelles resembles an electron-dense, hemi-ellipsoidal bowl with both of its acute ends removed. The bowl is loosely enclosed by a surrounding membrane that is best visualized when the cells are fixed with glutaraldehyde and post-fixed with osmium tetroxide. Gamma particles are absent in exponentially growing plants and disappear during or shortly after spore germination. In contrast, the particles in T. aureum do not have a surrounding membrane and are most conspicuous in sporangia with fully developed cell walls. It is not known if these particles contain

any nucleic acids or enzymes involved in cell wall synthesis, although the concentration of the particles directly beneath the cell wall is suggestive of such a role. T. aureum possesses an elaborate cell wall composed of thin circular scales (fig 8), a characteristic feature of thraustochytrids. However, similar unidentified structures associated with the cell wall have not been reported for any other members of this group.

Annulate Lamellae

According to Kessel (1965) annulate lamellae (AL) may be most abundant in rapidly proliferating and differentiating cells. Subsequently they have been observed in a wide variety of vertebrate and invertebrate germ cells, embryonic and fetal cells, virus-infected cells, and neoplasms (for reviews on the structure, function, and distribution of AL, see Kessel, 1968, 1970, 1973; and Wischnitzer, 1970). Although the biological significance of AL is poorly understood, the frequent association with endoplasmic reticulum suggests a functional role in protein synthesis or membrane proliferation. In some cases it has been shown that AL give rise to endoplasmic reticulum (Wischnitzer, 1970). The formation of AL can be induced in cell cultures by prolonged treatment with drugs that interfere with the function or structure of microtubules (DeBranbander and Borgers, 1975). In HeLa cells the induction of AL may be observed after only two hours when dissociated cells are allowed to aggregate (Liebrich and Paweletz, 1976).

In the present study AL were observed only in cells from anaerobic cultures (fig 28) and consist of stacks of parallel membranes interrupted

at periodic intervals by annuli. Speculation that AL in T. aureum represent an abnormal host response to a latent virus infection is based on two considerations: first, they appear under the identical conditions in which the formation of tubuloreticular structures are induced; and second, AL have never been previously observed in thraustochytrids. Actually, this may be the first report of AL in fungi. A search of the literature revealed that in at least three other instances investigators have reported the simultaneous appearance of tubuloreticular structures and AL (Popoff and Malinin, 1976; Hillman et al., 1977; and Watanabe et al., 1977). Interestingly, the three systems all involve malignant lymphoreticular cells in which a viral etiology is strongly suspected.

Crystalline Inclusions

The crystalline inclusions shown in figs 13-15 were observed only in cells from anaerobic cultures. Since these structures have not been reported in other thraustochytrids, it is believed that they are indicative of a virus infection, perhaps representing sites of aggregation of previously formed virions. Although similar crystals, often proteinaceous are readily found in several types of cells in healthy organisms (Fawcett, 1966; Wirgin et al., 1970), they are also characteristic symptoms of plant viruses (McWhorter, 1965). In fungi, compact aggregates of dense particles, the same size and density as ribosomes, and arranged in a crystal with a very regular three-dimensional spacing, were reported in a mutant strain of Penicillium claviforme (Metitiri and Zacharaiah, 1972). The inclusions always had straight sides and in

profile appeared as squares, rectangles or rhomboids. In later developmental stages of this mutant, dense particles, 65-75 nm in diameter, were visible. The particles were believed to be mycoviruses and were associated with a considerable amount of cell lysis. Evidence that the small, ribosome-like particles and associated protein crystals represent virus-related material comes from the fact that the ability to develop the crystalline inclusions can be transmitted from the mutant to the wild type. In T. aureum the probability that these crystalline inclusions are virus-related is further enhanced since they can be seen to be in direct contact with tubuloreticular structures (fig 13), which are also strongly suspected of being virus-related.

Other VLPs

Apparently the small 45-50 nm VLP particles (figs 26 and 27) are a separate class of mycoviruses than are the large 150 nm hexagonal shaped VLPs. A high proportion of the fungal strains in which viruses have been demonstrated contain more than one type of VLP, the different types being morphologically and serologically distinct (Hollings, 1978).

It is not known if the dense particles present in the cytoplasmic inclusions of cells grown at 34°C(fig 34) are viral in nature, although the lack of a uniform size for the particles tends to argue against this possibility. The inclusions themselves bear a superficial resemblance to cytolysomes, however no reaction product was visible (fig 41) when cytochemical staining for acid phosphatase was performed. The significance of the microtubular bundles present within these inclusion is unknown.

Concluding Remarks

This study provides a detailed description of the morphological alterations and high degree of sensitivity of *T. aureum* to environmental manipulation. It is highly probable that this primitive marine micro-organism harbors a latent virus whose recrudescense is induced under anaerobic conditions. The morphological data accumulated provides essential information on the intracellular relation of a fungal virus to the anatomy of the host cell. The elucidation of cell control mechanisms involved in virus activation as well as the biochemical and biophysical characterization of the VLPs awaits further study.

REFERENCES CITED

Adair, E. J., and Vishniac, H. S. 1958. Marine fungus requiring vitamin B_{12}. Science 127: 147-148

Alderman, D. J., Harrison, J. L., Bremer, G. B., and Jones, E. B. G. 1974. Taxonomic revisions in the marine biflagellate fungi: the ultrastructural evidence. Marine Biology 25: 345-357

Andres, G. A., Spiele, H., and McCluskey, R. T. 1972. Viruslike structures in systemic lupus erythematosus. In: "Progress in Clinical Immunology", vol. 1 (R. S. Schwartz, ed.) pp 23-44 Grune and Straton, New York

Andrews, J. H. 1976. The pathology of marine algae. Biol. Rev. 51: 211-253

Artemtchuk, N. F. 1972. The Fungi of the White Sea. III. Phycomycetes, discovered in the great Sama Strait of the Kandalakshial Bay. Veroff Inst. Meeresforsch. Bremerhaven 13: 231-237

Atkey, P. T., Barton, R. J., Hollings, M., and Stone, O. M. 1974. Rep. Glasshouse Crops Res. Inst., 1973 p 119

Atkinson, A. W., John, P. CC L., and Gunning, B. E. S. 1974. The growth and division of the single mitochondrion and other organelles during the cell cycle of Chlorella, studied by quantitative stereology and three dimensional reconstruction. Protoplasma 81: 77-109

Bahnweg, G., and Sparrow, F. K. 1972. Aplanochytrium kerguelensis gen. nov. spec. nov., a new Phycomycete from subartic marine waters. Arch. Mikrobiol. 81: 45-49

Banks, G. T., Buck, K. W., Chain, E. B., Balbyshire, J. E., Himmelweit, F. 1969. Penicillium cyaneo-fulvum virus and interferon stimulation. Nature 223: 155-158

Banks, G. T., Buck, K. W., Chain, E. B., Darbyshire, J. E., Himmelweit, F., Ratti, G., Sharpe, T. J., and Planterose, D. N. 1970. Antiviral activity of double stranded RNA from a virus isolated from Aspergillus foetidus. Nature 227: 505-507

Banks, G. T., Buck, K. W., Chain, E. B., Himmelweit, F., Marks, J. E., Tyler, J. M., Hollings, M., Last, F. T., and Stone, O. M. 1968. Viruses in fungi and interferon stimulation. Nature 218: 542-545

Baringer, J. R. 1971. Tubular aggregates in endoplasmic reticulum in herpes-simplex encephalitis. New Engl. J. Med. 285: 943-945

Baringer, J. R and Griffith, J. F. 1969. Experimental herpes encephalitis: crystalline arrays in endoplasmic reticulum. Science 165: 1381

Baringer, J. R., and Swoveland, P. Tubular aggregates in endoplasmic reticulum: evidence against their viral nature. J. Ultrastruct. Res. 41: 270-276

Barka, T., and Anderson, P. J. 1962 Histochemical methods for acid phosphatase using hexazonium pararosanillin as coupler. J. Histochem. Cytochem. 10: 741-753

Barr, D. J. S. and E. Hartmann 1977. Zoospore ultrastructure of Olpidium brassicae and Rhizophlyctis rosea. Can. J. Bot. 55: 1221-1235

Barry, D. W., Sullivan, J. L., Lucas, S. J., Dunlap, R. C. and Albrecht, P. 1976. Acute and chronic infection of human lymphoblastoid cell lines with measles virus. J. Immunol. 116: 89-98

Bartniki - Garcia, S. 1970. Cell wall composition and other biochemical markers in fungal phylogeny. In: "Phytochemical Phylogeny", (J. B. Harborn, ed.) pp 81-103, Academic Press, New York

Bellet, A. J. D. 1968. The iridescent virus group. Advances in Virus Res. 13: 225-246

Belsky, M. M., Goldstein, S., and Menna, M. 1970. Factors affecting phosphate uptake in the marine fungus Dermocystidium sp. J. Gen. Microbiol. 62: 399-402

Blinzinger, K. Simon, J., Magrath, D., and Boulger, L. 1969. Poliovirus crystals within the endoplasmic reticulum of endothelial and mononuclear cells in the monkey spinal cord. Science 163: 1336-1338

Booth, T. and Miller, C. E. 1968. Comparative morphologic and taxonomic studies in the genus Thraustochytrium. Mycologia 60: 480-495

Borré, E., Morgantini, L. E., Ortali, V., and Tonolo, A. 1971. Production of lytic plaques of viral origin in Penicillium. Nature 229: 568-569

Bozarth, R. F. 1972. Mycoviruses: a new dimension in microbiology. In: Environmental Health Perspectives, Experimental Issue no. 2, pp 23-29, U. S. Dept. Health, Education and Welfare, Washington, D. C.

Bozarth, R. F., Wood, H. A., and Nelson, R. R. 1972. Viruslike particles in virulent strains of Helminthosporium maydis. Phytopathology 62: 748

Breen, G. and Goldstein, S. 1975. Identification of the marine Phycomycete isolate "S-3". Mycologia 67: 177-180

Breese, S. S. and Graves, J. H. 1966. Electron microscopic observation of crystalline arrays of foot-and-mouth disease virus. J. Bacteriol. 92: 1835-1837

Bremer, G. B. 1974. Physiological responses of some thraustochytrid fungi. Veroff. Inst. Meeresforsch. Bremerhaven suppl. 5: 237-250

Bromberg, R. 1974. Mitochondrial fragmentation during germination in Blastocladiella emersonii. Developmental Biology 36: 187-194

Buck, K. W., Chain, E. B., and Himmelweit, F. 1971. Comparison of interferon induction in mice by purified Penicillium chrysogenum virus and derived double stranded RNA. J. Gen. Virol. 12: 131-139

Cantino, E. C., and Mack, J. P. 1969. Form and function in the zoospore of Blastocladiella emersonii. I. The gamma particle and satellite ribosome package. Nova Hedwigia 18: 115-148

Cantino, E. C., and Myers, R. B. 1973. The gamma particle and intracellular interactions in Blastocladiella emersonii. In: "Basic Mechanisms in Plant Morphogenesis", Brookhaven symposia in Biology. 25: 51-74

Cantino, E. C., and Mills, G. L. 1976. Form and function in Chytridiomycete spores, with emphasis upon Blastocladiella emersonii. In: The Fungus Spore (D. J. Weber and W. M. Hess, eds.) pp 501-577, John Wiley and Sons, New York

Cesarini, J. P. and Prunieras, M. 1972. Inclusions tubulaires type lupus dans le mélanome malin. Ann. Dermatol. Syphiligr. (Paris) 99: 525

Chandra, S. 1968. Undulating tubules associated with endoplasmic reticulum in pathologic tissues. Laboratory Investigation 18: 422-428

Chandra, S., and Stefani, S. S. 1976. A possible mode of formation of tubuloreticular structures. J. Ultrastruc. Res. 56: 304-311

Chapman, R. L. and Lang, N. J. 1973. Viruslike particles and nuclear inclusions in the red alga Porphyridium purpureum (Bory) Drew et Ross. J. of Phycology 9: 117-122

Darley, W. M., and Fuller, M. S. 1970. Cell wall chemistry and taxonomic position of Scizochytrium. Am. J. Bot. 57: 761

Darley, W. M., Porter, D., and Fuller, M. S. 1973. Cell wall composition and synthesis via Golgi-directed scale formation in the marine eucaryote, Schizochytrium aggregatum, with a note on Thraustochytrium sp. Arch. Mikrobiol. 90: 89-106

Day, P. R., and Anagnostakis, S. L. 1972. Heterokaryon transfer of the "killer" factor in Ustilago maydis. Phytopathology 62: 494

DeBrabander, M., and Borgers, M. 1975. The formation of annulated lamellae induced by the disintegration of microtubules. J. Cell Sci. 19: 331-340

Demsey, A. and Grimley P. M. 1975. Lymphocyte tubuloreticular structures studied by freeze-fracture and stereography. J. Microscopie Biol. Cell 24: 271-276

Diamond, L. S., and Mattern, C. F. T. 1976. Protozoal viruses. Advances in Virus Res. 20: 87-112

Docherty, J. J., and Chopan, M. 1974. The latent herpes simplex virus. Bacteriol. Rev. 38: 337-355

Eakin, R. M., and Brandenburger, J. L. 1975. Retinal differences between light-tolerant and light-avoiding slugs (Mollusca: Pulmonata) J. Ultrastruct. Res. 53: 382-394

Edwardson, J. R. 1966. Electron microscopy of cytoplasmic inclusions in cells infected with rod shaped viruses. Amer. J. Bot. 53: 359-364

Edwardson, J. R., Purcifull, D. E., Christie, R. G. 1968. Structure of cytoplasmic inclusions in plants infected with rod-shaped viruses. Virology 34: 250-263

Ehret, C., Savage, N., and Alblinger, J. 1964. Patterns of segregation of structural elements during cell division. Z. Zellforsch. Mikroskop. Anat. 64: 129-139

Farley, C. A. Banfield, W. G., Kasnic, G., and Foster, W. S. 1972. Oyster herpes-type virus. Science 178: 759-760

Fawcett, D. W., 1966. "The Cell". An Atlas of Fine Structure. pp 319-337. Saunders, Philadelphia, Pennsylvania.

Feorino, P.M., Hierholzer, J. C., and Norton, J. L. 1970. Viral isolation studies of inclusion positive biopsy from human connective tissue diseases. Arthritis Rheum. 13: 378

Fuller, M. S., Fowles, B. E., and Mclaughlin, D. J. 1964. Isolation and pure culture studies of marine Phycomycetes. Mycologia 56: 745-756

Gaertner, A. 1970. Beobachtungen uber die sporulation der dickwandigen sporangien von Thraustochytrium Kinnei Gaertner. Veroff. Inst. Meeresforsch. Bremher. 12: 321-327

__________. 1972. Characters used in the classification of thraustochytriaceous fungi. Veroff. Inst. Meeresforsch. Bremerh. 13: 183-194

Gaertner, A., Schaumann, K., and Ulken, A. 1974. Final discussion of the 2. International Symposion on Marine Mycology, 11.9-16.9. 1972 in Bremerhaven. Veroff. Inst. Meeresforsch. Bremerh. suppl. 5: 519-539

Gandy, D. G. 1969. A transmissible disease of cultivated mushrooms ("Watery Stipe"). Ann. Appl. Biol. 48: 427-430

Gandy, D. G. and Hollings, M. 1962. Die-back of mushrooms: a disease associated with a virus. Rep. Glasshouse Crops Res. Inst. 1961: 103-108

Gerber, P. 1972. Activation of Epstien-Barr virus by 5-bromo-deoxyuridine in "virus free" human cells. Proc. Natl. Acad. Sci. U. S. A. 69: 83-85

Gibbs, A., Skotnicki, A. H., Gardiner, J. E., Walker, E. S., and Hollings, M. 1975. A tobamovirus of a green alga. Virology 64: 571-574

Goldstein, S. 1963a. Development and nutrition of new species of Thraustochytrium. Amer. J. Bot. 50: 271-279

__________. 1963b. Morphological variation and nutrition of a new monocentric marine fungus. Archiv fur Mikrobiol. 45: 101-110

__________. 1963c. Studies of a new species of Thraustochytrium that displays light stimulated growth. Mycologia 55: 799-811

__________. 1973. Zoosporic marine fungi (Thraustochytriaceae and Dermocystidiaceae) Ann. Rev. Microbiol. 27: 13-26

Goldstein, S., and Belsky, M. M. 1963. B_{12} and B_1 auxotrophy of lower marine Phycomycetes. Archiv fur Mikrobiol. 47: 161-163

__________________________. 1964. Axenic culture of a new marine Phycomycete possessing an unusual type of asexual reproduction. Amer. J. Bot. 51: 72-78

Goldstein, S., Belsky, M. M., and Chosak, R. 1969. Biology of a problematic marine fungus, Dermocystidium sp. II. Nutrition and respiration. Mycologia 61: 468-72

Goldstein, S., Moriber, L., and Hershenov, B. 1964. Ultrastructure of Thraustochytrium aureum, a biflagellate marine Phycomycete. Mycologia 56: 897-904

Goodman, J. R., Sylvester, R. A., Talal, N., and Tuffanelli, D. L. 1973. Viruslike structures in lymphocytes of patients with systemic and discoid lupus erythematosus. Ann. Intern. Med. 79: 396-402

Grimley, P. M., Barry, D. W., and Schaff, Z. 1973a. Induction of tubular structures in the endoplasmic reticulum of human lymphoid cells by treatment with 5-bromo-2 -deoxyuridine. J. Natl. Cancer Inst. 51: 1751-60

______________________________. 1973b. Induction of "viruslike" tubular structures in the endoplasmic reticulum of human lymphoid cells treated with 5-bromodeoxyuridine (BUdR) (abstr.) Fed. Proc. 32: 964

Grimley, P. M., Barry, D. W., Schaff, Z. and Becker, K. E. 1973c. Lupus-type tubular structtres in human lymphoid cell cultures. Arth. Rheum. 16: 550

Grimley, P. M. and Schaff, Z. 1976. Significance of tubuloreticular inclusions in the pathobiology of human diseases. Pathobiol. Ann. 6: 221-257

Gyorkey, F., Min, K. W., Sinkovics, J. G., and Gyorkey, P. 1969. Systemic lupus erythematosus and myxovirus. New Engl. J. Med. 280: 333

Harrison, J. L., and Jones, E. B. G. 1974. Ultrastructural aspects of the marine fungus Japonchytrium sp. Arch. Microbiol. 96: 305-317

Hashimoto, K. 1970. Paramyxovirus-like inclusions in several skin diseases of unknown etiology (abstr.) Clin. Res. 18: 349

Hashimoto, K. and Chandler, R. W. 1972. Paramyxovirus-like inclusion in systemic lupus erythematosus. Acta Dermatovener (Stockholm) 52: 263-277

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

Helder, A. W., Feltkamp-Vroom, T. M., and VanLoghem, J. J. 1975. A comparative morphological study of tubular structures in human tissues and nucleocapsids of measles virus. J. Microscopie Biol. Cell 23: 1-16

Hillman, E. A., Charamella, L. J., Temple, M. J. and Elser, J. E. 1977. Biological characterization of an Epstein-Barr nuclear antigen-positive American Burkitt's tumor derived cell line. Cancer Res. 37: 4546-4558

Hoffman, L. R., and Stanker, L. H. 1976. Viruslike particles in the green alga Cylindrocapsa. Can. J. Botany 54: 2827-2841

Hollings, M. 1962. Viruses associated with a die-back disease of cultivated mushroom. Nature 196: 962-965

__________. 1978. Mycoviruses: viruses that infect fungi. Advances in Virus Res. 22: 1-53

Hollings, M., and Stone, O. M. 1971. Viruses that infect fungi. Ann. Rev. Phytopathol. 62: 823-825

Hooper, G. R., and Wiese, M. V. 1972. Cytoplasmic inclusions in wheat affected by wheat spindle streak mosaic. Virology 47: 664-672

Howatson, A. F. 1970. Vesicular stomatitis and related viruses. Advances in Virus Res. 16: 195-256

Hulanicka, B., Barry, D. W., and Grimley, P. M. 1977. Induction of tubuloreticular inclusions in human lymphoma cells (Raji line) related to s-phase treatment with halogenated pyrimidines. Cancer Res. 37: 2105-2113

Hurd, E. R. Eigenbrodt, E., Ziff, M., and Strunk, S. W. 1969. Cytoplasmic tubular structures in kidney biopsies in systemic lupus erythematosus. Arthritis Rheum. 12: 541-542

Hutchinson, T. E., Cantino, M. E., and Cantino, E. C. 1977. Calcium is a prominent constituent of the gamma particle in the zoospore of Blastocladiella emersonii as revealed by X-ray microanalysis. Biochem. Biophys. Res. Comm. 74: 336-342

Jensen, W. A. 1968. Cotton embryogenesis: The tube-containing endoplasmic reticulum. J. Ultrastruct. Res. 22: 296-302

Jenson, A. B., Spjut, H. J., Smith, M. N., and Rapp, F. 1971. Intracellular branched tubular structures in osteosarcoma. Cancer 27: 1440-1448

Jones, E. B. G. and Alderman, D. J. 1970. Althornia Crouchii, Gen. et sp. Nov., a marine biflagellate fungus. Nova Hedwigia 21: 381-400

Kazama, F. Y. 1972a. Ultrastructure of Thraustochytrium sp. zoospores. I. kinetosome. Arch. Mikrobiol. 83: 179-188

__________. 1972b. Ultrastructure of Thraustochytrium sp. zoospores II. striated inclusions. J. Ultrastructure Res. 41: 60-66

__________. 1973. Ultrastructure of Thraustochytrium sp. zoospores III. cytolysomes and acid phosphatase distribution. Arch. Mikrobiol. 89: 95-104

__________. 1974a. Ultrastructure of Thraustochytrium sp. zoospores IV. external morphology with notes on the zoospores of Schizochytrium sp. Mycologia 66: 272-280

__________. 1974b. The ultrastructure of nuclear division in Thraustochytrium sp. Protoplasma 82: 155-175

__________. 1975. Cytoplasmic cleavage during zoosporogenesis in Thraustochytrium sp. Ultrastructure and the effects of colchicine and D_2O. J. Cell Sci. 17: 155-170

Kazama, F. Y., and Schornstein, K. L. 1972. Herpes-type virus particles associated with a fungus. Science 177: 696-697

____________________. 1973. Ultrastructure of a fungus herpes-type virus. Virology 52: 478-487

Kessel, R. G. 1965. Intranuclear and cytoplasmic annulate lamellae in tunicate oocytes. J. Cell Biol. 24: 471-487

__________. 1968. Annulate lamellae. J. Ultrastruct. Res., suppl. 10: 5-82

__________. 1973. Structure and function of the nuclear envelope and related cytomembranes. In: "Progress in Surface and Membrane Science", vol. 6 (J. F. Danielli, M. D. Rosenber, and D. A. Cadenhead, eds.) pp 243-329 Academic Press, New York

Khandjian, E. W., Roos, U. P., Timberlake, W. E., Eder, L. and Turian, G. 1974. RNA viruslike particles in the Chytridiomycete Allomyces arbuscula. Archives of Microbiol. 101: 351-356

Khandjian, E. W., Roos, U. P. and Turian, G. 1975. Mycovirus d'Allomyces. Pathologia et Microbiologia 42: 250-251

Kimberg, D. V. and Loeb, J. N. 1972. Effects of cortisone administration on rat liver mitochondria. J. Cell Biol. 55: 635-643

Kobayashi, T. and Asboe-Hansen, G. 1972. Virus particles in lupus erythematosus. Acta dermato-venereologica 52: 425-437

Kobayashi, Y. and Ookubo, M. 1953. Studies on the marine Phycomycetes. Bull. Nat. Sci. Mus. (Tokyo) 33: 53-65

Koestner, A., Kasza, L., and Holman, J. E. 1966. Electron microscopic evaluation of the pathogenesis of porcine polioencephalomyelitis. Am. J. Pathol. 49: 325-337

Koltin, Y., and Day, P. R. 1976a. Inheritance of killer phenotypes and double-stranded RNA in Ustilago maydis. Proc. Natl. Acad. Sci. U. S. A. 73: 594-598

________________________. 1976b. Suppression of the killer phenotype in Ustilago maydis. Genetics 82: 629-637

Lange, L., and Olson, L. W. 1976. The zoospore of Olpidium Brassicae. Protoplasma 90: 33-45

Langenberg, W. G., and Schroeder, H. F. 1973. Endoplasmic reticulum-derived pinwheels in wheat infected with wheat spindle streak mosaic virus. Virology 55: 218-223

Lemaire, J. M., Jouan, B., Perraton, B., and Sailly, M. 1971. Perspectives de lutte biologique contre les parasites des cereales d'origine tellurique en particulier Ophiobolus graminis Sacc. Sciences Agronomiques Rennes 1971: 1-8

Lemke, P. A. 1976. Viruses of eukaryotic microorganisms. Ann. Rev. Microbiol. 30: 105-45

Lemke, P. A. and Nash, C. H. 1974. Fungal viruses. Bacteriol. Rev. 38: 29-56

Lemke, P. A., Nash, C. H., Pieper, S. W. 1973. Lytic plaque formation and variation in virus titre among strains of Penicillium chyrsogenum J. Gen. Microbiol. 76: 265-75

Lemke, P. A., Saksena, K. N., and Nash, C. H. 1976. Viruses of industrial fungi. In: "Genetics of Industrial Microorganisms", (K. D. Macdonald, ed.) pp 323-355 Academic Press, New York

Lhoas, P. 1971a. Infection of protoplasts from Penicillium stoloniferum with double-stranded RNA viruses. J. Gen. Virol. 13: 365-367

________. 1971b. Transmission of double-stranded RNA to a strain of Penicillium stoloniferum through heterokaryosis. Nature 230: 248-249

Liebrich, W., and Paweletz, N. 1976. Aggregates of HeLa cells: a source for annulate lamellae. Cytobiologie 12: 473-475

Locket, N. A. 1973. Retinal structure in Latimeria chalumnae. Phil. Trans. R. Soc. Lond. B, 266: 493-518

Lombard, C., Cabanie, P. and Izard, J. 1967. Images evoquant l'aspect de virus dans les cellules du sarcome de Sticker. J. Microscopie 6: 81-84

Lowy, D. R., Rowe, W. P., Teich, N., and Hartley, J. W. 1971. Murine leukemia virus: high frequency activation in vitro by 5-iododeoxyuridine and 5-bromodeoxyuridine. Science 174: 155-156

MacKin, J. G., Owen, H. M., and Collier, A. 1950. Preliminary note on the occurrence of a new protistan parasite. Dermocystidium marinum n. sp. in Crassostrea virginica (Gmelin). Science 111: 328-329

Margalith, M., Thornton, H., Narconis, R., Pinkerton, H., and Green, M. 1975. Studies on virus induction by 5-bromodeoxyuridine in nonproducer murine sarcoma virus-transferred 3T3 cells. Virology 65: 27-39

Markey, D. R. 1974. A possible virus infection in the brown alga Pylaiella littoralis. Protoplasma 80: 223-232

McWhorter, F. P. 1965. Plant virus inclusions. Ann. Rev. Phytopathol. 3: 287-312

Metitiri, P. O. and Zachariah, K. 1972. Viruslike particles and inclusion bodies in penicillus cells of a mutant of Penicillium. J. Ultrastruct. Res. 40: 272-283

Myers, R. B., and Cantino, E. C. 1974. "The Gamma Particle". 117 pp. Karger, Basel

Nemes, M. M., Tytell, A. A., Lampson, G. P., Field, A. K. and Hilleman, M. R., 1969. Inducers of interferon and host resistance. VII. Antiviral efficacy of double-stranded RNA of natural origin. Proc. Soc. Expt. Biol. Med. 132: 784-789

Ohman, P. 1974. Fine structure of the retinal pigment epithelium of the river lamprey (Lampetra fluviatilis, Cyclostomi). Acta Zoologica. 55: 245-253

Olive, L. S. 1975. "The Mycetozoans". 293 pp. Academic Press, New York

Osafune, T., Mihara, E., Hase, E. and Ohkuro, I. 1972. Electron microscope studies on the vegetative cellular life cycle of Chlamydomonas reinhardi Dangeard in synchronous culture. I. Some characteristics of changes in subcellular structures during the cell cycle, especially in formation of giant mitochondria. Plant and Cell Physiol. 13: 211-227

_______________________________________. 1975. Formation and division of giant mitochondria during the cell cycle of Euglena gracilis Z in synchronous culture. I. Some characteristics of changes in the morphology of mitochondria and oxygen-uptake activity of cells. Plant and Cell Physiol. 16: 313-326

Oshiro, L. S. 1973. Coronaviruses. In: "Ultrastructure of Animal Viruses and Bacteriophages": an atlas, (A. J. Dalton and F. Haguenau, eds.) pp 331-343, Academic Press, New York

Oshiro, L. S., Schieble, J. H., and Lennette, E. H. 1971. Electron microscopic studies of coronavirus. J. Gen. Virol. 12: 161-168

Pearson, B. R., and Norris, R. E. 1974. Intranuclear viruslike particles in the marine alga Platymonas sp (Chlorophyta, Prasinophyceae). Phycologia 13: 5-9

Perkins, F. O. 1969. Ultrastructure of vegetative stages in Labyrinthomyxa marina (=Dermocystidium marinum), a commercially significant oyster pathogen. J. Inverteb. Pathol. 13: 199-222

____________. 1970. Formation of centriole and centriole-like structures during meiosis and mitosis in Labyrinthula sp. (Rhizopodea, Labyrinthulida). J. Cell Sci. 6: 629-653

____________. 1972. The ultrastructure of holdfasts, "rhizoids", and "slime tracks" in thraustochytriaceous fungi and Labyrinthula spp. Arch. Mikrobiol. 84: 95-118

____________. 1973. Observations of thraustochytriaceous (Phycomycetes) and labyrinthulid (Rhizopodea) ectoplasmic nets on natural and artificial substrates - an electron microscope study. Can. J. Bot. 51: 485-491

____________. 1974. Phylogenetic considerations of the problematic thraustochytriaceous-labyrinthulid-Dermocystidium complex based on observations of fine structure. Veroff. Inst. Meeresforsch. Bremerh. Suppl. 5: 45-63

Perkins, F. O., and Amon, J. P. 1969. Zoosporulation in Labyrinthula sp.; an electron microscope study. J. Protozool. 16: 235-257

Perkins. F. O. and Menzel, R. W. 1967. Ultrastructure of sporulation in the oyster pathogen Dermocystidium marinum. J. Invert. Pathol. 9: 205-229

Pincus, T., Blacklow, N. R., Grimley, P. M. and Bellanti, J. A. 1970. Glomerular microtubules of systemic lupus erythematosus. Lancet 2: 1058-1061

Pollak, A. 1975. The induction of viruslike particles in the marine Phycomycete Thraustochytrium aureum. (Abstr.) Ann. Meet. Amer. Soc. Microbiol. 1975: 190

Popoff, N. A. and Malinin, T. I. 1976. Cytoplasmic tubular arrays in cells of American Burkitt's type lymphoma. Cancer 37: 275-284

Porter, D. 1972. Cell division in the marine slime mold, Labyrinthula sp., and the role of the bothrosome in extracellular membrane production. Protoplasma 74: 427-448

________..1974. Phylogenetic considerations of the Thraustochytriaceae and Labyrinthulaceae. Veroff. Inst. Meeresforsch. Bremerh. Suppl. 5: 19-44

Pothier, L., Uzman, B. G., Kasac, M. M., Saito, H., and Adams, R. A. 1973. Immunoglobulin synthesis and tubular arrays inthe endoplasmic reticulum in transplanted human tumors of lymphoid origin. Lab. Invest. 29: 607-613

Provasoli, L., McLaughlin, J. A. A., and Droop, M. R. 1957. The development of artificial media for marine alga. Arch. Mikrobiol. 25: 392-428

Rawlinson, C. J., Hornby, D., Pearson, V., and Carpenter, J. M. 1973. Viruslike particles in the take-all fungus, Gaeumannomyces graminis. Ann. Appl. Biol. 74: 197-209

Reanney, D. C. 1974. Viruses and evolution. Int. Rev. Cytol. 37: 21-52

Reynolds, E. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208-212

Roizman, B. 1965. An inquiry into the mechanisms of recurrent herpes infections of man. In: "Perspectives in Virology", vol. 4 (M. Pollard ed.) pp 283-301, Harper and Row, New York

Roos, U. P., Khandjian, E. W., and Turian, G. 1976. RNA virus in Allomyces arbuscula: Ultrastructural localization during the life-cycle. J. Gen. Microbiol. 95: 87-95

Rowe, W. P., Lowy, D. R., Teich, N., and Hartley, J. W. 1972. Some implications of the activation of murine leukemia virus by halogenated pyrimidines. Proc. Natl. Acad. Sci. U. S. A. 69: 1033-1035

Saksena, K. N. and Lemke, P. A. 1978. Viruses in fungi. In: "Comprehensive Virology", vol. 12 (H. Fraenkel-Conrat and R. R. Wagner, eds.) pp 103-143, Plenum Press, New York

Schaff, Z., Barry, D. W., and Grimley, P. M. 1973. Cytochemistry of tubuloreticular structures in lymphocytes from patients with systemic lupus erythematosus and in cultured human lymphoid cells. Lab. Invest. 29: 577-586

Schaff, Z., Heine, U., and Dalton, A. J. 1972. Ultramorphological and ultracytochemical studies on tubuloreticular structures in lymphoid cells. Cancer Res. 32: 2696-2706

Schnepf, E., Soeder, C. J., and Hegewald, E 1970. Polyhedral viruslike particles lysing the aquatic Phycomycete Aphelidium sp., a parasite of the green alga Scenedesmus armatus. Virology 42: 482-487

Schuster, F L. 1969. Intranuclear viruslike bodies in the amoeboflagellate Naegleria gruberi. J. Protozool. 16: 724-727

Schuster, F L. and Dunnebacke, T. H. 1971. Formation of bodies associated with viruslike particles in the amoeboflagellate Naegleria gruberi J. Ultrastruct. Res. 36: 659-668

______________________________. 1974a. Growth at 37°C of the EGs strain of the amoeboflagellate Naegleria gruberi containing virus-like particles. I. Nuclear changes. J. Invertebr. Pathol. 23: 172-181

______________________________. 1974b. Growth at 37°C of the EGs strain of the amoeboflagellate Naegleria gruberi containing virus-like particles. II. Cytoplasmic changes. J. Invertebr. Pathol. 23: 182-189

______________________________. 1974c. Viruslike particles and an unassociated infectious agent in amoebae of the genus naegleria. Ann. Soc. Belge Med. Trop. 54: 359-370

______________________________. 1976. Development and release of viruslike particles in Naegleria gruberi EGs. Cytobiologie 14: 131-147

______________________________. 1977. Viruslike particles in the amoeboflagellate Naegleria gruberi EGs. Microbiology 1977, Am. Soc. Microbiol. pp 579-582

Schuster, F. L., and Rechthand, E. 1975. In vitro effects of amphotericin B on growth and ultrastructure of the amoeboflagellates Naegleria gruberi and Naegleria fowleri. Antimicrobial Agents and Chemotherapy 8: 591-605

Sherman, L. A. and Brown, R. M. 1978. Cyanophages and viruses of eukaryotic algae. In: "Comprehensive Virology", vol. 12 (H. Fraenkel-Conrat and R. R. Wagner, eds.) pp 145-234, Plenum Press, New York

Siegenthaler, P. A., Belsky, M M., and Goldstein, S. 1967. Phosphate uptake in an obligately marine fungus: a specific requirement for sodium. Science 155: 93-94

Smith, R. D., and Deinhardt, F. 1968. Unique cytoplasmic membranes in Rous Sarcoma virus-induced tumors of a subhuman primate. J. Cell Biol. 37: 819-823

Sparrow, F. K. 1936. Biological observations on the marine fungi of Woods Hole waters. Biol. Bull. 70: 236-263

____________. 1960. "Aquatic Phycomycetes" 2nd ed. 1187 pp. University of Michigan Press, Ann Arbor, Michigan

____________. 1968. Remarks on the Thraustochytriaceae. Veroff. Inst. Meeresforsch. Bremerh. Suppl. 3: 7-18

____________. 1974. Mastigomycotina. In: "The Fungi" vol. 4B(G. C. Ainsworth, F. K. Sparrow, and A. S. Sussman, eds.) pp 61-73, Academic Press, New York

Splinter, T. A. W., Helder, A. W., Lucas, C. J., and Feltkamp-Vroom, T. M. 1975. Tubuloreticular structures in human lymphoid cell lines: a cell biological study. Brit. J. Exp. Pathol. 56: 124-132

Spurlock, B., Kaltine, V., and Freeman, J. A. 1963. Technical modifications in maraglas embedding. J. Cell Biol. 17: 203-207

Tinsley, T. W., and Harrap, K. A. 1978. Viruses of invertebrates. In: "Comprehensive Virology", vol. 12 (H. Fraenkel-Conrat and R. R. Wagner, eds.) pp 1-101, Plenum Press, New York

Uzman, B. G., Saito, H. and Kasac, M. 1971. Tubular arrays in the endoplasmic reticulum in human tumor cells. Lab. Invest. 24: 492-498

Van Lennep, E. W., and Lanzing, W. J. R. 1967. The ultrastructure of glandular cells in the external dendritic organ of some marine catfish. J. Ultrastruc. Res. 18: 333-344

Vishniac, H. S. 1955. Marine mycology. Trans. New York Acad. Sci. 17: 352-360

____________. 1956. On the ecology of the lower marine fungi. Biol. Bull. 11: 410-414

____________. 1961. A biological assay for thiamine in sea water. Limnol. Oceanogr. 6: 31-35

Watanabe, S., Komura, J., and Ofuji, S. 1977. Ultrastructural studies of epidermal lesions in pityriasis lichenoides chronica. British J. Dermatology 96: 59-66

Watanabe, S., Beard, C. W., and Triche, T. 1977. Annulate lamellae in four cases of diffuse lymphocytic lymphoma. J. Natl. Cancer Inst. 58: 777-780

Wergin, W. P., Gruber, P. J. and Newcomb, E. H. 1970. Fine structural investigations of nuclear inclusions in plants. J. Ultrastruct. Res. 30: 533-557

Whisler, H. and Travland, L. 1973. Mitosis in Harpochytrium. Arch. Protistenk. 115: 69-74

Wischnitzer, S. 1970. The annulate lamellae. Int. Rev. Cytol. 27: 65-100

Wood, H. A. 1973. Viruses with double-stranded RNA genomes. J. Gen. Virol. 20: 61-85

Wood, H. A. and Bozarth, R. F. 1972. Properties of viruslike particles of Penicillium chrysogenum: one double-stranded RNA molecule per particle. Virology 47: 604-609

Wool, S. H., and Held, A. A. 1976. Polyphosphate granules in encysted zoospores of Rozella allomycis. Arch. Microbiol. 108: 145-148

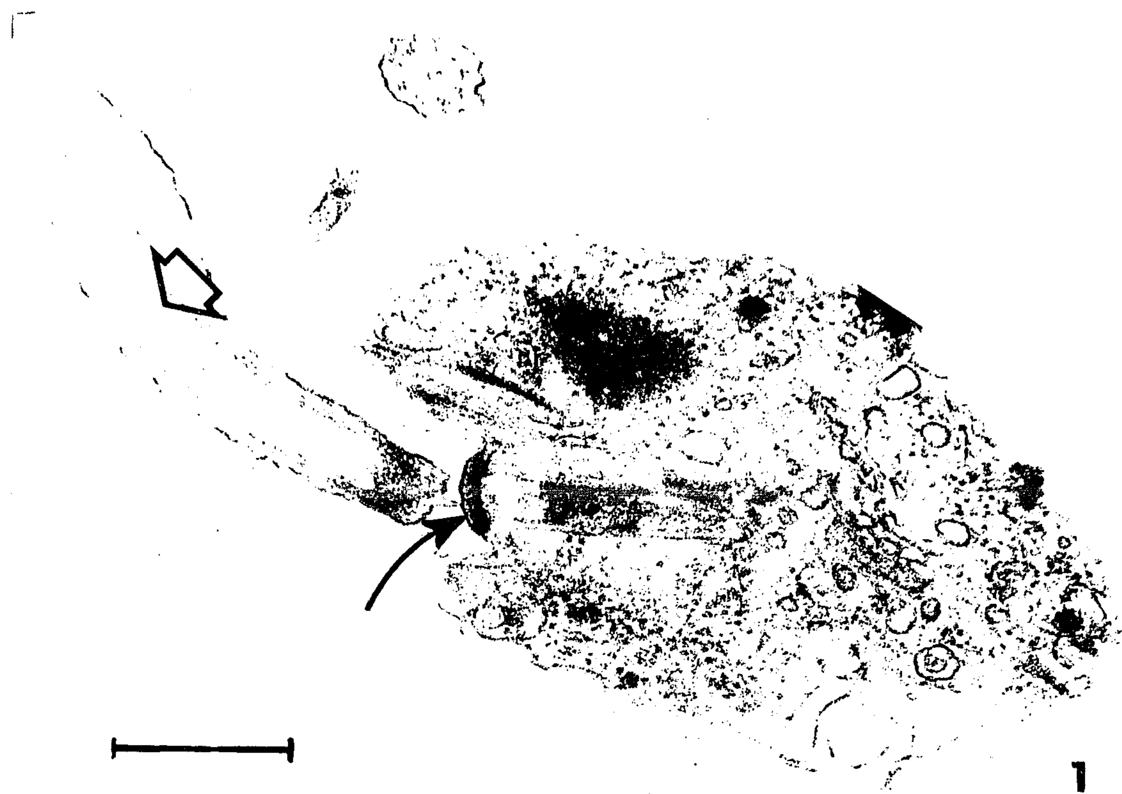
Zachariah, K. 1970. Lattice bodies and filament bundles in apothecial cells of the fungus Ascobolus. Can. J. Bot. 48: 1115-1118

Zachariah, K., and Anderson, R. H. 1973. On the distribution and development of lattice bodies in apothecial cells of the fungus Ascobolus. J. Ultrastruct. Res., 44: 405-420

Fig. 1. Longitudinal section of zoospore showing flagellar apparatus and associated structures, consisting of kinetosome granule (KG), terminal plate-basal disc region (arrow) and flagellar microtubules (open arrowhead). Bar = 0.5 µm. (x48,000)

Fig. 2. Zoospore displaying large branched mitochondrion (M). Bar= 1.0µm. (x18,500)

Fig. 3. Cross-section of an encysted zoospore showing single circular mitochondrion (M), nucleus (N), sagenogenetosome (SG), and centriole (arrowhead). Bar= 1.0 µm. (x24,000)


1

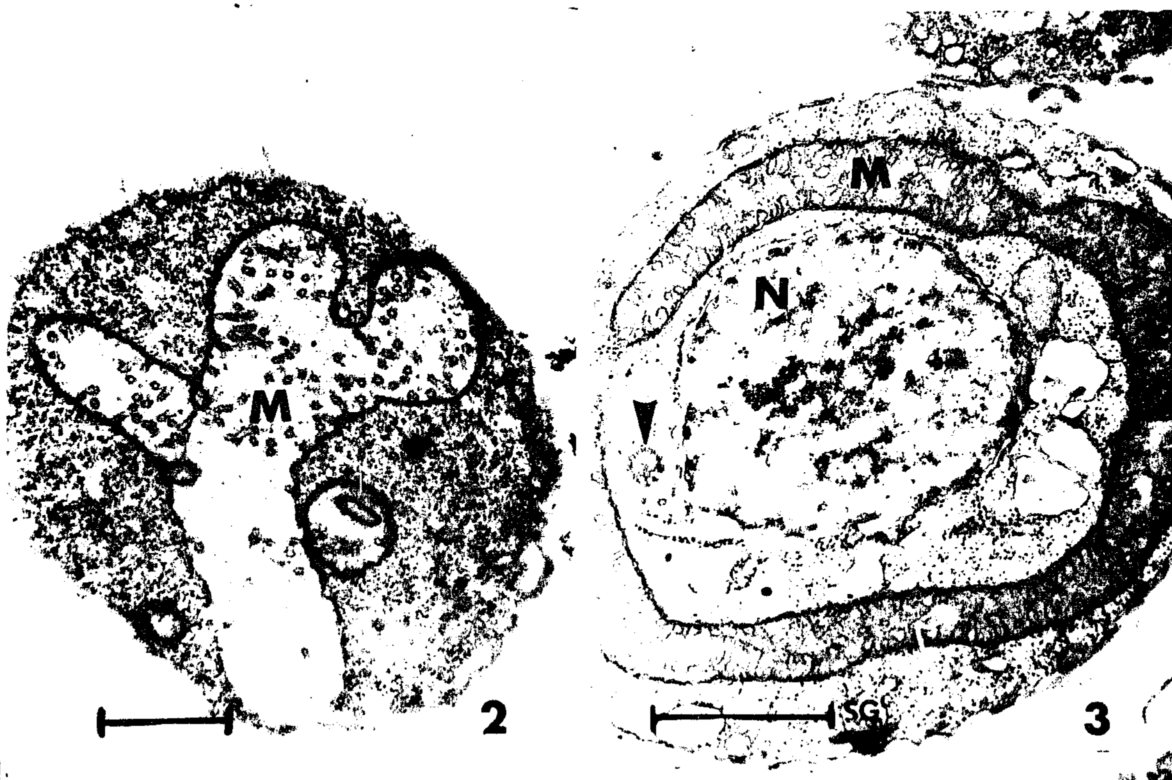
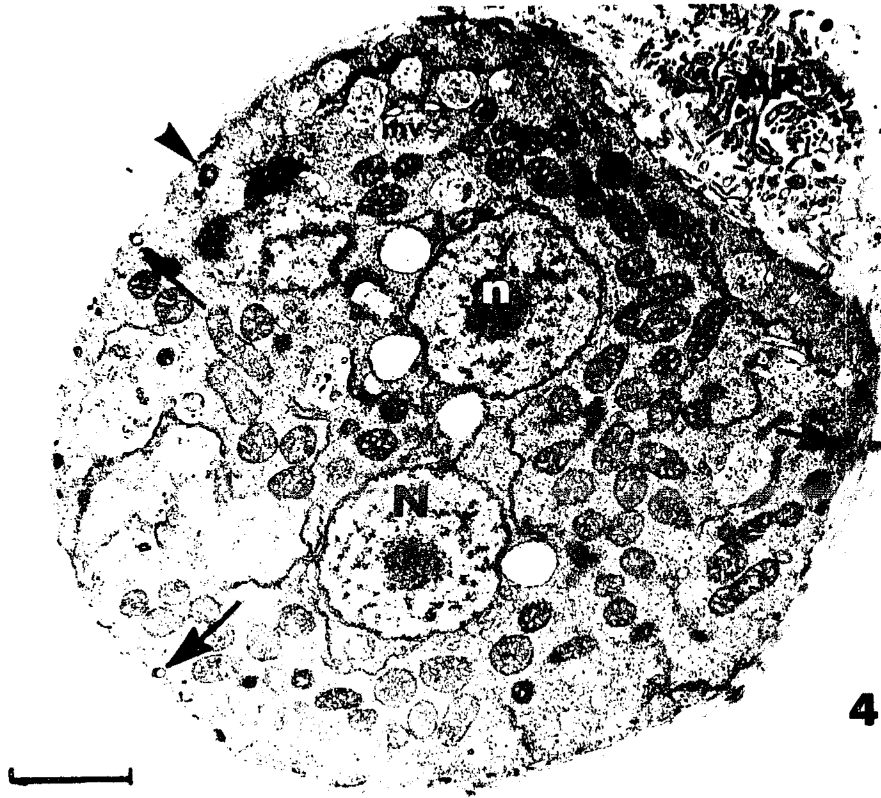

M
2
M
N
SG
3

Fig. 4. Section through a developing sporangium fixed approximately fifteen hours after encystment. At this stage of the developmental cycle nuclei (N) are undergoing mitotic divisions, nucleoli (n) are clearly discernible, the mitochondria become fragmented, multivesicular bodies (mv) are dispersed throughout the cytoplasm, there is a thickening of the cell wall, and gamma-like bodies (arrows) are visible. At one pole of the cell wall an apophysis (AP) consisting of ectoplasmic net elements forms. Associated with each nucleus is a single Golgi (g) and a pair of centrioles (arrowhead) Bar= 2.0 µm. (x7,7000)

Figs. 5-7. These micrographs represent three different tangential sections through the sagenogenetosome (SG). Fig. 5. shows a series of fused endoplasmic reticulum membranes converging at the sagenogenetosome. Bar= 0.25 µm. (x92,000). Fig. 6. Organelle is composed of electron-dense granules (arrow) lying directly beneath cell wall. Bar= 0.25 µm. (x80,000) Fig. 7. Periphery of organelle is composed of channels (arrow) continuous with the granular endoplasmic reticulum. Bar= 0.25 µm. (x80,000)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N
4

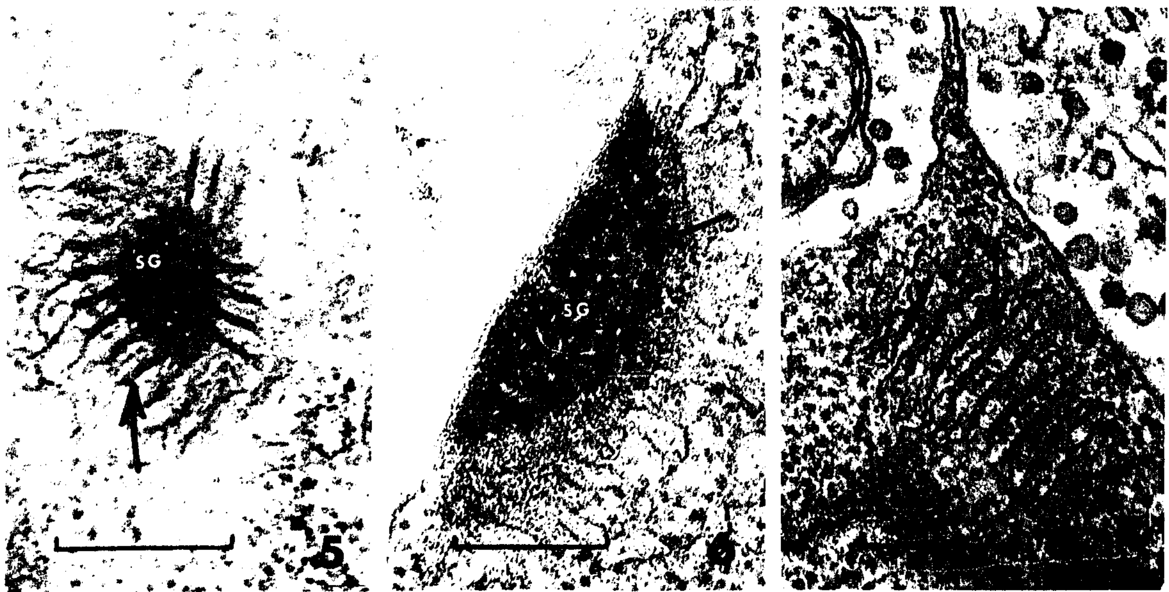

SG
5
SG

Fig. 8. Grazing section through cell wall (CW.) and adjacent cytoplasm. Thin circular scales (asterisks) are an integral component of cell wall, possibly transported through endoplasmic reticulum channels (arrow). Outer perimeter of cytoplasm contains many gamma-like bodies (open stars) in various configurations. Dark dense structures at top of micrograph are mitochondria (M). Bar= 0.5 µm. (x47,000)

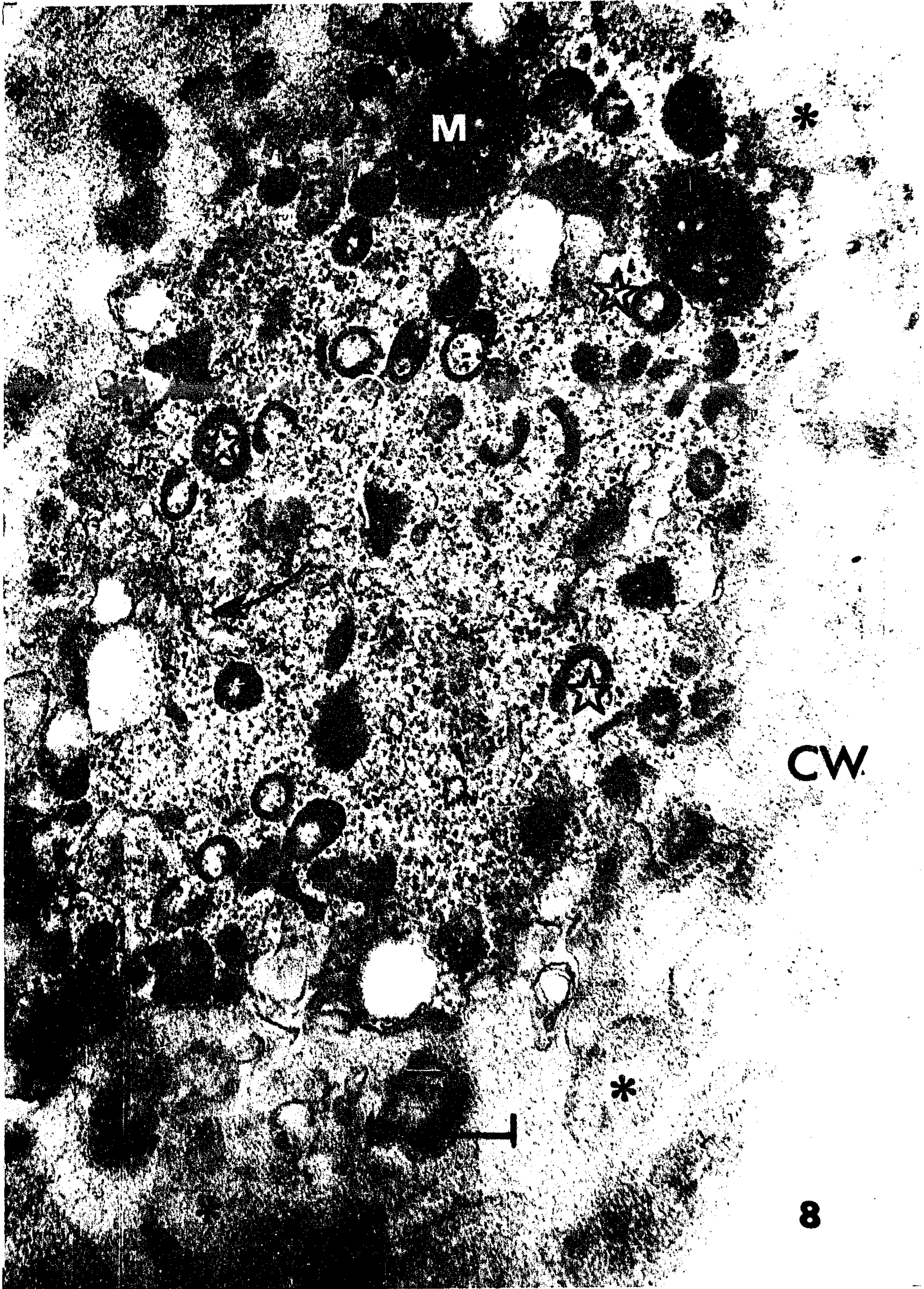

M
*
CW.
*
8

Fig. 9. Section through mature sporangium containing fully cleaved zoospores. Flagella are not yet evident as they emerge just prior to zoospore release. Encircled in zoospore (upper right corner) are a pair of centrioles. Arrowhead points to Golgi lying near indentation of nucleus (N). Bar= 2.0 um. (x15,700)

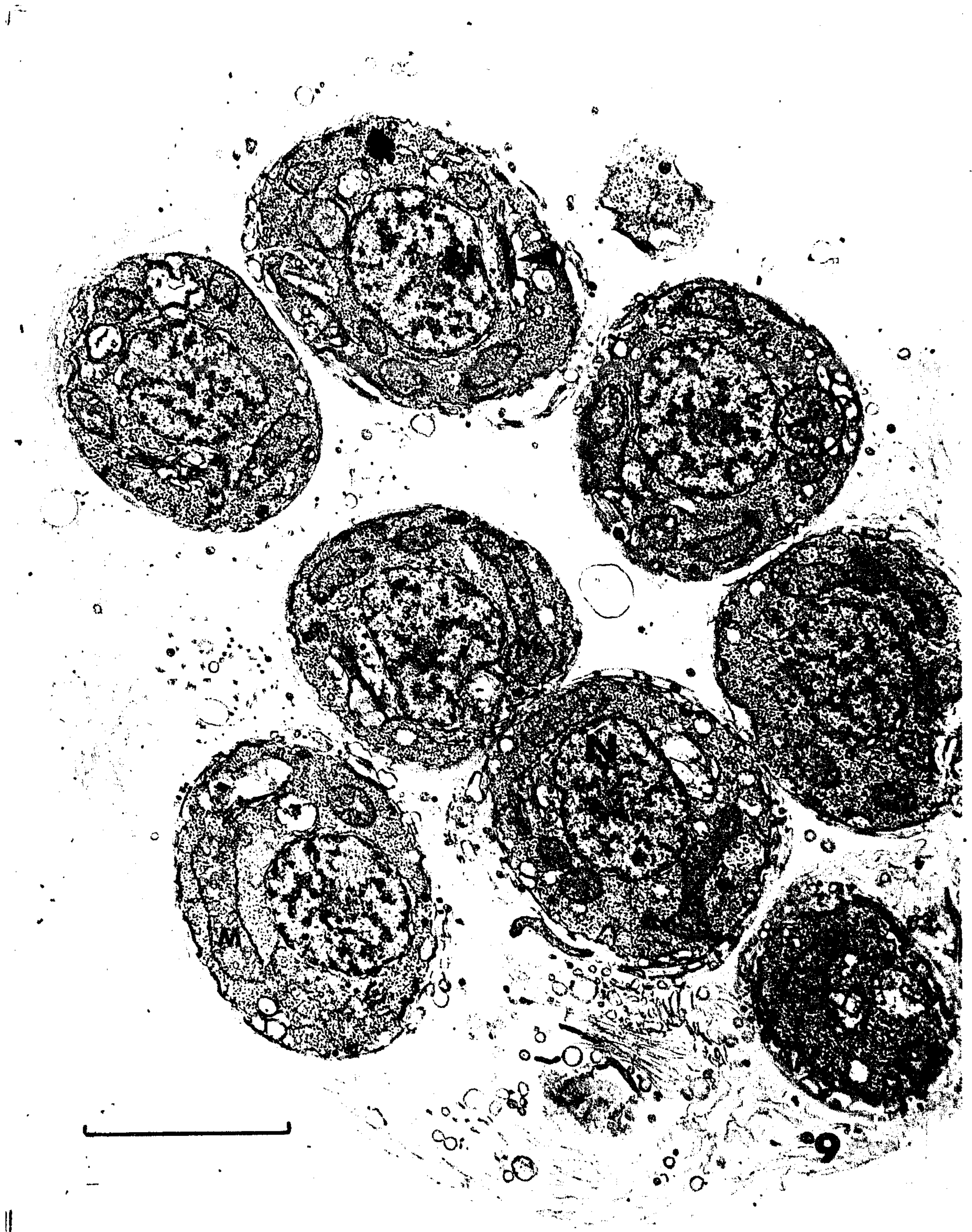
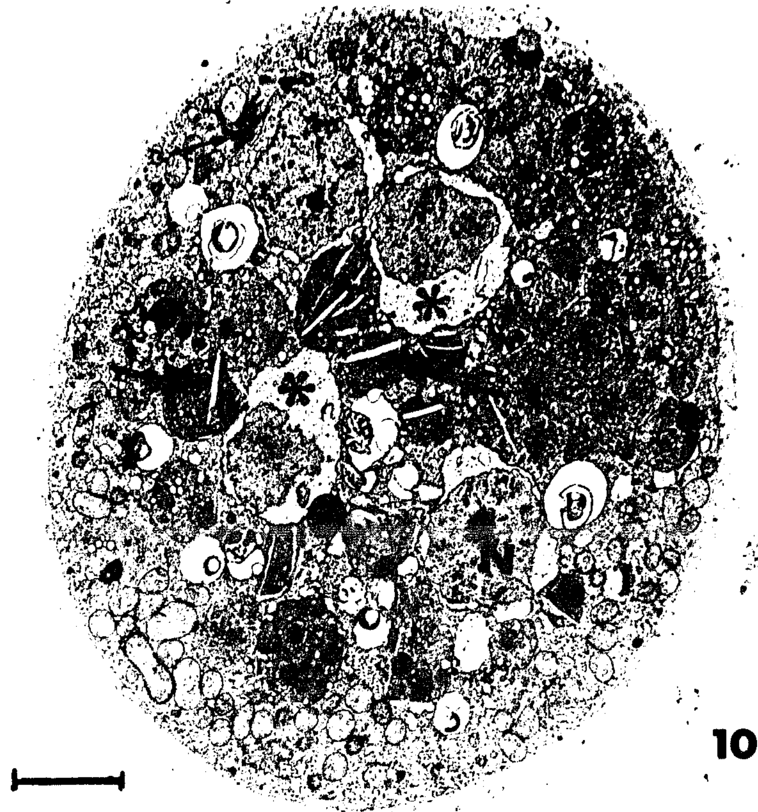
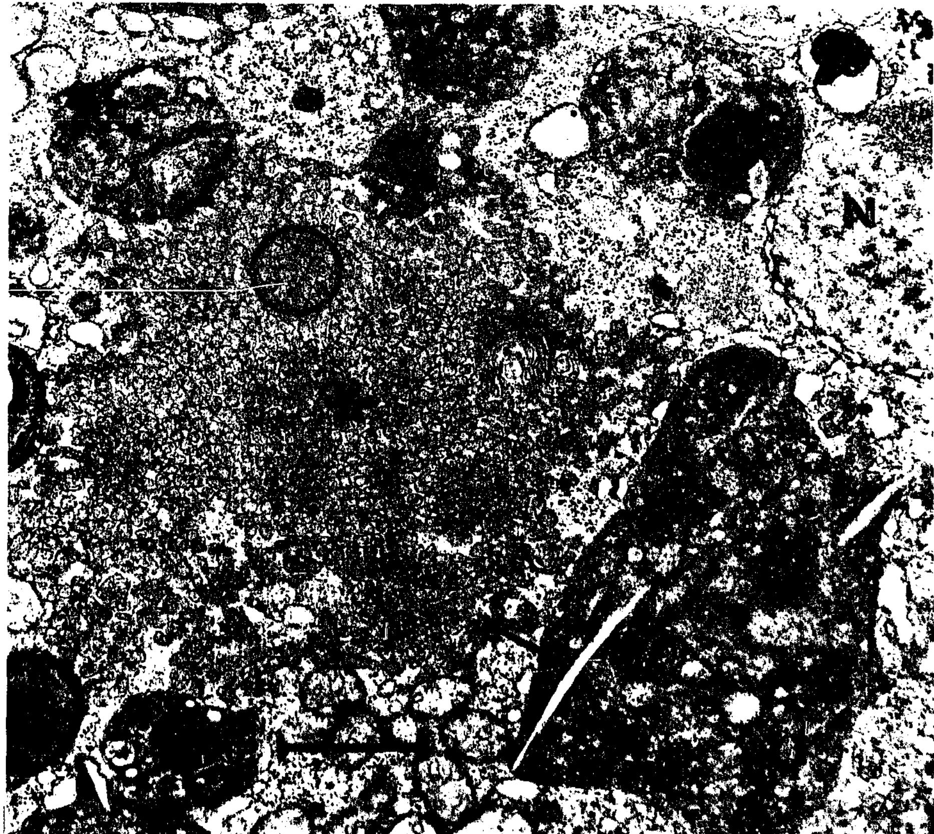

M
9

Fig. 10. Section through developing sporangium incubated 48 hrs. under anaerobic conditions. Space between nuclear envelope is grossly distorted (asterisk). Arrows point to empty spaces within cytoplasmic inclusions. Empty spaces probably represent crystalline structures lost during preparation for EM (See legends figs. 13-15) Also visible is a mass of tightly packed membranous tubules probably representing early stages in the formation of tubuloreticular structures. Bar= 2.0 µm (x7,300)

Fig. 11. Area from anaerobically cultured cell showing two kinds of inclusions at higher magnifications. Asterisk marks area of great ER proliferation and modification. Certain areas of this membranous mass are more electron-dense (circled area), the significance of which is not known. Arrow points to emptied crystalline formation within inclusion that also contains organelles in various stages of degeneration. Bar= 1.0 µm (x17,500)


N
10


N

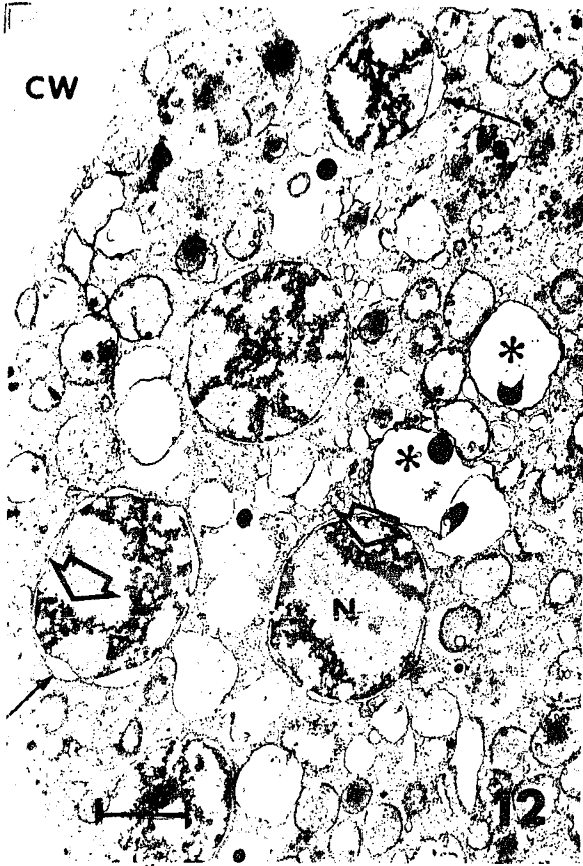
Figs. 12-15. Pathological effects observed after cells were incubated 48 hrs. under anaerobic conditions.

Fig. 12. Chromatin is highly condensed and marginated (open arrows), the nuclear envelope is swollen (arrows) and the cytoplasm is vaccuolated. Bar= 1.0 µm. (x11,600)

Fig. 13. Crystalline arrays are visible within inclusions. Close proximity of tubuloreticular structures (TRS) to crystalline material suggests a secretory function for the TRS. Bar= 0.5 µm. (x22,600)

Fig. 14. Section through inclusion shows crystalline material as well as early stages in the formation of 150 nm virus-like particles (arrow). Bar= 0.5 µm (x36,400)

Fig. 15. High magnification micrograph of several overlapping crystals within inclusion. Crystals consist of electron-dense rods crisscrossing to form a lattice with a center-to-center spacing of 26 nm. Bar= 0.25 µm. (x104,000)


CW
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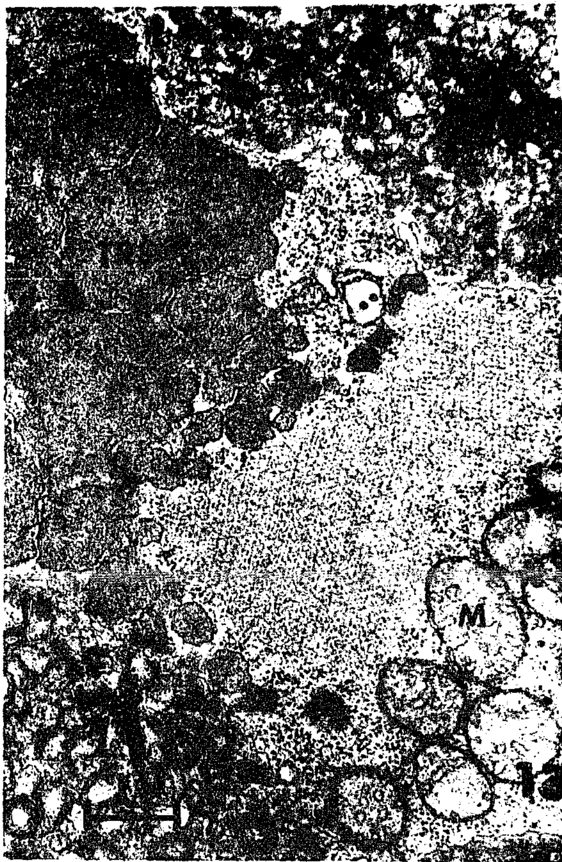
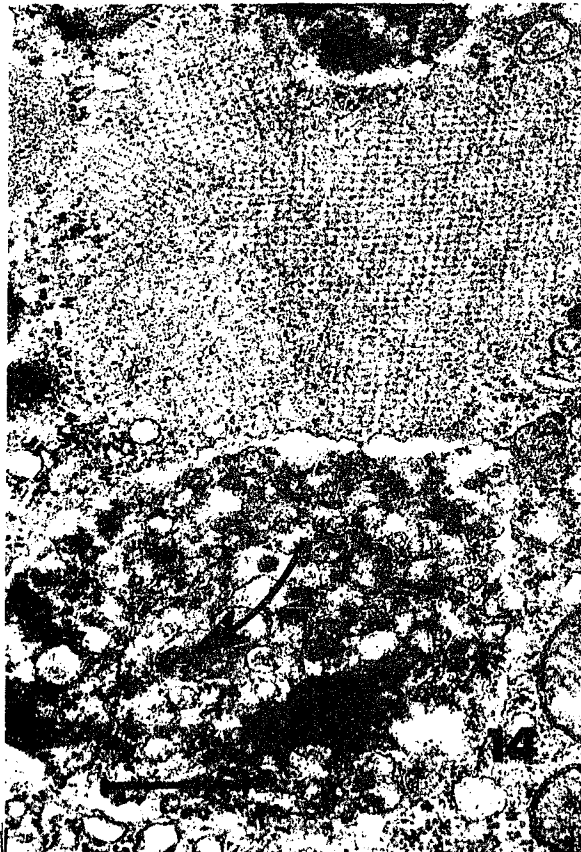
12


M
13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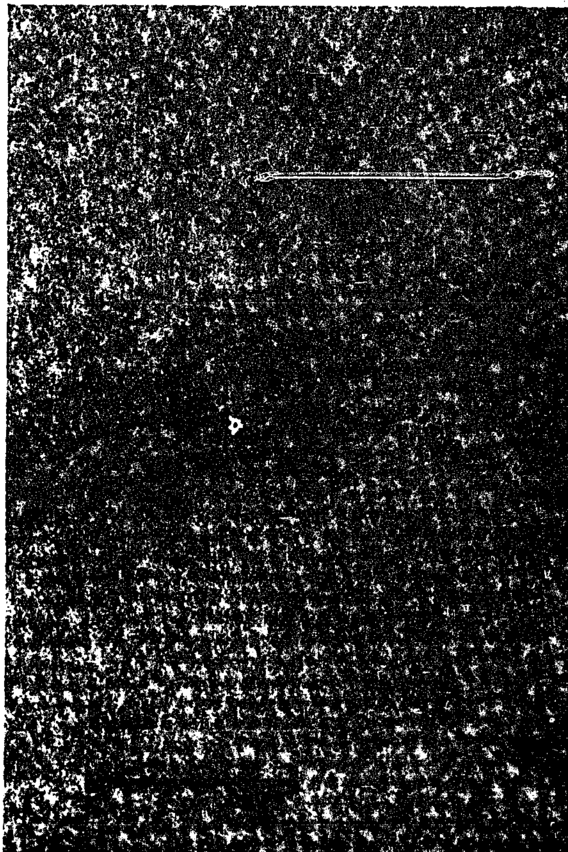


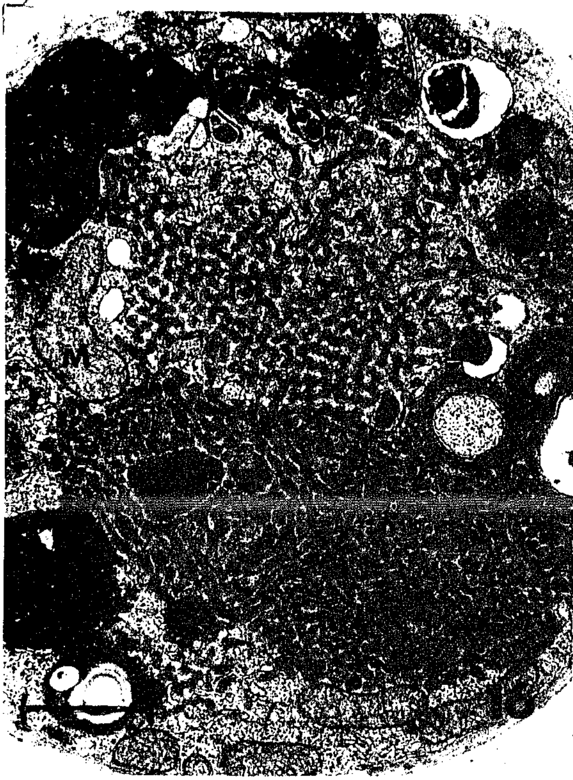
Figs. 16-19. Tubuloreticular structures in cells exposed to anaerobic conditions from 3-4 days.

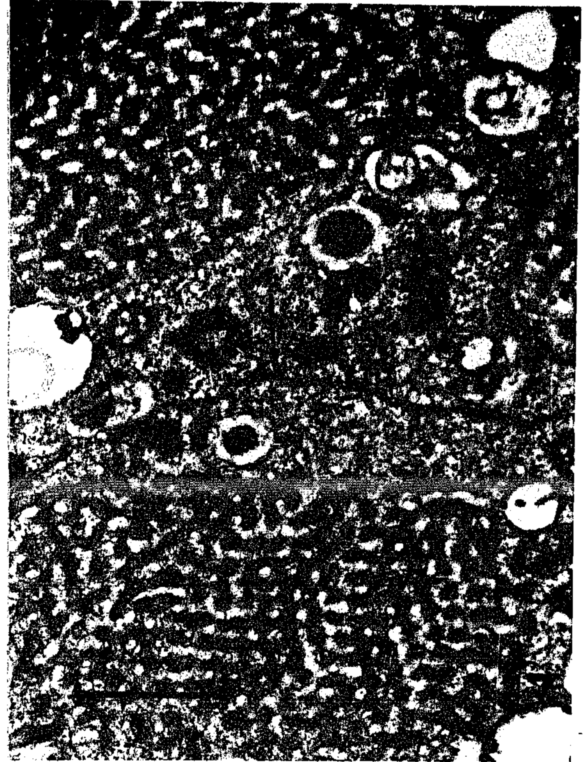
Fig. 16. A tangential section of a cell exhibiting extensive amounts of tightly packed TRS. Mitochondria (M) and normal granular ER in the surrounding areas remain relatively intact. The significance of the large osmiophillic bodies in the upper and lower left hand corners is unknown. The three small arrowheads indicate the point of demarcation between two separate inclusions of TRS, although there is no limiting membrane physically separating them. The changes in packing configuration and density are due to different orientation of these structures during sectioning. Bar= 0.5 µm. (x32,400)

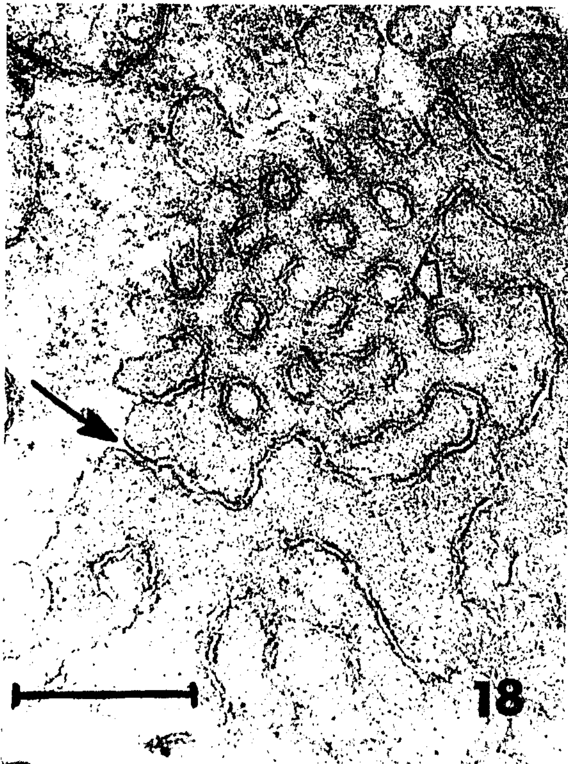
Fig. 17. A TRS displaying a geometric pattern consisting of a series of short anastomosing tubules. Arrow points to a conventional ER element with attached ribosomes. Bar= 1.0 µm. (x19,200)

Fig. 18. In this section TRS appear as circles measuring 80-115 nm in diameter corresponding in size to the inner diameter of the virus-like particles shown in figs. 20 and 21. Arrow shows point of continuity of TRS with expanded cisternae of the granular ER. Bar= 0.25 µm. (x46,000)

Fig. 19. This micrograph represents a longitudinal section through an inclusion containing TRS and illustrates the undulations and repeating patterns of individual tubular elements. There is no indication of any cross-linkages between adjacent tubules. Bar= 0.25 µm. (x63,800)



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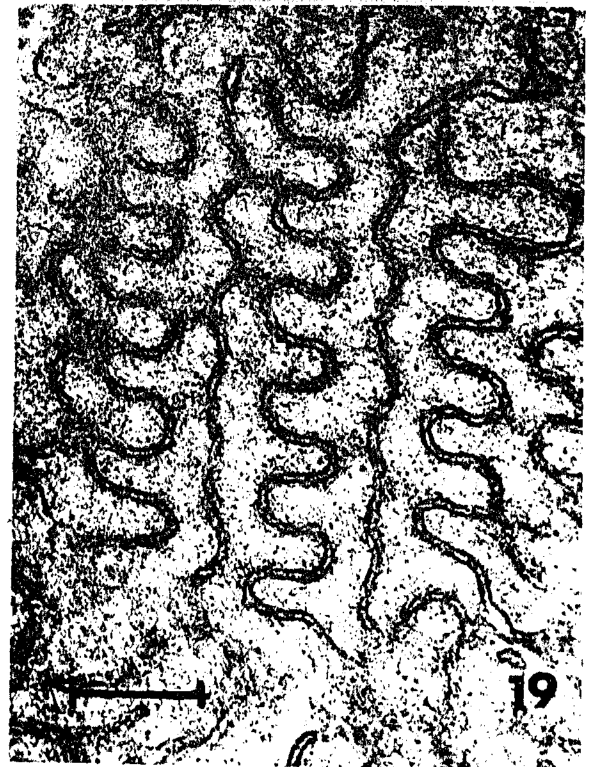

19

Fig. 20. A cell exposed to anaerobic conditions for 5 days. Most of the cellular organelles are no longer recognizable. Dispersed throughout the cell remnants are hexagonal-shaped virus-like particles 150 nm in diameter embedded in a "virogenic" stroma. The particles are either empty shells (open arrow) or filled with an electron-dense substance resembling in size and shape the nucleocapsids of the iridescent virus group. The striated cell wall (CW) remains intact and free VLPs outside the cell wall were never observed. Also embedded within the "virogenic" stroma are lipid bodies (L). Bar= 1.0 µm (x17,6000)

Fig. 21. VLPs at a higher magnification. Many of the particles have an electron-dense core and are enveloped by a triple layered shell, 20-25 nm thick, consisting of two unit membranes separated by an electron-lucent zone. Bar- 0.25 µm (x70,000)

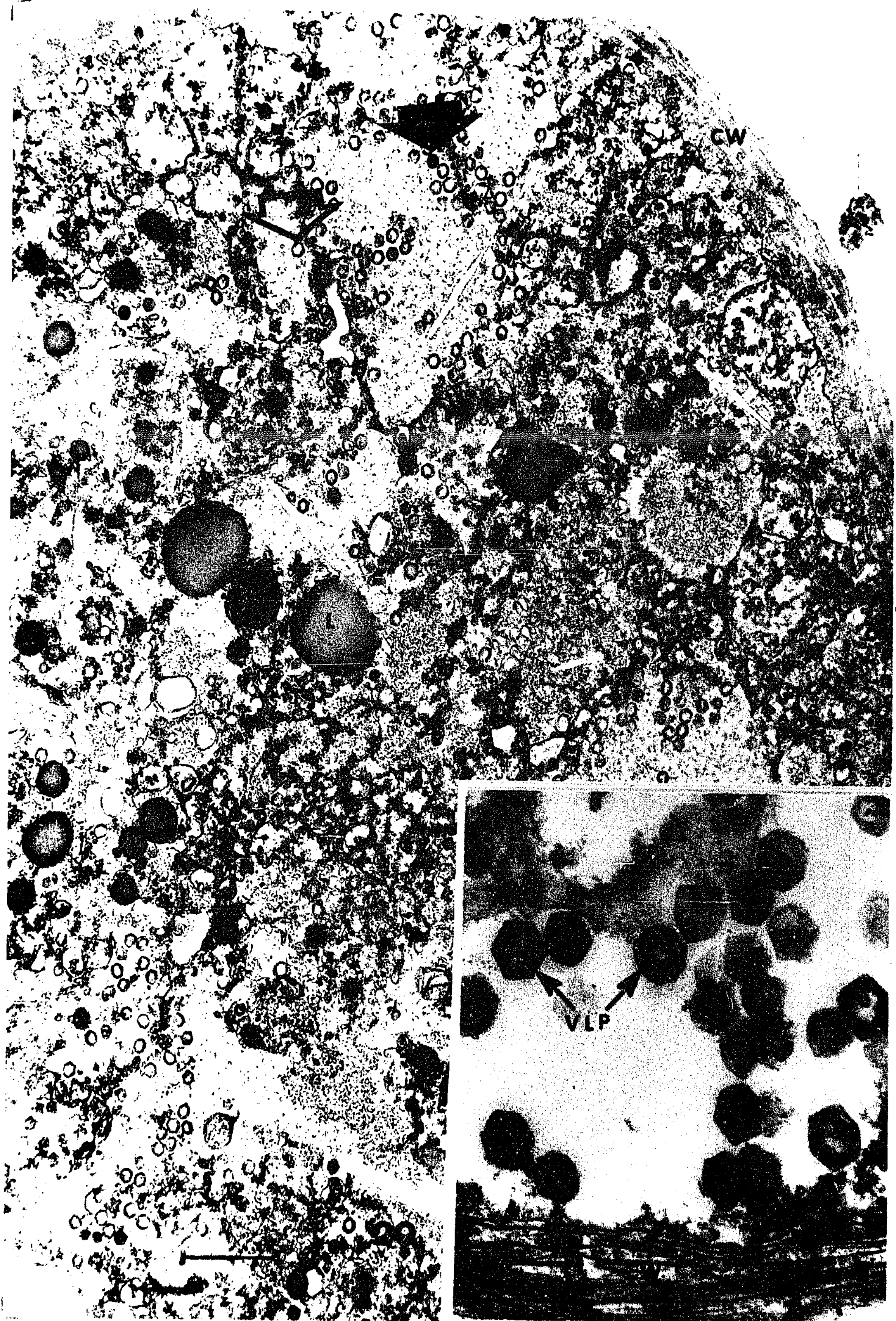
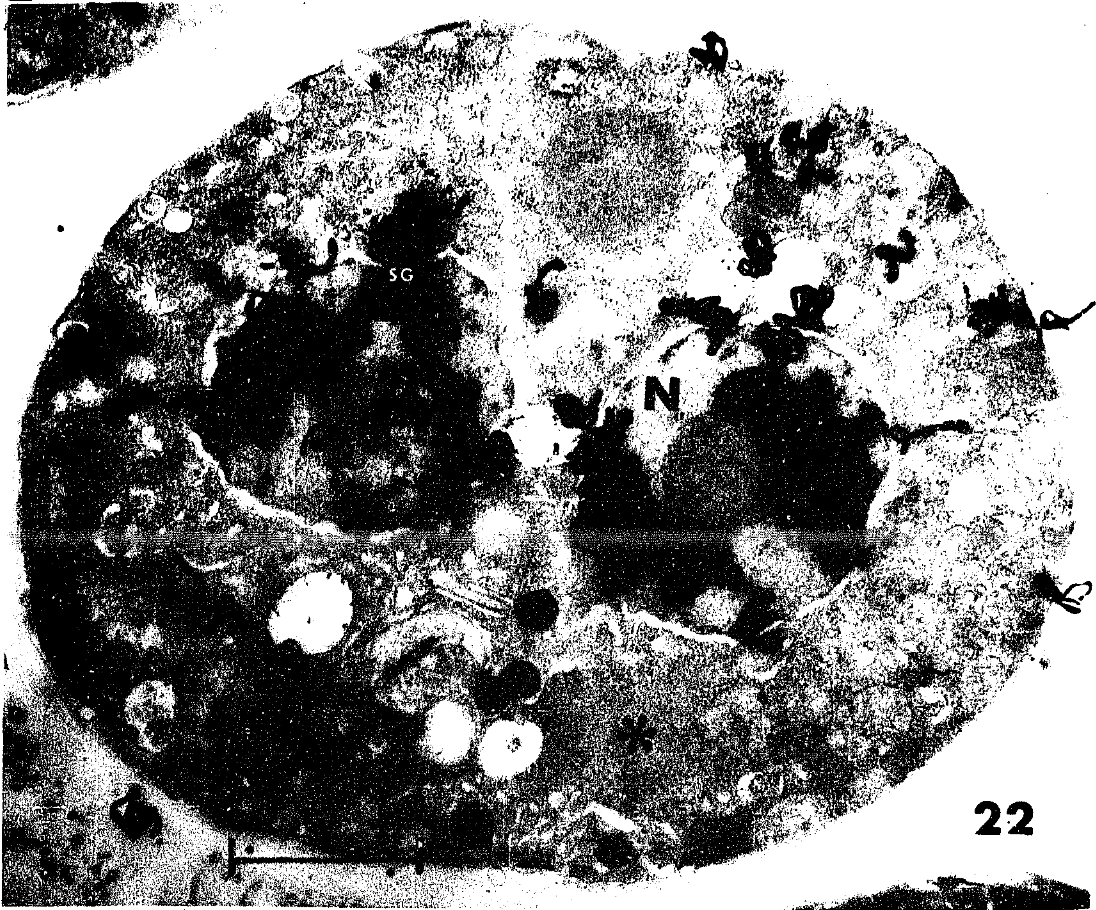

CW
L
VLP

Fig. 22. Autoradiograph of a cell pulsed for 20 hrs. under anaerobic conditions with 40 µCi/ml of tritiated thymidine and then reincubated, anaerobically, for an additional 48 hrs. Heavy incorporation of thymidine within the nuclei indicated an active synthesis of DNA under such conditions. Asterisk marks a crystalline inclusion, a characteristic observed in other cells similarly treated (see figs. 13-15). Bar= 0.5 µm. (x30,000)

Fig. 23. Autoradiograph of cell pulsed with tritiated uridine and cultivated for five days under anaerobic conditions. Numerous VLPs are present, some of which contain an electron-dense core. Labeling, although sparse, is present in areas in which normal cytoplasmic components have either lysed or degenerated. The fact that uridine incorporation is evident in the vicinity of VLPs when cells are pulsed with tritiated uridine but not with tritiated thymidine suggests that these VLPs possess an RNA core.

Fig. 24. Autoradiograph of tritiated uridine pulsed cell at higher magnification. Tracks in the nuclear emulsion are present in the vicinity of VLPs with electron-dense cores (arrows). Bar= 0.25 µm. (x68,000)


SG
N
22

23

LP
24

Fig. 25. Cells from 14 day anaerobic cultures were incubated aerobically for 48 hrs. in the presence of 40 µCi/ml of tritiated uridine. Shown here is an autoradiograph of a mature sporangium. Incorporation of label in the individual zoospores indicates that these are newly formed cells and that the effects of anaerobiosis are reversible. The location and appearance of centrioles (open arrow), Golgi bodies (solid arrows), mitochondria (M), nuclei (N), cell wall (CW), and apophysis (AP), resemble a normal mature sporangium as shown in figs. 4 and 9. Bar= 2.0 µm. (x12,000)

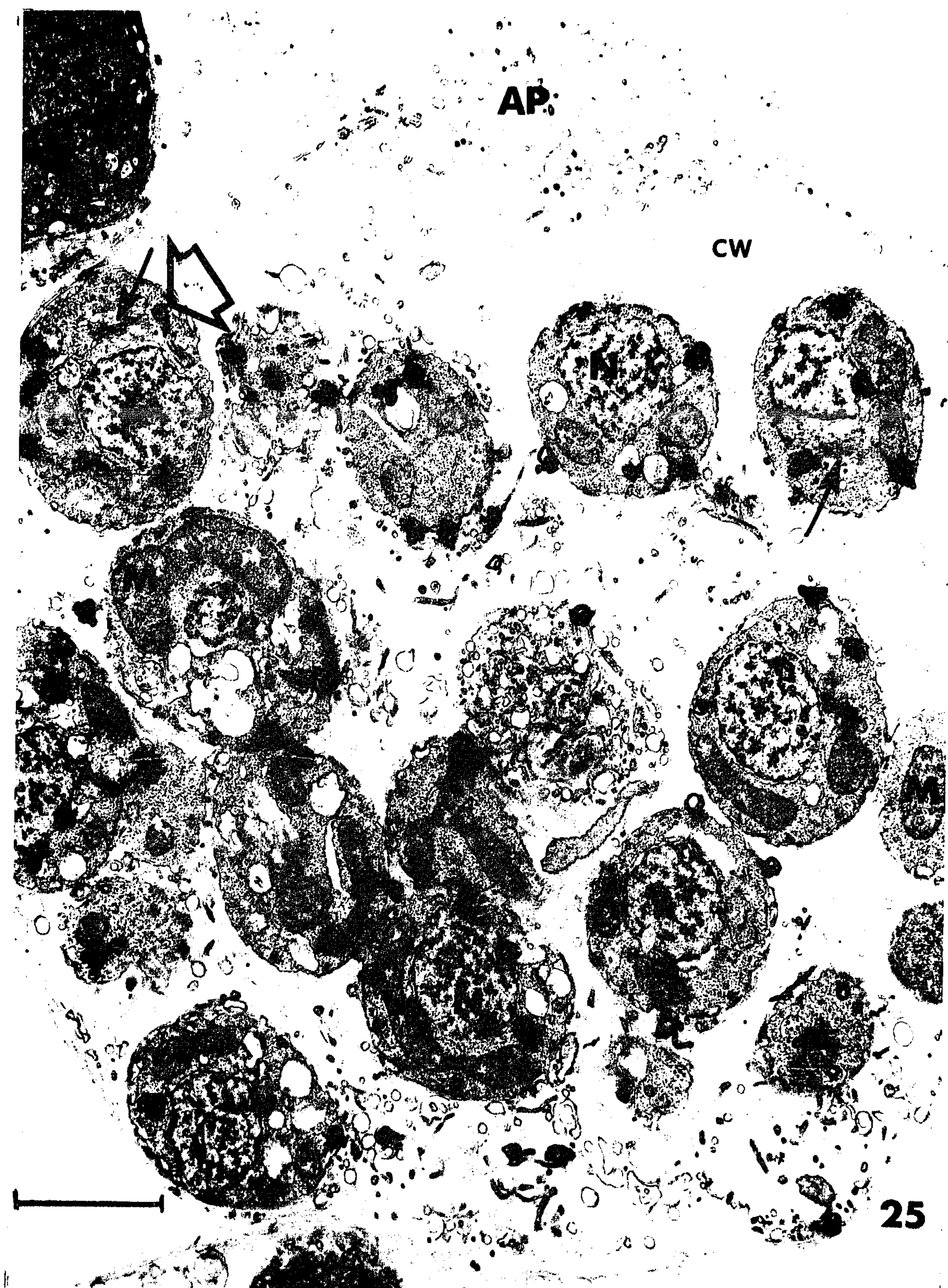
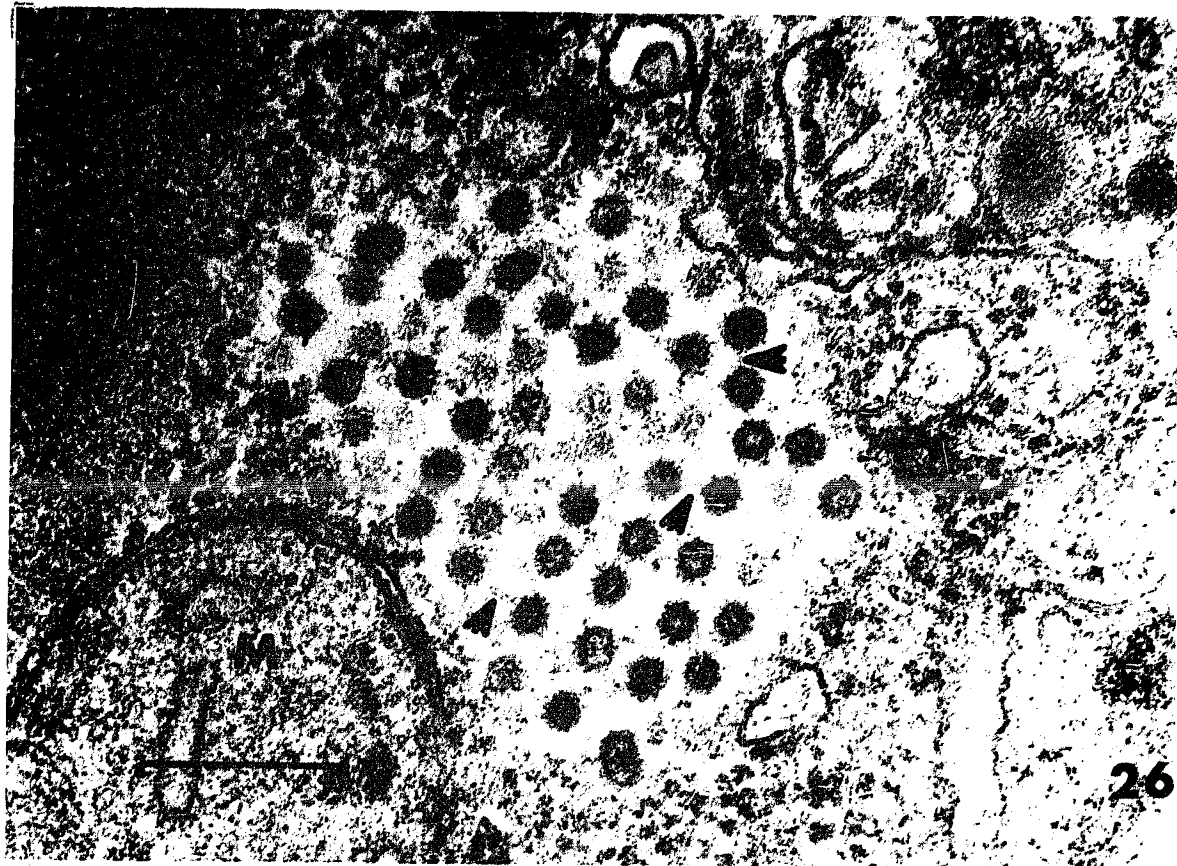
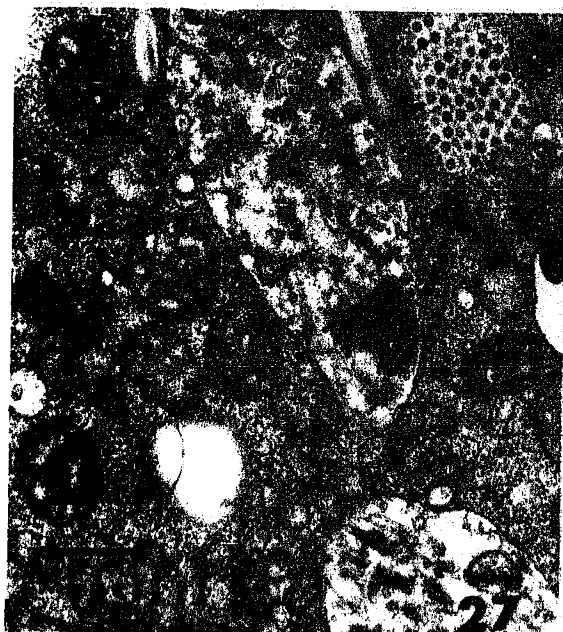

AP
CW
N
M
25

Fig. 26. A second class of viruslike particles infrequently seen in anaerobically grown cells. These particles are approximately 50 nm in diameter and bear no resemblance to the larger 150 nm particles. Fine strands projecting from the surface of the particles (arrowheads) serve to interconnect the particles. Bar=0.25 µm. (x104,000)

Fig. 27. Both 50 nm VLPs (Arrow) and tubuloreticular structures (TRS) are present in this section. Bar=0.5 µm (x22,000)

Fig. 28. Annulate lamellae (AL) were observed several times in anaerobically grown cultures. These pore-containing channels (arrows) of endoplasmic reticulum do not resemble the undulating tubules in the TRS. The mitochondria (m) appear more electron-dense than otherwise seen and may be indicative of early signs of cell degeneration. Bar=0.5 µm (x54,000)


M
26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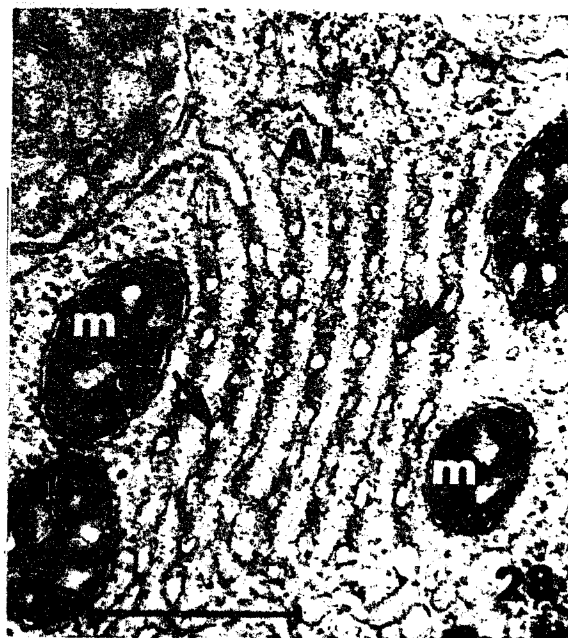

AL
m
m
28

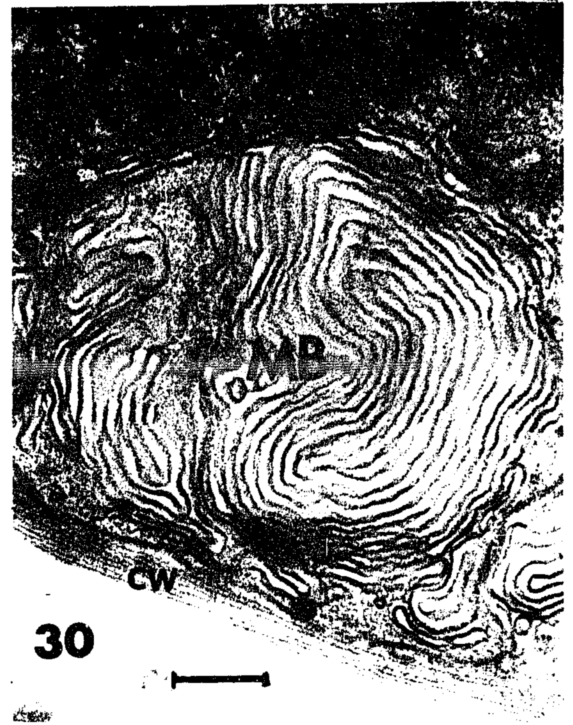
Fig. 29. Section of cell obtained from an aged culture. The presence of VLPs or other morphogenetic changes associated with anaerobic growth was not detected. Membranous configurations (arrows) are routinely observed in mitochondria (M) of senescent cells. Note the unusually large microtubules seen in both longitudinal (solid arrowheads) and cross-section (open arrow). Bar= 0.5 µm. (x26,500)

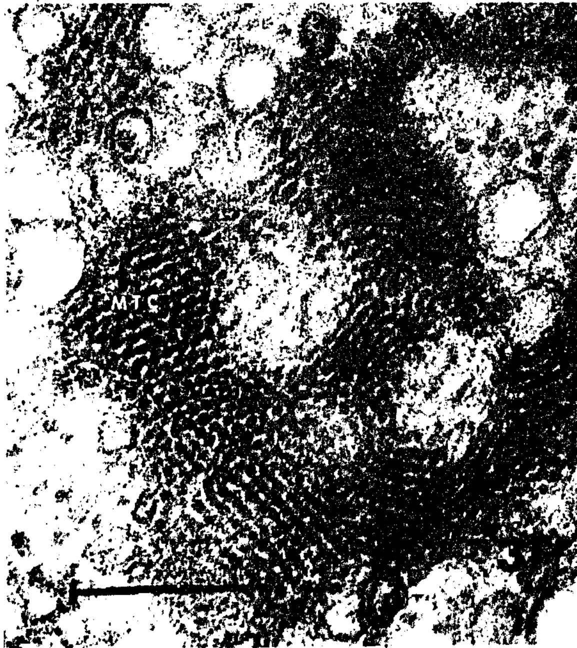
Fig. 30. Myelin bodies (MB) are occasionally observed in senescent cells. Bar= 0.25 µm. (x53,500)

Fig. 31. Microtubular complexes (MTC) consisting of electron dense tubules 16-18 nm in diameter arranged parallel to one another. These tubules or filaments are only about one-third the diameter of the microtubules shown in fig. 29. Bar= 0.25 µm. (x104,000)

Fig. 32. At times cytoplasmic contents degenerate and fragment when allowed to stand in their spent growth medium 3-4 weeks. Bar= 0.5 µm. (x24,500)

M
M
29


MP
CW
30


MTC

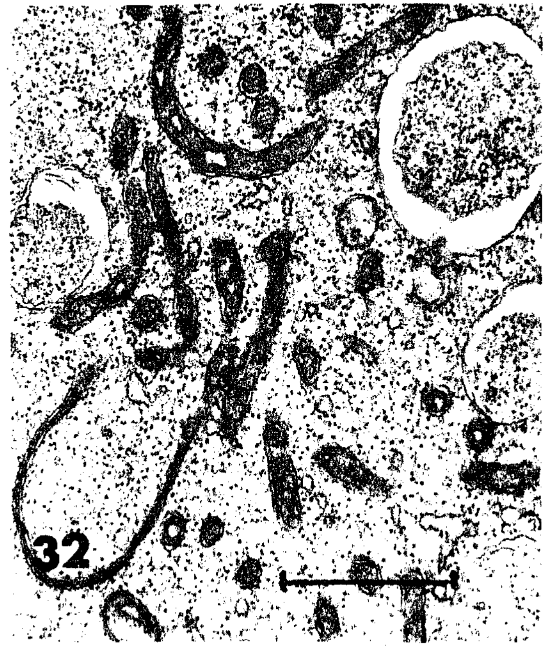
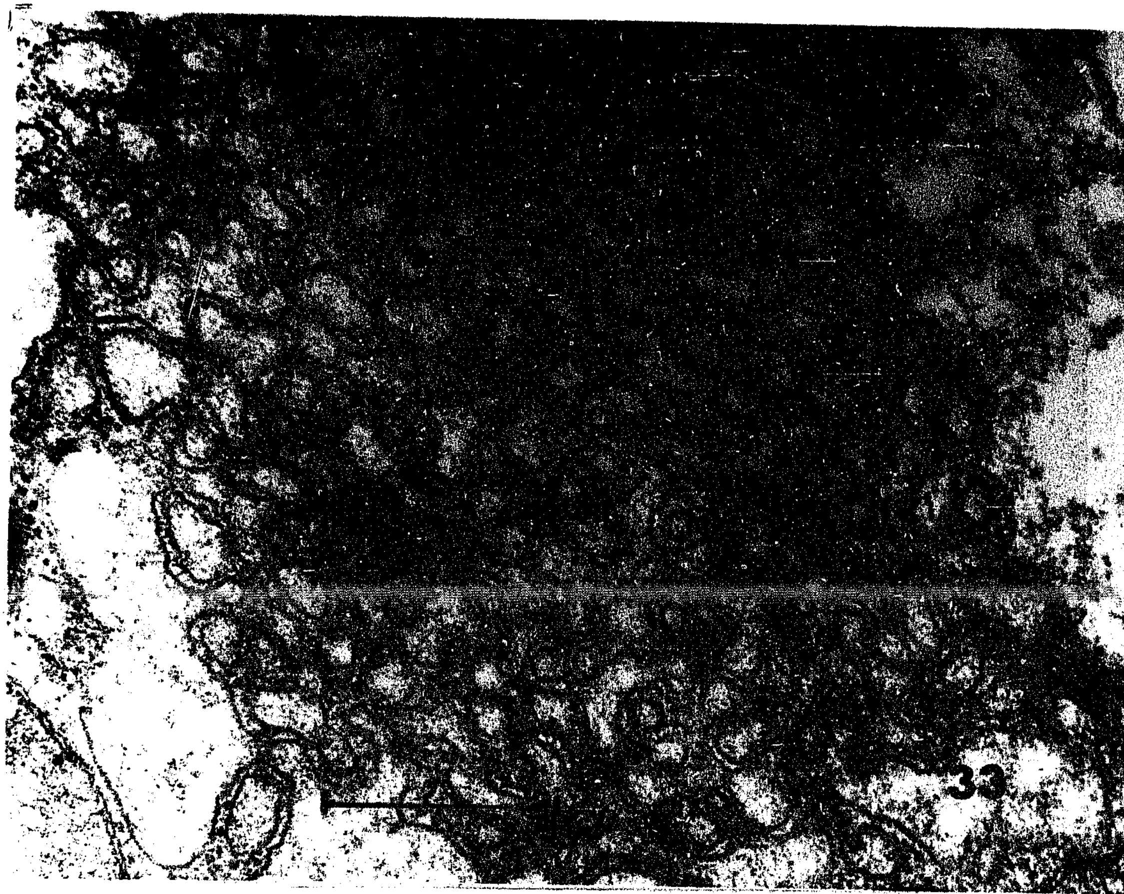

32

Fig. 33. Tubuloreticular structures (TRS) in a cell cultivated at 34°C for 4 days. These tubules appear swollen and the entire structure lacks the symmetry and compactness of the TRS observed in anaerobically cultivated cells (see figs. 16-19). Bar= 0.5 µm. (x62,000)

Fig. 34. The unusual cytoplasmic inclusions shown here are present in the majority of cells grown at elevated temperatures (34°C). Although they bear a superficial resemblance to cytolysomes, they are acid phosphatase negative (see fig. 41). The inclusions are limited by a unit membrane and contain numerous membrane fragments which are often observed to envelop electron-dense particles 35-55 nm in diameter. It is unknown whether or not the contents of these inclusions represent another class of virus-like particles. Bar= 0.25 µm. (x96,000)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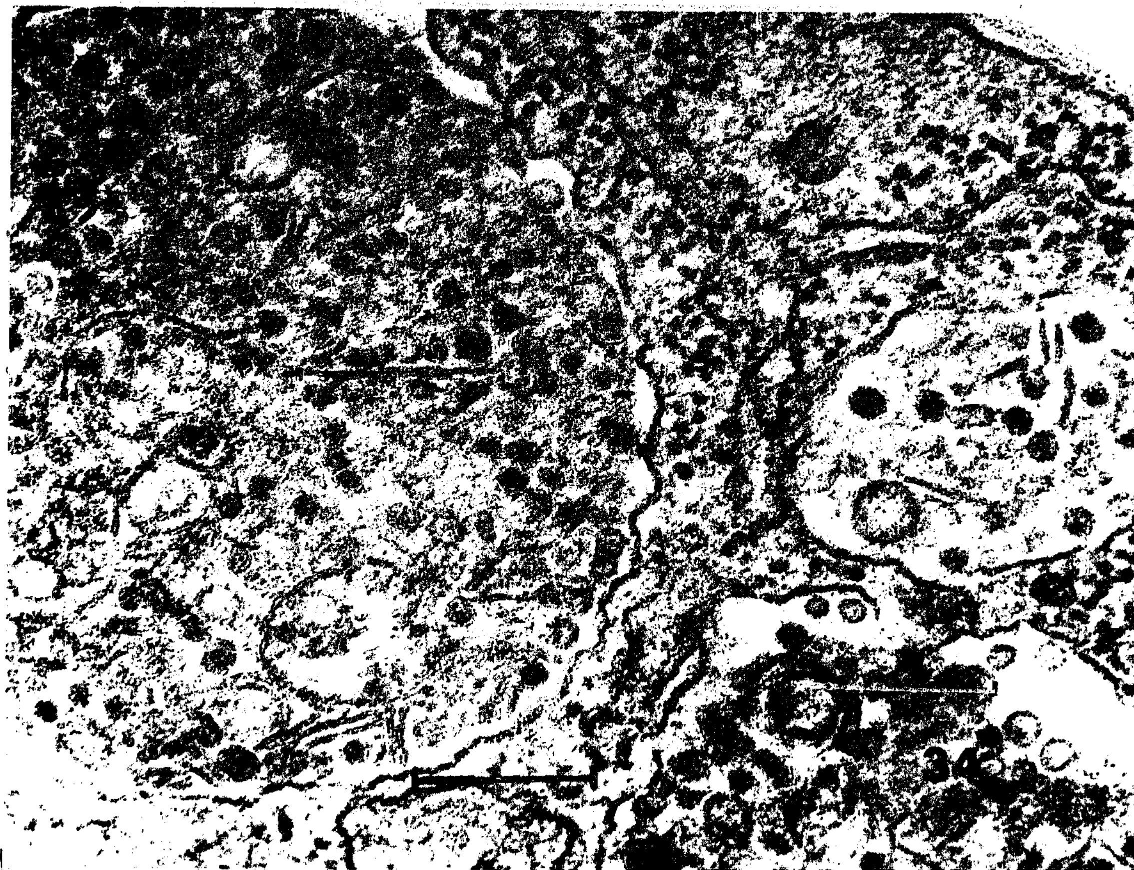
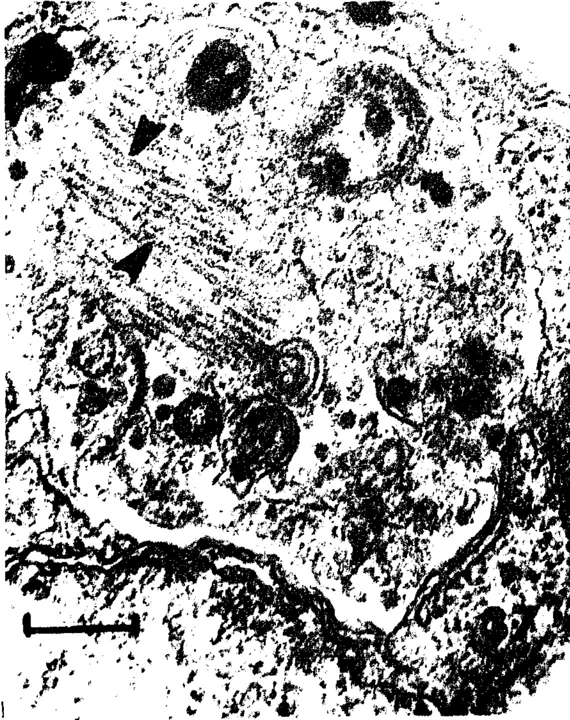

34

Fig. 35. Inclusions seen in cell grown at 34^{0}C often contain bundles of fused filaments (arrow). Bar = 0.25 µm. (x74,000)

Fig. 36. This micrograph along with fig. 35 are from a single section. An inclusion containing 50 nm VLPs is present. Bar= 0.25 µm. (x78,500)

Fig. 37. Microtubules (arrowheads) 16 nm in diameter are often observed in the cytoplasmic inclusions present in cells grown at 34^{0}C. Bar= 0.25 µm. (x60,000)

Fig. 38. Cross-section of microtubules within the high temperature induced inclusions. Arrows point to the fine bridges which serve to interconnect the microtubules. Bar= 0.1 µm. (x170,000)



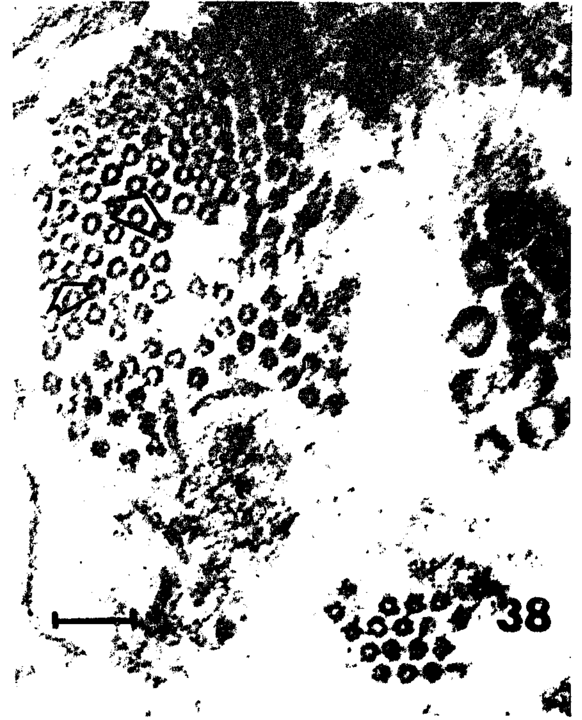

38

Fig. 39. Section of cell fixed after 4 days incubation in a candle-jar in which CO_2 levels were relatively high. When observed with a light microscope these cells had a "donut" appearance. Surrounding the vacuole are a normal complement of organelles generally observed in a developing sporangium including nuclei (N), mitochondria (M), Golgi bodies (G), and gamma-like bodies (arrowheads). Bar= 1.0 μm. (x16,000)

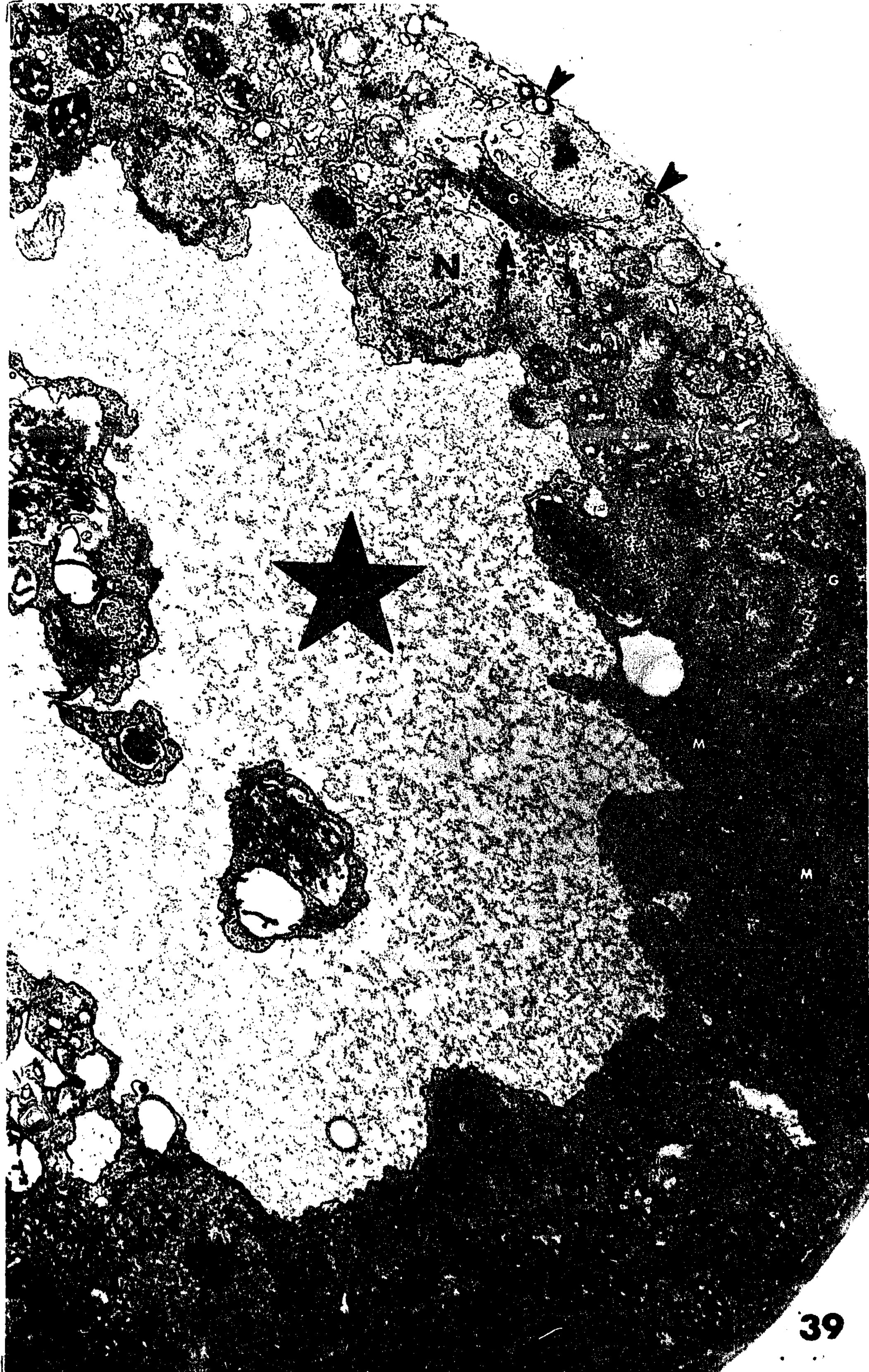

N
G
M
G
M
M
39

Fig. 40. Tubuloreticular structures (TRS) from cell incubated in medium containing 200 µg/ml of BU.dR. Bar= 1.0 µm. (x19,000)

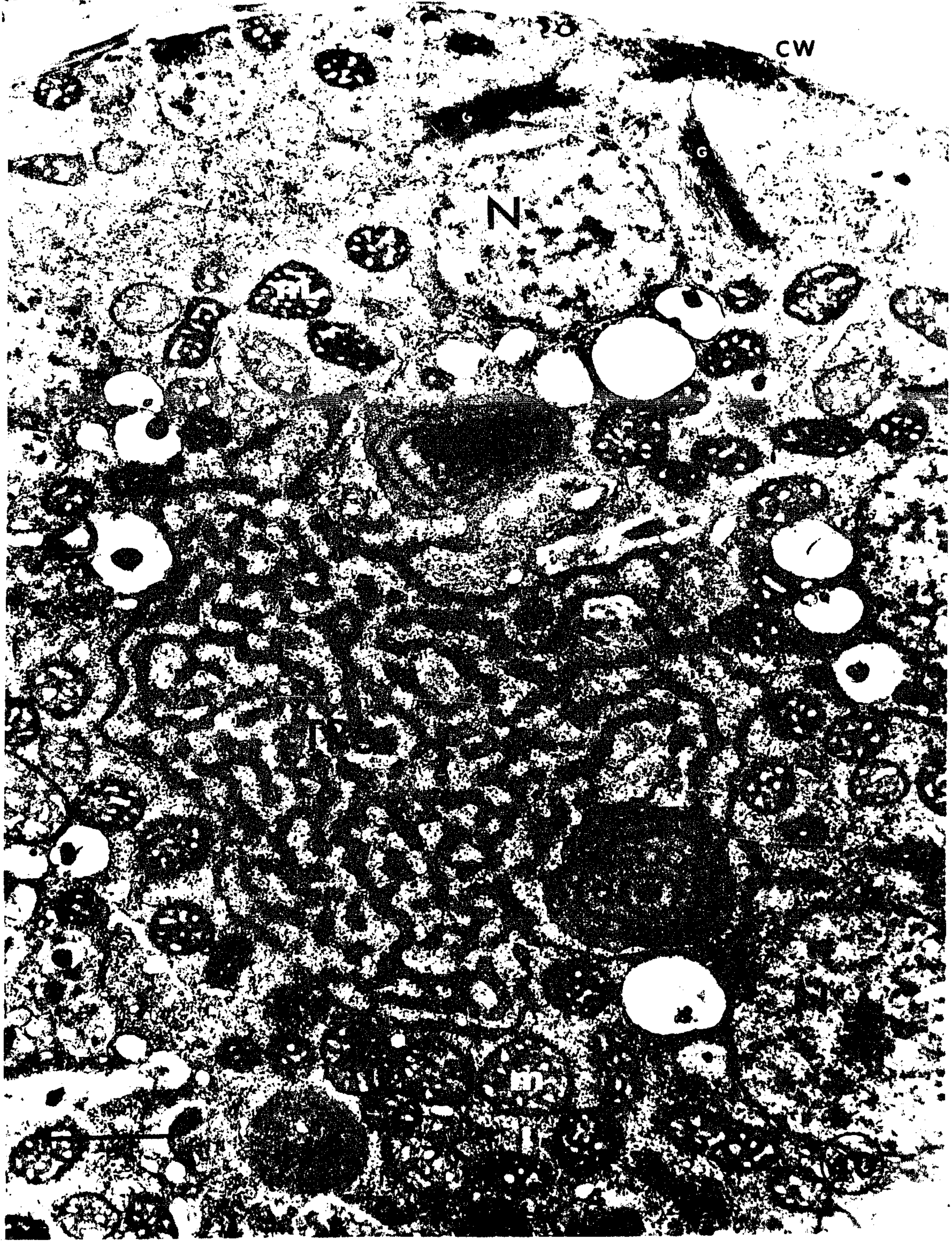
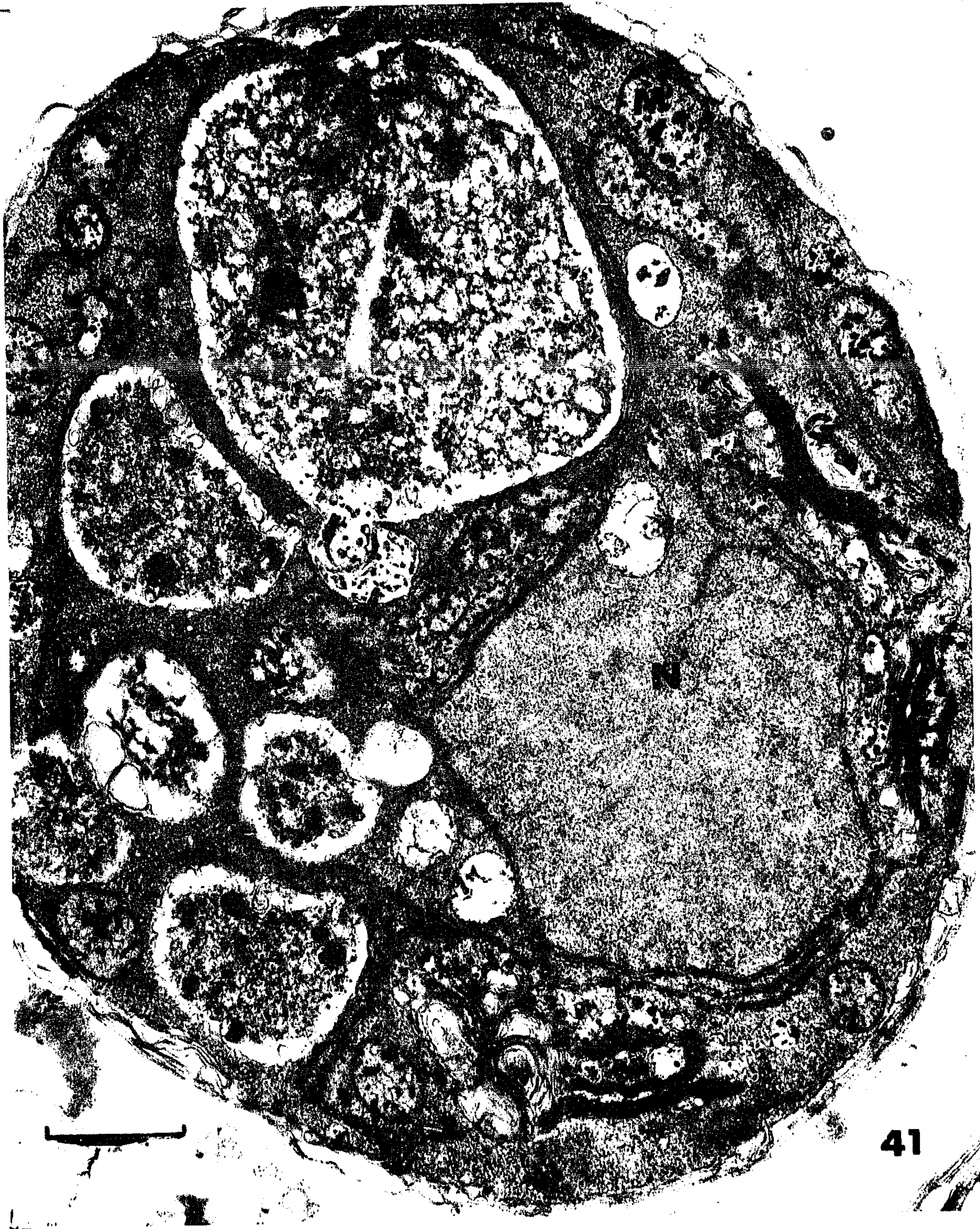
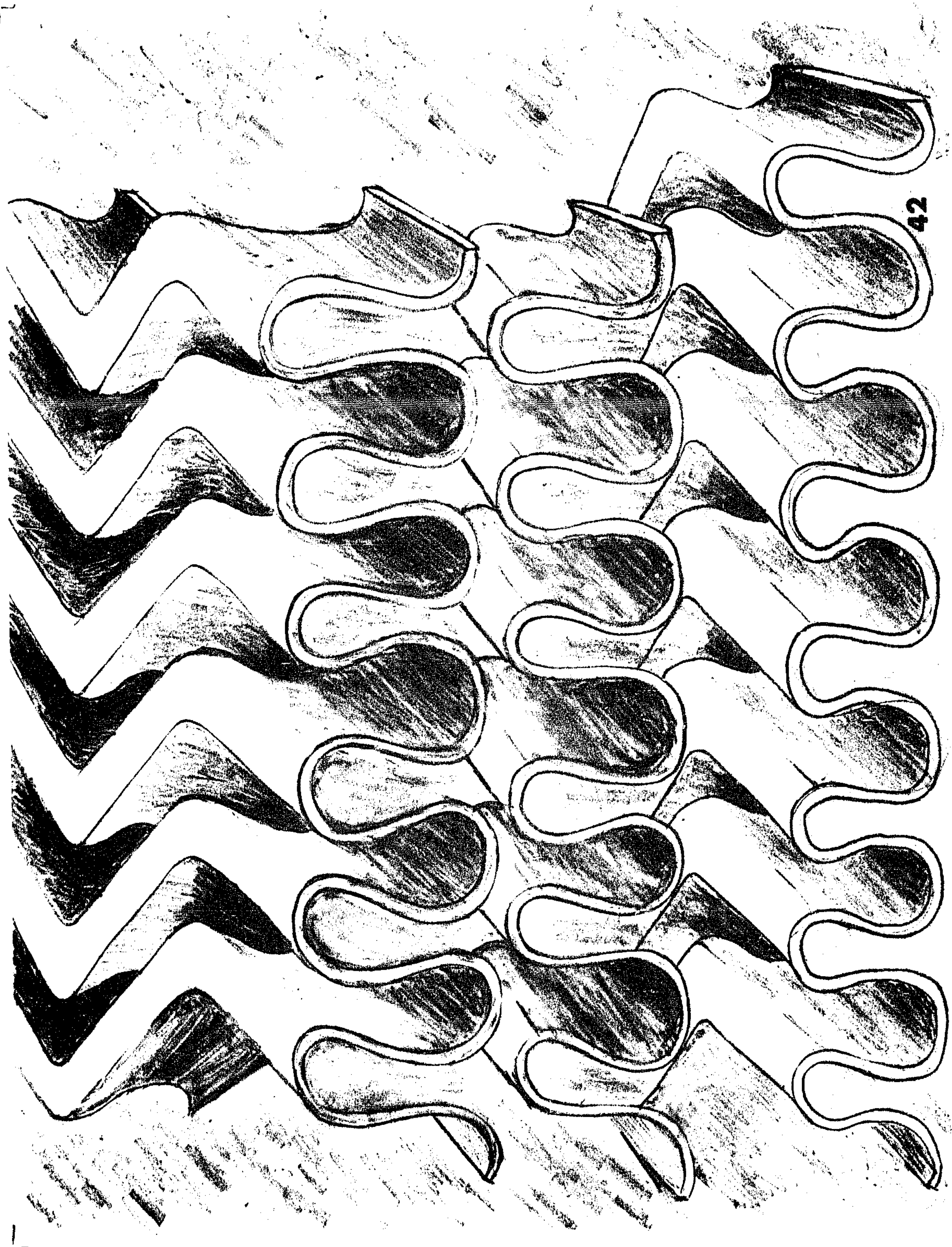

CW
N

Fig 41. Developing sporangium of cell grown at 34°C and incubated in medium to detect acid phosphatase activity. Large cytoplasmic inclusions typical of cells cultivated at elevated temperatures show few lead precipitates, while Golgi bodies (G) and mitochondria (M) appear acid phosphatase positive.

Bar = 0.5 um. x 42,200


N
41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