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REJUVENATION OF *MELOSIRA GRANULATA* (BACILLARIOPHYCEAE) RESTING CELLS FROM THE ANOXIC SEDIMENTS OF DOUGLAS LAKE, MICHIGAN. II. ELECTRON MICROSCOPY[1]

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ABSTRACT

Detailed cytological changes that accompany the rejuvenation of resting cells of Melosira granulata *were studied with the electron microscope. Dormant and viable cells that we previously classified as the condensed state generally contain definable chloroplasts, mitochondria, a nucleus and other cytoplasmic remnants. However, there appears to be a continuous cytoplasmic degradation spectrum and some cells which appear intensely colored with the light microscope have discontinuous chloroplast membranes and few other cytoplasmic remnants. Rejuvenation of viable dormant cells is initially accompanied by the accumulation of both lipids and polyphosphates. In the earliest stages of expansion, these storage products are dispersed throughout the cell. In later stages of expansion, the lipids appear to be coalesced into larger droplets which are easily identified at the light microscope level. The fully expanded stage is characterized by the normal complement of organelles and their arrangement at the periphery of the cells and central cytoplasmic bridge. These cells appear both anabolically and catabolically active as evidenced by the abundance of endoplasmic reticulum, ribosomes and secretory and lytic vesicles. Prior to cell division, both lipids and polyphosphates are reduced or absent in the cells. The ultrastructural features of the dormant, condensed state in resting cells of* M. granulata *are similar to those described for hypnospores. A rejuvenation sequence that produces cytological features common to resting state formation could provide a population of cells which could easily revert should environmental conditions become adverse.*

Key index words: diatom ultrastructure; Melosira; *rejuvenation; resting cells*

Although knowledge of the function and fate of resting spores of diatom populations has increased, little attention has been given to cells which function as a vegetative resting state. These resting cells differ

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from spores in several aspects. Spores can be more easily distinguished from vegetative cells in the light microscope by their external or frustular morphology. In some genera, resting spores resemble the parent vegetative cells. In others, spores and vegetative cells are morphologically dissimilar (Hargraves 1976, 1979). Generally spores are more heavily silicified than vegetagive cells (Hargraves and French 1983) or may possess frustular modifications such as the internal thecae described by von Stosch and Fecher (1979). Cells which are dormant but viable and have no known frustular modifications have been referred to as "physiologically resting cells" (Hargraves and French 1983). Perhaps because of the lack of differentiable morphology, the distribution and fate of resting cells have been studied very little. Malone et al. (1973) found that several species of pennate diatoms collected from red clay sediments in the North Atlantic bloomed when exposed to light at sea surface temperatures. Anderson (1975, 1976) studied the cytological and physiological characteristics of *Amphora* resting cells and found that the alga was capable of forming resting cells under adverse environmental conditions. These cells were also capable of reestablishing themselves under favorable growth conditions.

In our previous report (Sicko-Goad et al. 1986) we described with the light microscope cytological changes and ^{14}C uptake during the rejuvenation sequence of *Melosira granulata* resting cells. The following report is a detailed description of the fine structural changes that delineate this ordered rejuvenation sequence.

MATERIALS AND METHODS

Ten and 20 cm sediment samples, roughly corresponding to 15 to 30 years burial in the sediment, were collected and processed as previously described (Sicko-Goad et al. 1986). Cell types were individually distinguished by light microscopy and selected for sectioning. All photographs were taken of sediment samples that had been resuspended in light for less than 104 h. Thin sections of the epoxy-embedded material were cut with a diamond knife, collected on cleaned formvar coated 200 mesh copper grids and stained with aqueous uranyl acetate (Watson 1958). Sections were examined in a JEOL JEM 100B electron microscope operating at 80 KV. Permanent epoxy mounts were also made from the osmicated samples as reference slides.

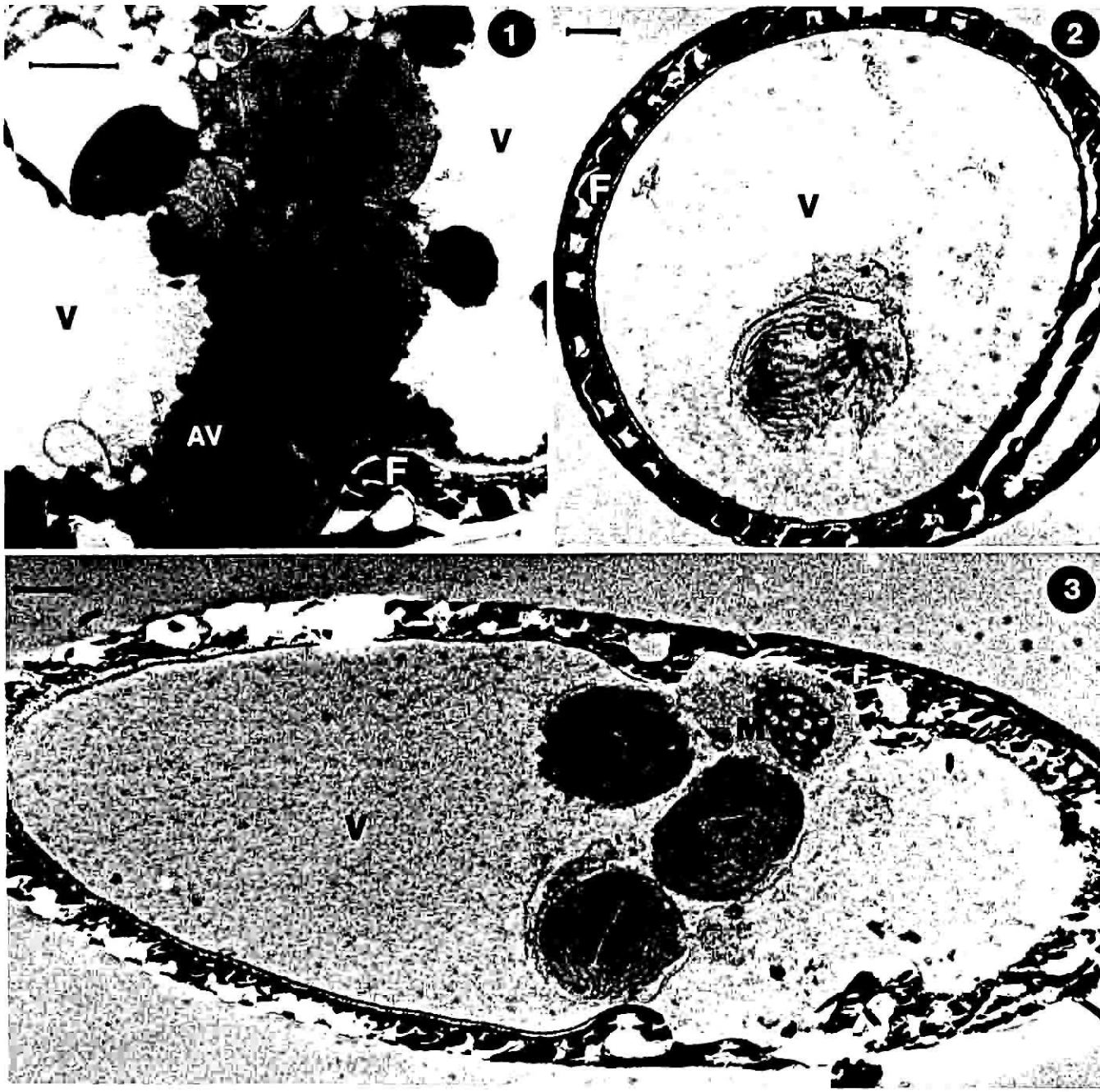
RESULTS

The ultrastructural differences between non-viable cells and cells which are in the condensed, dormant state appear to be minimal and can be best described as a continuous spectrum of degeneration. Our previous observations supported the assumption that the cell type containing dark brown cytoplasmic masses near the sulcus was the dormant condition. Individual cells in this state were identified in epoxy blocks sectioned and examined in the TEM. They were found to contain structures generally recognizable as chloroplasts, mitochondria, and other cytoplasmic remnants (Fig. 1). However, the cytoplasm is intensely electron dense and few thylakoids or mitochondrial cristae are readily discernible. Examination of cells that were not as intensely stained and which we classified as containing non-viable cytoplasmic fragments revealed that these cells also contained similar organelles but appeared to be in a more advanced state of degradation (Figs. 2, 3). The non-viable cell population consisted of cells with a range of morphologies from the advanced degraded condition consisting for the most part of barely recognizable organelles (Fig. 2), to cells that resembled the condensed state. However, the cytoplasm is less electron-dense, fewer organelles are present, and the chloroplast membrane is not intact (Fig. 3).

Upon resuspension from the sediment and exposure to light, resting cells (condensed state) are quickly transformed to a more metabolically active and recognizable form that we have described as the partially expanded state (Figs. 4, 5). This state is characterized by the proliferation of cytoplasm and organelles in the sulcus region and along the cell periphery. The cytoplasm is less dense and occupies a greater portion of this region. The nucleus and nucleolus become more prominent (Fig. 4) and there is a proliferation of Golgi and its associated vesicles (Fig. 5). Chloroplasts and mitochondria increase in number and have recognizable thylakoids and cristae. Concomitant with the proliferation of cytoplasmic components there is also an increase in abundance of storage products, particularly polyphosphate and lipid. These storage products are not easily identified with the light microscope during the early expansion stage because of their small size (Fig. 5). The greatest number of cytological changes occur with hours of exposure to light.

The partially expanded state is a transient condition with usually no more than 2% of rejuvenating cells in this category (Sicko-Goad et al. 1986). These cells quickly differentiate into a cell type that is indistinguishable from vegetative populations. The single most noticeable change that occurs in the cells as cytoplasmic expansion continues is the accumulation of polyphosphates and lipids. These storage products, in many instances, occupy most of the cell volume (Fig. 6).

The fully expanded stage is characterized by the normal complement of organelles and arrangement at the periphery of the cell and central cytoplasmic bridge (Fig. 7). Endoplasmic reticulum becomes fairly abundant (Fig. 6). Vesiculation increases as cells approach the division state (Fig. 6). Several types of vesicles are common. One class of vesicles appears to be derived from the vacuole membrane (Fig. 8). These fully formed vesicles are found both in the cytoplasm and central vacuole. Frequently, autophagic-like vacuoles (Fig. 10) and inclusions resembling multivesicular-like bodies (Fig. 11) are found in fully mature cells. A third common vesicle type



NOTE: Abbreviations used in figures: AV = autophagic-like vacuole; C = chloroplast; ER = endoplasmic reticulum; F = fistule; FV = fibrillar vesicles; G = Golgi; L = lipid; M = mitochondrion; MVB = multivesicular-like body; N = nucleus; P = polyphosphate; V = vacuole.

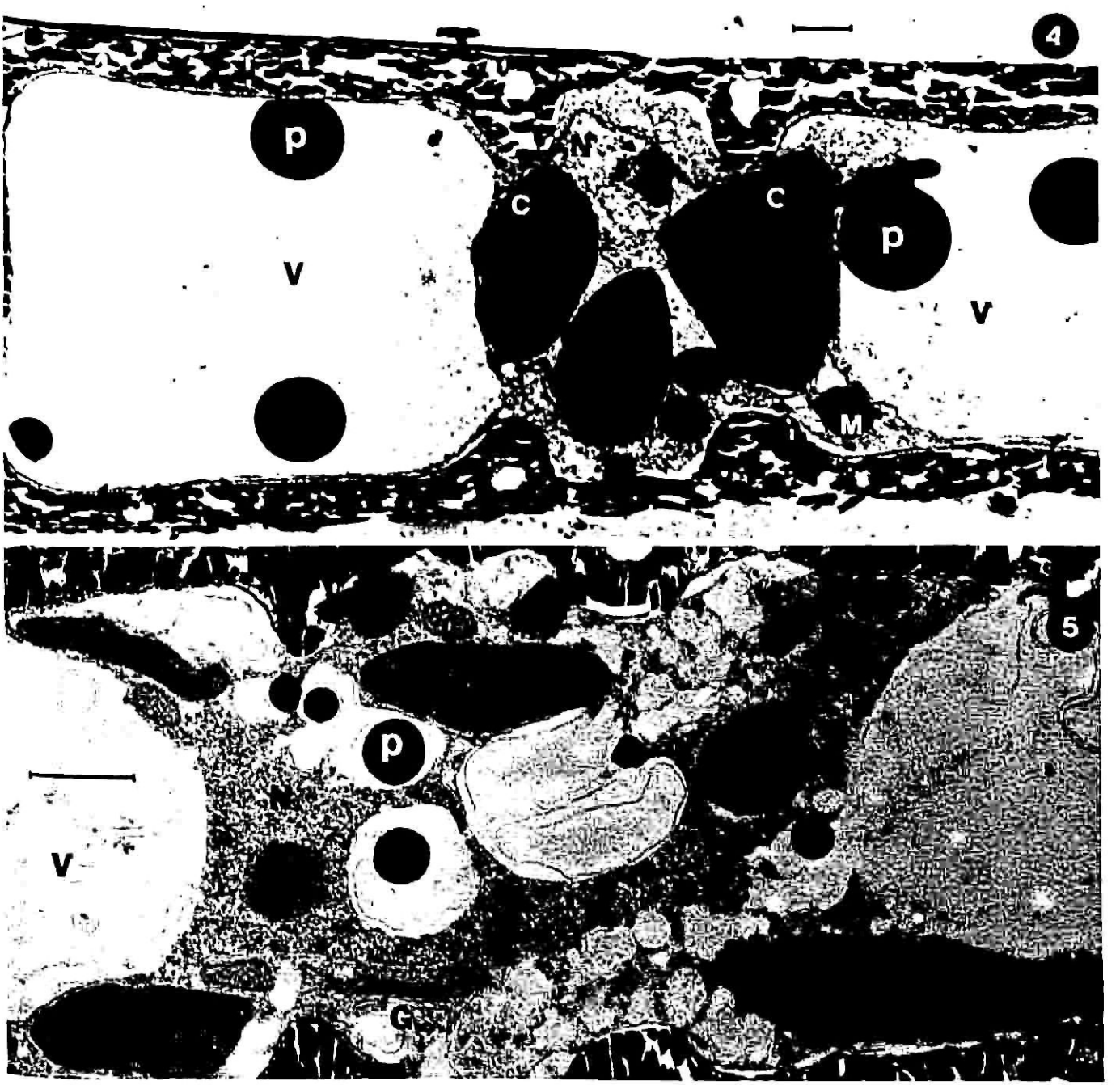
All magnification bars represent 1 μm with the exception of Figure 9 which is a 0.5 μm bar.

FIGS. 1–3. Dormant and non-viable cells. FIG. 1. Section through a cell identified to have an intensely colored cytoplasmic mass in the sulcus region of *Meloura* containing chloroplasts, mitochondria, some residual storage products, and an autophagic-like vacuole. FIG. 2. Cell in the advanced degraded (non-viable) category. Some chloroplast and cytoplasmic remains are evident. FIG. 3. Section through a cell believed to be non-viable. These cells look intensely colored in the light microscope, but lack extensive cytoplasm and have few organelles present.

is usually found in cells just prior to and during division. This vesicle is usually found near the Golgi and its contents are characterized by a mottled electron opaque appearance and in some instances appears fibrillar (Figs. 9, 7). Prior to cell division the amounts of storage products are greatly reduced. Degraded polyphosphate bodies are found frequently in dividing cells (Fig. 12).

DISCUSSION

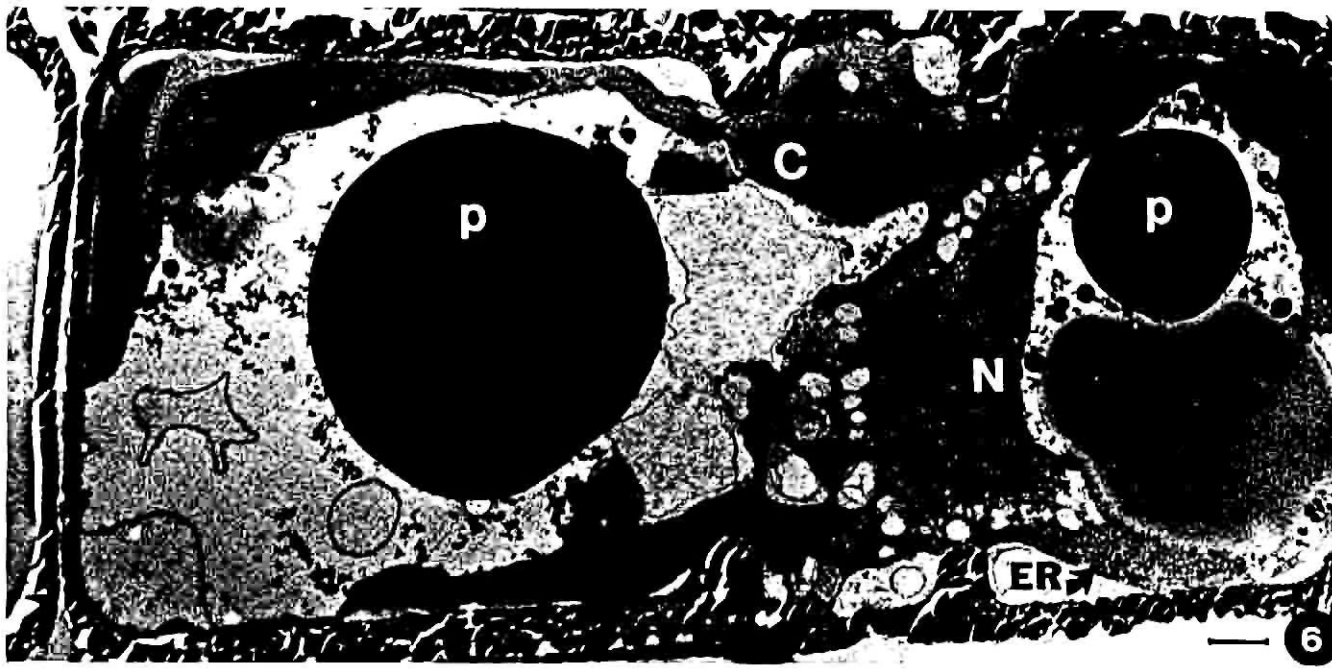
The most intriguing feature of resting cells is the apparent fine line that distingushes cytologically (and

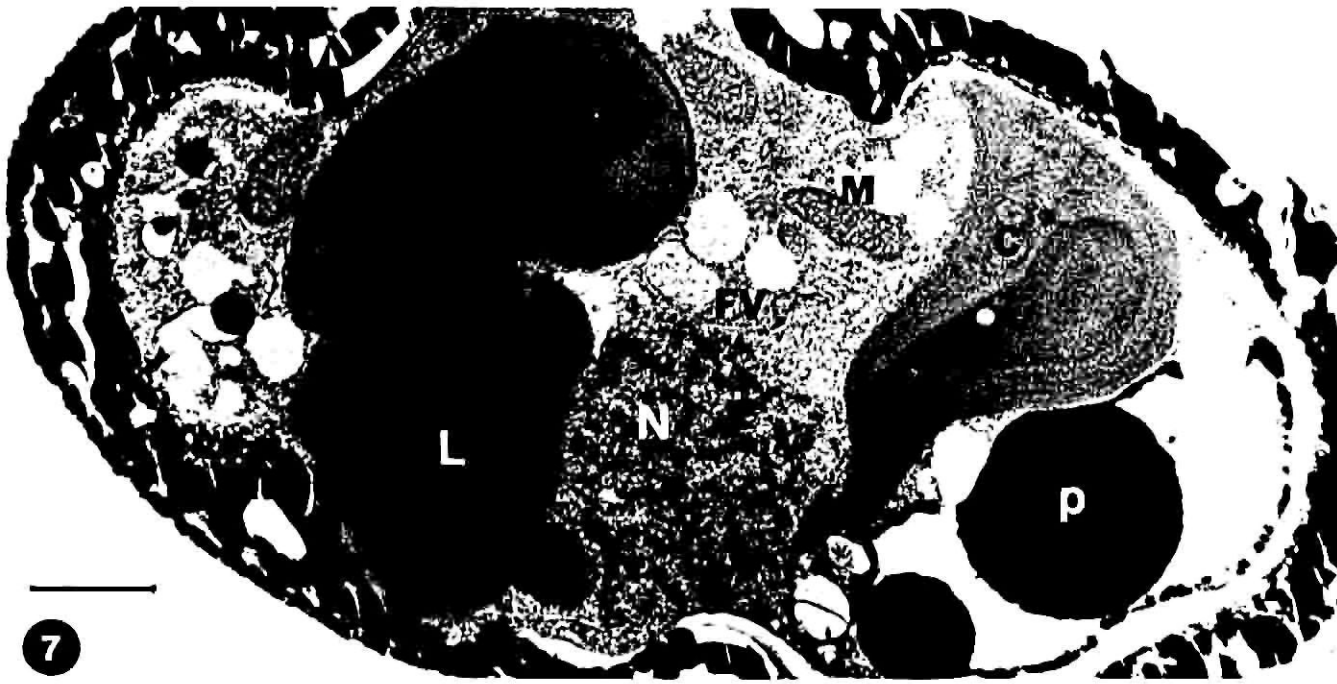


FIGS. 4, 5. Early expansion stage. Numerous small polyphosphate bodies are present. The cytoplasmic mass is still located in the sulcus region, although the chloroplasts are shown to be more elongated (Fig. 5). The nucleus and nucleolus are well defined at this early stage.

most probably physiologically) cells that are dormant and viable and those that are non-viable (i.e., unable to rejuvenate). In our previous report (Sicko-Goad et al. 1986), we demonstrated a consistent, but small, percentage of cells that remain in the condensed state during the rejuvenation experiments and are easily recognizable at the light microscope level. Ultrastructural observations presented in this report suggest that there is a continuous staging gradient, and although some cells may appear viable by cursory light microscope examination, they are most likely beyond their capability to resume active vegetative growth.

In terms of survival strategies a staging gradient could be quite advantageous to species which form resting cells rather than spores. Resting spore formation usually requires one or more cell divisions to yield the final dormant stage, whereas species that form resting cells can go through the maximum number of cell divisions to yield greater numbers of potentially dormant cells (Hargraves and French 1983). Consequently, rejuvenation "efficiency" (i.e.

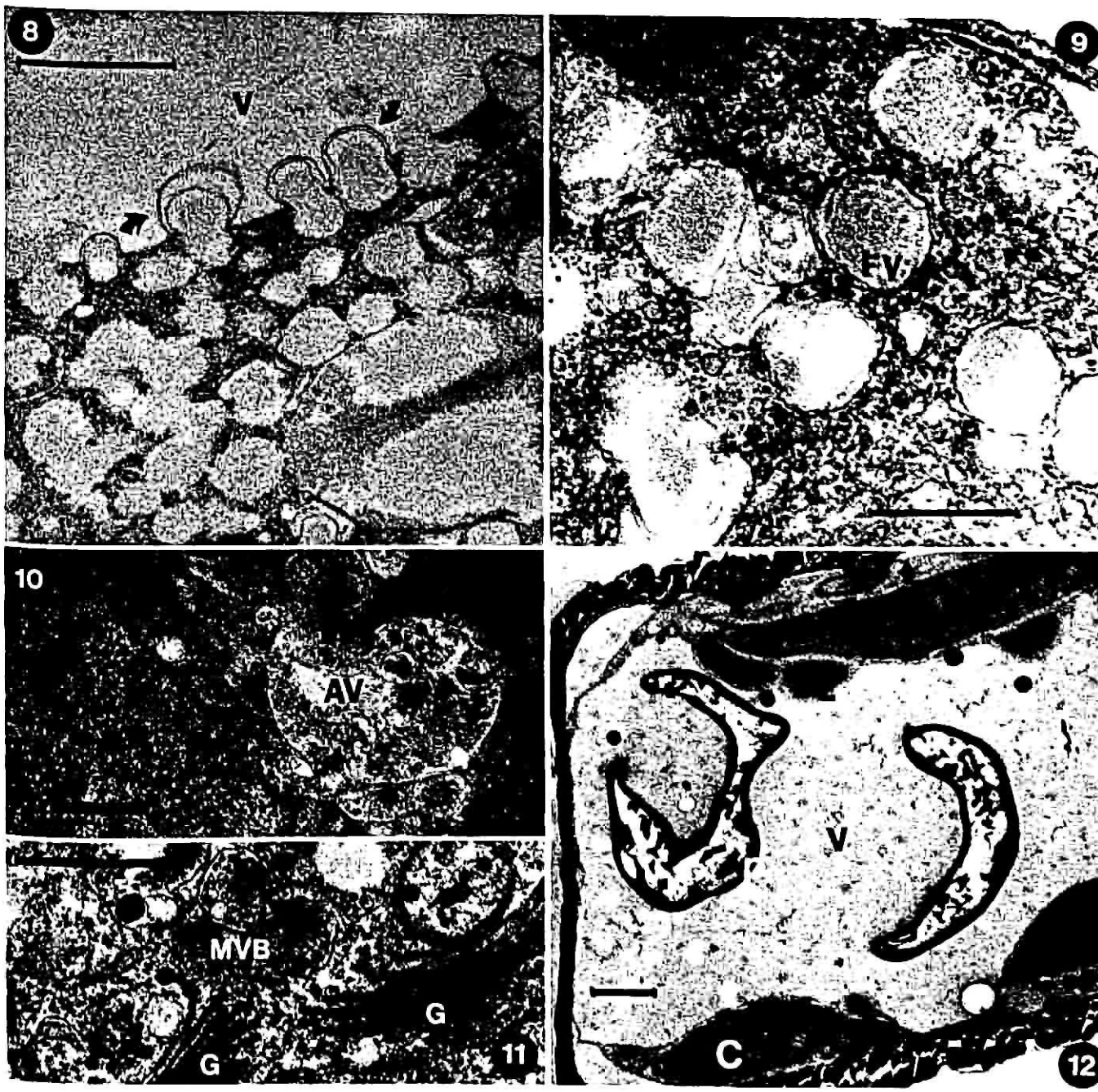




FIGS. 6, 7. Partially to fully expanded stages. These stages are characterized by further cytoplasmic proliferation and organelle placement at the cell periphery. Small polyphosphate and lipid granules are replaced by larger variety which occupys a significant portion of the cell volume. Numerous vesicles are located near the Golgi nuclear area.

the number of cells which can rejuvenate in a population) is less critical. Similarly, staging is also less critical, assuming that in a natural setting dormant cells may be the seed material for sudden and perhaps unexpected growth of certain populations. Resting cell populations continuously could be replenished to replace older populations in which rejuvenation efficiency is reduced. In view of their longevity of up to perhaps 20–30 years (Sicko-Goad et al. 1986), even if no more resting cells were produced, it is likely that a sediment mixing or perturbation phenomenon would occur that would bring resting cells back into favorable growth conditions.

The sudden onset of storage product accumulation initially was unexpected because increases are usually associated with onset of adverse environmental parameters such as nutrient or toxicant stress (Davis 1972, Sicko-Goad 1982), or senescence and/



FIGS. 8–12. Vesicles and storage products. FIG. 8. Higher magnification photo demonstrating vesicles that appear to be associated with the vacuole membrane. FIG. 9. "Fibrillar" vesicles found in the central cytoplasmic bridge. FIGS. 10, 11. Autophagic-like vacuole and multivesicular-like body found in completely differentiated cells. FIG. 12. Portion of a dividing cell showing reduced amounts of lipid and degrading polyphosphate bodies.

or spore formation (McLean 1968, Schlichting 1974, Anderson 1975, von Stosch and Fecher 1979, French and Hargraves 1980). However, Bisalputra and Antia (1980) demonstrated that reillumination of dark cultures of *Porphyridium* resulted in deposition of significant numbers of starch grains within three hours and they interpret this as indicative of early restoration of photosynthetic capacity in cells that survive prolonged darkness. The ^{14}C uptake patterns we reported for *Melosira granulata* (Sicko-Goad et al. 1986) also suggest that photosynthetic capacity is restored early and is very high during rejuvenation of *M. granulata* resting cells. The increase in storage product accumulation occurs at a point when the cells are not completely differentiated but have well organized chloroplasts. It is conceivable that cells could use the storage products for their rapid burst of growth and resumption of the vegetative

growth cycle. The formation of storage products during the initial stages of resting cell rejuvenation could also provide another survival strategy for *Melosira*. If the dormancy-rejuvenation sequence is indeed reversible, then formation of storage products at this early stage would provide additional seed populations for the formation of resting cells if environmental conditions should prove unfavorable for resumption of vegetative growth.

Another unusual cytological feature of rejuvenating cells is the great degree of vesiculation found in the expansion stage including the pre-division stage. Crawford (1973) described four types of small vesicles in *Melosira varians*. Three types were Golgi derived and were described as smooth, coated, and secretion vesicles. The fourth vesicle type, dense vesicles, were described as products of the nuclear envelope. Crawford frequently found what he termed vesicle complexes which we believe are similar to the structures we describe as multivesicular-like bodies (MVB). The presence of MVBs as well as residual bodies or autophagic-like vacuoles in *Melosira* is not surprising in view of the tremendous metabolic activity associated with rejuvenation. The presence of both secretory and lytic vesicles merely indicates that both anabolic and catabolic activities are occurring simultaneously and need not be separated either spatially or temporally (Matile 1975). Indeed lytic activity is not just associated with formation of the resting stage and compaction and reorganization of cytoplasmic components.

The vesicles that appear prior to division and are fibrillar in nature have also been described in *Caloneis* (Walker et al. 1979, Edgar 1980) and several other diatoms (Drum and Pankratz 1964, Stoermer et al. 1965, Taylor 1972). Although the fibrillar bundles appear in close association with the nuclear envelope in *Caloneis* (Walker et al. 1979), no direct evidence for their origination could be determined in *Melosira*, where the fibrous vesicles are usually located near the nucleus and Golgi.

The cytological transition between the dormant state and vegetative growth appears to be a continuous reversible gradient of both anabolic and catabolic activities. Features which are generally regarded as being indicative of the beginnings of senescence or dormancy are also associated with the rejuvenation process and general features of this reversible transition may be summarized as follows: (1) There is an accumulation of storage products prior to spore formation in many diatoms and during the rejuvenation sequence of resting cells before full resumption of vegetative growth; (2) There is extensive lytic activity prior to spore formation that most likely involves cytological compaction and conservation of essential organelles for entry into the dormant state. Likewise, during the rejuvenation sequence there is evidence of lytic activity which probably suggests remobilization of essential metabolites for growth. The presence of lytic activity need not suggest a detrimental catabolic state.

Diatom survival strategies include the formation of resting states in both the form of hypnospores or resting cells whose frustule structure has not been modified. There are cytological similarities between the compact brown mass in both spores and resting cells. Additional features of the germination or rejuvenation of resting state cells that insure the persistence of diatom populations are both non-synchronous "germination" and formation of storage products and lytic vesicles. Non-synchronous germination insures that not all spores will germinate under adverse environmental conditions. A rejuvenation sequence that produces cytological features which are common to resting cell formation could provide a population of cells that could easily revert to a resting state.

Although resting cells have not been studied extensively, we have observed other populations of diatoms in the sediments of Douglas Lake and Lake Michigan which appear to have the capacity to rejuvenate from a resting cell. However, these diatoms do not appear to survive prolonged burial like *Melosira*. It is likely that resting cells are common in many genera but have not been recognized.

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IRRADIANCE, DAYLENGTH AND TEMPERATURE EFFECTS ON ZOOSPOROGENESIS IN *COLEOCHAETE SCUTATA* (CHAROPHYCEAE)[1]

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ABSTRACT

Using a factorial design, we investigated the effects of 150 different combinations of irradiance, daylength and temperature on zoosporogenesis in Coleochaete scutata. *Analysis of variance (ANOVA) revealed that irradiance and daylength did not significantly influence the response, but that temperature was highly significant. Exposure of thalli to 20°C for one to several days is sufficient to induce zoospore production in* C. scutata *and several other northern temperature species of* Coleochaete. *Results of the factorial experiment correlate well with field observations on the seasonal occurrence of asexual reproduction in several Coleochaete species. A technique based on results of this factorial study is described for using zoospores to obtain morphologically normal, unialgal cultures of* Coleochaete *sp. It was concluded that the factorial approach to investigation of environmental control of zoosporogenesis can be a powerful tool for understanding natural algal population dynamics, as well as controlling growth and reproduction of algae in the laboratory.*

Key index words: Coleochaete; *irradiance; photoperiod; reproduction; temperature; zoosporogenesis*

The green alga *Coleochaete*, a freshwater littoral epiphyte, is of considerable phylogenetic interest due to its presumed relationship to the ancestry of land plants (Graham 1984). In order to study this genus in detail it is desirable to have cultures of as many species as possible. Eleven species of *Coleochaete* have been described (Printz 1964), but unialgal cultures of only two species (*C. scutata* and *C. nitellarum*) are generally available (ex. UTEX LB 610 and LB 1261, Starr 1978), and these are morphologically abnormal. Isolation of *Coleochaete* species is unusually difficult because field-collected thalli typically harbor a variety of tightly-attached epiphytes which can grow faster than *Coleochaete* under culture conditions, and overrun new isolates. The best technique for obtaining *Coleochaete* cultures thus appears to be zoospore isolation, but the conditions which promote zoosporogenesis in *Coleochaete* have not been determined previously. A factorial experimental approach has been successfully used to elucidate environmental control of zoosporogenesis in *Cladophora glomerata* (Hoffmann and Graham 1984) and *Ulothrix zonata* (Graham et al. 1985b). Here we report the results of a similar experiment designed to determine what effects the environmental factors of temperature, irradiance and photoperiod and their combinations might have on zoospore production in *Coleochaete scutata*. This information has been used to develop a technique for obtaining morphologically normal, unialgal isolates of various *Coleochaete* species.

MATERIALS AND METHODS

Collections of natural populations of *Coleochaete* and field temperature measurements were made during the growing season at Lake Tomahawk (Oneida Co., WI) (*Coleochaete scutata, C. soluta* and *C. pulvinata*), Fish Lake (Dane Co., WI) (*C. scutata, C. irre-*

[1] *Accepted: 10 October 1985.*
[2] Reprint requests.