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REJUVENATION OF *MELOSIRA GRANULATA* (BACILLARIOPHYCEAE) RESTING CELLS FROM THE ANOXIC SEDIMENTS OF DOUGLAS LAKE, MICHIGAN. I. LIGHT MICROSCOPY AND ^{14}C UPTAKE[1]

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ABSTRACT

Resting cells of Melosira granulata *(Ehr.) Ralfs were collected from the anoxic sediments of Douglas Lake, Michigan. Sediment containing* M. granulata *was inoculated into distilled water and incubated in a growth chamber for one week during which observations were made on the cytological differentiation process. Cells classified as "condensed," i.e. containing a dark brown cytoplasmic mass were identified as resting cells. The differentiation process consisted of a series of gradual cytological changes that included elongation of the cytoplasmic mass and recognition of definable organelles to the point where the cells were non-distinguishable from water column vegetative cells. Differentiating cells accumulated large polyphosphate and lipid granules. However, these granules disappeared just prior to cell division. The complete differentiation or rejuvenation sequence occurred in some cells in less than 24 h. However, not all dormant cells rejuvenated at the same time and it was observed that the lag period for rejuvenation increased with resting cell age (depth of burial in sediments). In the ^{14}C uptake studies, label was initially observed in condensed state cells. The label gradually progressed to the more differentiated forms. Total carbon uptake during the rejuvenation process was initially lower in the rejuvenating cells, but roughly equal to water column populations after 8 h, indicating a period of high metabolic activity in the rejuvenating cells between 1 and 8 h.*

Key index words: diatoms; Melosira; *rejuvenation; resting cells*

Extremely long periods of survival under inimical conditions are well known for a large number of algae and this topic has elicited much comment and considerable research (for reviews see Fogg 1969, Davis 1972, Schlichting 1974, Fryxell 1983). Although certain cyanobacteria are apparently able to survive desiccation in a vegetative condition, prolonged survival in aquatic habitats is usually associated with the formation of morphological modifications such as spores, cysts, or akinetes. Most species of eukaryotic algae capable of prolonged survival in aquatic habitats have specialized modifications of vegetative cells or specifically modified and resistant life-cycle stages.

Studies of prolonged darkness survival (Antia and Cheng 1970, Bunt and Lee 1972, Smayda and Mitchell-Innes 1974, Palmisano and Sullivan 1982) and desiccation (Bristol 1920, Evans 1958 and 1959, McLean 1967, Parker et al. 1969) are abundant. The most common mechanisms for survival under adverse conditions seem to be (1) formation of resting spores (Hendey 1964, Hargraves 1976); (2) formation of cysts (Wall et al. 1970, Sandgren 1983), and (3) formation of "resting cells" where there is no distinct external modification, but there is some, probably physiological, modification of the vegetative cell (Anderson 1975, Kalley et al. 1977, Bisalputra and Antia 1980, McLean 1969).

The classic study of Nipkow (1950) and later works by Lund (1954) and Stockner and Lund (1970) demonstrated that certain populations of the diatom genus *Melosira* are capable of surviving sinking and burial in sediments for periods of at least years and perhaps decades. These cells are able to survive sediment burial considerably longer than planktonic species of other major divisions that do have specialized survival cells. Furthermore, cells that survive burial can return to a state that is visually indistinguishable from "normal" vegetative cells within hours of being returned to suitable growth conditions. Rejuvenation of populations of "physiologically" resting cells of *Melosira granulata* (Ehr.) Ralfs obtained from the anoxic sediments of Douglas Lake, Michigan, and the sequence of cytological events that occur in this process are the subject of the following report.

MATERIALS AND METHODS

A core of Douglas Lake from South Fishtail Bay was obtained using a benthos corer at approximately 0530 on July 19, 1983. The core was kept in subdued light and sectioned at 1 cm intervals. Three milliliters of each core segment (including interstitial water) from depths of 1, 10, 20, and 30 cm was added to approximately 2 L of deionized water in non-toxic sterile rotary shaker bottles. Deionized water was used to provide the least nutrient-rich conditions possible. Similar rejuvenation responses

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occurred when resting cells from sediments were resuspended in filtered lake water. The bottles were put into a growth chamber on a rotary shaker platform with the growth chamber set at 18° C, a 16:8 h LD cycle, and a cool white light level of 50 $\mu E \cdot m^{-2} \cdot s^{-1}$. Samples were withdrawn at 15 min intervals for the first hour, hourly intervals for the first 8 h, 2 h intervals for hours 8–16, and subsequently every 8 h for 6 days. The light cycle of the growth chamber was initialized with placement of the shaker bottles into the chamber. The time interval between core collection and placement of samples into the growth chamber was ca. 30 min.

As samples were withdrawn, fixative was added to the sample tubes to give a final concentration of 1% paraformaldehyde, 1% glutaraldehyde, and 0.05 M cacodylate buffer at pH 7.2 (Lazinsky and Sicko-Goad 1979). Light microscope observations were made on both living and preserved samples with a Leitz Dialux 20 microscope with an optical system furnishing a numerical aperture of 1.32. For electron microscopy, samples were post-fixed in 1% osmium tetroxide in pH 7.2, 0.05 M cacodylate buffer for 1 h, dehydrated in a graded ethanol propylene oxide series, and embedded in Epon. Permanent epoxy mounts were also made from the OsO_4 post-fixed sample.

Water column samples from Douglas Lake containing populations of *Melosira* were also collected to compare the primary productivity of lakewater populations at the time sediment experiments were conducted with those populations rejuvenated from the sediments. Water column samples were collected from the epilimnion and transported back to the lab in the cold and dark. Average irradiance of water column samples at the time of collection was ca. 300 $\mu E \cdot m^{-2} \cdot s^{-1}$. Primary production was measured with the ^{14}C technique (Vollenweider 1974). Lakewater and sediment samples (1–3 mL of sediment and approximately 1½ L DDW) were incubated in 2-L bottles at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 1–104 h with 2–10 μCi of NaH $^{14}CO_3^-$. Upon completion of the incubation, the samples were preserved with either glutaraldehyde, paraformaldehyde and sodium cacodylate (Lazinsky and Sicko-Goad 1979), or 1% formalin. Immediately, these samples were filtered onto 0.45 μm membrane filters, placed on slides that have been vigorously washed in strong detergent, rinsed, and dipped in a solution containing 5 g × gelatin and 0.5 g chrome alum per liter (Knoechel and Kalff 1976), and then fumed with boiling acetone (Paerl and Stull 1979). Slides were dipped in NTB 3 photographic emulsion, exposed for 4–30 h, and developed for track autoradiography as described in Knoechel and Kalff (1976). The amount of carbon fixed per cell was calculated using pH and alkalinity measurements (Vollenweider 1974). Light and epifluorescent microscopy were used to count tracks and determine which cells contained autofluorescent chloroplasts.

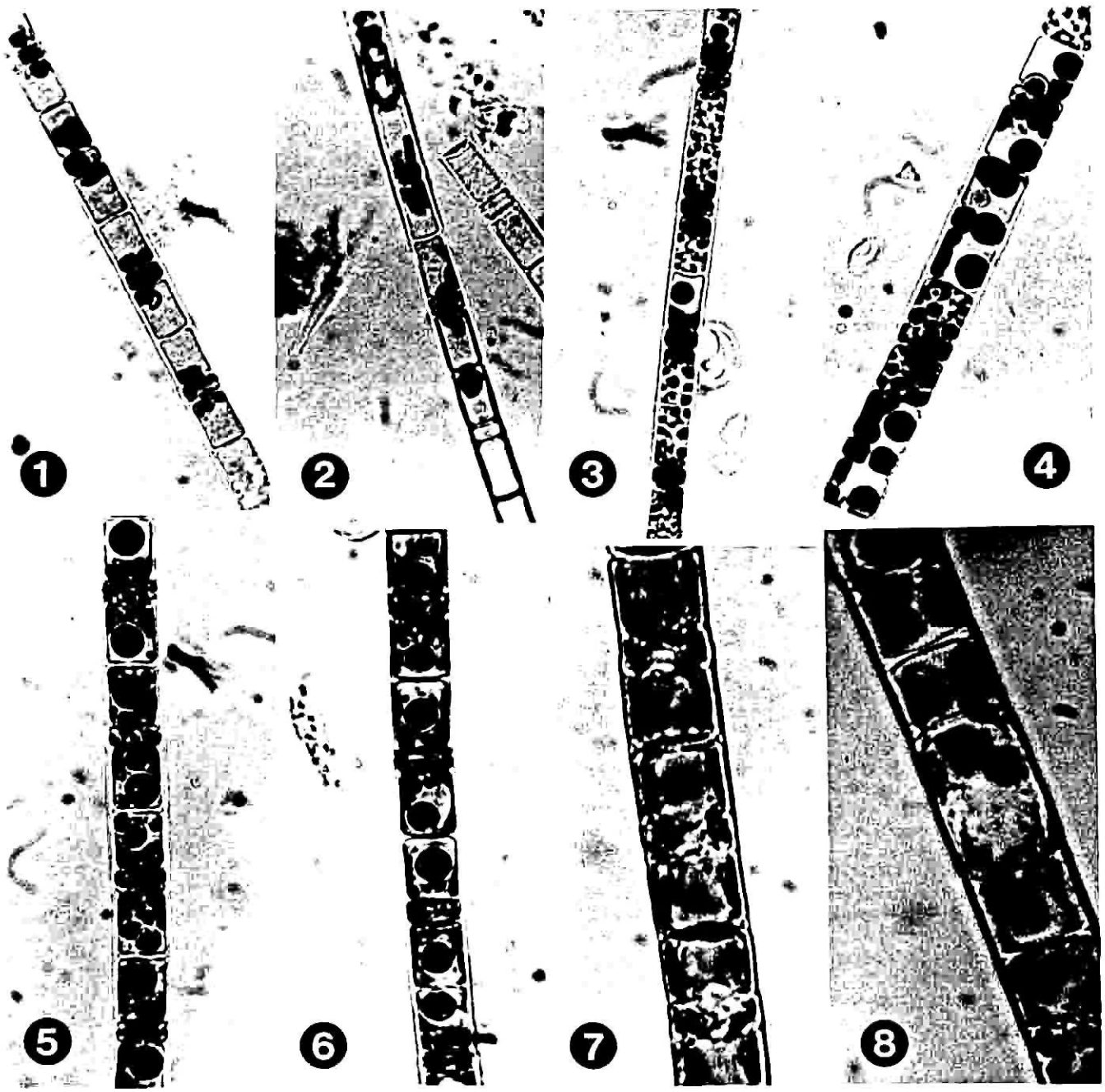
RESULTS

For discussion purposes throughout the text, we have described the four following cell types which are easily distinguished by light microscopy: (a) *Nonviable.* This category of cells is a mixture of two cell types including both empty frustules and cells containing small green globules or cytoplasmic fragments that may be weakly autofluorescent; (b) *Condensed.* Cells in this category have the protoplasm reduced to brownish globules with no visible substructure. The protoplasm is autofluorescent and is most frequently found in the sulcus region although it may be found occasionally adjacent to the valves. These cells are easily distinguished from cells with non-viable cytoplasmic fragments by their intense coloration (Fig. 1); (c) *Partially expanded.* Cells in this category are somewhat opaque with the cytoplasmic mass elongated and usually located near the sulcus; (d) *Fully expanded.* Cells are indistinguishable from normal vegetative cells. This category of cells, for counting purposes, also includes cells that are packed with storage products or are in the process of dividing.

The populations of cells classified as "condensed" appear to be the vegetative resting form of *Melosira.* This statement is based on light microscope observations and ^{14}C uptake determined by track autoradiography on which we elaborate below. An ordered sequence of rejuvenation stages was observed to occur within 24 h following resuspension of the sediment containing the resting cells. The initial step in the rejuvenation sequence is the expansion of cytoplasm in condensed cells from the intensely colored brownish compact globule located near the sulcus (Fig. 1). During this initial expansion stage, the cytoplasmic mass begins to lose its intense coloration and the chloroplasts become identifiable as discrete ovoid organelles still located near the sulcus (Fig. 2). The early expansion state is also characterized by the proliferation of lipids and polyphosphate. Small storage granules first appear scattered throughout the cell (Fig. 3). As cytoplasmic expansion progresses, the cells are characterized by what appear to be coalesced lipid granules (Fig. 4). At this stage, the cytoplasmic expansion and differentiation are incomplete but the chloroplasts assume a more parietal position. Further cytoplasmic expansion is then characterized by the appearance of large polyphosphate granules which often appear mottled (Fig. 5). The polyphosphate bodies were confirmed by lead-sulfide staining (Ebel et al. 1958). At this stage both polyphosphates and lipid may be present in the cell. Completely differentiated cells (i.e. those non-distinguishable from vegetative cells growing in the lake) may contain storage products (Fig. 6). However, just prior to (Fig. 7) and during cell division (Fig. 8), no storage products are present and when the cells are established as vegetative cultures (e.g. after a period of one week), lipid and polyphosphate granules are rare.

Cell counts of initial sediment samples (Table 1) demonstrate that the numbers of cells in the condensed state diminish with depth from approximately 32% in the surficial sediments to 7% at the 20 cm depth. Extrapolation of data from recent pollen profiles of Douglas Lake (Webb 1973, Andresen 1976) would suggest that the linear sedimentation rate is approximately 0.6 to 0.7 cm/year, making the 10 and 20 cm samples roughly 15 and 30 years old. It is also evident from Table 1 that the lag period for rejuvenation also increases with depth.

Cumulative carbon uptake data (Table 3 and Fig. 9) show that the population of 8 h cells has most of the carbon associated with the condensed and partially expanded states. With time, there is a net movement of label into cells that are fully expanded (including cells packed with storage products as well



FIGS. 1–8. Photomicrographs of stages of rejuvenation of *Melosira granulata* resting cells. FIG. 1. Condensed state cell. Note intense coloration of cytoplasmic mass in the sulcus region. FIG. 2. Early expansion stage. Chloroplasts are beginning to elongate although they are still located near the sulcus. FIG. 3. Early expansion stage accompanied by the accumulation of storage granules. These small storage granules are not easily identified at this stage. FIG. 4. As expansion proceeds, the lipids appear to coalesce into larger granules. FIG. 5. Polyphosphate granules become recognizable at the light microscope level and generally appear "mottled." At this stage they are easily differentiated from lipids. FIG. 6. Fully expanded cells are characterized by the arrangement of cytoplasm at the cell periphery and at the central cytoplasmic bridge. Storage products may still be present, although they are larger and fewer in number. FIGS. 7, 8. Cells just prior to (FIG. 7) and during division (FIG. 8) contain few or no storage granules. These cells are now indistinguishable from actively growing water column populations.

as dividing cells). The initial hourly uptake rates (Table 2) of actively growing water column populations of *M. granulata* are much higher than sediment samples initially placed into the light. However, after 8 h, water column and sediment cumulative rates are roughly equal (Table 3), indicating a period of high metabolic activity in the sediment samples between 1 and 8 h. Hourly uptake rates after 56 and 104 h of growth are essentially the same. The total cumulative carbon uptake data (Table 3) show that there is a continuous increase in carbon content with time up to 104 h, and that

TABLE 1. *Percent composition of* Melosira *rejuvenation experiments from resuspended sediments as a function of sediment depth and time. Results are presented as the mean (±1 SE). Refer to text for cytological description of categories.*

	Non-viable	Condensed	Partly expanded	Full expanded
Surficial				
t = 0	46.33 (±4.33)	32.33 (±1.76)	16.33 (±1.86)	5.0 (±1.53)
8	36.89 (±3.81)	17.38 (±6.22)	6.15 (±1.96)	39.57 (±5.21)
32	46.20 (±1.84)	6.43 (±5.61)	0.58 (±0.31)	46.78 (±5.49)
56	45.79 (±5.16)	0 (0)	0.56 (±0.59)	53.65 (±5.10)
80	26.36 (±5.74)	0 (0)	0.26 (±0.27)	73.39 (±5.79)
104	38.88 (±9.47)	0 (0)	0.26 (±0.29)	60.84 (±9.31)
152	30.03 (±5.26)	0 (0)	3.12 (±1.99)	66.86 (±3.28)
10 cm				
t = 0	67.33 (±8.68)	23.33 (±4.9)	9.0 (±3.5)	0.3 (±0.3)
8	64.16 (±7.59)	20.80 (±9.35)	2.02 (±1.37)	13.00 (±5.59)
32	87.79 (±7.12)	0.29 (±.28)	2.08 (±1.96)	9.82 (±7.92)
56	76.43 (±7.61)	2.11 (±1.65)	2.11 (±1.13)	19.34 (±7.06)
80	71.04 (±4.65)	0 (0)	0 (0)	28.96 (±4.65)
104	67.43 (±7.08)	1.43 (±.14)	0.29 (±.38)	30.86 (±7.25)
152	66.47 (±3.23)	0 (0)	0 (0)	33.53 (±2.70)
20 cm				
t = 0	91.67 (±31.18)	6.67 (±2.6)	1.67 (±1.2)	0 (0)
8	87.08 (±2.22)	12.92 (±2.22)	0 (0)	0 (0)
32	95.87 (±0.60)	4.12 (±0.60)	0 (0)	0 (0)
56	97.29 (±1.66)	2.71 (±1.66)	0 (0)	0 (0)
80	100 (±0)	0 (0)	0 (0)	0 (0)
104	84.34 (±9.88)	0 (0)	0.30 (±0.28)	15.36 (±9.62)
152	96.49 (±1.61)	0.64 (±0.63)	0.64 (±0.64)	2.23 (±2.25)

Melosira populations collected from the water column have similar carbon content. ^{14}C label is initially located in condensed and partially expanded cell types. However, as incubation time increases, the label is associated with the more advanced rejuvenation stages (Fig. 9).

DISCUSSION

Development of a mechanism allowing prolonged survival of diatoms could be of considerable selective advantage to populations growing in both temperate lakes and oceans. Although spores have generally been regarded as benthic resting stages of planktonic species (Gran 1912), evidence from natural populations would suggest that spores (as morphologically distinct entities) may not represent the sole source of "seed material" for the sudden onset of phytoplankton blooms. For example, spores are more commonly found in neritic species than in oceanic or freshwater species (Hargraves and French 1983). Hargraves and French (1975, 1983) demonstrated that diatom resting spores (hypnospores) persist in culture for only as long as two years and in the sediments for only a few weeks, although Garrison (1981) demonstrated the persistence of spores in Monterey Bay. Moreover, some important coastal phytoplankton species such as *Skeletonema costatum* and *Asterionella glacialis* do not form morphologically distinct resting cells. The survival of populations that form physiologically dormant cells may be more prolonged and efficient than species that form hypnospores. This may explain the persistence of viable cells at great depths in the ocean (Malone et al. 1973, Anderson 1975, 1976, Platt et al. 1983). The resting cell strategy also avoids metabolic and material loss, which occurs as a consequence of spore formation in diatoms. The problem associated with assuming that spores are a benthic resting stage is more pronounced in freshwater systems because resting spores are uncommon. Lund (1954, 1955, 1959) has presented evidence which suggests that

TABLE 2. *Hourly bicarbonate uptake rates for 1 h ^{14}C experiments as a function of growth time. Total includes all cell categories.*

Growth condition	Total hourly rate (pg C·cell^{-1}·h^{-1})
1 h	0.183 ± 0.025
56 h	0.35 ± 0.04
104 h	0.573 ± 0.065
1 h dark uptake	0.00028 ± 0.00028
1 h initial water column (*Melosira* population)	0.61 ± 0.05

TABLE 3. *Cumulative bicarbonate uptake as a function of long-term continuous exposure and growth. Total includes all cell categories.*

Time in culture	Cumulative uptake (pg C·cell^{-1}·h^{-1})
8 h	7.92 ± 0.60
16 h	10.07 ± 0.66
32 h	15.35 ± 0.59
56 h	24.05 ± 0.82
104 h	27.4 ± 0.85
8 h water column (*Melosira* population)	6.70 ± 0.95

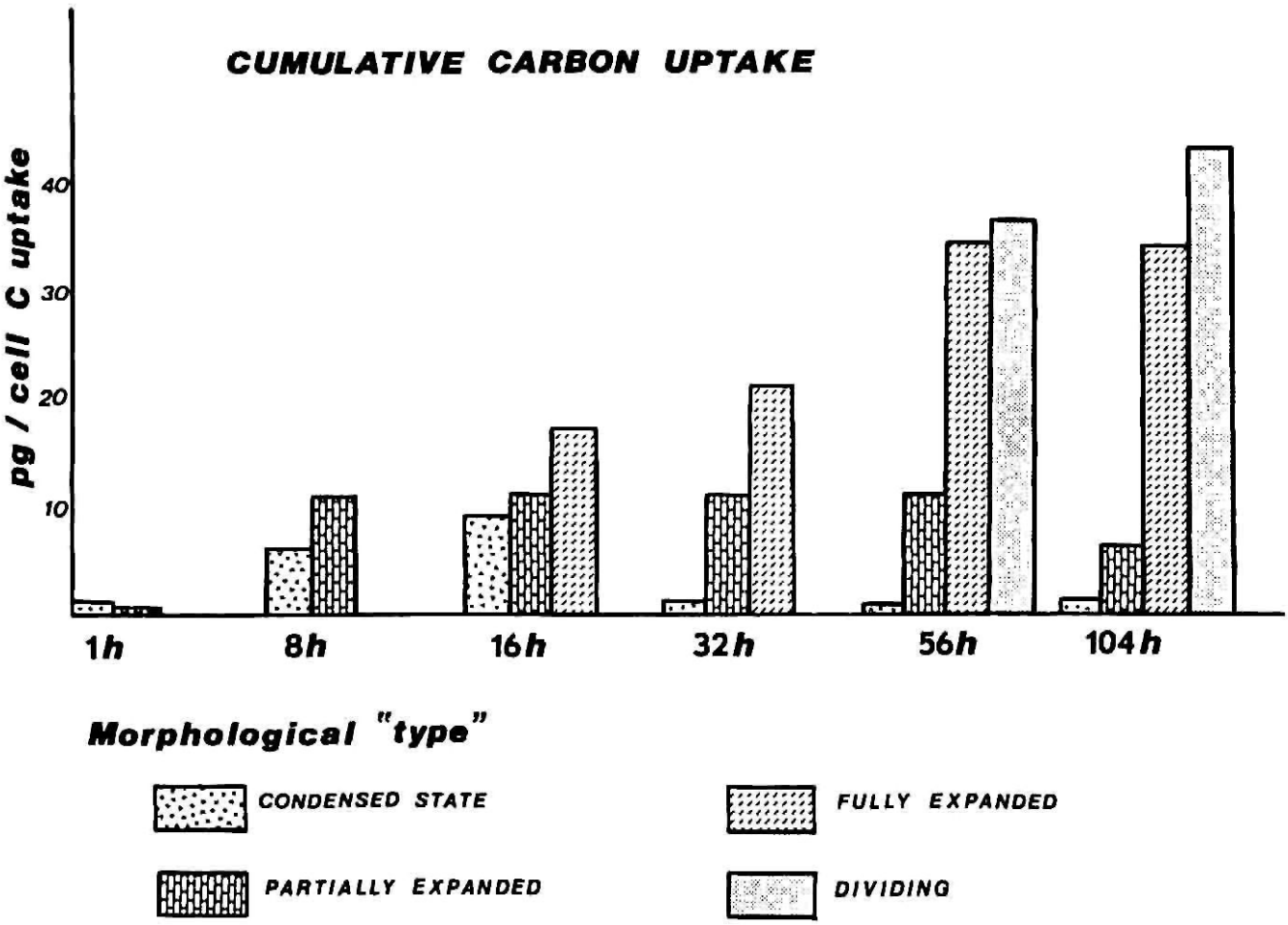


FIG. 9. Distribution of ^{14}C uptake in rejuvenated *M. granulata* cells as a function of time.

the perennation of *Melosira italica* subsp. *subarctica* in English lakes involves a regular cycle of growth during circulation, sinking into sediments, and entrainment into the plankton during the next circulation event. Lund (1949, 1950a, b, Lund et al. 1963) puzzled over massive blooms of *Asterionella formosa* followed by apparently complete disappearance from the water column and subsequent rapid assumption of dominance with return of favorable conditions. He also observed apparently living vegetative cells in surficial sediments.

Our data suggest that *Melosira* is indeed capable of surviving prolonged periods (up to perhaps two decades) in a dormant state that is externally nondistinguishable from a vegetative cell. The cells, which we have identified by both light microscopy and autoradiography to be the physiologically "resting" cells are, however, distinguished from vegetative cells by their distinctive cytology which consists of a condensed, dark brown cytoplasmic mass. Review of the literature on cytological descriptions of dormant cells (including spores) reveals that this description is also true for other species (Anderson 1975, Hargraves and French 1975, Hargraves 1979, Hoban et al. 1979), and both compaction and conservation of key organelles may be the key to prolonged survival in both resting spores and resting cells.

The resting cells of *M. granulata* in this study were taken from core depths that may correspond to 20 years of burial in anoxic lake sediments. This is significant from both the standpoint of time and dormancy conditions. These cells are considerably older than those previously shown to rejuvenate or germinate (e.g. Hargraves and French 1983). Furthermore, viable resting cells were obtained from anoxic lake sediments, while anoxia is considered an environmental factor that reduces spore viability (Davis et al. 1980, Hollibaugh et al. 1981). Our data on cumulative carbon uptake and hourly uptake rates during the rejuvenation sequence are remarkably consistent with physiological data presented for germinating spores of several diatoms (Hollibaugh et al. 1981). However, the semantics involved in comparing a true germination from a spore with rejuvenation of dormant cells makes the staging difficult to compare. In both *Melosira* and other diatoms (e.g. Anderson 1976, Hollibaugh et al. 1981), placement

of dormant cells under conditions that favor resumption of active growth results in an immediate, dramatic increase in carbon fixation. In *Melosira*, the ^{14}C uptake is initially associated with cells in the condensed state. As incubation time increases, the label is found in more advanced rejuvenation stages. *Melosira* is also somewhat unusual in that we found large increases in storage products during cytoplasmic expansion state and prior to cell division. Increases in storage products are usually associated with the formation of resting cells and spores (McLean 1967, 1969, Anderson 1975, von Stosch and Fecher 1979, Bisalputra and Antia 1980). However, we feel that it is not unreasonable to speculate that the rejuvenation (germination)-dormancy sequence is a continuum and reversible.

The increased lag period of rejuvenation with depth (i.e. age of dormant cells) is not unusual. Hollibaugh et al. (1981) reported similar results with three *Chaetoceros* species and it has been suggested (Anderson 1976) that staggered growth resumption as we observed, may be yet another survival mechanism, ensuring that a portion of viable cells are retained, should unfavorable growth conditions occur after resumption of growth.

Simonsen (1979) has postulated that resting spore formation is a primitive characteristic that has been retained by some species. In view of our findings and those of other investigators, we find this statement quite plausible considering the cytological and physiological characteristics that are common to rejuvenating resting cells and germinating spores. Populations that have no distinctive resting stage based on external morphology have probably gone unnoticed in natural assemblages, and further work both in marine and freshwater ecosystems needs to be done to determine the abundance and role of species with resting cells.

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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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