Planta (Berl.) 130, 159–167 (1976)

Planta

Studies on the Biochemistry and Fine Structure of Silica Shell Formation in Diatoms

Division Cycle and Chemical Composition of *Navicula pelliculosa* during Light-Dark Synchronized Growth * **

W.M. Darley [1], C.W. Sullivan [2], and B.E. Volcani [3]
Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093, USA

Summary. Cell division in *Navicula pelliculosa* (Bréb.) Hilse, strain 668 was synchronized with an alternating regime of 5 h light and 7 h dark. Cell volume and dry weight increased only during the light period. DNA synthesis, which began during the third h of light, was followed sequentially by mitosis, cytokinesis, silicic acid uptake, cell wall formation, and cell separation. Silicification and a small amount of net synthesis of DNA, RNA and protein occurred during the dark at the expense of carbohydrate reserves accumulated during the light period. Cells kept in continuous light, after synchronization with the light-dark regime, remained synchronized through a second division cycle; the sequence of morphological events was the same as that in the light-dark division cycle, but the biosynthesis of macromolecular components changed from a stepwise to a linear pattern. The silicon-starvation synchrony was improved by depriving light-dark synchronized cells of silicic acid at the beginning of their division cycle, then resupplying silicic acid to cells blocked at wall formation.

Introduction

The use of synchronized cultures of microorganisms is well established as a means of investigating the morphological and biochemical events of the cell cycle (Tamiya, 1966; Pirson and Lorenzen, 1966). Light-dark (L-D) synchronizing regimes have been commonly used to study various aspects of development and metabolism in several marine diatoms: Werner (1966) and Werner and Pirson (1967) used a L-D regime to synchronize cultures of *Cyclotella cryptica*; Jørgensen (1966) reported the photosynthetic rate and pigment changes in *Skeletonema costatum* ; Epply et al. (1967) investigated the morphological events and cell composition during the division cycle of *Ditylum brightwellii*. Coombs et al. (1967a) measured changes in morphology and in levels of nucleoside triphosphates in *Cyclindrotheca fusiformis*; while Darley and Volcani (1969) and Sullivan and Volcani (1973a, b) investigated the silicon requirement for DNA synthesis in this organism.

Because diatoms require silicon for formation of the siliceous shell and for growth (Darley and Volcani, 1969), they may be synchronized by silicon-deprivation (Lewin et al., 1966; Busby and Lewin, 1967). Previous studies based on the silicon-starvation synchrony of the freshwater diatom, *Navicula pelliculosa* (Lewin et al., 1966; Coombs et al., 1967b, 1967c) have shown that during the silicon-starvation period cell metabolism declines; mitosis and cytokinesis take place but cell wall formation is blocked and the cells fail to separate. When silicic acid is supplied, energy metabolism is immediately reactivated; a 3–4 h period of rapid silicic acid uptake is followed by wall formation and synchronous cell separation.

Since only silicon uptake and cell separation are synchronized, however, the silicon-starvation synchrony is useful for studying cell metabolism and silicification during cell wall formation but not during other stages in the division cycle. Moreover, the studies of Lewin et al. (1966) and Coombs et al. (1967c) showed that the cell population is not morphologically uniform at the end of the starvation period, and that arresting the division cycle at this

* Portions based on a thesis submitted by W.M.D. to the University of California, San Diego in partial fulfillment of the requirements for the PH.D. degree

** *Abbreviation:* L=light, D=dark

[1] *Present address:* Department of Botany, University of Georgia, Athens, Georgia 30602

[2] *Present Address:* Department of Biological Sciences, University of Southern California, Los Angeles, CA 90007

[3] To whom reprint requests are to be addressed

juncture results in extensive alterations in cellular metabolism. The L-D synchronies of diatoms, on the other hand, synchronize the entire vegetative division cycle, allowing all stages to be investigated. Furthermore, as compared to silicon-starvation, the L-D synchronizing mechanism may impose less physiological stress.

It has been suggested (Baker and Schmidt, 1964) that the L-D system may also impose at least a degree of stress, raising the question whether the observed events accurately reflect the 'normal' division cycle; i.e., whether the events occur in successive generations not exposed to synchronizing treatment.

This paper compares three synchronizing regimes: the conventional L-D method; a "continuous light" synchrony in which cells synchronized by the L-D regime are returned to continuous light for 2 division cycles; and a "combined" silicon-starvation synchrony, obtained by subjecting L-D synchronized cells to silicon deprivation. The correlation between morphological events and changes in chemical composition in *N. pelliculosa* under each of these regimes is reported.

Materials and Methods

Cultures. Axenic, clonal cultures of *N. pelliculosa* (Bréb.) Hilse (Strain No. 668, Indiana University Culture Collection, Bloomington, Ind. USA) were maintained on a freshwater Bacto-Tryptone (Difco) medium (FWT) (Lewin et al., 1966).

Light-Dark Synchronization. Synchronization was carried out in FWGL medium as described previously (Darley and Volcani, 1971).

Continuous Light Synchronization. Cultures synchronized by the L-D regime were kept in continuous light for two successive cycles, with dilution after the first separation burst, as in the L-D regime.

Combined Synchronization. Young synchronized cells from h 12 of the L-D synchrony (at the end of the dark period) were harvested by centrifugation at 4,000 g, 5 min, 20° C, washed twice with silicic acid-free (FWGL) medium and resuspended to 3×10^6 cells/ml in the same medium contained in a 4 l clear polycarbonate bottle (Nalge). The culture was incubated for 12 h in continuous light under conditions described for the L-D synchronization. To induce synchronous cell wall formation and cell separation, a 2% solution of filter-sterilized $Na_2SiO_3 \cdot 9H_2O$ (adjusted to pH 8.5 with 1N HCl) was then added to give a final concentration of 0.42 mM $Si(OH)_4$.

Cell Number and Volume. Cell counts were made as previously described (Coombs et al., 1967b). Cell volume was measured with a Celloscope Electronic Particle Counter, model 101 (Particle Data Instrument Co., Elmhurst, Illinois) equipped with a 76 μ orifice. The data from the counter were analyzed with a Nuclear Data Multichannel Analyzer (Model 110) with a Teletype printout. The cell volume given represents the peak value of the size distribution and approximates the mean cell volume.

Microscopy. Nuclear staining was carried out according to Coombs et al. (1967a). Nuclei were observed under oil in a Leitz microscope with a Wratten series VI filter.

Cells were scored as mature (one nucleus/large cell) or biprotoplastic (two daughter protoplasts within the parent frustule, each with one nucleus); 100 to 200 cells were scored for each sample. For electron microscopy cells were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 6.8) for 1.5 h at 4° C followed by 1% OsO_4 under the same conditions for 3 h. After dehydration in ethanol and propylene oxide, the specimens were embedded in Epon 812. Sections were cut with a diamond knife and stained with methanol uranyl acetate and lead citrate (Lauritis et al., 1968). Electron micrographs were taken with a Siemens Elmiskop I.

Biochemical Methods. All centrifugations were carried out at 800 g for 3 min; optical densities were measured on a Zeiss PMQ II spectrophotometer using a 1 cm cell. DNA was determined on samples containing $2–3 \times 10^7$ cells, by the fluorometric method of Holm-Hansen et al. (1968) using an Aminco-Bowman spectrophotofluorometer; highly polymerized calf thymus DNA (Sigma) served as the standard. RNA was measured on samples containing $3–6 \times 10^8$ cells as described previously (Combs et al., 1967b), except that the cells were pre-extracted twice in each of the following solvents: absolute methanol (25° C), 5% trichloroacetic acid (0° C), 95% ethanol (25° C); purified yeast RNA served as the standard. Protein was determined by the procedure of Lowry et al. (1951) on samples containing $4–6 \times 10^6$ cells; crystalline bovine serum albumin (Pentex Biochemicals, Inc.) served as the standard. Total carbohydrate was determined by the phenol-sulfuric acid method (Dubois et al., 1956) as described previously (Coombs et al., 1967b) on samples containing $3–9 \times 10^7$ cells; glucose served as the standard. Pigments were estimated by the trichromatic method using the equations of Strickland and Parsons (1965) on cell samples containing $6–12 \times 10^7$ cells. Lipid content was measured as solvent-extracted carbon (Holm-Hansen et al., 1967) on samples containing $7–10 \times 10^7$ cells. The dry weight of lipid was calculated by multiplying the lipid carbon by the factor 1.7, and was measured by filtering 20–30 ml of culture through dried and tared Millipore filters (0.45 μm, 25 mm in diameter). The filters were rinsed with distilled H_2O, dried at 80° C for 12 h, cooled in a dessicator over P_2O_5-KOH and weighed to the nearst 0.05 mg. Silicic acid uptake was measured as depletion of silicic acid from the medium (Coombs et al., 1967b), except that the cells were removed by filtration through a membrane filter (0.45 μm Millipore).

Results

Light-Dark Synchrony. Cells in the mid-exponential growth phase subjected to a 24 h dark period, continued to divide for approximately 8 h, presumably because diatoms contain enough reserve material to support a 50–80% increase in cell number (Werner, 1966; Coombs et al., 1967b). Thus calculations of the optimal photoperiod (Cook and James, 1960) based on cellular reserve energy alone could not be used. By the end of the dark period cells had apparently exhausted their endogenous reserves and the culture consisted predominantly of small young cells. To determine the minimum light duration required for one cell division, the young cells, in separate vessels, were exposed to varying photo-periods. It was found that 5 h of light followed by 7 h dark supports a 100%

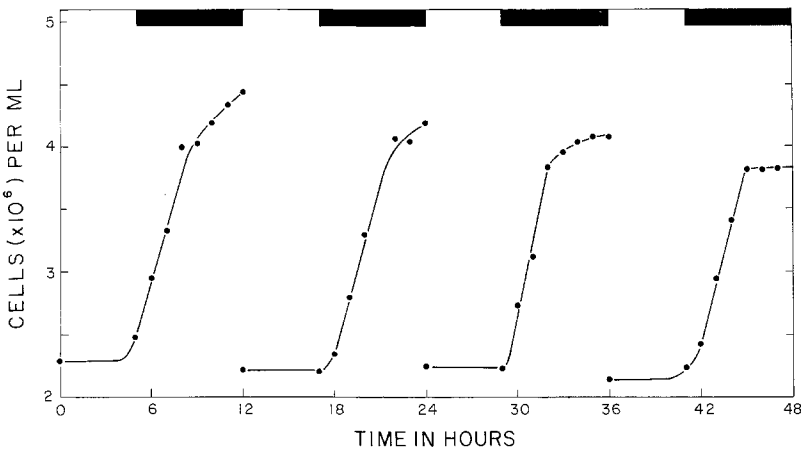

Fig. 1. Change in cell number of *N. pelliculosa* during 4 successive L-D cycles (cycles 4 through 7). Shaded bars at the top denote dark periods

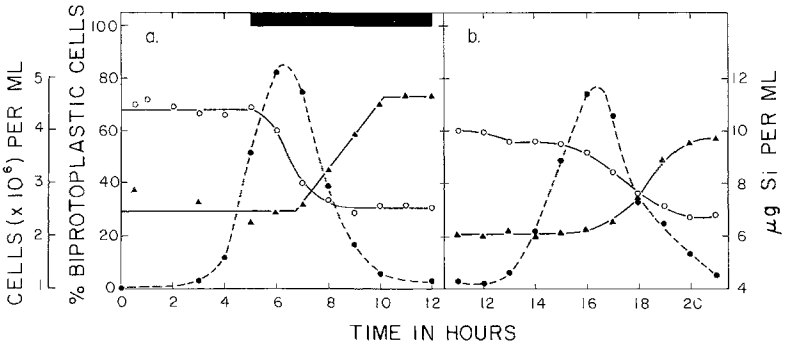

Fig. 2a and b. Nuclear division (●), silicon uptake (○), and cell number (▲) in *N. pelliculosa* during the L-D and continuous-light division cycle. (a) L-D; shaded bar at the top denotes dark period. (b) Second cycle in continuous L. Culture placed in L at 0 h and diluted at h 11

increase in cell number; this increase is confined to the dark period and depends on the duration of the previous light period. The resulting 12 h (5L:7D) synchronized division cycle is about 50% longer than the doubling time (8 h) of *N. pelliculosa* grown in continuous light.

After 5 consecutive L-D cycles, the cultures had reached maximum synchronization; the time course of cell increase for cycles 4 through 7 is shown in Figure 1. Separation bursts ranged from 2 to 4 h, with the midpoint occurring between h 7 and 10 of the L-D cycle. Approximately 88% (range 75% to 100%) of the cells divide at each burst. This culture was maintained for 19 repeated cycles without apparent loss of synchronization. For morphological and chemical analysis only cycles 5 through 19 were used.

Morphological Development during the L-D-Division Cycle. Data on the timing and sequence of morphological development were correlated with the changes in cell biochemical composition. Cell volume increased linearly from 37 μm^3 to 78 μm^3 during the light period, and rose slightly to 80 μm^3 during the first h of darkness, after which cell separation began. But by the end of the dark period, volume of the daughter cells had decreased from the initial value of 37 μm^3 to 34 μm^3.

The temporal relationship between nuclear division, cytokinesis, silicic acid uptake, and cell separation is shown in Figure 2a. Nuclear division and cytokinesis began at h 4; at the end of the light period (5 h), silicic acid uptake, which can be considered as signifying cell wall formation (Busby and Lewin, 1967; Coombs and Volcani, 1968b), began and lasted for three h. Cells tok up an average of 1.5 µg Si/10^6 cells (range 1.2 µg to 1.8 µg/10^6 cells). Cell separation was initiated as silicic acid uptake ceased, after the cell walls were completed.

These morphological events were also studied in the electron microscope (Fig. 3). During the light period, "young", newly-separated cells (Fig. 3a) grow to "maturity" (Fig. 3b) and undergo mitosis and cytokinesis; at the beginning of the dark period, they form "biprotoplastic" cells (Fig. 3c). During silicic acid uptake, new walls are initiated within membrane-bound (silicalemma) vesicles (Reimann et al., 1966) on the cytoplasmic side of the plasmalemma at the new cell interface (Fig. 3d). As each daughter cell completes a new wall (Fig. 3e and f) a new plasmalemma appears beneath the maturing wall. The two daughter cells, which still adhere to each other, are termed "paired cells." The difference between the biprotoplastic and paired cells could not be determined with the light microscope and both types were

therefore scored as "biprotoplastic" cells in acetocarmine stained material for light microscopy. Wall formation is completed about halfway through the dark period and the cells separate. A similar series of morphological events has been deduced from sectioned cells from an exponential culture of *Gamphonema parvulum* (Dawson, 1973).

Chemical Composition during L-D Division Cycle. Dry weight per ml of culture increased linearly during the light period (from 25.5 µg/ml to 50 µg/ml) and remained constant during the dark period. Synthesis of nucleic acids and protein is shown in Figure 4. DNA synthesis, which began at h 3, was chiefly limited to a 3 h period; it increased only slightly during the next 2 h and then at h 9 began to level off in the step-fashion typical of a synchronized cell population. The course of RNA and protein synthesis was almost identical, and was largely confined to the light period with only a very slight rise during the dark.

Both carbohydrates and lipids (Fig. 4b) increased only during the light period, but while the carbohydrates showed a very sharp rise in the light and almost as sharp a decrease with the onset of the dark period, lipid content remained relatively steady throughout the dark period falling only slightly by its end. Total carotenoid synthesis also occurred only in the light (Fig. 4c) but synthesis of chlorophyll a continued almost unabated during the dark period, while that of chlorophyll c, after an initial levelling at the onset of the dark, continued its sharp rise (Fig. 4c).

As the figures demonstrate, all the cell components studied almost doubled during the 12 h division cycle, corresponding to the doubling in cell number, Although the carbohydrate content of the culture nearly quadruped during the light, the decrease during the dark was such that this component, too, had doubled by the end of the dark period. Moreover, while it might appear that over a series of cycles the cell gradually accumulated carbohydrates, in fact the overall increases in carbohydrate during the alternate cycles was less than 100%. In one experiment, the increase in carbohydrate/ml during the 12 h cycle averaged 133% for the odd-numbered cycles and only

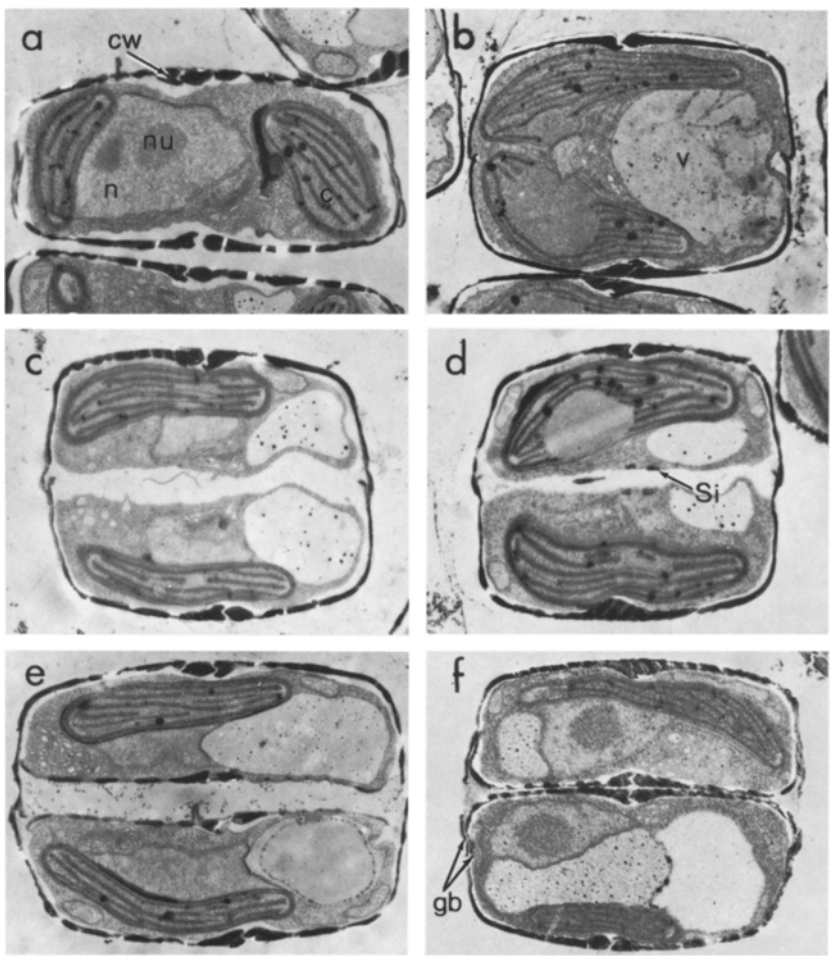

Fig. 3a–f. Electron micrographs of transverse sections of *N. pelliculosa* during the L-D division cycle. (a) Young cell. (b) Mature cell. (c) Biprotoplastic cell immediately following cytokinesis. (d) Biprotoplastic cell with initial stages of silica deposition (Si). (e) Paired cell with nearly complete cell walls. (f) Paired cell with complete cell walls including girdle bands (*gb*). *cw* cell wall, *n* nucleus, *nu* nucleolus, *c* chloroplast, *v* vacuole. × 14,250

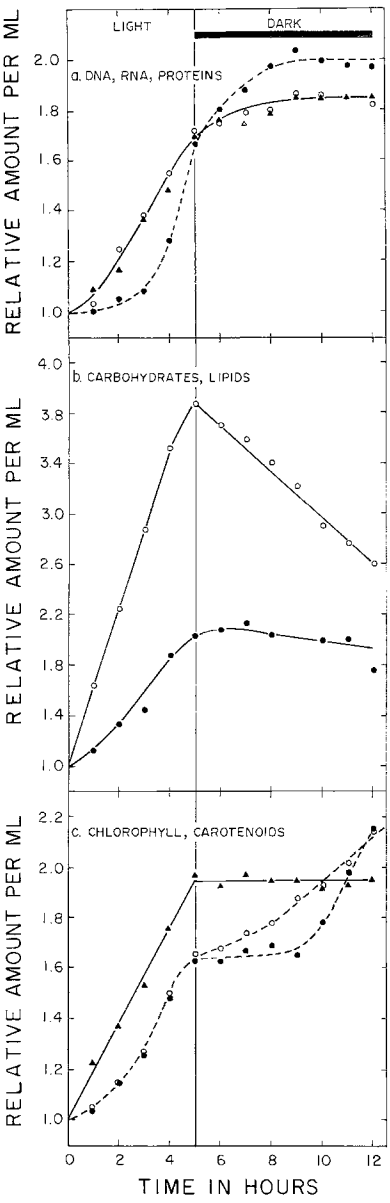

Fig. 4a–c. Chemical composition of *N. pelliculosa* during L-D division cycle. (a) DNA (●), RNA (○), proteins (▲). (b) Carbohydrates (○), lipids (●). (c) Chlorophyll a (○), chlorophyll c (●), carotenoids (▲). The factors for converting the relative amount to μg/ml of culture (2×10^6 cells/ml at h 0) are: DNA 0.17; RNA 0.91; proteins 12.4; carbohydrates 3.45; lipids 3.61; chlorophyll a 0.27; chlorophyll c 0.046; carotenoids 0.26. The shaded bar at the top denotes the D period

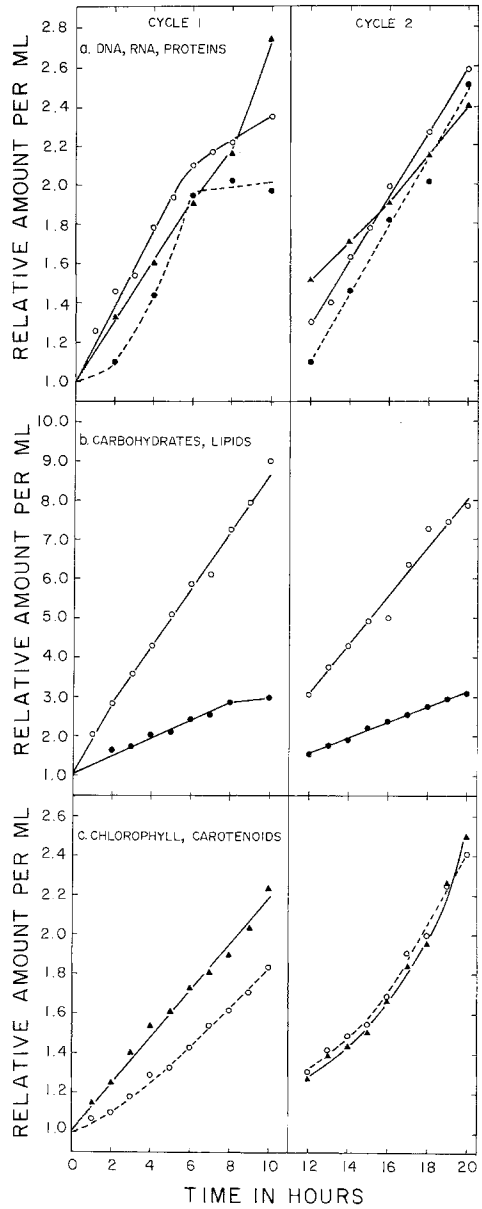

Fig. 5a–c. Chemical composition of *N. pelliculosa* during two successive cycles of synchronized growth in continuous L. The culture was diluted at h 11. (a) DNA (●), RNA (○), proteins (▲). (b) Carbohydrates (○), lipids (●). (c) Chlorophyll a (▲), carotenoids (○). The factors for converting the relative amount to μg/mg of culture (2×10^6 cells/ml at out 0) are: DNA 0.14; RNA 0.85; proteins 10.3; carbohydrates 3.5; lipids 3.6; chlorophyll a 0.312; carotenoids 0.312

80% for the even-numbered. Apparently the cells were enriched in carbohydrate during one division cycle and depleted during the next; hence the net carbohydrate content doubled when an average of several cycles is considered.

Morphological Development and Chemical Composition of Synchronized Cells in Continuous Light. To see whether the L-D synchronization imposes a noticable degree of stress, morphological development and changes of chemical composition observed in the L-D regime, were compared with those in synchronized cultures kept in continuous light for two successive division cycles. During the first 6 h in the light, cell volume increased linearly from 38 μm^3 to 82 μm^3; after cell separation, daughter cells were 43 μm^3 and began immediately to increase in volume. The temporal relationship between nuclear division,

cytokinesis, silicic acid uptake and cell separation was essentially similar to that of the L-D synchronization (Fig. 2b).

During the first division cycle in continuous light, as Figure 5a shows, DNA synthesis occurred in step-fashion, but during the second cycle, synthesis was linear. Net synthesis of RNA and protein were essentially linear during the major part of both cycles, with minor rate changes during the latter part of the first cycle. During both cycles, the increase in carbohydrate content was remarkable, especially during the first cycle when it was more than 800%. Lipid content of the culture increased by about 200% in the first cycle and 150% in the second (Fig. 5b). However, the relative increase in both lipids and carbohydrate content was about the same as in the L-D synchrony. The net synthetic rate of chlorophyll a and of total carotenoids was relatively constant during the first cycle but tended to rise as the second cycle progressed (Fig. 5c).

The general trends in net synthesis of cellular components were similar in both the L-D and continuous light synchronies; the chief difference was that in the L-D system the increases tended to be stepwise with a leveling-off during the dark period, while in the continuous light regime they were linear, indicating a return to an exponential type of growth pattern in the absence of the synchronizing dark period.

Combined L-D Starvation Synchrony. Studies carried out in the silicon-starvation synchrony by Lewin et al. (1966) and Coombs et al. (1967c) showed that at the end of the starvation period the cultures contained both "biprotoplastic" and paired cells, even though the subsequent division burst, after silicon was supplied, indicated physiological uniformity. It was suggested that silicon deprivation arrested cell development at various stages during wall formation and that the heterogeneity of the culture at the end of the starvation period was due to its normal heterogeneity at the start of the period. It seemed likely, therefore, that if an already synchronized culture were deprived of silicon, the culture might be more homogenous when development was arrested and a better synchronized division might be attained after silicon was resupplied.

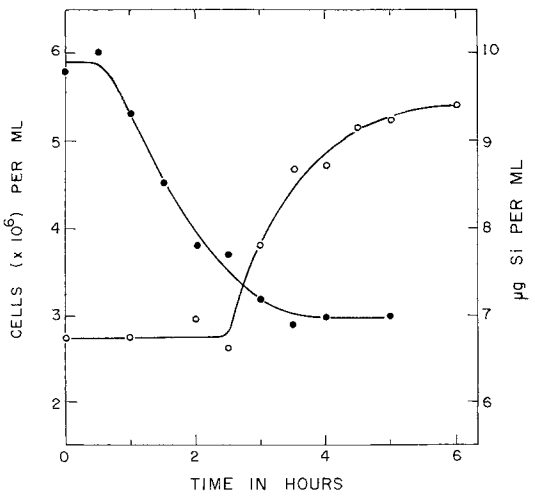

Fig. 6. Silicic acid uptake (●) and cell separation (○) of *N. pelliculosa* in combined L-D starvation synchrony. Silicic acid was added to the culture of h 0

Such a synchronization procedure was tested by placing young L-D synchronized cells in a silicic acid-free medium in the light for 12 h. At the completion of cytokinesis, further development was blocked by lack of silicon for wall formation. Of stained cells taken at the end of the starvation period, more than 95% were "biprotoplastic". Electron microscopy of thin sections showed that when the starvation medium is virtually free of silicic acid, only a barely-initiated cell wall can be seen in the raphe region of the cell; when the starvation medium contained small amounts of residual silicic acid (perhaps freed from dead cells), wall formation had been advanced before the silicic acid was exhausted (not shown).

The introduction of silicic acid to the starved cells (Fig. 6) was followed almost immediately by a 2.5 to 3.0 h period of silicic acid uptake; cell separation began at about h 2.5 and continued for a 3 h period. Increase in cell number averaged 85%. The periods both of silicic acid uptake and of cell separation were slightly shorter than those in the usual silicon starvation synchrony (Coombs et al., 1967b), indicating a higher degree of synchrony.

Preliminary studies on the chemical composition of the cells during the combined synchrony showed that the course of net biosynthesis was similar to that in the first cycle of the continuous-light synchrony until cell development was arrested; after that it resembled the pattern of the silicon-starvation synchrony as reported by Coombs et al. (1967b).

Discussion

Using the synchronization index (SI) of Scherbaum (1962) the three synchronization regimes in *N. pelliculosa* were compared with each other and with L-D regimes in other diatoms. An SI of 0.53 was previously found for the silicon starvation synchrony in *N. Pelliculosa* (Coombs et al., 1967b), while those for L-D regime and the combined synchrony in this study were 0.55 and 0.61 respectively. These figures compare favorably with those reported for L-D regimes in other diatoms which averaged 0.59 with a range

of 0.14 for *S. costatum* (Jørgensen, 1966) to 0.82 for *D. brightwellii* (Paasche, 1968).

Since, in the silicon-starvation synchrony, cell development of *N. pelliculosa* halts after cytokinesis, at the wall formation stage, the cells are blocked before cell separation can take place; upon the provision of silicic acid the cell walls are formed and the cycle is completed resulting in synchronized cell separation. In the L-D synchrony, on the other hand, the normal cycle is completed about halfway into dark period; the result at the end of this period is a fully synchronized population of young cells ready, upon the provision of light, to resume their normal cycle.

The developmental sequence of events in cells synchronized by both the L-D and continuous light synchronies of *N. pelliculosa* confirms results obtained in the silicon-starvation synchrony of this organism (Coombs et al., 1967c). Cellular growth, as measured by increase both in dry weight and in volume, is limited for the most part of the light period and is terminated by the following sequential events: (1) doubling of DNA content, (2) mitosis, (3) cytokinesis, (4) silicic acid uptake and polymerization to form a new wall for each of the two daughter cells, and (5) cell separation. This pattern is not affected by the L-D regime, as demonstrated by the fact that it is not significantly changed when synchronized cells are maintained in continuous light for two successive division cycles; exponential growth is resumed during the second cycle. The processes occurring after cytokinesis in the L-D synchrony are the same as those in the silicon-starvation synchrony (Coombs et al., 1967c). Morphological development and chemical composition of cells in the three synchronies described in this paper were similar; however, smaller daughter cells were produced by the L-D synchrony, because the later stages of the division cycle took place in the dark with consequent lack of photosynthesis and growth.

Synthesis of cellular components in the L-D and in the continuous-light synchronies was essentially similar, the chief difference being the change from a stepwise increase of components in the former to a linear increase in the latter. This shift may result from the fact that cell division occurs in the light; thus the "biprotoplastic" and pair daughter cells continue photosynthesis and, in effect, begin a new division cycle prior to their physical separation.

This early development is also reflected by the fact that during the first division cycle in continuous light, daughter cells are released at h 19, while during the second cycle, they are released at h 17. Moreover, when the L-D synchronized cells are placed in continuous light, exponential growth is gradually resumed, as is indicated by the continuing linear increase in DNA in the second cycle, which resembles that found during exponential growth (Coombs et al., 1967b).

The resumption of an exponential growth-pattern during the second cycle may also account for the greater increase in cell components during this cycle as compared to the first. That exponential cultures are characterized by high levels of all major cell components except DNA, is shown by the prolonged period of cell division and subsequent near-doubling in cell number that occurs during the first 8 h after such cultures are placed in the dark (Darley and Volcani, 1971).

In the silicon-starved cultures of *N. pelliculosa*, lipids serve as the storage product (Coombs et al., 1967b). In the L-D synchronies of this organism, however, carbohydrates accumulated during the light appear to provide a reserve carbon and energy source for the synthesis of DNA, RNA, protein, and chlorophyll during the dark period. Carbohydrate metabolism may also provide the energy source presumably required for silicic acid uptake and deposition.

Earlier studies (Coombs et al., 1967b, 1967c; Coombs and Volcani, 1968a) suggested that silicon metabolism might be closely integrated with general cell metabolism, and it was thought that this might be verified in the L-D synchrony. We assumed that the L-D synchrony could provide information on the rates of synthesis at various stages of the division cycle. In this synchrony, however, the changes in net-synthetic rates caused by the light-dark transition are difficult to distinguish from those attributable to cell wall formation. Since the effects of the light-dark transition are eliminated in the continuous-light synchrony, the relation between cell wall formation and biosynthetic capacity should be more apparent. However, except for a slight interruption of carbohydrate accumulation during the division period, net biosynthetic rates showed no appreciable decline during silicic acid uptake and subsequent cell wall formation.

The present study clearly contradicts earlier reports that in L-D synchronized cultures of *N. pelliculosa*, silicic acid uptake occurs at a constant rate throughout the division cycle (Lewin, 1961, 1962). In the present L-D synchrony, silicic acid uptake was rapid and of short duration, beginning at the start of the dark period and lasting for only 3 h; moreover, the trend was similar though less marked in the second cycle of the continuous-light synchrony.

Synthesis of major cell components in *N. pelliculosa* were similar to those reported for various species of *Euglena* and *Chlorella*, with which most of the L-D synchronies have been conducted. Most of the components assayed in *N. pelliculosa* (DNA, RNA, protein, carbohydrates, carotenoids, and chlorophyll)

are synthesized in the light, with a slight continuing increase during the first part of the dark period, although chlorophyll synthesis is not completed until the end of the dark period. With the exception of the chlorophyll synthesis, this pattern is similar to that reported for *E. gracilis* (Edmunds, 1965; Cook, 1966) and *Ch. pyrenoidosa* (Ruppel, 1962). However, Kates and Jones (1967) reported that in *E. gracilis* and *Chlamydomonas reinhardtii*, RNA and protein synthesis are confined entirely to the light period; carotenoids, in both *E. gracilis* (Edmunds, 1965; Cook, 1966) and *N. pelliculosa*, and chlorophyll, in *E. gracilis* are also synthesized only in the light. However, Paasche (1968) reported that in L-D synchronies of *D. brightwellii*, using photoperiods of 6, 10, or 16 h, chlorophyll synthesis was completed during the first 10 h of the division cycle regardless of the duration or intensity of illumination. The accumulation of carbohydrates in the light, and their utilization during the dark in *N. pelliculosa* is also similar to the pattern reported for *E. gracilis* (Cook, 1966), *Ch. pyrenoidosa* (Ruppel, 1962), and *D. brightwellii* (Epply et al., 1967).

Results with the continuous light synchrony suggest that the "dark starvation" does not result in physiologically abnormal cells. It must be considered, of course that the "stress" of the dark period may persist through several generations grown in light. Nevertheless, although the cell cycle is synchronized by disrupting cellular metabolism prior to cell division, the fact is that in nature diatoms are subjected to dark periods during their cell cycle. It is interesting to note that in *N. pelliculosa*, Si deprivation of young cells (combined synchronization) did not block subsequent development prior to mitosis as is the case with *C. fusiformis* (Darley and Volcani, 1969). It is possible, however, to arrest development in *N. pelliculosa* prior to mitosis if silicic acid is removed from the culture medium during the final stages of wall formation in the previous cycle (Darley, 1969). The differences in these two diatoms with respect to the Si requirement in the division cycle is apparently more one of sensitivity rather than of a fundamental nature.

The authors wish to thank Dr. O. Holm-Hansen for the lipid carbon analysis, Dr. R.W. Eppley for the determination of cell size distributions, and Juta Kiethe and Mary-Lou Chiappino for expert assistance with electron microscopy. This investigation was supported by Grants GM-1065 and GM-08229-8-9, from the National Institutes of Health, USPHS.

References

Baker, A.L., Schmidt, R.R.: Further studies on the intracellular distribution of phosphorous during synchronous growth of *Chlorella pyrenoidosa*. Biochim. Biophys. Acta **82**, 336–342 (1964)

Busby, W.F., Lewin, J.C.: Silicate uptake and silica shell formation by synchronously dividing cells of the diatom *Navicula pelliculosa* (Bréb.) Hilse. J. Phycol. **3**, 127–131 (1967)

Cook, J.R.: Photosynthetic activity during the division cycle in synchronized *Euglena gracilis*. Plant Physiol. **41**, 821–825 (1966)

Cook, J.R., James, T.W.: Light-induced division synchrony in *Euglena gracilis* var. *bacillaris*. Exp. Cell Res. **21**, 583–589 (1960)

Coombs, J., Halicki, P.J., Holm-Hansen, O., Volcani, B.E.: Studies of the biochemistry and fine structure of silica shell formation in diatoms. Changes in concentration of nucleoside triphosphates during synchronized division of *Cylindrotheca fusiformis* Reimann and Lewin. Exp. Cell Res. **47**, 302–314 (1967a)

Coombs, J., Darley, W.M., Holm-Hansen, O., Volcani, B.E.: Studies on the biochemistry and fine structure of silica shell formation in diatoms. Chemical composition of *Navicula pelliculosa* during silicon-starvation synchrony. Plant Physiol. **42**, 1601–1606 (1967b)

Coombs, J., Halicki, P.J., Holm-Hansen, O., Volcani, B.E.: Studies on the biochemistry and fine structure of silica shell formation in diatoms. II. Changes in concentration of nucleoside triphosphates in silicon-starvation synchrony of *Navicula pelliculosa* (Bréb.) Hilse. Exp. Cell Res. **47**, 315–328 (1967c)

Coombs, J., Volcani, B.E.: Studies on the biochemistry and fine structure of silica shell formation in diatoms. Silicon-induced metabolic transients in *Navicula pelliculosa* (Bréb.) Hilse. Planta (Berl.) **80**, 264–279 (1968a)

Coombs, J., Volcani, B.E.: Studies on the biochemistry and fine structure of silica shell formation in diatoms. Chemical changes in the wall of *Navicula pelliculosa* during its formation. Planta (Berl.) **82**, 280–292 (1968b)

Darley, W.M.: Silicon requirements for growth and macromolecular synthesis in synchronized cultures of the diatoms *Navicula pelliculosa* (Brébisson) Hilse and *Cyclindrotheca fusiformis* Reimann and Lewin. Ph. D. Thesis, Univ. Calif., San Diego 1969

Darley, W.M., Volcani, B.E.: Role of silicon in diatom metabolism. A silicon requirement for deoxyribonucleic acid synthesis in the diatom *Cylindrotheca fusiformis* Reimann and Lewin. Exp. Cell Res. **58**, 334–342 (1969)

Darley, W.M., Volcani, B.E.: Synchronized cultures: diatoms. In: Methods in enzymology, Vol. XXIII, Part A, pp. 85–96. Ed.: San Pietro, A. New York: Academic Press 1971

Dawson, P.A.: Observations on the structure of some forms of *Gomphonema parvulum* Kütz. III. Frustule formation. J. Phycol. **9**, 353–365 (1973)

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F.: Colorimetric method for determination of sugars and related substances. Analyt. Chem. **28**, 350–356 (1956)

Edmunds, L.N., Jr.: Studies on synchronously dividing cultures of *Euglena gracilis* Klebs (Strain Z). II. Patterns of biosynthesis during the cell cycle. J. Cell. Comp. Physiol. **66**, 159–181 (1965)

Eppley, R.W., Holmes, R.W., Paasche, E.: Periodicity in cell division and physiological behavior of *Ditylum brightwellii*, a marine planktonic diatom, during growth in light-dark cycles. Arch. Mikrobiol. **56**, 305–323 (1967)

Holm-Hansen, O., Coombs, J., Volcani, B.E., Williams, P.M.: Quantitative micro-determination of lipid carbon microorganisms. Analyt. Biochem. **19**, 561–568 (1967)

Holm-Hansen, O., Sutcliffe, W.H., Sharp, J.: Measurement of deoxyribonucleic acid in the ocean and its ecological significance. Limnol. Oceanogr. **13**, 507–514 (1968)

Jørgensen, E.G.: Photosynthetic activity during the life cycle of synchronous *Skeletonema* cells. Physiol. Plantarum **19**, 789–799 (1966)

Kates, J.R., Jones, R.F.: Periodic increases in enzyme activity in synchronized cultures of *Chlamydomonas reinhardtii*. Biochim. Biophys. **145**, 153–158 (1967)

Lauritis, J.A., Coombs, J., Volcani, B.E.: Studies on the biochem-

istry and fine structure of silica shell formation in diatoms. IV. Fine structure of the apochlorotic diatom *Nitzschia alba* Lewin and Lewin. Arch. Mikrobiol. **62**, 1–16 (1968)

Lewin, J.C.: Silicon as an essential element for diatom cultures. In: Recent advances in botany 1, pp. 253–254. Toronto: Univ. Toronto Press 1961

Lewin, J.C.: Silicification. In: The physiology and biochemistry of algae, p. 445–455. Ed.: Lewin, R.A. New York: Academic Press 1962

Lewin, J.C., Reimann, B.E., Busby, W.F., Volcani, B.E.: Silica shell formation in synchronously dividing diatoms. In: Cell synchrony studies in biosynthetic regulation, p. 169–188. Eds.: Cameron, I.L., Padilla, G.M. New York-London: Academic Press 1966

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**, 265–275 (1951)

Paasche, E.: Marine plankton algae grown with light-dark cycles. II. *Ditylum brightwellii* and *Nitzschia turgidula*. Physiol. Plantarum **21**, 66–77 (1968)

Pirson, A., Lorenzen, H.: Synchronized dividing algae. Ann. Rev. Plant Physiol. **17**, 439–458 (1966)

Reimann, B.E.F., Lewin, J.C., Volcani, B.E.: Studies on the biochemistry and fine structure of silica shell formation in diatoms. II. The structure of the cell wall of *Navicula pelliculosa* (Bréb.) Hilse. J. Phycol. **2**, 74–84 (1966)

Ruppel, H.G.: Untersuchungen über die Zusammensetzung von *Chlorella* bei Synchronisation im Licht-Dunkel-Wechsel. Flora (Jena) **152**, 113–138 (1962)

Scherbaum, O.H.: A comparison of synchronized cell division in protozoa. J. Protozool. **9**, 61–64 (1962)

Strickland, J.D.H., Parson, T.R.: A manual of sea water analysis. Second edition, revised. Queens Printer, Ottawa, Canada. Bull. Fisheries Res. Board, Can. **125**, 117–124 (1965)

Sullivan, C.W., Volcani, B.E.: Role of silicon in diatom metabolism. II. Endogenous nucleoside triphosphate pools during silicic acid starvation of synchronized *Cylindrotheca fusiformis*. Biochim. Biophys. Acta **308**, 205–211 (1973a)

Sullivan, C.W., Volcani, B.E.: Role of silicon in diatom metabolism. III. The effects of silicic acid on DNA polymerase, TMP kinase and DNA synthesis in *Cylindrotheca fusiformis*. Biochim. Biophys. Acta **308**, 212–229 (1973b)

Tamiya, H.: Synchronous cultures of algae. Ann. Rev. Plant Physiol. **17**, 1–26 (1966)

Werner, D.: Die Kieselsäure im Stoffwechsel von *Cyclotella cryptica* Reimann, Lewin und Guillard. Arch. Mikrobiol. **55**, 278–308 (1966)

Werner, D., Pirson, A.: Über reversible Speicherung von Kieselsäure in *Cyclotella cryptica*. Arch. Mikrobiol. **57**, 43–50 (1967)

Received 14 January; accepted 26 January 1976