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The Effect of Synchronizing Dark Period on Populations of *Scenedesmus quadricauda*

V. Zachleder, J. Doucha, Eva Berková and I. Šetlík

Laboratory of Biotechnology, Institute of Microbiology, Czechoslovak Academy of Sciences, Třeboň*

Dedicated to Academician S. Prát on the occasion of his 80th birthday

Abstract. Populations of *Scenedesmus quadricauda* grown in a continuous chemostatic regime and synchronized by light and dark intervals were exposed to continuous illumination. The effect of light on synthetic and reproduction processes during the time of the omitted dark period and in the subsequent cell cycle was studied. In general, the sequence of cellular processes and their mutual coupling remain the same as in the darkened population. Synthetic processes and photosynthetic activity are depressed during the period of protoplast fission also in the light. The synchronizing effect of the dark period in chlorococcal algae consists in reducing the developmental variability in the population. The developmental state of daughter cells at the end of the dark interval varies in the span of two (as a rule) or four (at maximum) genomic cycles, while at the end of the light period this variability comprises up to eight genomic states. The more advanced autospores start the next cycle with a greater lag.

Additional index words: cell cycle, nuclear division, cellular division, DNA replication, RNA synthesis, chlorophyll synthesis, photosynthesis.

Several authors have attempted to define what attributes should characterize a good synchronous culture of a cell population (Tamiya *et al.* 1961, Baker and Schmidt 1964, Zeuthen 1964, Lorenzen 1970). The conclusions do not converge and this diversity of opinions is likely to persist. The criteria will inevitably change with various objects and with the purpose for which the culture is used. This variety of situations will be also reflected in the choice of the most adequate synchronizing procedure. Mitchison (1971) presents a brilliant critical survey of the numerous techniques available for the establishment of synchronous populations by either induction or selection (according to the classification introduced by James 1966). The synchronization of algal populations by alternation of light and dark periods belongs clearly to the induction category. It could be specified as a physiological induction method since the light-dark and dark-light transitions represent

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* *Address:* Opatovický mlýn, 379 81 Třeboň, Czechoslovakia.

a situation that algae are physiologically adapted to cope with. Consequently, in contrast to many other induction methods (*cf.* MITCHISON 1971) no major disturbance in the mechanisms controlling cell development need be expected.

Although the first synchronous cultures of chlorococcal algae were obtained by the selection method (IWAMURA 1955, HASE *et al.* 1957, TAMIYA *et al.* 1961) synchronization by light-dark (LD) cycles is at present the most widely used with these algae. The main reason for its popularity is the simplicity of handling such cultures and the ease with which populations can be maintained synchronous using this procedure for long periods of time. Both these features are based on the specific character of the cell cycle in chlorococcal algae, which includes several consecutive nuclear cycles. We have explained elsewhere (ŠETLÍK *et al.* 1972) that while the populations synchronized by LD cycles display reasonably high synchrony in cell division, the synchrony of their nuclear cycles is rather poor (*cf.* also LORENZEN 1970, SULEK 1972). This fact will be further documented in the present paper.

Even if its influence is characterized as being "physiological" the insertion of the dark interval will no doubt modify in one way or another the course of cell cycle events in the synchronized cells as compared with cells growing in continuous light. Thus, when the light-dark synchronized populations of algae are used for cell cycle studies, the questions that should be answered are the following: What is the nature of the synchronizing effect of the dark interval and what degree of synchrony can be induced by it? Is the sequence of essential cell cycle events the same in the synchronized as in the non-synchronized populations and are the same causal links preserved? Do processes, observed to occur during the dark interval in synchronized cultures have a similar course if the synchronized culture is exposed to continuous light? (*cf.* BAKER and SCHMIDT 1964, SCHMIDT 1966). In this paper we present some evidence that might help to find correct answers.

Material and Methods

Synchronous cultures of *Scenedesmus quadricauda* TURP. (BRÉB.) were grown in chemostatic continuous cultures. The culture equipment is described and its parameters are given elsewhere (ŠETLÍK 1967, 1968, DOUCHA and VENDLOVÁ 1970). In short, vessels with plate-parallel glass walls were used, with thickness of the algal suspension column 18 mm, the useful culture space 1 or 2 l and the rate of aeration about 60 cm min^{-1}. The irradiation on the surface (E_S) was 150 W m^{-2} PhAR from one side and the mean irradiance of the cells was controlled by suspension density which, in turn, was determined by the flow rate (D). In stabilized synchronous cultures grown at a constant flow rate (D = $\bar{\mu}$) the variation in absorption of the suspension layer during the cycle was reasonably small although the absorption properties of the cells vary considerably (KUBÍN and DOUCHA 1976).

Growth temperature in most experiments was 30 °C, aeration gas mixture contained 1.5% of CO_2 and the nutrient solution used in this laboratory for chlorococcal algae (ŠETLÍK 1968) was used with either urea or nitrate as a source of nitrogen.

The analytical procedures are also described elsewhere (DOUCHA and ŠETLÍK 1969, ŠETLÍK *et al.* 1969, ZACHLEDER and ŠETLÍK 1969, BERKOVÁ and DOUCHA 1970, ZACHLEDER *et al.* 1970) in more detail. The dry weight of algae per unit volume of culture was determined by spinning down 10 ml samples, washing the cells twice with distilled water and drying them immediately in the weighed centrifuge tubes at 105 °C for 24 h. RNA and DNA content in the cells was assessed in perchloric acid extracts by direct spectrophotometry and by the diphenylamine reaction, respectively, as described in more detail in LUKAVSKÝ *et al.* (1973). The analysis started again from 10 ml samples of the suspension. The pellet after the nucleic acid reaction was used for

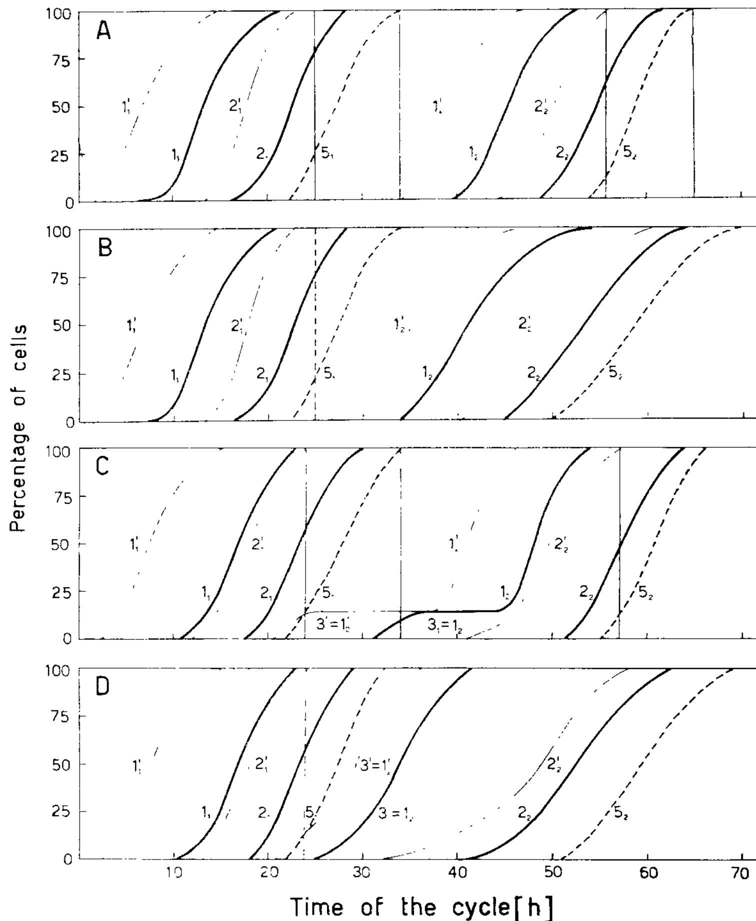

Fig. 1a

Fig. 1. The effect of the LD (A, C, E, G) and LL (B, D, F, H) regimes on the nuclear and cellular division and their inductions. The mean specific growth rate ($\bar{\mu}$) was 0.045 h^{-1} for cultures A, B, 0.05 h^{-1} for cultures C, D, 0.10 h^{-1} for cultures E, F and 0.125 h^{-1} for cultures G, H; t = 30 °C; E_s = 150 W m^{-2} for all cultures. Cultures E, F were grown on a medium with urea as nitrogen source; for the rest the medium with nitrate as nitrogen source was used.
1′, 2′, 3′ — courses of induction of the first, second and third nuclear divisions. The induction curves for the second and third nuclear divisions coincide with induction curves for cellular

protein determination for which a slightly modified method of LOWRY *et al.* (1951) was used. The quantity of photosynthetic pigments in acetone extracts of disintegrated algae and their *in vivo* absorbancy was measured and evaluated as described by KUBÍN and DOUCHA (1976). Starch was assayed by colorimetric measurements of the iodine complex using the procedure described by DUYNSTEE and SCHMIDT (1967). Nuclei were visualized for counting by a slightly modified procedure of PESHKOV and RODIONOVA (1964) and by the quick fluorochromic procedure using acridine orange (ZACHLEDER *et al.* 1974).

Photosynthesis was measured in small samples taken from the synchronous population and diluted as required to obtain a standard low absorption in the measuring vessel. Changes in oxygen concentration were recorded with a Clark type electrode as described by ŠETLÍK *et al.* (1973), in the apparatus designed by BARTOŠ *et al.* (1975).

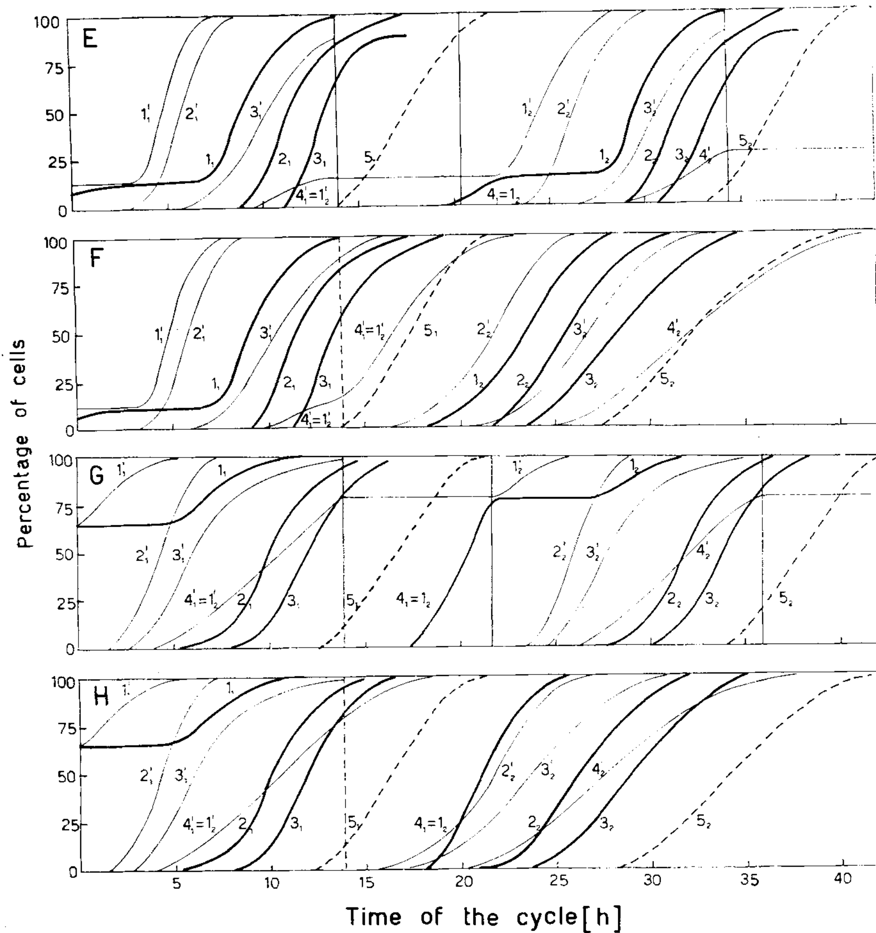

Fig. 1b

division into quadruplet and octuplet autocoenobia. Division into doublet autocoenobia did not occur at the culture conditions used. 4′ = 1′ — course of the fourth induction of nuclear division which is the first induction for the next cell cycle (in following figures it is labelled as 1′). 1, 2, 3 — course of consecutive nuclear divisions (2, 4, 8 nuclei). 4 = 1 — course of the nuclear division which is first of the next cycle (in following figures it is labelled as 1). 5 — course of liberation of autospores (in following figures it is labelled as 4). Subscripts 1 and 2 at the numbers designate curves belonging to the first and second cell cycle, respectively.

The significance of the term "induction" for various reproductive processes was explained and the method for its assessment was described by Šetlík *et al.* (1972): The induction of nuclear and cellular division was assessed by a modified version of recording the "potential cell number" originally devised by Wanka (1959) and extensively used by Tamiya *et al.* (1961). The procedure consists in removing samples from the parent culture at regular intervals and incubating them in the dark until all the division processes induced are terminated. The number of daughter cells thus formed (or, in our case, also the number of the nuclei appearing without subsequent cell division) is referred back to the time when the sample was withdrawn from the culture. In analogy the "induction" value of the content of some cellular components (DNA, RNA, starch) was determined on dark incubated samples.

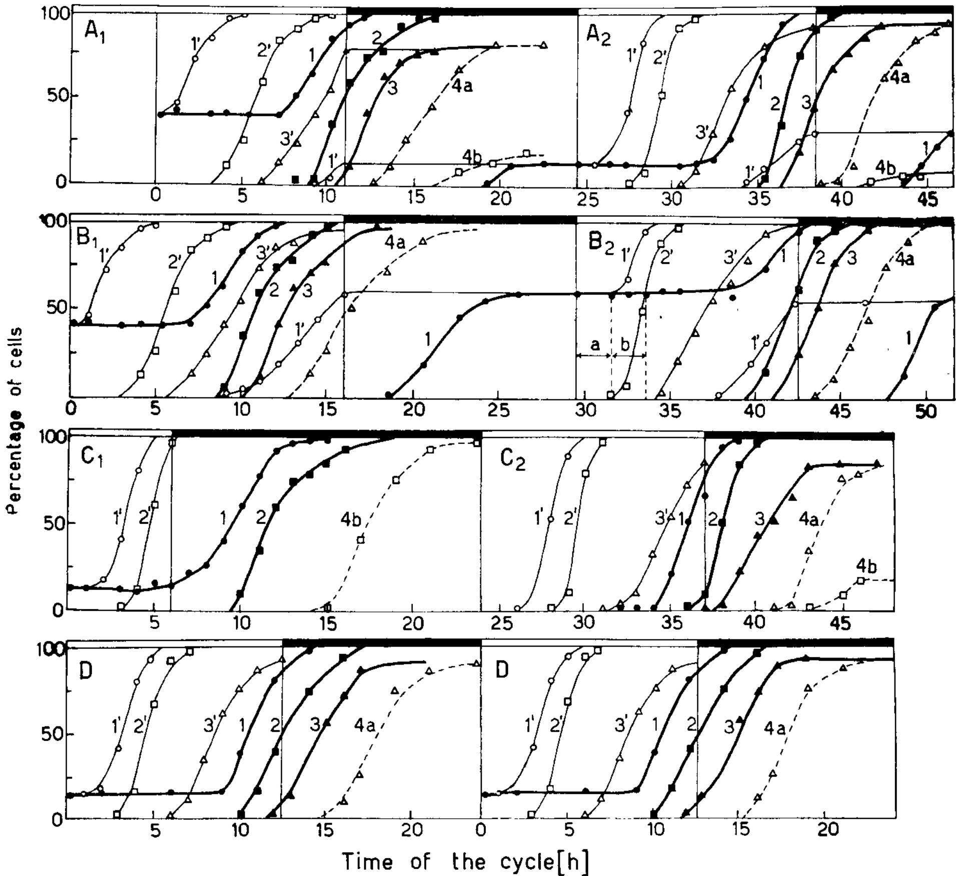

Fig. 2. The effect of the dark period started at various stages of the cell cycle on the nuclear and cellular divisions and their inductions.
A and B: The same parent octuplet population darkened early (A) and late (B). The standard time of darkening would be around the 14th h. Culture conditions: $\bar{\mu} = 0.11\ h^{-1}$; t = 30 °C; $E_s = 150\ W\ m^{-2}$. C_1 — a fast growing population darkened after the quadruplet induction was terminated. When cell division was terminated, another fast growing cycle was started (C_2). D — a twin population to that shown in C_1 grown in light until the end of the octuplet cycle. The same diagram of nuclear events is drawn twice to make comparison with both C_1 and C_2 possible. 4a — percentage of released octuplet autocoenobia, 4b — percentage of released quadruplet autocoenobia. For the labelling of other curves see Fig. 1. The meaning of segments *a* and *b* is explained in the text.

Results

Since a more detailed map of cell cycle markers for chlorococcal algae is still lacking, we have proposed to use the successive nuclear divisions and their inductions (Šetlík *et al.* 1972) for the basic characteristic of the cycle. The progress of a given nuclear division or any other event in the population is characterized by a variability that results in the sigmoidal cumulative curves (Fig. 1). We call them starting time distribution (STD) curves for the respective process, since they reflect the distribution of cells

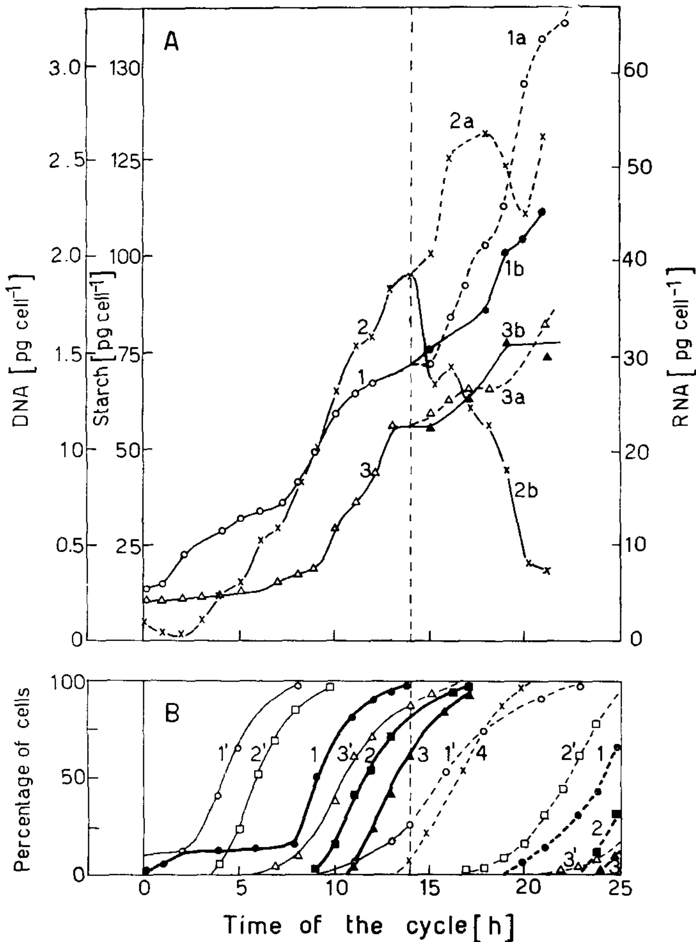

Fig. 3. The variations in the content of DNA, RNA and starch in the cells (A) and diagram of nuclear events (B) in a fast growing population divided at the end of the light period into the LD and LL variants. Culture conditions: $\bar{\mu}$ = 0.10 h^{-1}; t = 30 °C; E_s = 150 W m^{-2}.
A: 1 — RNA, 2 — starch, 3 — DNA; 1a, 2a, 3a — the course of syntheses in population growing in light (LL cycle); 1b, 2b, 3b — corresponding processes in the population darkened at the end of the cycle (LD cycle). B: Labelling of the curves as in Fig. 1.

into classes characterized by different times in the cycle when the process starts.

Fig. 1, A through H, shows pairs of STD diagrams for synchronous populations of *Scenedesmus* grown at low (A through D) and high (E through H) growth rates, which were split at the end of the first cycle into two cultures each, one grown further under the standard light-dark (LD) synchronizing conditions and the other in continuous light (LL). In all variants lights were switched off in the LD cycles at the moment when autospores release started in the population.

No effect of continuous illumination on the liberation of daughter coenobia can be seen in any case. If the time period separating the first division of

nuclei (STD curves 1_1 and 1_2) is examined*, it is found to be shorter in the LL cycles of all variants. The curves 1_2 are shifted forward over a distance that is shorter (A, B, and G, H), of equal length (E, F) or longer (C, D) than the dark interval. This can be understood in view of the following facts: (1) During the period of protoplast fission the rate of many synthetic processes is depressed. (2) The darkened cells make some progress in their development at the expense of material and energy reserves (starch) so that starting the next cycle they are further on the developmental scale but without reserves, which they must replenish. (3) The dark interval also causes some retardation in the start of the development of autospores liberated in the dark. The factors mentioned under (1) and (2) tend to make the advancement of the first nuclear division in the LL cycle less than the length of the dark period in the corresponding LD cycle, while factor (3) works in the opposite direction. The resultant effect will therefore depend on the relative importance of each of these components in a particular case.

The distances between the midpoints of homologous STD curves of nuclear division are about the same in the LD and LL cycles, and the same is true in general of the corresponding induction curves. The conspicuous extension of the $1_2' \rightarrow 2_2'$ and $1_2 \rightarrow 2_2$ intervals in the LL cycle D as compared with these intervals in the LD cycle C is not (only) the result of the absence of the dark interval; the same dilution rate was applied to the LD and LL cycle and, as a result of dilution of LD cycle during the dark period,

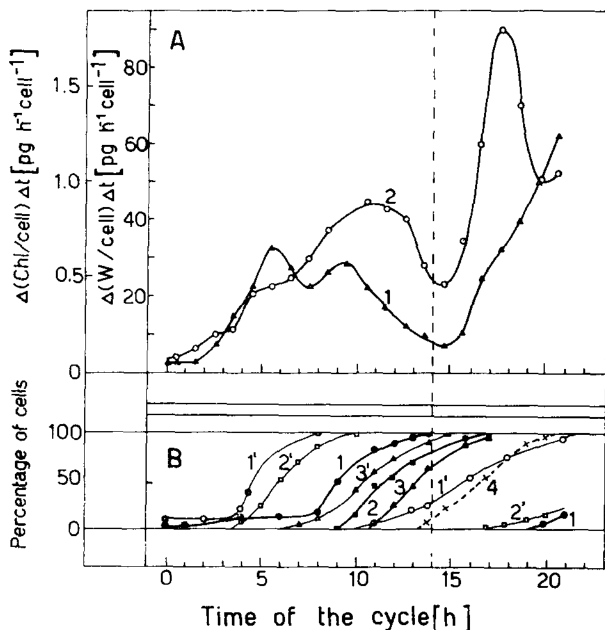

Fig. 4. The rates of chlorophyll synthesis (1) and dry weight accumulation (2) in the LL variant of the octuplet culture of Fig. 3 (part A). Nuclear events and autospore liberation are shown in part B. Labelling of the curves in part B as in Fig. 1.

* Whenever the distance of two STD curves is considered without further specification in this paper, it is the distance of their midpoints that is meant.

the LL cycle begins with a higher density. In other variants this fact was partly compensated by one step dilution at the time when autospore release became terminated. In cycle C this dilution was omitted. On the other hand the greater distance between curves $1'_2$ and $2'_2$ in the LL cycle F as compared with the second LD cycle E is the consequence of the different conditions to which autospores released in the dark and in the light are exposed. The competence of the cells to enter nuclear division if incubated in the dark, traced by the induction curves, results from both the developmental stage of the cell and its accumulated energy reserves. The autospores released in light are rich in the latter, but are not much more advanced developmentally than the autospores in the dark. Consequently, if incubated in the dark (as for the assessment of induction) they show up as potentially more developed. If growing further in the light, they have to put the energy influx from photosynthesis into growth and development, while the developmentally advanced autospores after the dark period divert more of it into stored reserves. Thus, after some time of growth in light both types of cells become less different as to their capacity for further development in the dark. Accordingly, the position of the second induction curve ($2'_2$) is not very different in the corresponding LD and LL cycles.

It may appear unexpected that in the second cycles of all LL variants the STD curves of nuclear division inductions run consistently less ahead of STD curves of nuclear division than in the corresponding LD cycles. Shorter distance between the induction of nuclear division and its actual occurrence in the cycle means less available reserves in the cells and/or slower growth rate. Both conditions characterized the LL cycles as compared

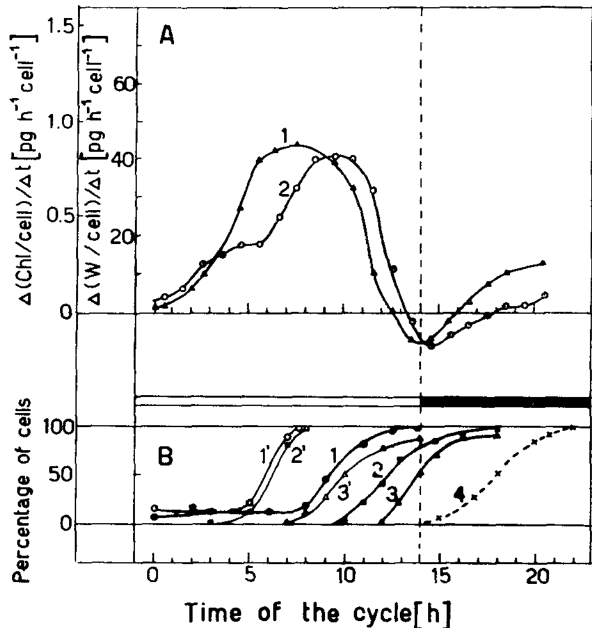

Fig. 5. The rates of chlorophyll synthesis and dry weight accumulation (part A) and nuclear evenst and autospore liberation (part B) in an octuplet LD cycle. Culture conditions: $\bar{\mu} = 0.10\ h^{-1}$; $t = 30\ °C$; $E_s = 150\ W\ m^{-2}$.
1 — the rate of chlorophyll synthesis per cell d(Chl/cell)/dt;
2 — the rate of dry weight accumulation per cell d(W/cell)/dt.
Labelling of the curves in part B as in Fig. 1.

with LD reference cycles in the experiments described here. The dilution rate was the same in both alternatives and in the LD cycles dilution continued even during the dark period. Thanks to the higher average irradiation obtained over the whole cycle the concentration of the LL population increased to higher values than in the LD cultures even in those experiments in which a one-step dilution was undertaken at the time when autospore release was terminated.

The variance of all STD curves is considerably greater in the LL cycles than in the LD cycles (Fig. 1) thus indicating the incipient desynchronization of the population. Hence the synchronizing effect of the dark period consists in retarding the development of the more advanced cells relative to the less advanced ones, *i.e.* the earliest released autospores begin the next cycle with a greatest lag in development while the latest are least retarded. The validity of this statement is illustrated *e.g.* by Fig. 1A and 1B. The tops of curves 2_1 and 1_2 characterizing the late cells are at a similar distance in the LDL and the LL cycles, while the bottom part of curve 1_2 which represents the most advanced subpopulation starts in the LL cycle much earlier than in the LD cycle. The difference is still more pronounced with the corresponding induction curves $2'_1$ and $1'_2$ in which the enhancement in the bottom parts amounts to nearly half the time interval separating the tops of the two curves.

In continuous light the most advanced autospores (Fig. 1B) need about half the time required by the tardiest ones to develop from the second nuclear division in the first cycle (curve 2_1) to the first nuclear division induction (curve $1'_2$) of the second cycle. The advanced autospores nearly reach this point in the first light period of the LDL cycle and, evidently, they manage to complete the rest during the dark period so that a very small amount of light in the next cycle makes them competent to nuclear division. They are therefore only halted in development by the dark period.

This differs from what is seen in other parts of Fig. 1. Curves in parts C and D indicate clearly that apart from the delay of further development for the length of the dark period, the latter causes a definite lag in development at the start of the light period. Curve $1'_2$ in the LDL cycle does not start immediately upon light is turned on but some four to five hours later, which is more than the time required by the advanced autospores released in light to proceed from 2_1 to $1'_2$. A very similar effect is observed with the fast growing octuplet*) cycle in parts E and F of Fig. 1. Both these cycles (C and E) also demonstrate that retardation caused by the dark period is the same for cells that entered the dark period before first nuclear division was induced in them as for those cells for which the darkening came after this point. The second nuclear division and its induction are in both cases traced by smooth curves ($2'_2$ and 2_2) which in their bottom parts include cells starting the second light cycle with two nuclei.

The same may be observed consistently in all similar cycles, *e.g.* in those shown in Fig. 2A and B. The latter demonstrate in addition that the time

*) In the following text two-, four-, and eight-celled coenobia will be also called doublets, quadruplets and octuplets, respectively, and the consecutive divisions will be designated in the same way.

at which the dark period starts has no appreciable influence on its synchronizing effect. In Fig. 2A lights have been switched off five hours earlier than in Fig. 2B. In A, there is a small proportion of binucleate cells in the four-celled coenobia (15%) while in B more than one half of cells in the purely octuplet population are binuclear. Except for this difference, or in spite of it, the course of nuclear events in the subsequent cycles is similar. The first cycle illustrated in parts A and B of Fig. 2 was preceded by one darkened at the time (most often used in our experiments) when autospores release started in the population. The situation obtained after the dark period is intermediary to that for the second cycles in A and B.

A logical extension of the last experiment is the very early darkened cycle in Fig. 2C. The lights were switched off as soon as the quadruplet induction (curve 2′) was terminated in the tardiest cells. Consequently, a population consisting of four-celled coenobia is present at the start of the next cycle. When it was made to grow at a high specific growth rate (through high mean irradiation density) the course of events was hardly different from that in the first cycle, which started with octuplet coenobia. For comparison the twin-population from the first cycle that was darkened after octuplet induction is drawn (twice) in part D of Fig. 2. It is also interesting to compare the early darkened, fast growing cycle (Fig. 2C) that yields quadruplet coenobia with a quadruplet yielding, slow growing cycle, which can be darkened only upon the release of the first autospores.

The following general statements can be deduced from the above results. When a population growing in the light is darkened, cells of all age groups develop further at the expense of their reserves. The maximum progress that reproductive processes can make in the dark is three nuclear divisions plus the corresponding cellular divisions (*cf.* the bottom part of curve $4'_1 = 1'_2$ in Fig. 1G and the top part of curve 3′ in Fig. 2, B_2). The rate of processes in the sequence leading to nuclear division is about the same in the dark as in the light (demonstrated by equal distances of homologous STD curves). The occurrence of a given nuclear division step (*e.g.* from 2 to 4 nuclei) can be postponed somewhat in the light as compared with its position in the dark, if initiation of the next division step takes place in the light (*cf.* Fig. 2, C_1 and D). This has a retarding influence on some processes in the nuclear division sequence.

The variability in the rate of processes leading to initiation of nuclear division among the cells in the population is greater than the variability of the actual rate of these processes. This causes *e.g.* the consecutive nuclear division inductions to desynchronize more (the STD curves to get flatter) than the actual nuclear divisions (*cf.* Fig. 2, B_1 and B_2). The last statement suggests that apart from being more variable the time required to accumulate sufficient reserves for a given nuclear division sequence is much shorter than the time necessary for its realization. A particularly suitable example is again provided by Fig. 2B. At the end of the dark period separating the two cycles (B_1 and B_2) a fairly heterogeneous population of autospores is present. The most advanced cells corresponding to the lowest part of curve 1 are binuclear and most probably bigenomic, nearly prepared to divide their two nuclei into four. The mean cells in the population are either binuclear and with unigenomic nuclei (just below the horizontal segment of curve 1)

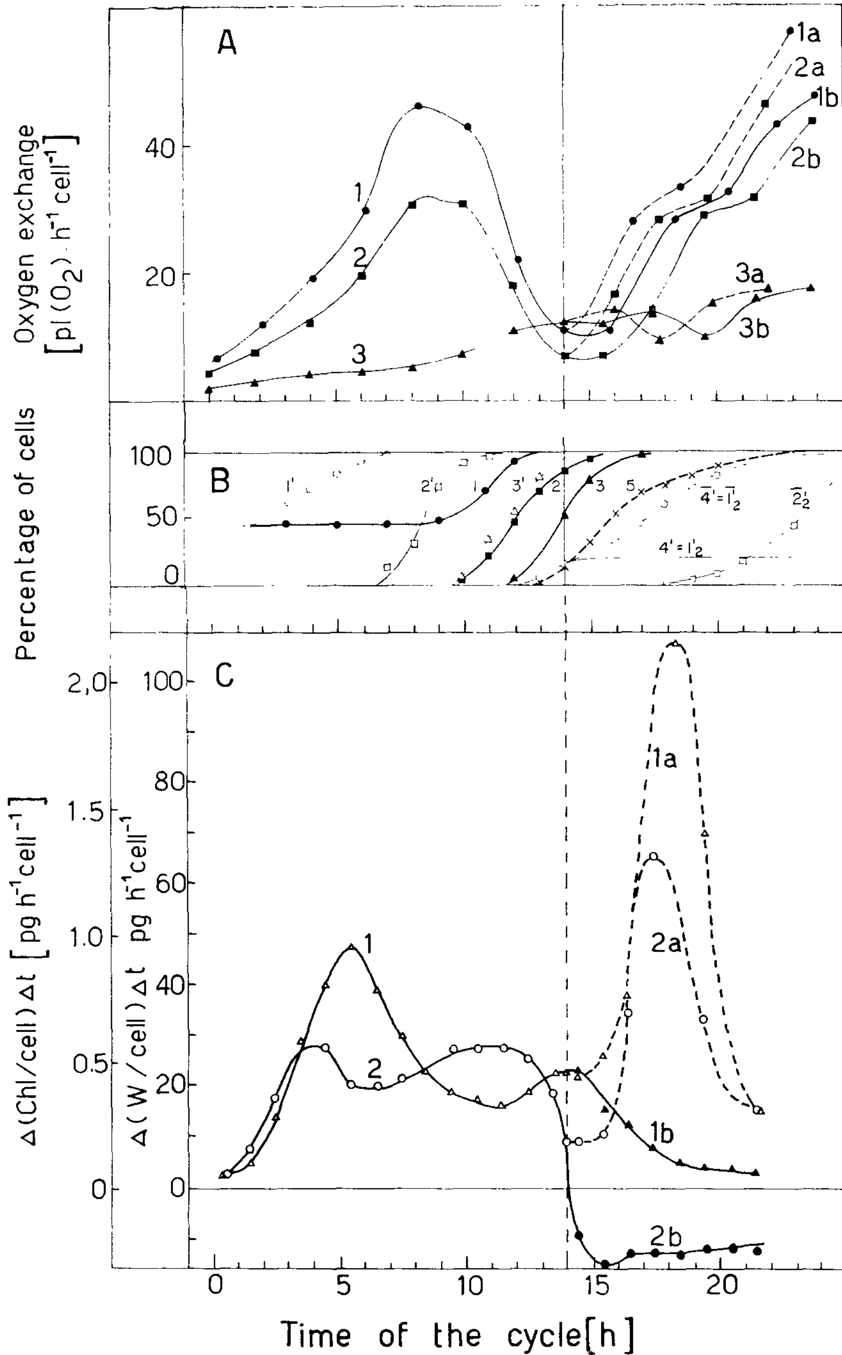

Fig. 6. Rates of photosynthesis, respiration, dry weight accumulation and chlorophyll synthesis in an octuplet cycle. At the 14th h of the standard cycle the population was split into the illuminated and darkened part. Culture conditions: $\bar{\mu} = 0.10\ h^{-1}$; t = 30 °C; $E_s = 150\ W\ m^{-2}$.

or uninuclear and bigenomic (just above the horizontal segment of curve 1). Finally the tardiest cells, characterized by the utmost tops of the STD curves are uninuclear and unigenomic. For both uninuclear and binuclear bigenomic cells the reserves required to realize the nuclear division step are acquired in the time interval (a); the time interval (b) is the additional time that unigenomic cells of both types need to accumulate reserves for another genome duplication and the corresponding transcription and translation processes. Both curves 1′ and 2′ are rather steep since the cells were aligned to a state with more or less exhausted reserves at the end of the dark period. Curve 3′ shows that the fast cells (bottom) acquire reserves required for the next nuclear division much faster than the slow cells (top). One can assess, of course, only the competence to divide that is so much advanced in the fast cells. Evidence to assume that it is also the initiation proper that starts earlier in the fast cells comes from such situations as illustrated *e.g.* in Fig. 1B, D and E (comparing curves 2_1 or 3_1 and 1_2). In any case the enhancement of actually performed steps, *e.g.* nuclear division, is less than that of their induction (in the advanced cells as compared with the tardy ones).

Fig. 3 shows the changes of DNA, RNA and starch amounts per cell in a fast growing octuplet yielding synchronous population that was split at the end of the cycle into a darkened subpopulation and another growing further in the light. As can be expected, the light and dark curves for starch content display the most dramatic difference in their course. In the dark the reserves become exhausted to the negligible amount characteristic of the autospores. In the light the slight retardation of starch accumulation at the time of protoplast fission (see caption to Fig. 3) is followed by further increase and a depression corresponding to that seen in young autospores at the start of the cycle. In both situations this depression occurs at the time of vigorous RNA synthesis. The latter is also retarded at the time of protoplast fission, but afterwards, it runs much faster in the light than in the dark. Nevertheless, even the dark increase is substantial showing that the genomes in the autospores go on with transcription. As a result, a new wave of DNA replication is apparently initiated in a fraction of the population. Increase in DNA for the period analyzed is about the same in the two variants although a somewhat different course is observed.

Variation in the rate of dry weight (W) and chlorophyll (Chl) synthesis for the LL subpopulation of this experiment is shown in Fig. 4. On the rate curves the depression of synthetic activity during protoplast fission is better seen than on the concentration curves of Fig. 3. The maximum rate of W increase coincides with the fast RNA synthesis in Fig. 3, while the rate of Chl synthesis increases steadily, proportionately to the amount of RNA present. What course of d [W]/dt and d [Chl]/dt can be expected in a LD cycle is shown in Fig. 5. The data come from another cycle than data of

A: 1 — photosynthesis measured at 105 W m^{-2} PhAR, 2 — photosynthesis measured at 50 W m^{-2} PhAR, 3 — respiration; 1a, 2a, 3a — rates of syntheses of the population growing in light (LL cycle), 1b, 2b, 3b — rates of syntheses of the population growing in dark (LD cycle).
B: diagram of nuclear divisions, their inductions and autospore liberations. Labelling of the curves as in Fig. 1. C: 1 — the rate of chlorophyll synthesis per cell d(Chl/cell)/dt; 2 — the rate of dry weight accumulation per cell d(W/cell)/dt; 1a, 2a — LL cycle, 1b, 2b — LD cycle.

Fig. 4, and the degree of synchrony was better in this case. As a result, the drop in both rates at the time of protoplast fission is more pronounced. Since the minima on the curves occur still in light, it is clear that their depth has nothing to do with the ensuing dark interval.

The rates of W and Chl synthesis for a population split at the end of the cycle into a light and a dark variant are compared with the rates of photosynthesis in Fig. 6. The synthetic rates show similar relations as in the foregoing examples except for the fact that the d [Chl]/dt curve also goes through a maximum coinciding with that on the d [W]/dt curve. We have shown earlier (BERKOVÁ *et al.* 1972) that the minimum in photosynthetic activity (PSA) occurring at the end of the light period of the cycle parallels the frequency of cells in the population in which protoplast fission occurs. Fig. 6 shows that there is little difference in this variations of PSA between populations that divide in the dark and in light. From the comparison of parts A and C of the figure it can also be deduced that the increase in the PSA up to the level equal to its maximum in light also occurs in the dark where no appreciable synthesis of Chl takes place. This process therefore represents only a recovery of the photosynthetic apparatus present in mother cells. The steeper increase of curves 1 and 2 (part A) in the upper part of the double wave reflects the formation of new portions of the chloroplast, as indicated by the peak of Chl synthesis rate (curve 1, part C).

Fig. 7 illustrates the changes of starch content in the split population of Fig. 1 C and D. It is remarkable that cells whose growth is strongly limited by light accumulate nevertheless a substantial amount of starch through nearly the whole cycle. The temporary increase in the induction values for starch occurs just at the time when the induction to the second nuclear division starts in the population. It appears that cells which have not sufficient reserves for the whole nuclear division step do not attempt it and stop in development while still containing noticeable reserves.

Fig. 8 presents data from an experiment in which analyses of DNA, RNA and protein were made during the whole course of a LL cycle. The LD cycle

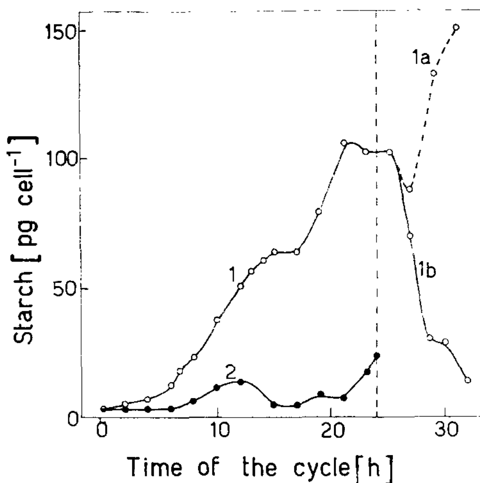

Fig. 7. Variation of starch content in the LD and the corresponding LL cycle. Nuclear diagrams for the same populations are shown in Fig. 1, C and D.
1 — starch content per cell, 1a — the LL variant, 1b — the LD variant; 2 — the "induction" values of starch content.

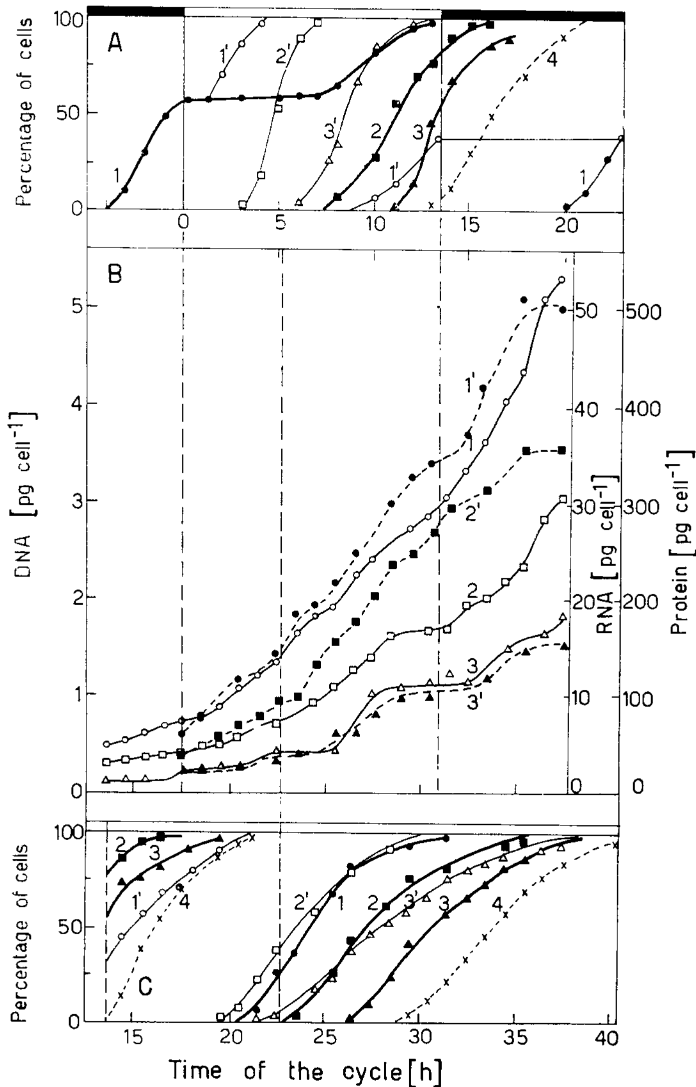

Fig. 8. The variations in the content of DNA, RNA and protein in the cells (B) and diagrams of nuclear events for a synchronized population (A) and one in which one dark time was omitted (C). At the beginning of the dark period a fast growing octuplet population was split into an illuminated and darkened part. The course of syntheses in the continuously illuminated culture (LLL) is compared with that in the preceding synchronized cycle (DLD). The curves are drawn in such position that the courses of DNA synthesis in DLD and LLL cycles approximately coincide.

A: Nuclear events in the DLD cycle. B: Syntheses of RNA (1), protein (2), and DNA (3) in the standard DLD cycle; dashed curves with primed symbols trace the course of syntheses in the continually illuminated variant. C: Nuclear events in the LLL cycle. Labelling of the curves in parts A and C as in Fig. 1.

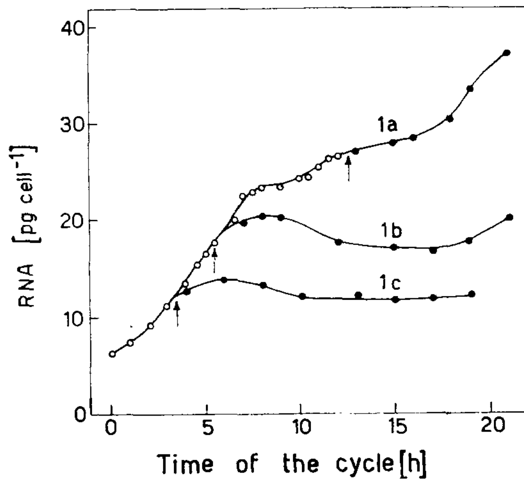

Fig. 9. Variations in the content of RNA in *Scenedesmus* cells in an octuplet cycle that was darkened after the light periods of various lengths. Culture conditions: $\bar{\mu}$ = 0.10 h^{-1}; t = 30 °C; E_t = 150 W m^{-2}. Open circles — RNA synthesis in the light; 1a, 1b, 1c (full circles) — RNA synthesis after darkening; arrows indicate time of darkening. Corresponding nuclear events are shown in Fig. 2 C_1 and D.

characteristic for the synchronized population is also represented. The nuclear events for the latter are shown in part A, those for the LL cycle in part C. The time scales are mutually shifted so as to obtain best fit of the DNA/cell curves in both cycles. This condition is met when the start of the DL cycle is fixed at about four hours after the start of the LL cycle, *i.e.* when this population continues to develop in the light while its reference cycle was darkened. The amount of RNA and protein approach similar values at the end of the period analyzed but their courses are far from coinciding. The synthesis rates are lower in the LL than in the LD cycle. This would not be paradoxical, if stabilized populations in continuous culture were compared, one exposed to LD cycle, the other in continuous light and both growing with the same $\bar{\mu}$. Such a situation, however, does not prevail during the first cycle growing in light. In the present case the primary cause of the deviation is perhaps again a greater density of the population in the LL regime resulting from the fact that the dilution rate has not been increased to compensate for the dilution of the LD culture during the dark period. Otherwise the course of synthetic events in the dark and in the light is in agreement with all the results presented above.

Discussion

Perhaps the most striking and readily observable effect of the dark period is its synchronizing influence. This has two components in chlorococcal algae. The first is linked with the fact that several nuclear cycles may constitute the cell cycle. Consequently, the cells in the population may be found in any of the *n* steps performed before cell division is reached under the given growth conditions. And they have always sufficient reserves to reach the lowest state by division processes occurring in the dark. It appears that in *Scenedesmus quadricauda* more energy is required to pass from nuclear division to protoplast fission than in the various *Chlorella* strains. As a result, binuclear cells are often formed when processes run in the dark and reserves become exhausted. This may happen either just upon the nuclear division

step or in the course of further development of the binuclear cell that may even lead to the subsequent genome replication. In such cases the population of the cells following a dark interval is anything but homogeneous. It contains two different nuclear states each in two genomic states, *i. e.* four different genomic states altogether. The maximum number of genomic states that can be present in the population is eight: four nuclear states (one, two, four and eight nuclei) and each in two genomic states again If such variability exists at the end of the light period and the dark period ends with four genomic states, the variability becomes reduced by one half. If the above highly variable population yields through division in the dark only uninuclear cells (with one or two genomes) the asynchrony drops to one fourth of the value before the dark interval. All situations actually encountered will fall between these two extremes, so that asynchrony drops one half to one fourth through the division processes in the dark interval.

The other synchronizing factor is the variable lag inflicted to autospores in various developmental stages. It appears that the lag is proportional to the development the cells have taken in the dark. One could speculate on an unstable component essential for development whose amount produced is proportional to the realized progress in transcription but which is decomposed in the dark. In analogy with other cell cycle studies an unstable messenger or ribosomal RNA could be considered. Although some decline in the RNA concentration is occasionally observed in the darkened population (*cf.* Fig. 9), this is most often followed by another wave of synthesis at the time new autospores are formed. A net decrease in RNA amount is therefore not very important. Another possibility is that only inactivation occurs. The active state of the ribosomes is known to depend markedly on the energy charge in the cell (*cf.* BOULTER *et al.* 1972). The cells at the end of the dark period are consistently very poor in reserves. Therefore a fraction of the ribosomes present may be found in the inactive state and it may cost energy to put them again to work.

In any case RNA synthesis in the cells dividing in light runs very fast as soon as it passes the temporary depression accompanying the period of protoplast fission. In combination with high energy supply from photosynthesis and from high reserves this may constitute a condition of high activity. On the other hand at the beginning of the DL cycle (*cf.* also ŠETLÍK *et al.* 1972, ZACHLEDER *et al.* 1970) a slow start of RNA synthesis is often observed and energy supply is also sluggish.

The striking retardation of all synthetic processes during protoplast fission is interesting since in other eukaryotic cells this condition usually accompanies mitosis (*cf.* MITCHISON 1971), which is as a rule tightly coupled with cell division. This coupling is dissociated in cells of *Scenedesmus quadricauda* in which as a rule protoplast fissions set in only after the last nuclear division occurred. It appears, therefore, that the major part of the inhibition of synthetic processes is linked with protoplast and not with nuclear division.

Turning back to the questions raised in the introduction it appears that our results provide a least definite answer as to the primary process in the sequence of synthetic events which are affected in the dark. This will require further experiments; some suggestions for their planning may come from the evidence presented in this paper.

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V. ZACHLEDER, J. DOUCHA, E. BERKOVÁ, I. ŠETLÍK (Třeboň): **Vliv synchronizujících období tmy na populace *Scenedesmus quadricauda*.** — Biol. Plant. **17** : 416—433,, 1975.

Populace *Scenedesmus quadricauda*, kontinuálně kultivované v chemostatu a synchronisované obdobími světla a tmy jsme vystavili trvalému osvětlení. Sledovali jsme jeho vliv na průběh syntetických a reprodukčních pochodů během období, v němž kultura bývá zatemněna a ještě v průběhu následného buněčného cyklu. Současně jsme analysovali populace dále synchronisované. Ukázalo se, že sled buněčných pochodů a jejich vzájemné vztahy zůstávají na světle v podstatě stejné jako u zatemňované kultury. Stejně jako u zatemňované kultury dochází na trvalém světle k poklesu rychlosti makromolekulárních syntéz a fotosyntetické aktivity během dělení protoplastu. Synchronisační efekt období tmy u chlorokokálních řas spočívá v obnovování poměrně úzké vývojové variability synchronisované populace. Variabilita ve stupni vývoje se u dceřiných buněk na konci období tmy snižuje a pohybuje se obvykle v rozpětí jednoho jaderného cyklu a nanejvýše v rozpětí dvou cyklů. Vše nasvědčuje tomu, že u autospor, uvolněných ve tmě, dochází na počátku dalšího cyklu k zadržení vývojových pochodů, které je tím větší, čím větší úsek vývoje autospory předtím prodělaly ve tmě.

BOOK REVIEW

O'CONNOR, M., WOODFORD, F. P.: **Writing Scientific Papers in English.** — Elsevier-Excerpta Medica - North Holland, Amsterdam-Oxford-New York 1975. 108 pp. Dfl. 21.00.

The rapid progress of science is reflected in an upsurge of valuable information supplied by scientific papers. The presentation of the reports, however, is often not adequate to their content; their value is depreciated by stilted, verbose and inaccurate writing.

The European Association of Editors of Biological Periodicals (ELSE) jointly with its Style Manual Committee have presented the first of a series of manuals which should provide the writers of scientific papers with instructions and advice of how to prepare papers of required standard. In nine chapters, wittily written, successive steps are outlined that the writers will take from planing a report to its final proof reading.

The book is complemented by five important appendices, containing Units of measure and their abbreviations, General abbreviations and symbols, Abbreviations used in biochemistry and taxonomy; the last appendix that brings a list of expressions to avoid and those to prefer will be particularly welcome by scientists whose mother tongue is not English. The Committee intends to prepare companion booklets with guidelines for special language groups and for the different scientific branches. Bibliography of references cited and recommended additional reading and a subject index are enclosed.

The book should be on the shelves of every research institute and the suggestions given should be followed by all "who want readers to understand, remember and even enjoy their papers".

J. ČATSKÝ (*Praha*)