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The Effect of Light on DNA Synthesis and Related Processes in Synchronous Cultures of *Chlorella*

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Summary. In autotrophic cultures of *Chlorella* synchronised by alternating light and dark periods of 16:8 hours the DNA content duplicated normally 4 times successively during the S_m phase, i. e. between the 10th and 18th hour after the beginning of the light period. This finding together with electron microscopical observations revealed that one duplication of the DNA and of the nuclei per cell proceeds every 110 minutes. All nuclei of a cell seem to undergo successive DNA syntheses and nuclear divisions synchronously. The rate of DNA synthesis was independent from illumination. On appropriate reduction of the light period the last duplication cycle fell out and the average final spore number per cell was accordingly lower.

If a culture was transferred to darkness or low light intensity 3 hours before the normal end of the light period the release of spores was promoted by approximately $1^1/_2$ hours, provided a strong decrease of metabolically accessible carbohydrates was prevented by either an additional short illumination during the dark period or by continuing the weak light.

A possible explanation for the shortening of the cell development is that, by passing over one DNA duplication and one protoplast division, the cell can enter sooner the respective subsequent developmental stages.

Synchronous cultures of unicellular algae have been widely employed in biochemical studies of the cellular life cycle soon after adequate methods for maintaining synchronous growth had been developed. The results were recently reviewed by TAMIYA (1966) and PIRSON and LORENZEN (1966). Most successful use was made in determining the time sequence of the different steps in cell development and the biochemical processes connected with these steps (e.g. IWAMURA, 1955; WANKA, 1959; LORENZEN and RUPPEL, 1960; SOEDER and RIED, 1962). More recently evidence has been provided that enzymes which are engaged in synthesis of DNA precursors are produced just before DNA synthesis (SHEN and SCHMIDT, 1966; JOHNSON and SCHMIDT, 1966).

In *Chlorella* cultures synchronised by repeating cycles of 16 hours light and 8 hours darkness DNA accumulation takes place between the 10th and 18th hour. During this period the DNA content of the cell becomes multiplied several fold in contrast to the single duplication in a

normal division cycle in growing tissues. We will therefore use the designation S_m in order to indicate the difference from the conventional S period. DNA synthesis was found to proceed also in the dark, but the final DNA content was lower, i.e. fewer DNA duplications took place when the illumination was terminated after 12 hours instead of after the usual 16 hours (WANKA, 1962b). From the view point of regulation it is of interest to know whether the lower DNA content is due to a reduced rate of DNA synthesis during an S_m phase of normal duration or whether the S_m phase itself is terminated sooner when cells are transferred to darkness after 12 hours. In the latter case it would seem possible that the subsequent developmental steps are slightly advanced. A slight advancement of the release of spores in *Chlorella* strain *7-11-09* by a shortening of the light period has already been reported by SOROKIN (1960) and by PIRSON (1962). Moreover, a promoting effect of darkness on protoplast division was observed in *Chlorella* strain *211-8b* (WANKA, 1965). The advantage of this *Chlorella* strain lies in the fact that S_m and sporulation are separated by a longer time interval and can therefore be studied more individually. The main aim of the present study was to point out correlations between DNA synthesis and other processes involved in cell division.

Methods

Chlorella strain *211—8b* from the algae collection of the Institute of Plant Physiology of the University of Göttingen has been grown as described previously (WANKA, 1965). Alternating periods of 16 hours light and 8 hours dark were used. The light intensity was 10 kilolux from the two opposite directions, temperature was 30° C. Cultures were bubbled with air containing 0.5—0.75% CO_2. Protoplast division was studied by use of the technique introduced by SOEDER and RIED (1962). The method for extraction and determination of DNA was the same as described in previous work (WANKA, 1962a; 1962b). For electron microscopy the cells were fixed as indicated and dehydrated by graded alcohol. Electron micrographs were taken with a Philips EM 100 electron microscope.

Results

a) DNA Synthesis and Nuclear Division

During the S_m phase the DNA content increases 16 fold on the average. The course of DNA synthesis was followed during the main part of this period. From the linear and semilogarithmic plots given in Fig. 1 it is obvious that DNA increases exponentially from the 12th until almost the 16th hour. The most adequate explanation for this is that the rate limiting factor in DNA synthesis is the amount of template available. From the slope of the semilogarithmic plot a duplication time of 105 min can be obtained. Under the conditions used in our experiment duplication times varied between 105 and 115 min. Longer apparent

duplication times under the same conditions probably mean that the synchrony of the cell growth is not complete.

The rate of DNA synthesis was unchanged for $2^1/_2$ more hours if the light period was terminated after $12^1/_2$ hours. Then it slowed down and after another $1^1/_2$ hours the DNA content remained constant; see Fig. 2 A. In the control with 16 hours light the strong DNA increase continued up to the 17th hour and the final DNA content was about $1^1/_2$ times as high. This strongly suggests that the enzymic DNA polymerization is not limited by light dependent processes as it was found to be the case

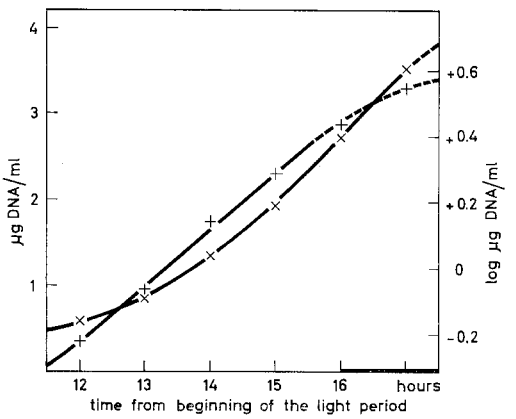

Fig. 1. Increase of the DNA content during the main period of DNA synthesis in a synchronous culture. +——+ logarithmic plot; ×——× linear plot

for RNA synthesis (WANKA, 1962b; SENGER and BISHOP, 1966) but that the initiation of further DNA duplications will cease earlier in cultures which are transferred to darkness before the end of the normal light period of 16 hours. Previous observations revealed that, as a consequence of a decrease of the light intensity, induction of cell divisions ceases for several hours (WANKA, 1959). In order to examine whether DNA synthesis might be effected in a similar way as by a transfer to darkness, a culture was transferred from 2×10 kilolux to 2×1.5 kilolux after 13 hours. As Fig. 2 B shows the effect on the increase of the DNA content is very similar to the effect of a light-dark shift. In both cases about 25% of the final DNA amount were synthesized during the $1^1/_2$ hour when the rate of synthesis slowed down. The independence of DNA synthesis from photosynthesis is in agreement with the finding that it is not inhibited by 3-(3,4-dichlorophenyl)-1,1 dimethylurea (SENGER and BISHOP, 1966).

Spore formation was previously shown to be completely inhibited by colchicine if applied 14 hours after the onset of illumination (WANKA, 1965). It was concluded from this findings that the cells must be mono-

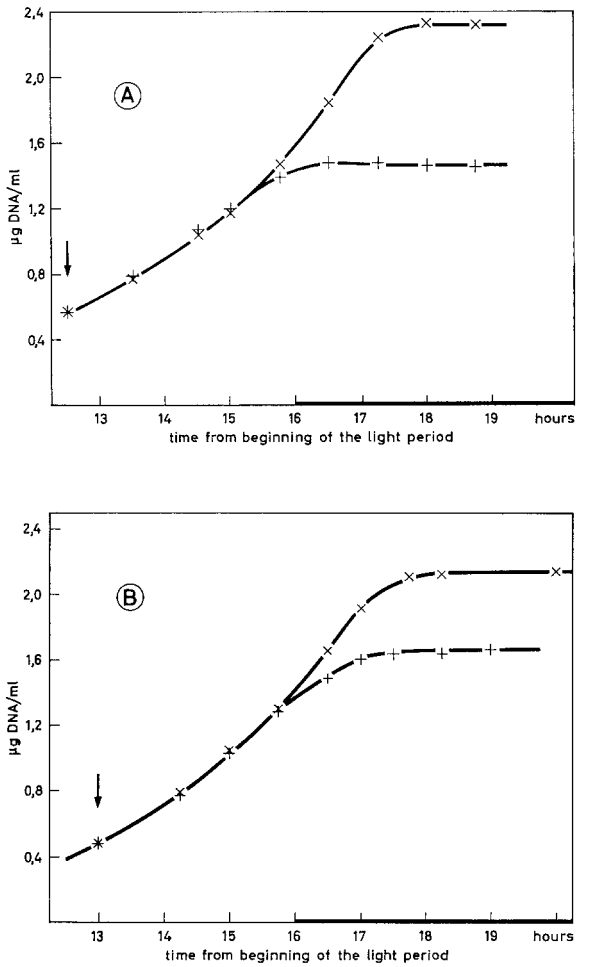

Fig. 2 A. Response of DNA synthesis on advanced termination of the light period. +——+ light period terminated after $12^1/_2$ hours (↓), ×——× control (16 hours light)

Fig. 2 B. Response of DNA synthesis on decrease of the light intensity during S_m phase. +——+ light intensity was reduced from 2×10 kilolux to 2×1.5 kilolux after 13 hours of illumination (↓); ×——× control

nucleate up to this time. This conclusion, however, was not confirmed by recent electron microscopical studies. At least 50% of the cells were found to be already dinucleate after $13^1/_2$ hours and there were even a few per cent of tetranucleate cells like the one shown in Fig. 3. Since the average DNA content per cell has increased about 3 fold by this time,

the most acceptable interpretation seems that DNA duplication and nuclear divisions alternate regularly during the S_m period.

In sections containing two or more nuclei, either all of them were dividing completely synchronously (10 unequivocal cases were observed

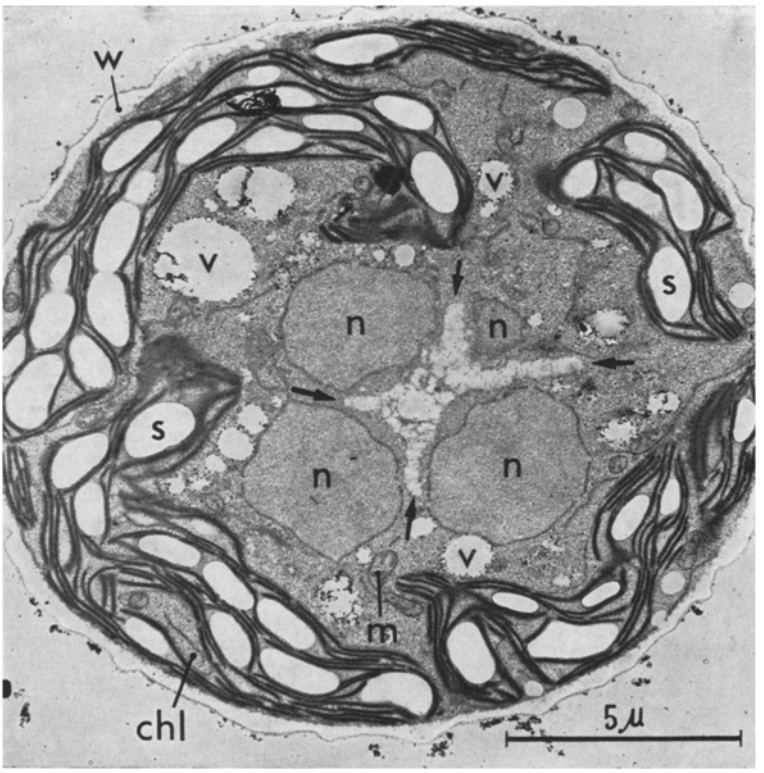

Fig. 3. Electron micrograph of a tetranucleate cell. After $13^1/_2$ hours of growth the cells were fixed for 2 hours in 2% glutar aldehyde at 0° C and post-fixed, for 1 hour in 1% $KMnO_4$ according to LUFT (1956). Sections were post-stained with lead (REYNOLDS, 1963). *chl* chloroplast; *m* mitochondria; *n* nuclei; *s* starch grains; *v* vacuoles; *w* cell wall; arrows indicate the planes of the beginning protoplast divisions

so far; see Fig. 4 A) or all were in the resting state (estimated to approximately 90%). In dividing nuclei the chromatin is condensing in a equatorial plane comparable to the metaphase. It subsequently divides into two portions which move to the poles of the strongly elongating nucleus (Fig. 4 B). Apparently nuclear division comes off by amitosis. Final cleavage stages were not found until now. On $KMnO_4$ fixation the intact nuclear membrane was found during the whole division cycle. A cyto-

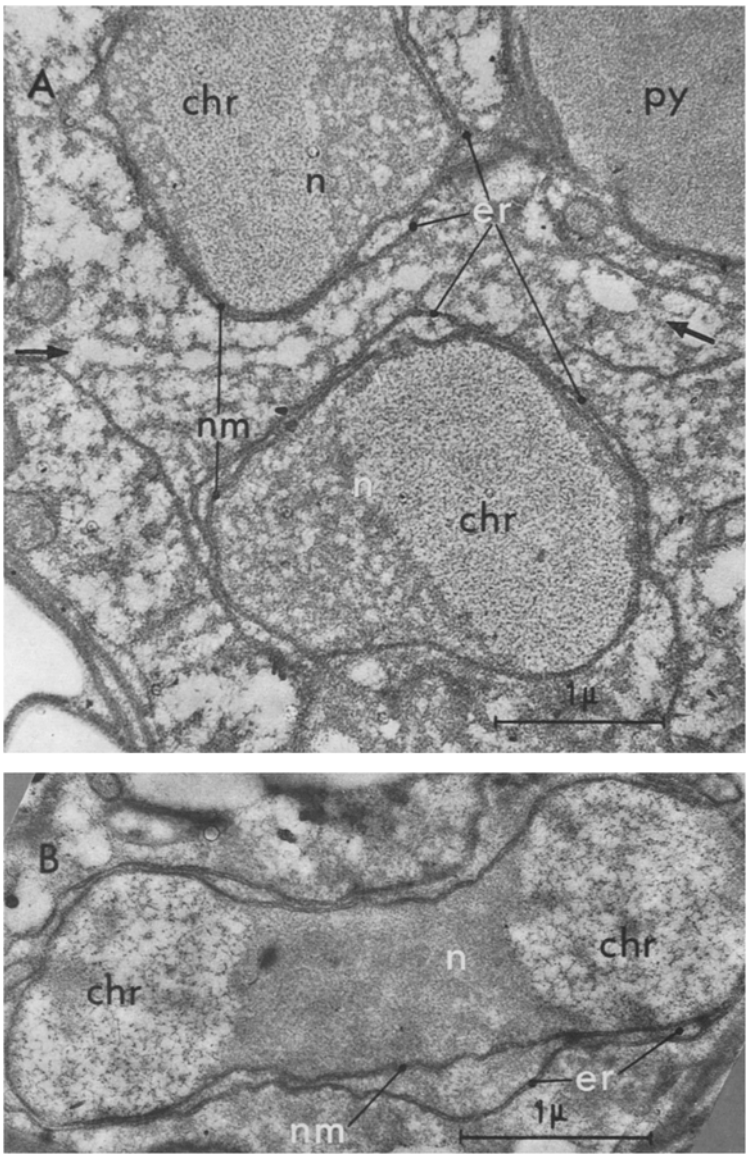

Fig. 4. Electron micrographs of dividing nuclei taken after $14^1/_2$ hours of growth. *chr* condensed chromatin; *er* cytoplasmic membranes; *nm* nuclear membranes; *py* pyrenoid. A. Two synchronously dividing nuclei. Fixation 15 min in $KMnO_4$ (LUFT, 1956) at 30° C, followed by 1% OsO_4 for 1 hour at 0° C and 2% aquous solution of uranyl acetate for 2 hours. Poststained with lead. B. Late division stage. Fixation: 1 hour in 1% $KMnO_4$ at 0° C

plasmic membrane surrounding the nucleus almost completely was found to be typical of nuclear division and for this reason is useful for identification in case of doubt due to unfavourable orientation of sections.

b) Protoplast Division and Release of Spores

Electron micrographs showed that protoplast division ensues already after 13 hours but it usually took 2 more hours until dyads could be found by the technique of plasmolysis as a result of completion of the first division plane. It could further be observed that the lay-out of the

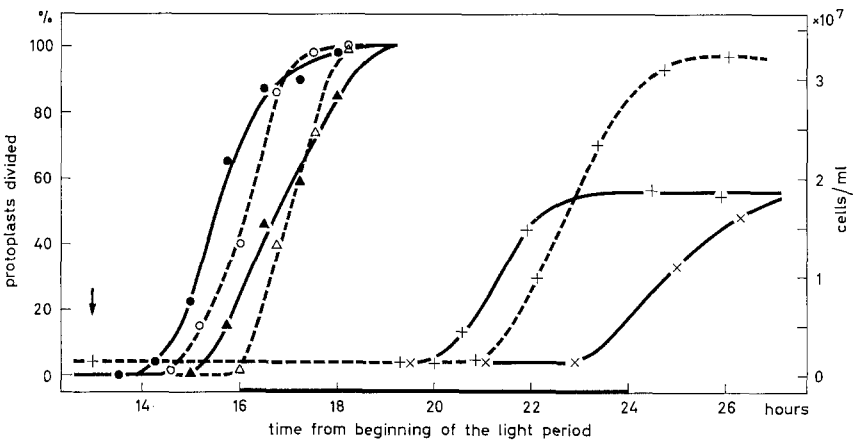

Fig. 5. Response of protoplast division and liberation of spores on advanced termination of the light period. Light period terminated after 13 hours (↓): •——• dyad stages completed, ▲——▲ tetrad stages completed, ×——× cell number, + ——+ cell number on application of an additional light period from 16 until $18^1/_2$ hours after the onset of illumination. Control (16 hours light): o-----o dyad stages completed, △-----△ tetrad stages completed, + -----+ cell number

second plane begins long before the first one is completed (Fig. 3). A slight advancement of the protoplast division usually appeared if the cells were transferred to darkness or weak light after 13 hours. However, the experiments were poorly reproducible with regard to the extent of advancement, varying between 0 and 1 hour. The slight advancement of the dyad and tetrad stages shown in Fig. 5 and 6 are representative for an average experiment. In contrast the release of spores seemed even more or less retarded under the same conditions. The retardation, however, seems to be the consequence of less favourable nutritional conditions due to longer duration of the dark time prior to the breakage of the cell wall, or to the lower light intensity during the last 3 hours of the normal light period. This became clear when in another culture the reduction of

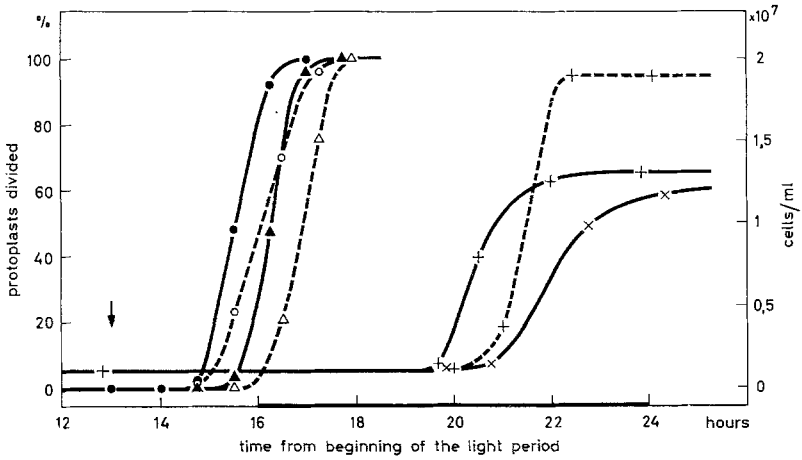

Fig. 6. Response of protoplast division and liberation of spores on reduction of the light intensity. Light intensity was reduced from 2×10 kilolux to 2×1.5 kilolux 13 hours after the beginning of the light period: •——• dyad stages completed, ▲——▲ tetrad stages completed, ×——× cell number, +——+ cell number obtained on continuing the weak light during the normal dark period. Control: The same signs as used in Fig. 4

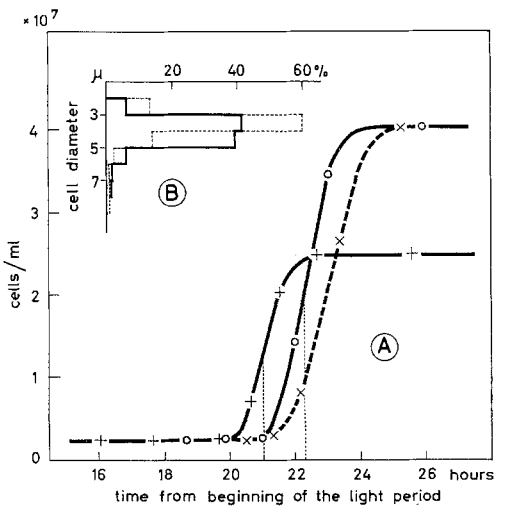

Fig. 7 A. Response of spore liberation on reduction of the light intensity. ×---× control: 16 hours light of 2×18 kilolux and 8 hours dark; o——o 16 hours 2×18 kilolux and 8 hours 2×1.5 kilolux; +——+ 13 hours 2×18 kilolux and 11 hours 2×1.5 kilolux

Fig. 7 B. Response of the cell size on reduction of the light intensity. Cell diameters were measured with a Coulter Counter 23 hours after onset of the illumination when the release of spores was almost completed: ⋯ 16 hours strong light and 8 hours weak light; ⌐¬ 13 hours strong light and 11 hours weak light

the light period was compensated by exposure of the cells to a second light period of $2^1/_2$ hours beginning at the 16th hour or, in the case of the weak light experiment, by continuing the weak illumination beyond the normal duration of 16 hours.

In both cases the release of spores occurred approximately $1^1/_2$ hours before the control, but the final cell number was equal to the one found in the normal transfer experiment, i.e. about $^2/_3$ of the control. If a culture is transferred to the weak light after 16 hours the release of spores appears only slightly earlier than in the control, whereas the culture transferred after 13 hours is still another $1^1/_2$ hour in advance (Fig. 7). In the first case the cell number was equal to the control, whereas with only 13 hours strong light the cell number is approximately $60^0/_0$ of it, but as can be seen from the size distribution given in Fig. 7B the cells are slightly larger. An average cell volume of 69 μ^3 was calculated for the spores obtained after 13 hours strong light and of 48 μ^3 for the ones obtained after 16 hours strong light.

Discussion

The cyclic alternation of light and dark periods belongs to the most convenient approaches for maintaining synchronous growth in unicellular algae. In *Chlorella* the first 8 hours of a 16 hours lasting light period were found to have purely trophical function, whereas in the subsequent 6 to 8 hours light exerts a strong stimulation of cell division, the times depending to a certain degree on culture conditions (WANKA, 1959, 1965; LORENZEN and RUPPEL, 1960). In the overall induction two different phases have to be distinguished. One is the primary induction of cell division leading usually to the formation of 4 spores per dividing cell, and the other is the increase of the number of spores per cell, which is effectuated if the cells remain in the light after the primary induction. The final numbers of spores for each cell were found to belong to the series 2^n ($n = 1, 2, \ldots$; see SOEDER and RIED, 1962; LORENZEN, 1965). This can only be understood if the involvement of a special regulation mechanism is assumed. Such a mechanism might for example intervene in DNA synthesis in a way which guarantees that if DNA replication occurs, it will do so in all DNA equivalents of the cell that have already arosen from preceding replications. Since the DNA equivalents are distributed to the daughter nuclei by nuclear divisions which soon follow every individual S phase, the simultaneous initiation of nuclear DNA replications would seem to be controlled by extranuclear forces. The cytoplasmic control of the initiation of DNA synthesis in *Stentor* and in *Amoeba proteus* has recently been demonstrated by nuclear transplantation experiments (PRESCOTT and GOLDSTEIN, 1967; DE TERRA, 1967).

Mononucleate *Chlorella* cells contain about 10^{-13} g DNA during G_1 period (WANKA, 1965). This is equivalent to 2 DNA double helices of 1.5 cm length, a size which is also found for chromatids of higher organisms. If chain growth would proceed from one point only at the speed calculated by CAIRNS (1963) for other organisms at a temperature of 37° C, one duplication would require 3 to 16 hours. Therefore either the chain growth in *Chlorella* has to be much faster, or the total DNA amount must be divided into subunits which can replicate simultaneously if the duplication has to be completed in less than 110 min. The residual time available for nuclear division may in any case be expected to be rather limited. This makes it easy to understand, why micrographs on nuclear divisions have not yet become available in spite of a reasonable number of electron microscopical studies on *Chlorella* (MURAKAMI *et al.*, 1963; SOEDER, 1964, 1965; BISALPUTRA *et al.*, 1963; ASHTON *et al.*, 1963; MERCER *et al.*, 1962; RODRIGUEZ-LOPEZ, 1965). The assumption is furthermore compatible with the finding that DNA synthesis continues for $1^1/_2$ hours, i.e. about 80% of the duration of one duplication cycle after cessation of initiations of new DNA replications. Whereas RNA synthesis is strongly affected by illumination, DNA synthesis proceeds completely independent of it and thus seems to rely essentially on endogenous substrate. The strong endogenous respiration found between the 12th and 22nd hour (RIED *et al.*, 1963) might therefore have a well defined function in DNA replication and cell division. One can easily imagine that it would be damaging for the cell if the supply of precursors would suddenly cease during DNA replication. In this light the advantage of a mechanism which guarantees the completion of a DNA duplication independent of external factors becomes obvious.

After a full light period the development in the dark seems to proceed at an optimal rate, since the promoting effect of illumination was found to be rather small (see also LORENZEN and SCHLEIF, 1966). However, the effect is quite remarkable in cultures which are transferred to darkness or weak light after 13 hours. This might be the consequence of a strong decrease of the carbohydrate content (RUPPEL, 1962) especially that of starch (MÜLLER, 1961) in the dark. It is therefore advisable to study correlations between time and extent of DNA synthesis on the one hand and time of liberation of the spores on the other by a transfer to weak light after 13 hours. It then becomes obvious that the termination of the cell cycle is advanced by about 1 to 2 hours. It seems possible that, by an earlier termination of the DNA synthesis, the cell will sooner enter the next developmental stage i.e. protoplast division, and a second promoting effect may result from the fact that in a large number of cells the last protoplast division is passed over. Whether the first does exist is not yet certain, however, because of the poor reproducibility of the

advancement of the dyad and tetrad stages in the transfer experiments. Also the response of protoplast division to external conditions seems to be very strong (e.g. CO_2; see SOEDER *et al.*, 1964).

The retardation of cell division by light of *Chlorella vulgaris* (TAMIYA *et al.*, 1961) cannot be explained in the same way, since almost identical final cell numbers were obtained in the dark and in the light. Whether the explanation is valid for a similar retarding effect of the light on cell division in *Chlorella* strain *7-11-09* (PIRSON, 1962; SOROKIN, 1960) cannot be decided since the final cell numbers, necessary for an adequate interpretation, were not reported. SOEDER *et al.* (1966) have recently found a considerable retardation of the release of spores by illumination when the cultures were aerated with a gas mixture containing 5% CO_2 but not at low CO_2-concentrations. The findings of SOROKIN (1960) and TAMIYA *et al.* (1961) are most probably due to a similar inhibition by the combined action of light and high CO_2-concentrations.

In the preceding paper (WANKA, 1965) it was found that cell division is completely inhibited by colchicine if added at a time when cells are already tetranucleate according to the present finding. Therefore, the conclusions which have been based on the assumption that colchicine specifically inhibits nuclear division have to be abandoned in favour of the division pattern discussed above. This is also in better agreement with the findings of SOEDER (1962) and of LORENZEN and SCHLEIF (1966). Colchicine must thus inhibit nuclear and protoplast division. A publication on this object is in preparation.

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