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Effects of 3,4-benzopyrene on the course of cell cycle events in the chlorococcal alga *Scenedesmus quadricauda*

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Abstract. Synchronous cultures of the chlorococcal alga *Scenedesmus quadricauda* were grown under optimal growth conditions. The mean length of their cell cycle was approximately 20 h. The cultures were treated at the start, at the 4th, and 8th hour of the cell cycle with 3,4-benzo(a)pyrene (BP) in the range of 0.1–0.5 μg ml^{-1} of final concentration. A period about 4 h was found within which no inhibitory effects could be detected even at the highest BP concentrations used. In presence of BP the rates of RNA and protein syntheses gradually decreased until complete inhibition of net syntheses occurred. In a similar way chlorophyll synthesis was inhibited, and this was followed by gradual degradation of the chlorophyll. The higher the concentration of BP the more rapid the decrease of the rates of syntheses and the earlier their complete inhibition. At low BP concentrations while DNA replications were initiated, the number of replications was lowered. At higher concentrations the initiations of DNA replications were delayed or completely suppressed. Syntheses of saccharides were the least inhibited processes in presence of BP. Starch synthesis was slowed down at the end of the cell cycle and fructose synthesis (free and sucrose bound) was even stimulated later in the cell cycle. The release of daughter coenobia, and protoplast fissions were most susceptible to BP treatment, being affected at concentrations which produced no measurable disturbances of macromolecular syntheses. At BP concentrations at which the inhibition of macromolecular syntheses occurred, the delay or suppression of mitoses was observed.

Key words: Chlorophyta – 3,4-Benzo(a)pyrene – Cell cycle – *Scenedesmus*.

Abbreviations: BP = 3,4-benzo(a)pyrene; PhAR = photosynthetically active radiation

Introduction

3,4-Benzo(a)pyrene (BP) and other polycyclic aromatic hydrocarbons are well known as powerful carcinogens (Selkirk 1977). This is one of the reasons why a considerable amount of information has recently been published about their metabolism and their interactions with cellular constituents (Pezzuto et al. 1978; Cohen et al. 1980). In spite of the fact that the most of the metabolic intermediates have been recognized (Freudenthal et al. 1975), the critical biological event is so far unknown. There is an increasing body of evidence that DNA could be a critical cellular target (Meehan et al. 1977; Belvedere et al. 1980), but other targets such as RNA or proteins cannot be excluded (Ivanovic et al. 1978).

While much work has been carried out on the effects of BP on animal cells there is little information on influence on plant cells. There is evidence, however, that plants can accumulate and metabolise BP into toxic or nontoxic substances in a similar way to animal cells (Durmishidze et al. 1974; Harms et al. 1977; Trenck and Sandermann 1978).

Apart from the work of Bartholomew et al. (1979), little information has been published on the effects of BP on the cell cycle in animals, and there is virtually no information on the effects on algal cells.

The present study concerns the effects of BP on the course of reproductive and synthetic processes during the cell cycle of the chlorococcal alga *Scenedesmus quadricauda* in synchronized cultures. This alga was chosen because previous work had produced a large amount of information concerning its cell cycle and physiological characteristics (Šetlík et al. 1972; Zachleder et al. 1975; Zachleder and Šetlík 1982).

This paper describes experiments in which the cells of *Scenedesmus quadricauda* were grown

under optimal conditions and BP concentrations varied in order to study the effects of this chemical on various events in the cell cycle.

Material and methods

Organism. The chlorococcal alga *Scenedesmus quadricauda* (Turp.) Bréb. strain Greifswald/15 was obtained from the culture collection kept at the Institute of Experimental Botany, Prague, Czechoslovakia (now at the Institute of Botany, Třeboň, Czechoslovakia).

Culture conditions. The cultures were synchronized by alternating light and dark periods (14:10 h). The suspensions of synchronous cells of *Scenedesmus quadricauda* were cultivated in plateparallel vessels illuminated from one side. In some experiments glass cylinders of 400 ml volume were used. Incandescent lamps (500 W) served as a light source. Irradiance at the surface of the cultivation cuvettes was approximately 180 W m^{-2} photosynthetically active radiation. Carbon dioxide concentration in the aerating gas mixture was maintained at between 1.5 and 3% (v/v). The inorganic nutrient solution was that described by Šetlík (1968). The culture cuvettes were submersed in a water bath at a constant temperature of 30° C. Batch cultures or semicontinuously and continuously diluted cultures were used for experiments. Details of culture equipment and conditions were the same as those described by Doucha (1979). Continuous illumination was employed during experiments with BP.

Determination of induction curves. The samples were withdrawn at one- or two-hour intervals during the growth of synchronous cultures and aerated in the dark at 30° C. The samples were fixed by iodine kalium-iodide solution at the end of the control cell cycle and the percentages of the binuclear cells, four-celled, eigth-celled daughter coenobia and undivided mother cells were estimated. The values were plotted against the time at which the samples had been darkened. The curves obtained are termed the induction curves. The significance of the term "induction" for various reproduction processes was explained and the method for its determination was described by Šetlík et al. (1972).

Cell number. The number of cells, or the fractions of induced quadruplet and octuplet daughter coenobia, and undivided mother cells were counted in the Bürker counting chamber (produced by Meopta, Czechoslovakia).

Staining of nuclei. Nuclei were fluorochromed by acridine orange and observed through the fluorescent microscope. Details of the procedure were described elsewhere (Zachleder et al. 1974).

Dry matter. The suspension of algae (10 ml) was centrifuged and the sediment of the algae was washed with distilled water and dried at 105° C for 24 h in an oven. The whole procedure took place in 10 ml centrifuge tubes in which the dry matter was also weighed.

Chemicals. All chemicals used for the analyses were of analytical grade. The DNA, RNA, casein, and albumin for calibration assays were obtained from Serva Heidelberg Ltd, FRG. Other chemicals were supplied by Lachema Ltd, Prague, Czechoslovakia.

Total nucleic acid assay. The procedure of Wanka (Wanka 1962) as modified by Lukavský et al. (1973) was employed in the assay of total nucleic acids. The samples were centrifuged in 10 ml centrifuge tubes, which also served for storage of the samples. The sediments of the algal cells sampled were stored under 1 ml of ethyl alcohol at −20° C.

The algae were extracted 5 times with 0.2 N perchloric acid in 50% ethyl alcohol for 50 min at 20° C and extracted three times with an ethanol-ether mixture (3:1) at 70° C for 10 min. Such pre-extracted samples can be stored in ethyl alcohol. Total nucleic acids were extracted and hydrolysed by 0.5 N perchloric acid at 60° C for 5 h. After hydrolysis, concentrated perchloric acid was added to reach the final 1 N concentration of perchloric acid in the sample. Absorbance of total nucleic acids in the supernatant was read off at 260 nm.

DNA assay. The light-acctivated reaction of diphenylamine with hydrolyzed DNA, as described by Decallonne and Weyns (1976), was utilized. Diphenylamine reagents (4% diphenylamine in glacial acetic acid, w/v) mixed with the samples of total nucleic acid extracts at the ratio of 1:1, were illuminated from two sides with fluorescent lamps (Tesla Z 40 W). The incident radiation from each side was 20 W m^{-2}. After 6 h of illumination at 40° C, the differences were identified between the absorbance at 600 nm and that at 700 nm.

RNA content calculation. The RNA content was calculated as a difference between the total nucleic acid content and the DNA content.

Protein assay. The sediment remaining after nucleic acid extraction was used for protein content determination. A Lowry's Folin-phenol method was employed in protein estimation (Lowry et al. 1951).

Chlorophyll assay. The amounts of chlorophylls *a* and *b* present was assessed in acetone extracts of disintegrated algae. The calculation of the chlorophyll content from absorbances measured at 645, 664, and 720 nm was carried out the method of Mc Kinney (1941). The procedure, modified for *Scenedesmus quadricauda,* has been described by Lukavský et al. (1979).

Fructose assay. The method of Roe et al. (1949) was used for the gross fructose content determination (both free and sucrose-bound fructose) with the following modification.

A paste of algal cells was disintegrated by vortexing with 1 cm^3 of glass beads (diameter 250–350 μm) in 0.3 ml of 80% ethyl alcohol for 3 min. Disintegrated algae were extracted three times with 80% ethyl alcohol for 20 min at 70° C. The extracts were collected and made up to 10 ml with 80% ethyl alcohol; 4 ml of the extract diluted in this way were mixed with 4 ml of benzene. After centrifugation and cooling (4° C for 1 h) the benzene was sucked off. From the water phase, 1 ml was taken and 0.5 ml of resorcinol reagent (100 mg resorcinol and 250 mg thiourea in 100 ml of acetic acid) and 3.5 ml of 30% HCl were added. The reagent mixture was stirred and heated at 80° C for 10 min, cooled and measured immediately at 520 nm. Calibration was carried out with fructose at each stage of the analytical procedure.

Starch assay. The following modification of the method of McCready et al. (1950) was used. The sediment remaining after fructose analysis served for starch content determination. Three ml of 30% perchloric acid were added to the sediment, stirred for 15 min, and centrifuged. This procedure was repeated three times and perchloric acid was added to collected supernatants to obtain of a final volume of the extract of 10 ml. 1 ml of the extract was cooled to 0° C; 5 ml of anthrone reagent (200 mg of anthrone in 72% H_2SO_4) was then added and stirred. The mixture was kept in a water bath at 100° C for 7 min. It was then cooled to 20° C and the absorbance was measured at 630 nm. Calibration was carried out simultaneously with the sample analysis and glucose served as a standard chemical for calibration. The measured values were multiplied by 0.9 to get calibration curve for starch determination.

Modifications of the fructose and starch assays were devised by J. Doucha (Třeboň, Czechoslovakia) – personal communication.

The measurement of irradiance. In order to define light conditions in suspensions of varying density and irradiated by different incident irradiance, the mean irradiance (I) was determined from the irradiance at the surface of the culture vessel (I_i) and from irradiance transmitted through the suspension (I_t) (Doucha 1979):

$I=\frac{I_i-I_t}{\ln I_i/I_t}$. The values of irradiance are expressed in W m^{-2} PhAR.

The incident (I_i) and transmitted radiation were measured by a phytoactinometer devised and constructed in the author's laboratory (Třeboň, Czechoslovakia) by Š. Kubín (Kubín 1971).

Benzopyrene treatment. 3,4-Benzopyrene was supplied by FERAK, Ltd. West Berlin. Stock solution of 100 µg BP per ml of acetone was used for dosage of BP into experimental cultures. Acetone was also added to the controls in these experiments. Various final concentrations of BP (0.1–0.5 µg ml^{-1}) were applied because of a different sensitivity of various cell cycle events and macromolecular syntheses. Very low solubility of benzo(a)pyrene in water (0.004 µg ml^{-1} – Davis et al. 1942) is strongly improved by using of a BP stock solution in acetone, then added to water. These solutions are opalescent, but stable for several days. Under these conditions BP is not crystallized again or absorbed by the glass-wall of the vessel (Wittenburg and Abarzua unpublished results). Assuming that BP even if not completely solubilized was homogeneously dispersed in algal suspensions because they were vigorously stirred, we express the amount of added BP in µg per ml of algal suspension. Furthermore, also the total volume of suspension and cell number are given in legends to figures.

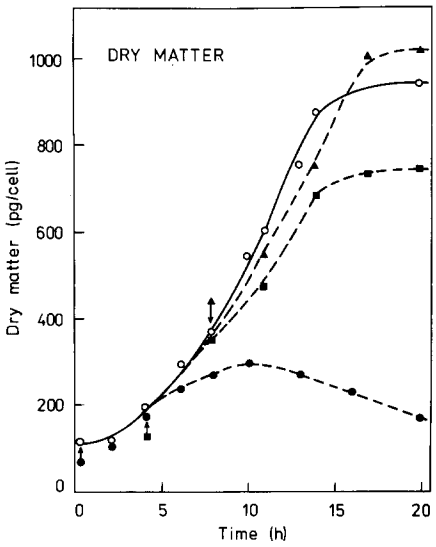

Fig. 1. The course of dry matter increase in control and BP-treated synchronous cultures of *Scenedesmus quadricauda*. Benzopyrene (BP) was added at the beginning (●), at the 4th (■), and at the 8th (▲) hour of the cell cycle in concentration 0.3 µg ml^{-1}. Time of addition is illustrated by vertical arrows with corresponding symbols. The control culture (○), and BP-treated cultures grew at incident irradiance 200 W m^{-2} photosynthetically active radiation (PhAR). Mean irradiance was 120 W m^{-2}. Plateparallel cuvettes (2,000 ml volume) served as a culture vessels. The cultures were not diluted during the experiment. Initial absorbance at 750 nm and cell number were 0.1 and $1.5 \cdot 10^6$ ml^{-1}, respectively

Results

3,4-Benzopyrene was added to the experimental cultures at the beginning of the cell cycle or at the 4th and 8th hour of the cell cycle, respectively. The latter times were chosen because the quadruplet and octuplet inductions of cell division were attained at these times (Zachleder et al. 1975). The present studies suggest that there is a period of a few hours (approx. 4 h) between the addition of BP and the first response of the cells of *Scenedesmus quadricauda* (Figs. 1, 2, 3).

Growth processes

Dry matter. The addition of BP at the beginning of the cell cycle produced a gradual decrease in the rate of dry matter growth. The results of the experiment where the increase in dry matter stopped after completion of the second third of cell cycle (10 h of light) are illustrated in Figs. 1 and 3. The application of BP later in the cell cycle (after 4 or 8 h of light) had little, if any, effect on the increase of the dry matter (Fig. 1).

RNA and protein syntheses. At high concentrations of BP net synthesis of RNA and protein is prevented. However, even at lethal concentrations of BP this inhibition does not occur until 3 or 4 h after the BP application (Fig. 3). The time after which the synthesis of RNA and protein is inhibited is dependent on the concentration of BP. At low BP concentrations synthesis of RNA and proteins is slowed down during the cell cycle but not completely stopped (Fig. 2). In addition, the application of BP later in the cell cycle has less of an inhibitory effect on RNA protein synthesis.

Chlorophyll synthesis. The syntheses of the principal photosynthetic pigments (chlorophylls *a* and *b*) appear to be the processes most effectively inhibited by BP. This inhibition seems to be coupled with the blockage of protein synthesis, because the inhibition of chlorophyll syntheses occurs simultaneously with that of protein synthesis (Figs. 2, 3). After the chlorophyll synthesis had ceased, a gradual degradation of the chlorophylls started and the cells became bleached.

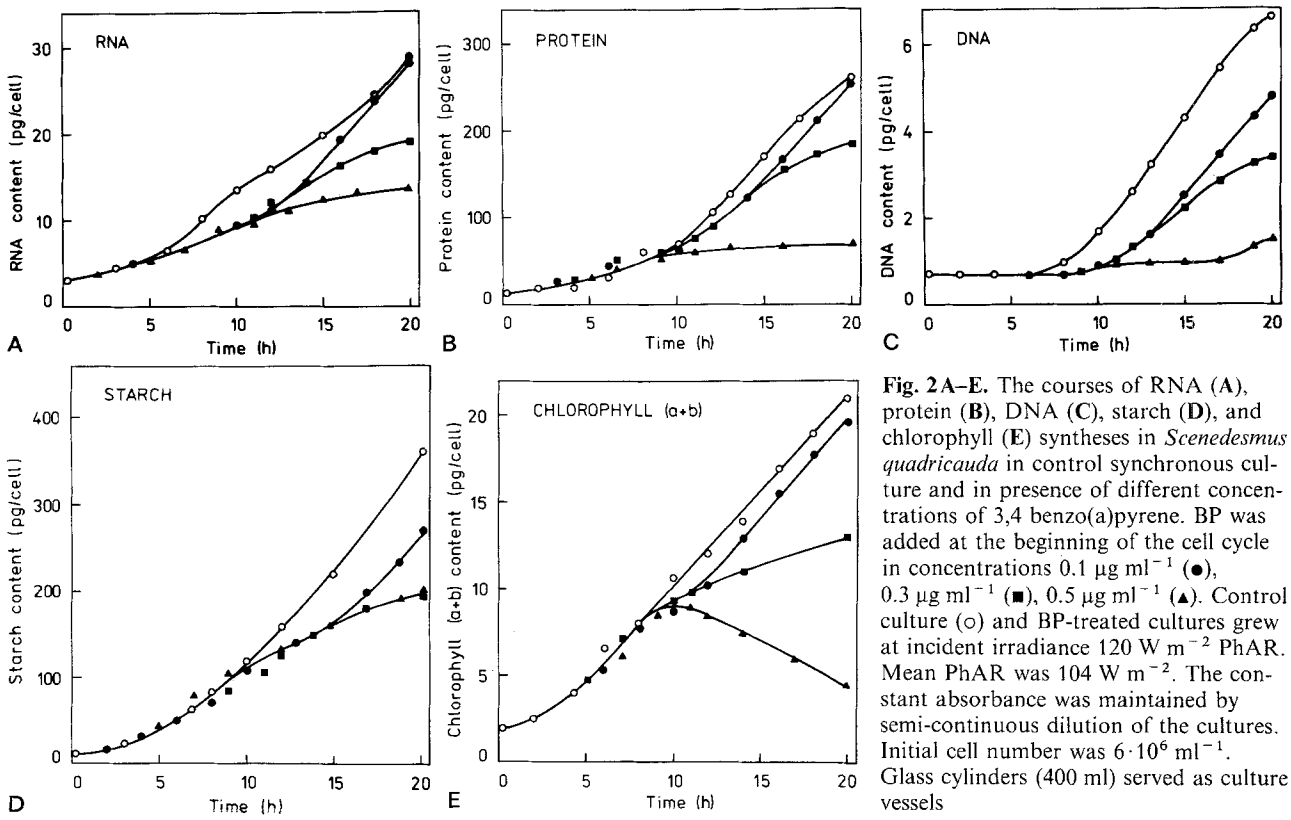

Fig. 2A–E. The courses of RNA (**A**), protein (**B**), DNA (**C**), starch (**D**), and chlorophyll (**E**) syntheses in *Scenedesmus quadricauda* in control synchronous culture and in presence of different concentrations of 3,4 benzo(a)pyrene. BP was added at the beginning of the cell cycle in concentrations 0.1 μg ml^{-1} (●), 0.3 μg ml^{-1} (■), 0.5 μg ml^{-1} (▲). Control culture (○) and BP-treated cultures grew at incident irradiance 120 W m^{-2} PhAR. Mean PhAR was 104 W m^{-2}. The constant absorbance was maintained by semi-continuous dilution of the cultures. Initial cell number was $6 \cdot 10^6$ ml^{-1}. Glass cylinders (400 ml) served as culture vessels

Function of the chloroplast

Syntheses of starch and fructose. It appears that starch syntheses is not directly inhibited by BP. The rate of starch synthesis declined late in the cell cycle after all the other macromolecular syntheses had been inhibited by BP (Figs. 2, 3). The cessation of starch synthesis at the end of the cell cycle seems to be a consequence of a gradual loss of viability of the cells.

The rate of photosynthesis was not measured during the present experiments. However, the unchanged rate of starch synthesis indicates that at least some of the photosynthetic activities of the chloroplast are rather unaffected in the presence of BP.

The same conclusion is drawn from the investigation of gross (free and sucrose bound) fructose synthesis. Figure 4 shows that this synthesis remains unchanged or may even be stimulated if the cultures are treated with BP.

Surprisingly, the syntheses of starch and fructose were found to continue for a few hours even if the amount of chlorophylls *a* and *b* was markedly decreased (Figs. 2, 3, and 4).

Reproductive processes

DNA replication. From Figs. 2 and 3 it is evident that DNA synthesis is allowed to be initiated in presence of low concentrations of BP. The onset of DNA replication occurs with a delay in comparison to the control. At time when DNA replication occurs the other macromolecular syntheses (RNA, protein) are usually inhibited by BP. A higher dose of BP is required for DNA inhibition than for inhibition of RNA and protein syntheses. DNA synthesis can be strongly deppressed however, if high BP concentrations are applied (Fig. 2).

Nuclear, protoplast division and daughter-cell release. The extent of inhibitory effects of BP can be easily regulated either by changing the concentrations of BP or by applying BP at different times during the cell cycle. The present studies have employed both of these features in order to distinquish the effects of BP on various reproductive processes.

The later events of the cell cycle are more susceptible BP treatment. Therefore, the release of daughter cells was the first markedly affected event

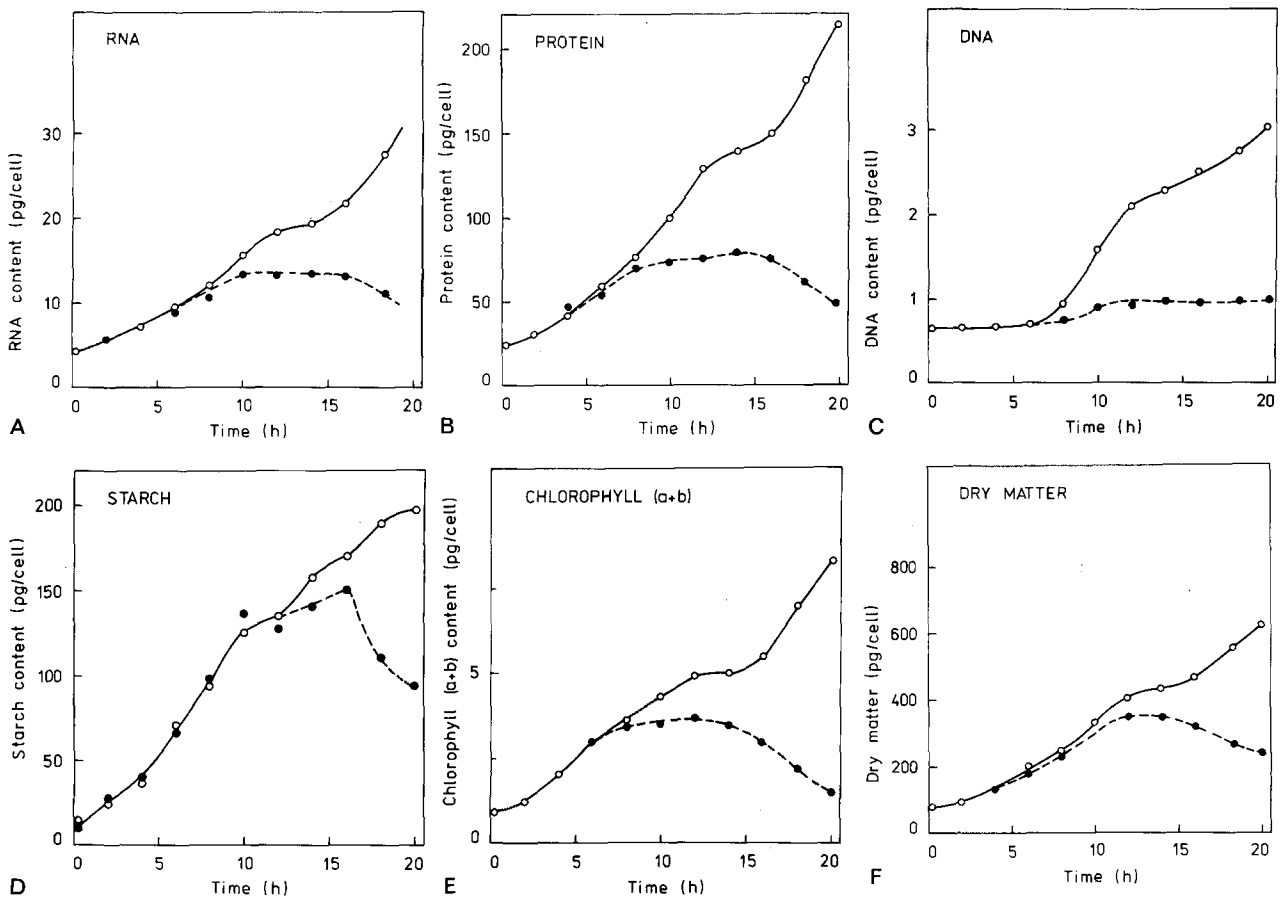

Fig. 3A–F. The courses of RNA (**A**), protein (**B**), DNA (**C**), starch (**D**), chlorophyll (**E**) content, and dry matter (**F**) increase in control and BP-treated synchronous cultures of *Scenedesmus quadricauda*. Control culture (o——o) and BP-treated culture (●– – –●) grew at incident irradiance 140 W m^{-2} PhAR. Mean irradiance was 104 W m^{-2}. BP was added at the beginning of the cell cycle in concentration 0.3 μg ml^{-1}. The constant absorbance (absorbance at 750 nm = 0.3) was maintained by semi-continuous dilution of cultures. Initial cell number was $6 \cdot 10^6$ ml^{-1}. Plateparallel cuvettes (1,000 ml) served as culture vessels

under conditions when no BP effects on macromolecular syntheses of cell-cycle characteristics were measurable. Monstrous or deformed daughter coenobia were released at the lowest effective concentrations of BP. With increasing concentration of BP or earlier application of high BP concentration, the release of the daughter cells is gradually suppressed. However, division of the protoplasts still takes place. Further increase of BP concentration causes the inhibition of protoplast fission. Simultaneously, it brings about a delay in mitoses. The changes in the courses of reproductive processes when BP was added at two different times during the cell cycle (the 4th and 8th h) are illustrated in Fig. 5.

If BP was added in the middle of the cell cycle (the 8th h) (Fig. 5B) mitoses were delayed. The delay increases with the length of time BP is present within the cell cycle, and therefore the third mitosis was more delayed than the first one. About 25% of cells in the population were prevented from dividing under the given conditions. On the other hand, the nuclear divisions were prevented only in a small percentage of the cells, and so four and eight nuclei were observed in undivided mother cells.

If BP was applied earlier in the cell cycle (the 4th h) all the inhibitory effects of BP previously described, were more pronounced. For example, as can be seen from Fig. 5C, only 60% of the population was able to divide and the majority of released daughter coenobia were deformed. Undivided cells were binuclear or tetranuclear, i.e. the third and second mitoses were partially suppressed. The application of BP at the beginning of the cell cycle (not shown in Fig. 5) caused complete inhibition of daughter cell release, protoplast fission, and octuplet nuclear division (the third mitosis). Qua-

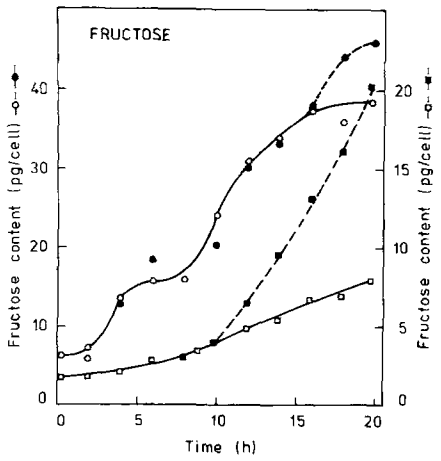

Fig. 4. The courses of fructose synthesis in control and BP-treated synchronous cultures of *Scenedesmus quadricauda*. Control cultures (□, ○) and BP-treated cultures (■, ●) grew at incident irradiance 140 W m^{-2} and 200 W m^{-2} PhAR and corresponding mean irradiances were 108 and 120 W m^{-2}, respectively. BP was added at the beginning of the cell cycle in concentrations 0.1 μg ml^{-1} (■) and 0.6 μg ml^{-1} (●). The cultures were grown in plateparallel cuvettes (1,000 ml) semi-continuously diluted to maintain absorbances at 750 nm about 0.1 and 0.6 and initial cell number was $1.9 \cdot 10^6$ ml^{-1} and $12.7 \cdot 10^6$ ml^{-1}, respectively

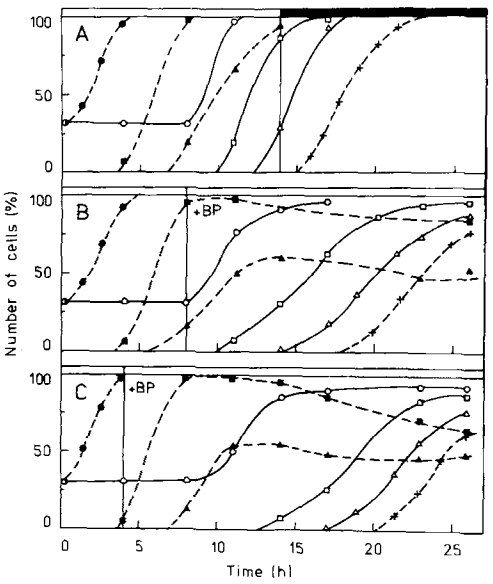

Fig. 5. The courses of nuclear divisions, their inductions and daughter cell release in control and BP-treated synchronous cultures of *Scenedesmus quadricauda*. Control culture events are illustrated in *panel A*. BP was added at the 8th hour (*panel B*) and at the 4th hour of the cell cycle (*panel C*) in concentration 0.3 μg ml^{-1}. Time of addition is illustrated by vertical lines. Light and dark periods are marked by white and black strips above panels. ●, ■, ▲ – the courses of induction curves for the first, second, and third mitoses, respectively. ○, □, △ – the courses of the first, second, and third nuclear divisions, respectively. + – the course of daughter cell release. Cultivation conditions see Fig. 1

druplet nuclear division was blocked only partially, and the population of cells consisted of bi- and tetranuclear cells. The inhibition of cell and nuclear division was accompanied by the strong inhibition of DNA and RNA synthesis. In addition, protein synthesis was severely depressed.

Discussion

The present results leave no doubt that 3,4-benzo(a)pyrene drammatically effects the cell cycle events in the chlorococcal alga *Scenedesmus quadricauda*. Daughter-cell release, protoplast fissions and mitoses are prevented, and a gradual inhibition of total RNA, protein, and DNA syntheses occurs in the presence of effective concentrations of BP.

The question arises as to which of the processes are primarily inhibited by BP. A survey of relevant literature supports that DNA replication could be the main target. BP inhibited the replication of phage (Hsu et al. 1977) and greatly reduced DNA synthesis in mammalian cells (Bartholomew et al. 1979). On the contrary, the present findings that RNA and protein syntheses usually ceased before DNA replication had started, indicate that the inhibition of DNA itself does not play an important role in the observed inhibition of RNA and protein syntheses.

Furthermore, in previous experiments with *Scenedesmus quadricauda* (unpublished results) DNA replication was prevented during the cell cycle by 5-fluorodeoxyuridine. However, RNA and protein syntheses and other growth and synthetic processes were allowed to continue till the end of the cell cycle in the same way as in a control culture. These results suggested that the absence of DNA replication itself does not provide a sufficient explanation for the suppression of RNA and protein syntheses in BP treated cultures.

It would appear therefore that the inhibition of transcription activity of DNA, and not the inhibition of DNA replication itself could be decisive in suppression of RNA and protein syntheses. This is in agreement with the work of Leffer et al. (1977), who have found that BP produces the loss of the template function of DNA in transcription by *Escherichia coli* RNA polymerase.

Further evidence against the involvement of inhibition of DNA on RNA and protein syntheses comes from present experiments with a low concentration of BP. In these experiments DNA replication was allowed to be triggered on but RNA and protein syntheses were gradually suppressed even under these conditions.

The inhibition of the transcription activity of DNA seems to provide the best explanation for this suppression. A shortage of mRNA could cause either a partial or complete inhibition of protein synthesis. It has been reported that concommitant protein synthesis was necessary for DNA synthesis in *Chlorella* (Wanka and Moors 1970). From this point of view DNA replication need not have been a primarily BP-inhibited process and if the inhibition of DNA occurred at high concentrations it was probably a secondary result of the inhibition of the synthesis of essential proteins. Accordingly, it might be assumed that the inhibition of RNA synthesis could be a secondary effect of the lack of specific proteins, since a gross RNA synthesis in *Chlorella* has been shown to depend on a concommitant protein synthesis as well (Wanka and Schrauwen 1971).

The present studies suggest that protein synthesis is the most severely affected process in presence of BP, compared with other macromolecular syntheses. Apart from the above mentioned inhibition of the transcription activity of genome, other additive mechanisms of protein inhibition by BP should also be considered. For example, Grunberger et al. (1980) found that active BP formed adduct with mRNA and that the modified mRNA did not form initiation complexes with ribosomes as effectively as it did the unmodified mRNA.

It is also possible that a direct inactivation or modification of protein molecules can be caused by a covalent binding of BP metabolites to them (Anderson 1979; MacLeod et al. 1979). The same could be said for RNA molecules (Ivanovic et al. 1978; MacLeod et al. 1979).

Although it has not been possible to establish a precise molecular mechanism by which BP effects macromolecular syntheses, it is apparent that the most important result of BP treatment is the blockage of protein synthesis. The inactivation of certain proteins or the prevention of their synthesis is likely to happen even at such low concentrations of BP which were found to cause no disturbances in the gross increase in RNA, DNA and protein content.

It is assumed that the inhibition of a cell division is caused by BP interference with some of the required proteins and the inhibition or inactivation of these proteins would not be detectable by the measuring of a gross protein increase. This assumption is supported by findings of MacLeod et al. (1982) that BP strongly binds to tubulins and some structural proteins.

We have found that the inhibitory effect of BP in *Scenedesmus* cells occurred a relatively long time (3–4 h) after BP application. The lag phase could be required for a simple uptake of BP or for its metabolism into biologically active compounds. What is a real cause of this lag phase remains to be solved. Nevertheless, the lag time between BP application and its inhibitory activity may provide an explanation as to why the inhibitory effects of BP are lower if it is applied later in the cell cycle.

The observation of the delay in the inhibitory activity of BP is consistent with present finding that the sensitivity to BP of various events is dependent on the cell cycle position and increases towards the end of the cell cycle. Howell et al. (1975) described that most of the transition points for various chloroplast activities in the volvocal alga *Chlamydomonas reinhardtii* were to be found in the first half of the cell cycle. No such data was obtained for *Scenedesmus* cells, but it is probable that the majority of chloroplast protein synthesis also takes place within the first half of the cell cycle. On the basis of this assumption, it is tempting to speculate that in the BP-treated cultures, the demand for proteins for photosynthetic activity was saturated before a sufficient quantity of fully biologically active BP had been present in the cells. The finding that the synthesis of fructose and starch were the least affected processes supports this idea.

A most interesting finding was that starch synthesis was slightly affected in BP-treated cultures even if the content of chlorophyll had been decreasing as a consequence of BP inhibition. This finding indicates that the chloroplast synthetic activity in *Scenedesmus* is unaffected by BP during one cell cycle and it is well adapted to the lowering of chlorophyll content.

In contrast to chloroplast activity, most of the events leading to protoplast fission and daughter cell release have transition points at the end of the cell cycle in the diatom *Cylindrotheca fusiformis* (Okita and Volcani 1980), *Chlamydomonas* cells (Howell et al. 1975), and probably also in *Scenedesmus* cells. This implies that the proteins required for these events are synthesized late in the cell cycle. It is proposed, therefore, that the added BP is in a sufficient quantity and fully biologically active at this time and so the probability of inhibition of proteins by BP is greatest for those synthesized late in the cell cycle. This suggestion is compatible with the observation that the sensitivity of cell cycle processes to BP increases in reverse order to their order in the cell cycle.

Aromatic carbohydrates, especially polycyclic ones, damage susceptible eucaryotic cells that ranges from cytotoxicity to mutation and the in-

duction of malignant transformation. Our findings do not provide any evidence of a possible relation of the observed BP inhibitory effects to carcinogenesis or mutagenesis. In our experiments, we did not observe any mutants, so we assume that the observed inhibitory effects of BP are most probably related to its cytotoxicity. This is common to most aromatic hydrocarbons. In view of the lack of detailed studies on the effects of other aromatic carbohydrates on algal cell-cycle events, it is difficult to compare the inhibitory effect of BP with the effects of other, especially non-carcinogenic aromatic carbohydrates. It is the effect of aromatic carbohydrates on photosynthesis that has been studied most in algal cells. Kusk (1980, 1981a, b) found that cytotoxicity of aromatic hydrocarbons increased in the following order: benzene, naphtalene and phenanthrene. The toxicity of aromatic hydrocarbons can be assumed to increase with the number of carbon atoms in their molecules. The finding that the toxic concentrations of benzopyrene were lower than those of phenanthrene (Kusk 1981b) is consistent with this assumption.

All hydrocarbons studied (Kusk 1980, 1981a, b) or their derivates (Rensen 1975) have inhibitory effects on photosynthesis which increase with the time of incubation. On the other hand, we have found chloroplast synthetic activity to be the least and probably not primarily inhibited process by BP. This results are consistent with the finding (Cerniglia 1980) that 5 mg g^{-1} of the non-carcinogenic herbicide diquat (9, 10 dihydro-8, 10-diazino(a)phenanthrone) was required to produce 50% inhibition of oxygen evolution and only 15–30 μg g^{-1} produced 50% reduction of growth. Other aromatic carbohydrates than BP may therefore also affect macromolecular synthesis in the first place, rather than photosynthesis.

It may be concluded that our and other authors' results with algal cells leave open the question in which way the inhibitory effects of BP on algal cell-cycle events could be related to its carcinogenic effects observed in higher organisms.

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