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THE EFFECT OF 3,4-BENZOPYRENE ON THE GROWTH OF CHLORELLA, STRAIN 366

1. INTRODUCTION

Numerous polycyclic aromatic hydrocarbons (PAH) are considered as strong cancerogens [2]. Their occurrence in the environment, a hazard to the health of humans and animals, is most probably the result of their emission into the atmosphere with industrial pollution. Other possibilities such as, for example, the suggestions about endogenous synthesis of PAH by the higher and lower plants, are continuously under investigation. Higher plants [9] and the recently-investigated green algae [13] growing in the industrial areas, contain considerably higher amounts of PAH as compared with the same plants cultivated in conditions where the pollution of the air by these hydrocarbons is lower. Payer et al. [13] suggest that endogenous synthesis of PAH, demonstrated in laboratory experiments with Chlorella vulgaris [1], may also take place in plants, but with such limited yield that the effect is completely masked by the accumulation of the same compounds from the environment. The possibilities of the synthesis of PAH by plants are very strongly related to the role of these chemicals in the plant metabolism. Gräf and Nowak [8] affiliate this possibility upon the action of PAH as the potential stimulators of plant growth. These authors found the stimulating effect of many PAH present in the plants on the growth of some species of higher plants and green algae. As the quantitative criterion of the observed stimulation of the algal growth they applied microscopic comparison of the size of algal cells, growing in a medium with and without hydrocarbons.

The purpose of our studies was to estimate the influence of one of

the strongest cancerogens of the PAH, 3,4-benzopyrene (BP), on the growth of unicellar algae (Chlorella, strain 366), in the aspect of stimulation of the multiplication of the algae cells in the experimental culture. This investigation was also undertaken to compare the results obtained with those in our second paper [14] related to the effect of some organic solvents on the growth of the same algae.

2. MATERIAL AND METHODS

The Chlorella algae, strain 366 [11], were cultivated on liquid media saturated with benzopyrene (~0.5 μg/dm³) and similar media, prepared in the same way, but without BP (standard media).

The saturation of the media with BP (reagent grade pure, Fluka AG, Buchs SG, Switzerland) was carried out by mixing the hydrocarbon suspension with the medium (1 mg BP in 5 dm³), in the dark, for about 48 hours. All the media were sterilized in the autoclave (pressure 1.2 Atm, temperature 120°C, 20 minutes); the sterilization of the media with BP was carried out after their saturation with hydrocarbon.

The amount of BP in the media prepared was determined by use of spectrofluorometry (spectrofluorometer Hitachi MPF-4, λ of excititation 384 nm, λ of measured fluorescence 405 nm) in the cyclohexane extracts. It was also found that the sterilization of the cultures with BP had no significant effect on the amount of the dissolved hydrocarbon.

The origin of the algae used, the composition of the medium and the conditions for maintaining the culture were the same as in our previous paper [14]. The growth of algae in the cultures was estimated by direct counting of the algal cells in a Bürker chamber. Each culture in the BP saturated medium (experimental culture) was conducted in identical conditions, parallel to culture in the standard medium (control cultures).

3. RESULTS

The results obtained were presented in Table 1 and two figures (Figs. 1 and 2) differing in the scale of value of the algal growth factor (ratio N/N_o, where N and N_o are the numbers of the algal cells on a given day of the cultivation and the initial number of cells on the first day of cultivation, respectively). The progress of the individual experimental cultures (Figs. 1 and 2) was, in each case, compared to the averaged progress of three control cultures. The results presented in Table 1 show the means of the final values of N/N_o in the phase of stationary growth of the culture, the percentage enhacement of the value N/N_o

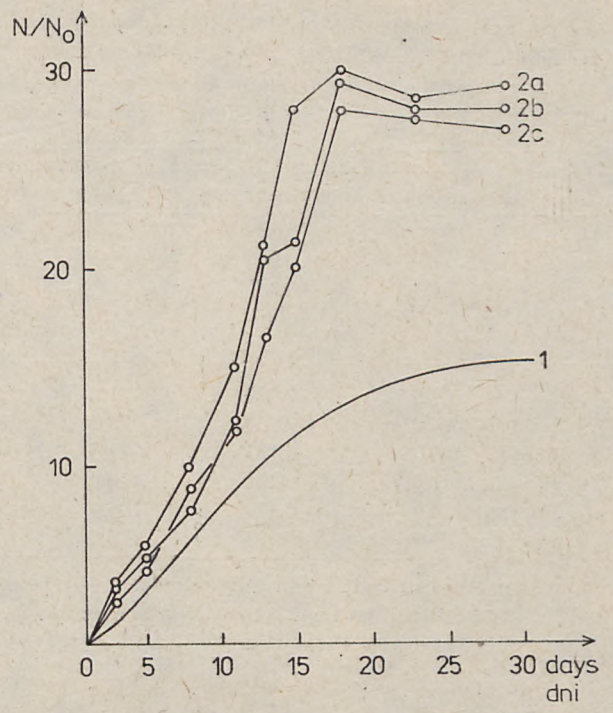

Fig. 1. The growth of Chlorella algae, strain 366, cultivated in a medium saturated with 3,4-benzopyrene (~0.5 µg BP/dm³), as compared with the control cultures
1 — the averaged rise for three control cultures
2a, b, c — three cultures with BP

Rys. 1. Wzrost glonów Chlorella, szczep 366, hodowanych na pożywkach wysyconych 3,4-benzopirenem (~0.5 µg BP/dm³) w porównaniu z hodowlami kontrolnymi
1 — średnia z trzech hodowli kontrolnych
2a, b, c — trzy poszczególne hodowle z BP

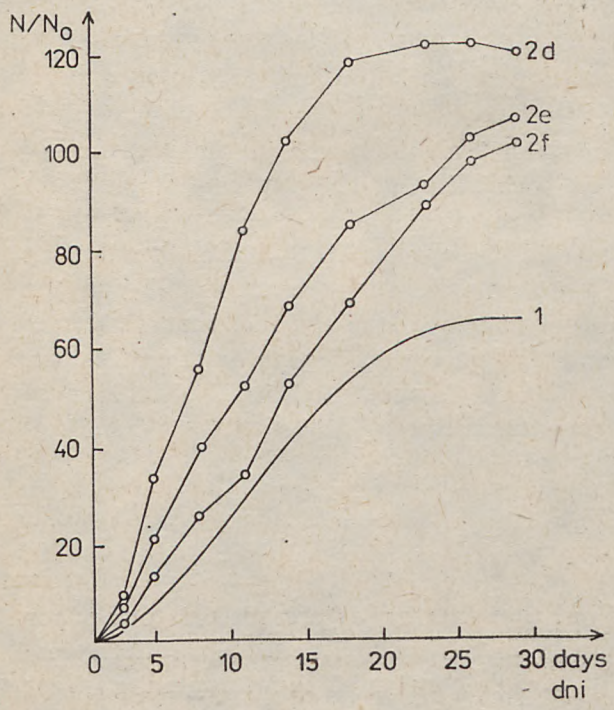

Fig. 2. The growth of Chlorella, strain 366, cultivated in a medium saturated with 3,4-benzopyrene (~0.5 µg BP/dm³) as compared with the control cultures
1 — the averaged rise for three control cultures
2d, e, f, — three cultures with BP

Rys. 2. Wzrost glonów Chlorella, szczep 366, hodowanych na pożywkach wysyconych 3,4-benzopirenem (~0.5 µg BP/dm³) w porównaniu z hodowlami kontrolnymi
1 — średnia z trzech hodowli kontrolnych
2d, e, f — trzy poszczególne hodowle z BP

Table 1. Comparison of the growth of Chlorella algae, strain 366, cultivated in a medium saturated with 3,4-benzopyrene (~0.5 μg BP/dm³) and in the standard media

Tab. 1. Porównanie wzrostu glonów Chlorella, szczep 366, hodowanych na pożywkach wysyconych benzopirenem (~0.5 μg BP/dm³) oraz na pożywkach standardowych

No. Lp.	Control culture[a] Hodowle kontrolne[a]		Experimental culture[b] Hodowle badane[b]		(~0.5 μgBP/dm³) (~0.5 μgBP/dm³)
	N/N_0	N [days⁻¹] [doba⁻¹]	N/N_0	N/N_0 enhacement podwyższenie N/N_0 [%]	k [days⁻¹] [doba⁻¹]
1	15.0	0.091	29.0	193.3	0.201
2	14.0	0.133	28.5	196.5	0.186
3	16.0	0.123	27.5	171.8	0.159
4	72.5	0.201	120.0	166.6	0.211
5	70.0	0.159	105.0	175.0	0.301
6	60.0	0.123	100.0	151.5	0.333

[a] Designated in Figs. 1 and 2 by number *1*. — Oznaczone na rys. 1 i 2 liczbą *1*

[b] Designated in Figs. 1 and 2 by successive numbers *2a, b, c* (Fig. 1) and *2d, e, f* (Fig. 2). — Oznaczone na rys. 1 i 2 kolejnymi liczbami *2a, b, c* (rys. 1) oraz *2d, e, f* (rys. 2)

in the cultures investigated, as compared with the control cultures and the constants of the algal growth rates. The above constants are calculated on the third day of cultivation according to the formula [6]:

$$k = 1/t \cdot \log N/N_o$$

where t is time.

It results from Figs. 1 and 2 that algal growth in the presence of BP in the medium is higher at each stage of the experimental culture than in the control culture. In the phase of stationary growth (Table 1) this increase in the experimental culture is 51.5 - 96.5% greater as compared with the control. Furthermore, very characteristic for the stimulation of algal growth influenced by BP is the fact that the effect of growth intensification appeares from the very beginning of cultivation. Significantly, the calculated values of k presented in Table 1 are in each case higher for the experimental cultures.

4. DISCUSSION

It seems worth while to point out that the results presented in our paper give further experimental support to the macroscopic observations described by Gräf and Nowak [8], who demonstrated the stimulating effect of some PAH, and in particular BP, on the growth of unicellular algae.

In the evaluation of our results the experimental course of the BP effect on algal growth seems to be particularly essential in the initial stage of cultivation. Comparison of the culture with BP with those presented in our previous paper [14] for cultures with hexane, cyclohexane and benzene shows that in contrast to the solvents investigated, BP does not give rise to noticeable retardation in algal growth.

The facts described suggest that BP has no toxic influence on algae, also illustrating the effect of this hydrocarbon from the point of view of the adaptation of algae to the presence of BP in the medium. It would seem that algal cells are subjected to the effect of BP stimulating the culture growth, without the necessity to adapt to the presence of hydrocarbon, in contrast to the similar effect observed for cyclohexane [14]. This might mean that in contrast to cyclohexane, BP does not change, but only intensifies the metabolism of the maturation and multiplication specific for the algal cells.

Thus the results presented in our paper would suggest that BP and other PAH, as suggested by Gräf and Nowak [8] and by Borneff et al. [1], are endogenous substances synthetized by plants. Apart from the qualitative observations indicating the intensification of cell multiplying (Chlorella vulgaris), Gräf and Nowak also demonstrated an increase in the size of algal cells in the presence of PAH, especially in the case of Ankistrodesmus orauni and Scenedesmus obliqus. This type of changes should probably be related to the deeper interference in the algal cell metabolism, which cannot be excluded, particularly in the experimental conditions applied by Gräf and Nowak [8] where much higher concentrations of BP, very long period of cultivation or transfers of the culture to fresh medium containing PAH were applied.

The observed increase in the size of the cells and intensification of their multiplication (to a greater extent than that of these phenomena conditioned by the possible endogenous synthesis of PAH in plant cells), can be considered to be analogous in character to the neoplastic growth in plant [12] or even animal tissues. This would seem to be suggested by the facts established by Gräf and Nowak [8], that of the PAH tested, stimulation effect of the growth of algae and higher plants is the strongest in the case of those hydrocarbons which simultaneously show the strongest cancerogenous effect on animals.

Treating the described effects of the influence of PAH on algae as reflecting the analogous character to the processes of neoplasmatic growth, could be correlated with many observations related to the metabolism of PAH in plant and animal cells.

Experiments with cell cultures derived from plants [10] or vertebrates [5] and also with alveolar macrophages [3, 4] or bacteria [7] show that BP is metabolized to oxygenated derivatives.

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WPŁYW 3,4-BENZOPIRENU NA WZROST GLONÓW CHLORELLA, SZCZEP 366

Streszczenie

Oceniono ilościowo powodowaną przez 3,4-benzopiren (BP) stymulację wzrostu glonów Chlorella (szczep 366), hodowanych w płynnych pożywkach, wysyconych badanym węglowodorem. Opierając się na obserwowanym przebiegu hodowli z BP, w porównaniu z opisanymi w drugiej naszej pracy [14] doświadczeniami, dotyczącymi hodowli z heksanem, cykloheksanem i benzenem, stwierdzono brak jakiegokolwiek toksycznego oddziaływania BP na badane glony. W przedstawionej pracy przedyskutowano problem zjawiska intensyfikacji namnażania oraz wzrostu rozmiarów komórek glonowych [8], wywoływanego działaniem niektórych policyklicznych węglowodorów aromatycznych, w aspekcie kancerogenności wielu tego typu węglowodorów.

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