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# The Growth Stimulating Effect of 5-bromouracil and Uracil on Chlorococcal Algae

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**Abstract.** The growth stimulating effect of 5-bromouracil and uracil on two strains of chlorococcal algae has been found in cell colonies grown from single cells, which were inoculated onto an agar medium. Analyses of the effect recorded in the cell cycles after treatment have revealed that the growth stimulating effect required four, or more, cell cycles to become evident. This has been proved by the number of autospores released from the treated cells and by the length of the lag phase after inoculation. The differences between the control and the treated population in some experiments with 5-bromouracil in *Chlorella* and with uracil in *Scenedesmus obliquus* have been visible by the unaided eye, whereas in some other experiments, they have been proved statistically. Growth stimulation has not been found only in a small number of experiments. The inhibition of growth induced by 5-bromouracil has been recorded in one experiment with *Scenedesmus quadricauda.*

5-bromouracil attracted attention first of all as a potential cytostaticum (ROY-BURMAN 1970). It was also used as an incorporable analogue of bases of nucleic acids in numerous mutation experiments with phages and bacteria (FREESE 1963, HOTZ and REUSCHEL 1967, and others). The incorporation of 5BU into nucleic acids was found to sensitize some organisms for the following treatment with radiation mutagens (LION 1968, 1970) or with some chemical mutagens (KONDRATIEV and SKAVRONSKAYA 1972). When applied to some higher plants, 5BU showed a growth stimulating effect (ŠORMOVÁ *et al.* 1960a,b, BALATKOVÁ and TUPÝ 1972). IWAMURA and MUTO (1964) reported the growth stimulating effect in a culture of Chlorella cells after the addition of 5BU into the liquid medium. They also proved the presence of 5BU in some fractions of nucleic acids which had been isolated from the same culture after a certain period of growth in the medium with 5BU. The induced resistance to uracil derivatives in a blue-green alga *Anacystis nidulans* (BAZIN 1971) and in a green alga *Chlamydomonas reinhardtii* (HIPKISS 1968) has been shown recently.

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On trying to sensitize the cells of three strains of chlorococcal algae for the following treatment with a radiation mutagen — UV-light (to be published elsewhere), we have also found the stimulating growth effect of 5BU. It has been manifested by the size of the cell colonies grown from single cells, which were treated before inoculation onto the surface of an agar medium. Also U applied in some control experiments has shown a similar growth stimulating effect.

## Material and Methods

### Organisms

Three strains of chlorococcal algae suitable for laboratory as well as mass cultivation were used: *Chlorella kessleri*, strain LARG-1 (from the Collection of Algal Strains at the Laboratory of Radiation Genetics, Institute of General Genetics, Academy of Sciences USSR, Moscow), *Scenedesmus obliquus*, strain LHOTSKÝ 1966/7, and *Scenedesmus quadricauda*, strain GREIFSWALD/15 (both from the Collection of Algal Strains of our laboratory). All the strains were kept at our laboratory as bacteria-free cultures on a minimal as well as on a complete agar medium.

### Methods

The cultures of chlorococcal algae used in the described experiments were first of all adapted to the growth conditions in the liquid medium (for its composition see NEČAS 1971). The continuous irradiance was about 10 $Wm^{-2}$ PhAR, the density of the culture approximated 0.2 $gl^{-1}$, and the temperature was maintained between 23 and 25 °C. The culture was diluted to about 0.05 g $l^{-1}$ after 4 or 5 cell cycles and to a part of it an alkaline solution of recrystallized 5BU was added (synthetized in the Institute of Organic Chemistry and Biochemistry of the Czechosl. Acad. of Sci., Prague, and supplied through the courtesy of Dr. Fučík). It was added in surplus, 0.5 or 1.0 mg $ml^{-1}$. In some control experiments, U (Lachema, Brno) was used instead of 5BU in the same quantities. The control and the treated variants were incubated for 48 hours (almost two cell cycles) under the same light and temperature conditions as the pre-cultivation. The high content of both substances in the culture even after incubation was proved by spectrophotometry within the area of specific absorption. The control as well as the treated populations of algal cells were inoculated onto a 2% agar medium in Petri dishes containing the same nutritive solution as was used in the pre-cultivation (1/4 of contentration in the agar medium, 1/8 in the liquid medium). The algal cells in the Petri dishes grew under continuous irradiance of 15 W $m^{-2}$ (I), 10 W $m^{-2}$ (II), 7.5 W $m^{-2}$ (III) PhAR, and temperature conditions (28 to 30 °C).

The number of autospores released from single cells in cell division was recorded in the cell cycle after inoculation onto the agar medium in Petri dishes. The length of the lag phase was ascertained according to the number of cell cycles run during 72 h after inoculation. The growth rate of the cell colonies was determined according to their size after the same period of growth (given in the captions to the Figures) in the control as well as in the treated variants.

## Results

### Growth Rate of the Cell Colonies

In most of the experiments, the diameters of the cell colonies were significantly different in both the control and the treated variants after the same time of growth. Fig. 1 shows the growth stimulating effect of 5BU in *Chlorella*. Fig. 2 gives the effect of U on *Sc. obliquus*; the effect of 5BU on the same strain was very similar, but the picture in the first cell cycles was different. The growth inhibition induced by 5BU in one experiment with *Sc. quadricauda* is indicated in Fig. 3. This experiment proved also the significant interaction of the effect of 5BU and the light intensity upon the culture in

*Abbreviations used: Sc.* = *Scenedesmus;* 5BU = 5-bromouracil; U = uracil; MM = minim medium; CM = complete medium.

the Petri dishes. In the experiments with *Chlorella*, the high pH in the culture medium with 5BU (pH = 8.0 to 8.3) could not be the cause of growth stimulation as one control variant in the medium with the same pH and without 5BU has proved. This experiment was no more repeated in the two Sc. strains. We also studied the influence of repeated washing of the treated cells of *Sc. obliquus* in distilled water before inoculation. Almost the same stimulating growth effect became evident in the washed and the unwashed variants, respectively.

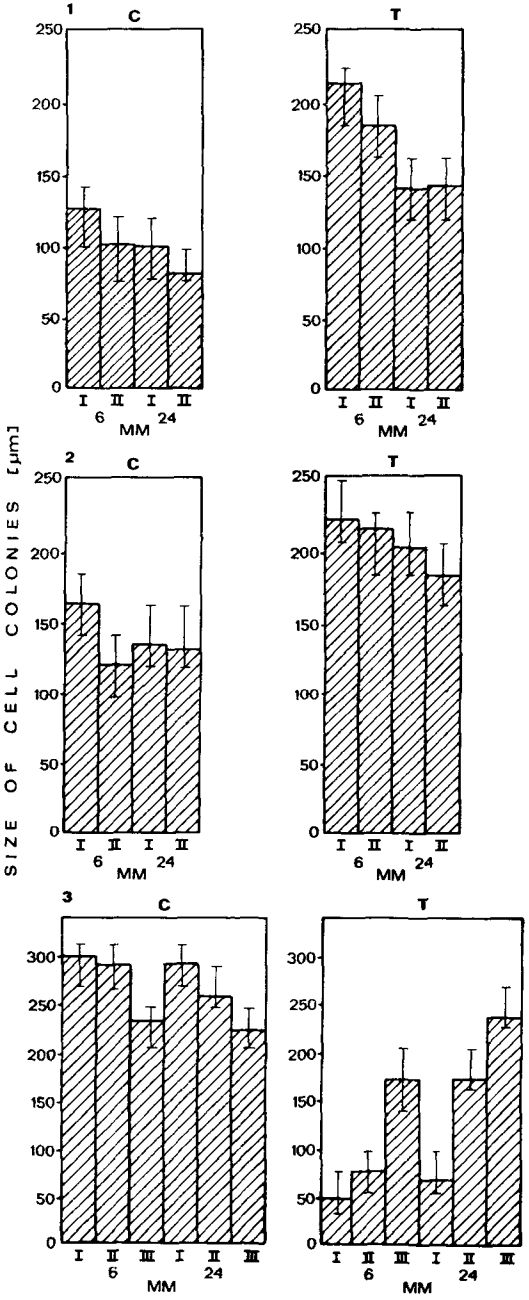


Fig. 1. Size of cell colonies in the control (*C*) and in the 5BU-treated (*T*) variants of a *Chlorella kessleri* culture after 9 days of growth on the surface of an agar medium.

Fig. 2. Size of cell colonies in the control (*C*) and in the U-treated (*T*) variants of a *Sc. obliquus* culture after 8 days of growth on the surface of an agar medium.

Fig. 3. Size of cell colonies in the control (*C*) and in the 5BU-treated (*T*) variants of a *Sc. quadricauda* culture after 10 days of growth on the surface of an agar medium. I = 15 W $m^{-2}$, II = 10 W $m^{-2}$, III = 7.5 W $m^{-2}$ irradiance with PhAR (400—700 nm).

**Number of Autospores**

Fig. 4 indicates that the stimulating growth effect caused in *Chlorella* by 5BU cannot be explained by an increased production of autospores in the first cell cycle after treatment. The increased production of autospores apparently shared in the stimulating growth effect of U in *Sc. obliquus* ( Fig. 5B); this was not the case with the application of 5BU to the same strain (similarly as with *Chlorella*;

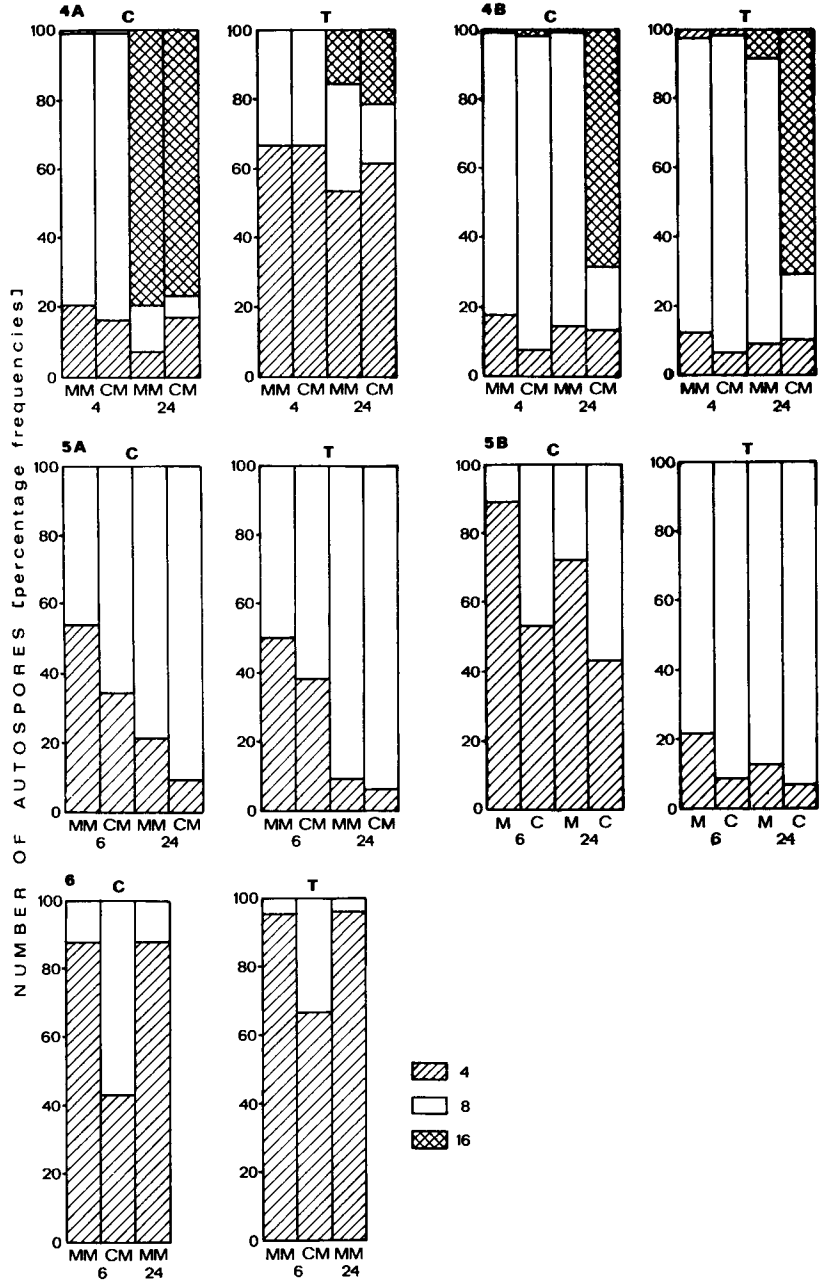


Fig. 4. Numbers of autospores released from cells of the control (*C*) and of the 5BU-treated (*T*) variants in the first cell cycle on the surface of an agar medium in a *Chlorella kessleri* culture which displayed a later stimulating growth effect on cell colonies. (*A*, *B* = two different experiments).
Fig. 5. Numbers of autospores released from cells of the control (*C*) and of the treated (*T*) variants in the first cell cycle on the surface of an agar medium in a *Sc. obliquus* culture which displayed a later stimulating growth effect on cell colonies. *A* = treated with 5BU; *B* = treated with U.
Fig. 6. Numbers of autospores released from cells of the control (*C*) and the 5BU-treated (*T*) variants in the first cell cycle on the surface of an agar medium in a *Sc. quadricauda* culture which showed a later growth inhibiting effect on cell colonies. I, II, III — explained in Fig. 3.

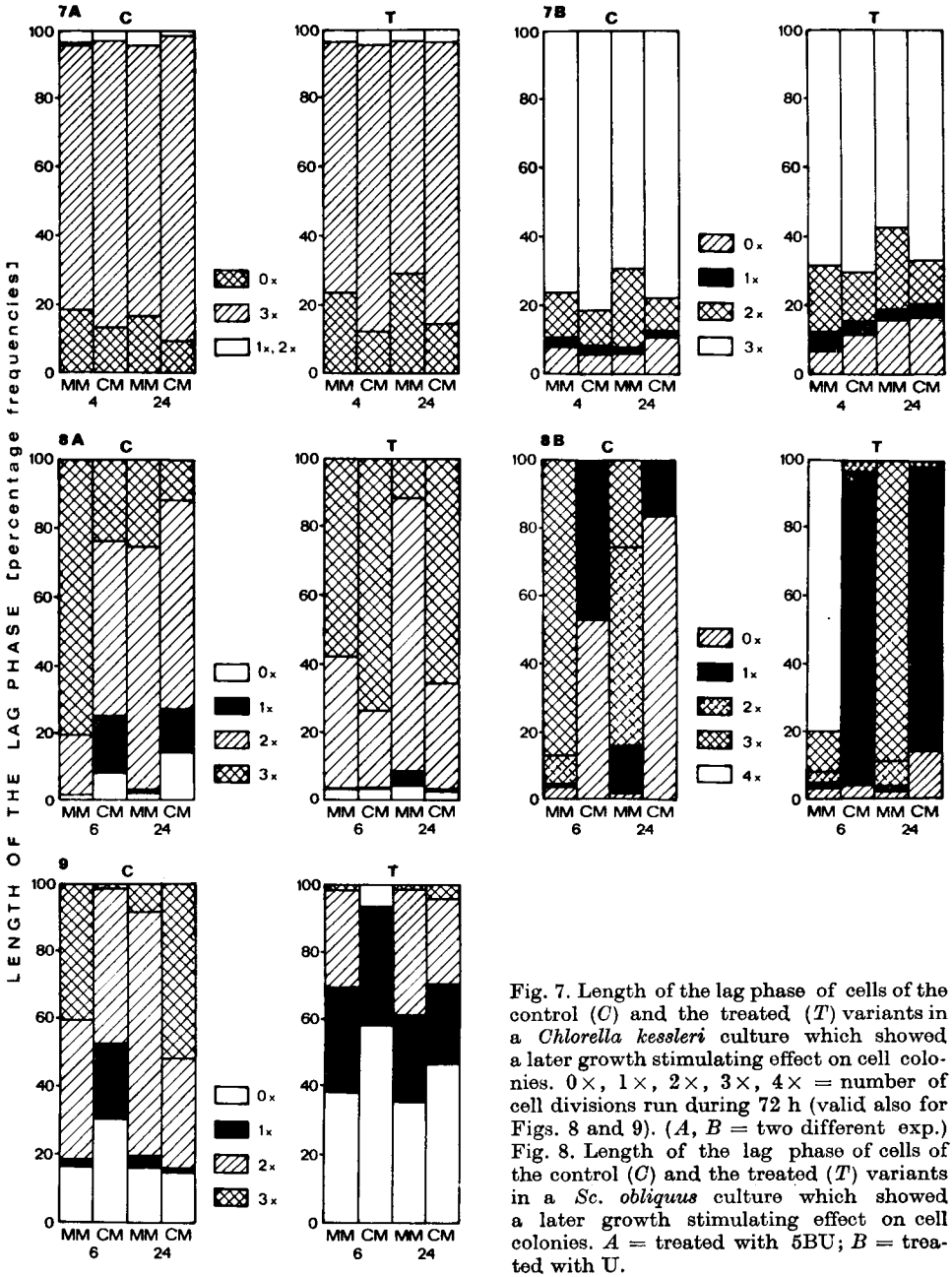


Fig. 7. Length of the lag phase of cells of the control (*C*) and the treated (*T*) variants in a *Chlorella kessleri* culture which showed a later growth stimulating effect on cell colonies. 0×, 1×, 2×, 3×, 4× = number of cell divisions run during 72 h (valid also for Figs. 8 and 9). (*A*, *B* = two different exp.)

Fig. 8. Length of the lag phase of cells of the control (*C*) and the treated (*T*) variants in a *Sc. obliquus* culture which showed a later growth stimulating effect on cell colonies. *A* = treated with 5BU; *B* = treated with U.

Fig. 9. Length of the lag phase of cells of the control (*C*) and the 5BU-treated (*T*) variants in a *Sc. quadricauda* culture which showed a later growth inhibiting effect. (I, II, III — explained in Fig. 3.)

For all Figs.: 6, 24 = time [h] for which the culture was placed in the dark immediately after treatment.

Fig. 5A). The inhibiting growth effect of 5BU in *Sc. quadricauda* was obviously caused, at least in part, by the decreased production of autospores (Fig. 6). From the mentioned results it follows that the increased production of autospores in the cell cycle after the treatment shared in the observed growth stimulation in some cases only and cannot be therefore a generally valid explanation of this effect.

#### Length of the Lag Phase

A shortened lag phase of the cells, which had been inoculated after treatment onto an agar medium, could be also one of the causes of the observed growth stimulation of the cell colonies. As one may see in Fig. 7, neither the lag phase was shortened significatly in the *Chlorella* cell poulation. On the contrary, in some experiments it was rather prolonged. Therefore, even in this case, there is no generally valid explanation of the recorded growth stimulation induced by 5BU. The same concerned *Sc. obliquus* (Fig. 8A); but U shortened significatly the lag phase of the cells of *Sc. obliquus*. There seem to be two different mechanisms of growth stimulation induced by the two substances. The prolongation of the lag phase shared significantly (Fig. 9) in the inhibiting growth effect of 5BU in *Sc. quadricauda*. The interaction between the effect of 5BU or U and the light intensity in the culture on the surface of an agar medium was not so significant as was the case with the growth inhibition in the colonies of *Sc. quadricauda* (Fig. 3).

The present study describes only several experiments, nevertheless, they give a general picture of all the results obtained. They suggest that each of the substances used is able to induce a stimulating or inhibiting growth effect on various algal strains or on one strain placed under specific conditions. This seems to be valid also for U which has not shown any inhibiting effect in the experiments. In some cases, it was possible to trace the influence of the two substances already from the first cell cycle after the treatment, in others, only from the subsequent cycles. In such a way, it was possible to find the differences between the action of the two substances.

## Discussion

The growth stimulation of algal colonies grown on an agar medium from U-fed cells is understandable if we take into account that U is an important component of ribonucleic acids and takes part in some enzyme systems of the cell. The influence of U may be followed as soon as the first cell cycle after treatment. However, its effect does not decline in further cell cycles and seems to be still increased, when we would rather expect the source of U to be exhausted. Thus it is necessary to search for some other explanation. The way how the observed growth stimulation by 5BU, which has emerged after analysis in the first cell cycle immediately after treatment, is manifested, rather points at a mechanism of induction that requires two or three cell cycles to be fully operative.

A less important difference between the effects of U and 5BU makes us consider the fate of the bromine atom in the molecule of 5BU and its relation to the assumed induction mechanism. First a debromination of the molecule

5BU and after that again a preferential uptake of the molecules of U would come into account. However, IWAMURA and MUTO (1964) have proved that 5BU enters into the cells and is incorporated into nucleic acids.

In the recently published papers dealing with the growth stimulation induced by 5BU or U in plants (ŠORMOVÁ *et al.* 1960a, b, BALATKOVÁ and TUPÝ 1972) no decisive explanation is given on the mechanism of the effect of the two substances. The present results also do not allow yet to explain the proper mechanism of the observed growth stimulation of algae induced by U or its 5-bromoderivative. Nevertheless, they bring forward the important ascertainment that the stimulating growth effect by these two substances also exists in those cases in which it cannot be recorded in the first cell cycle after the treatment. In fact, the number of autospores produced in the first cell cycle after the treatment and the length of the lag phase were either the same as in the control or even negatively influenced in the treated variants. Some previous papers (NEČAS 1970, 1971a,b) give the importance of these parameters for the analysis of the growth stimulating effect in algae observed on the cell colonies grown on the surface of an agar medium.

The investigation of the physiological parameters of the responses of algae to the action of the two substances studied and the fact that they cause a covering effect upon the following UV-irradiation (to be published elsewhere) demand a chemical tracing of the fate of both molecules in algal cells. The presence of U in the cells still for a longer time after their transfer from a medium that contained one of the two substances was proved in several experiments by the specific absorption in UV-light. It ought to be decided by detailed physiological and biochemical investigation if it is possible to speak about an inductive character of the stimulating mechanism induced by the two substances compared. BALATKOVÁ (1972) points out the controversy of the published results of research on the stimulating and inhibiting growth effects of both substances in various organisms. The results of this work bring forward a similar controversy. Nevertheless, again positive results are repeated here which confirm the fact that, under certain conditions, both substances may induce a growth stimulating effect. It is necessary to pay further attention to the matter and to search for an explanation of the controversies recorded in different experiments with two substances.

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Podle rychlosti růstu buněčných kolonií chlorokokálních řas z jednotlivých buněk (coenobií) naočkovaných na povrch agarového media v kontrolní a ovlivněné variantě pokusů byl zjištěn růstový stimulační vliv 5-bromuracilu a uracilu. Analysy tohoto účinku v několika prvých buněčných cyklech, jak se projevil u naočkovaných buněk (coenobií), ukázaly, že proběhnou čtyři až pět buněčných cyklů než se stimulační účinek zřetelně projeví. Ukázalo se to na počtu autospor, produkovaných ovlivněnými buňkami v prvém buněčném cyklu po naočkování na agarové medium a na délce lag fáze, vyjádřené počtem buněčných cyklů, které proběhly ve třech dnech. Rozdíly mezi kontrolními a ovlivněnými populacemi byly v některých pokusech s *Chlorelou* a *Scenedesmuus obliqus* patrné pouhým okem a v jiných se ukázaly po statistickém zhodnocení. Jen v malém počtu případů byly neprůkazné. Růstový inhibiční účinek 5-bromuracilu byl zjištěn v jednom pokusu se *Scenedesmus quadricauda*.