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Effects of Simazine on the Blue-Green Alga *Anacystis nidulans*

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The use of herbicides for selective control of weeds has drawn a considerable amount of interest among scientists. Inspite of wide and persistant use of herbicides, their physiological effect on plants are not clearly understood. We are apparently far off in elucidating the biochemical basis of plant-herbicides interaction; therefore, this type of study has become a much more prominent feature of herbicide research (AUDUS 1974). The removal of herbicides from environment and aquatic systems is also a difficult problem for scientists. It is known that algae are used for the removal of herbicides from aquatic environments, and herbicides are known to be metabolized by various aquatic plants algae (BINGHAM 1973). However, how herbicides are metabolized by blue-green algae is not clearly understood. This study attempts to fill the gap in understanding blue-green algae and herbicide interactions with the use of the electron microscope.

The blue-green algae of the family cyanophyceae have varied developmental and physiological characteristics (GNATT and CONTI 1969, PANKRTAZ and BOWEN 1963, and WOLK 1973). Such differentiation involves changes in cytochemical and physiological characteristics of the vegetative cells, spores, and heterocysts. The study of the physiological nature may, therefore, be expected to remain variable of phenonmena like photosynthesis, growth rate, movement and nitrogen fixation, when an external factor like herbicide is incorporated. Variation introduced by the addition of herbicide into the growth medium, will certainly bring about changes in algal metabolism. The purpose, therefore, for this work was to study the effects of simazine on one of the species of blue-green alga, (cyanobacteria) Anacystis nidulans, and determine which organelles were affected in the presence of herbicide.

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MATERIALS and METHODS

A unialgal culture of Anacystis nidulans, Drouet, No. 1550, was obtained from Indiana University culture collection and was grown and maintained in Bristol's solution (NICHOLS and BOLD, 1965) at 25-28 C under continuous day light type, fluorescent light (3500 lx), and kept in uniform suspension by shaking at 80 cycles/min. The above procedure was followed for stock as well as treated cultures.

A 10-day culture with an optical density (O.D.) of 0.9 at 678 nm was treated with simazine at a concentration of 10^{-5}M in Bristol's solution and was incubated under the above conditions. Aliquots (5ml) were removed after 0,12,24,48, and 53 hr and processed for electron microscopy. Similar cultures without simazine were used as the control.

The presence of ribonucleic acid (RNA) was estimated by OGUR and ROSEN technique (1950) in control as well as in cells treated for 48 hr with simazine.

For electron microscopy (E.M.) the cells were fixed in 6% glutaraldehyde for 3 hr followed by post fixation in 1% buffered osmium tetroxide for 1.5 hr (0.2M cacodylate buffer, pH 7.0 was used for both fixatives). The cells were dehydrated in ascending grades of ethyl alcohol and were embedded in Spurr's low viscosity medium (SPURR, 1969). Sections were cut either with glass or a diamond knife on a Sorvall-Mt-2 microtome. The sections were stained with uranyl acetate (saturated solution in 50% ethyl alcohol) followed by lead citrate (REYNOLDS and BOWEN, 1963) and were examined with a Phillips 200 electron microscope.

To study the effect of simazine on phycobiliprotein (PBP), control as well as simazine treated cultures were prepared in duplicate sets. Cells grown for 10 days were used for the experiment. For dark incubation, experimental cultures in 100 ml erlenmeyer flasks were wrapped in aluminium foil and placed in a dark box. After 8 and 15 hr of respective dark incubation, both sets were grown for the next 24 hr in continuous fluorescent light. The pigments of the treated and control cells were recorded at 678 nm for chlorophyll-a; at 550 nm for phycoerythrin; at 610 nm for phycocyanin; at 650 nm for allophycocyanin on a Beckman Model 24 Spectrophotometer before and after dark incubation.

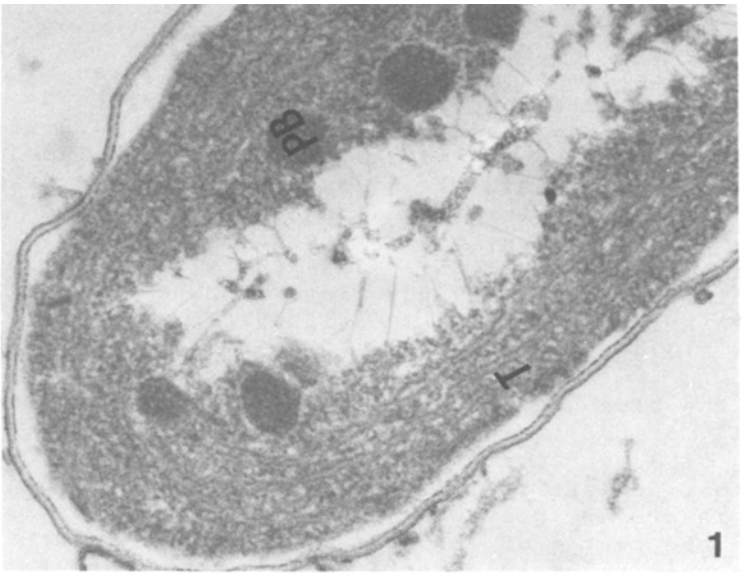
RESULTS and DISCUSSION

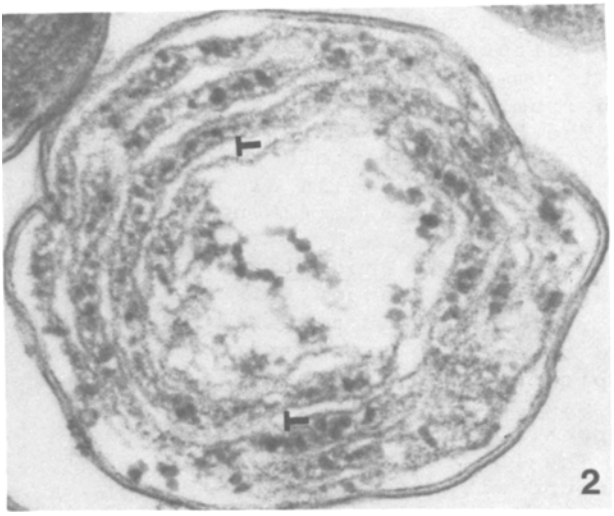
The cytoplasm of most species of blue green algae contain many inclusion of various size, shape and appearance. Among these, polyhedral bodies, (PB) also known as Carboxysomes, and the photosynthetic lamellae, thylakoids, are prominent and easy to recognize (fig 1). The function of thylakoids, along with photosynthesis, is also to produce phycobiliproteins necessary for cell metabolism (BOGORAD 1975). The PB are vital for cell activities and have many functions (CODD AND STEWART 1976, PANKRTAZ and BOWEN 1963, SHIVELY et al. 1973, SHIVELY 1974 and STEWART and CODD 1975).

In the blue-green algae, the thylakoids are seen generally around the periphery of the cell in the form of 1,2, or 3 concentric rings placed equidistant from each other and are filled with a homogenous colloidal mass. The PB are polygonal in shape and are normally seen in the center of the cell near nuclear material. They vary in size and often appear round or angular due either to thickness of section or plane of sectioning. The number of PB seen in a series of E.M. observations in a young (10 day) culture varied from 1 to 6 per cell but 1 to 3 were most common. In an old (3 week) culture numbers varied from 2 to 4, but 2 PB per cell were most common. The length of PB in the young control cells was 66 to 267 nm and in old culture, 80 to 360 nm. Simazine treated young cells also showed variation in length ranging from 94 to 426 nm.

The effects of simazine on Anacystis were noticeable first on thylakoids and then on PB. The mode of reaction after 12 hr of treatment appeared to be: (a) thylakoids appeared to develop granularity, (b) granular bodies became conspicuous over thylakoids and, as action of simazine became severe (48 hr after treatment) thylakoids appeared empty, distorted, and functionless (fig 2). The PB became larger, fewer, and appeared membrane bound with increase in time of treatment. The death of a cell was indicated by empty distorted thylakoids, depletion of RNA and disintegration of PB last, compared to other cell organelles. Separate biochemical measurements showed also that RNA content in simazine treated cells after 48 hr was less than the control.

We observed that stock cultures of Anacystis (3 weeks and older) showed a membrane around PB. It is not known why a membrane surrounds PB in old cultures. Nevertheless, membrane formation around PB is reported in many species of blue-green algae (WULLENWEBER et al.





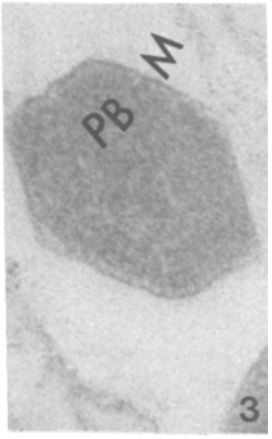


Fig 1. Blue-green alga Anacystis nidulans. Normal cell showing Thylakoids (T) and Polyhedral bodies (PB). X 62,500.

Fig 2. 48 hr after simazine treatment. Note depletion of RNA from cell and distortion of thylakoids (T). X 90,000.

Fig 3. 53 hr after simazine treatment. Note Polyhedral bodies (PB) surrounded by membrane (M). X 160,000.

1977). Membrane formation around PB among simazine treated young cell (fig 3) similar to that seen in untreated cells of 3 week old culture was also observed. Electron microscopic observation on simazine treated cultures, 12 hr after the treatment, showed granular bodies of different stain density originating from PB (fig 4). Preliminary E.M. stereology measurements suggest that marked areas are of different configurations and may be protein in nature (unpublished data).

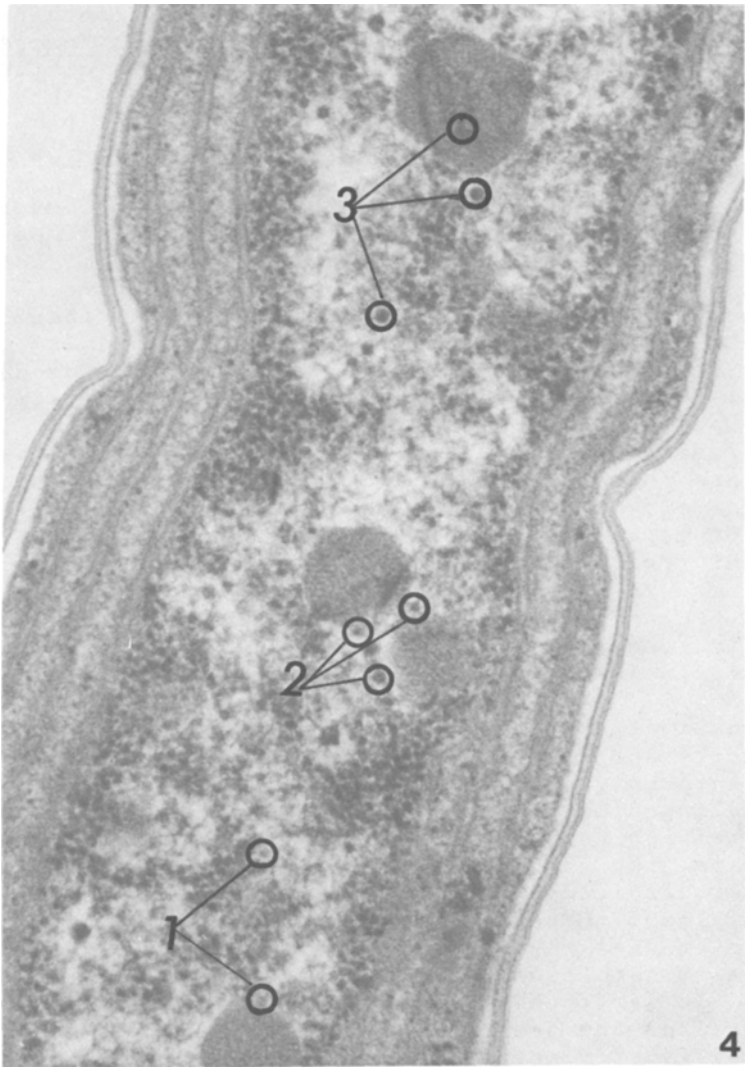


Fig 4. Anacystis nidulans 12 hr after simazine treatment showing granular bodies (marked 1,2 and 3) originating from PB X 114,000.

A complex phenomenon of formation of phycobiliprotein (PBP), the principal receptors for photosynthesis in the blue-green algae, plays a very decisive role in governing life activity. The major catagories of PBP are: the blue phycocyanins (PC) allophcocyanins (APC) and red phycoerythrins (PE). The ratio of one PBP to another and to chlorophyll varies in blue-green algae, depending upon the color of light in which they are growing (BOGORAD 1975). OHKI and FUJITA (1978) showed that the blue-green alga Tolypothrix tenuis grown in the dark contains PC and APC but not PE. We found that cells of Anacystis nidulans treated with simazine and grown in continuous day light type fluorescent light after 15 hr dark incubation, showed negligible amount of PE at 580 nm.

We observed a difference in chlorophyll content in control and simazine treated cultures when measured at 678 nm (table 1). These values in terms of optical density (O.D.), also suggest growth activity of living cells by way of chlorophyll synthesis; i.e. increase in chlorophyll content represents active growing cells. Thus, the difference in O.D. was believed to be due either to reduction in number of cells or a stationary phase which algal cells may have encountered in the presence of simazine. But, it appears more appropriate to consider a reduction in the number of cells because of (i) progressive depletion of RNA and (ii) loss of metabolic activity observed with increase in time of treatment (fig 2). The reduction in number of cells was further suggested by observing a reduction in PE, PC and APC values with an increase in time of simazine treatment, where as respective values increased in the control (fig 5).

Observations made so far are some of the efforts to understand the algae-herbicide interaction. No single technique tried so far with other herbicides (unpublished data) have provided a pattern of reactions ascertaining how herbicides affect algal growth. At the same time, no observations have been ascertained whether herbicides are accumulated or degraded by blue-green algae. Nevertheless, investigations carried out so far, reveal that there is definitely a regulatory effect of herbicides, depending upon the concentration used. It was found that the higher the concentration, the greater the destruction. This indicates a need for analysis of metabolic pathways in algae in the presence of herbicides. Such a study may establish whether the action of herbicide is a primary cause of death or a chain of reactions leading to death as a

Fig. 5. Photosynthetic pigments measured in the control and simazine treated cultures. Note, the treated cultures show no increase in the pigments compared to the control.

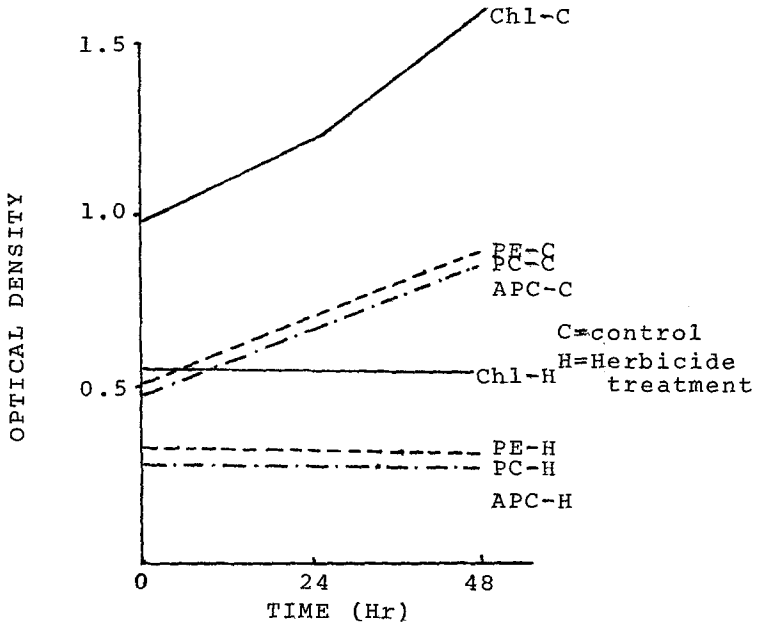


TABLE I

Chlorophyll content measurement in the control and simazine treated culture of Anacystis nidulans at 678 nm.

CULTURE	TIME (HR)[a]			
	0	24	48	72
CONTROL	0.616	0.884	1.072	1.077
SIMIZINE TREATED	0.532	0.542	0.578	0.569

[a]Grown in day light type fluorescent light. Increase in chlorophyll content indicates increase in number of cells.

secondary event. In the present study, use of E.M. has established correlations between herbicidal induced changes and the normal physiology of algae. It is also possible to correlate and compare action of herbicides under controlled experimental conditions and results can be substantiated. It remains to be seen, what happens if environmental conditions are changed.

Under experimental conditions, simazine at 10^{-5}M concentration is effective by disrupting photosynthetic functions. However, whether the presence of simazine affects the function of thylakoids and PB directly, or not is yet to be determined. In conclusion, we find that simazine is causing death in algal cells by imbalancing the function of thylakoids and in turn making PB incapable of performing their functions. What these functions are remains to be undertood.

ACKNOWLEDGEMENT

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