USE OF ULTRAVIOLET RADIATION TO ACHIEVE BACTERIA-FREE ALGAL CULTURE

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Green and blue-green algal cultures were exposed to ultraviolet (UV) radiation at varying intensities and times. No bacterial growth was observed for the first 64 hours when treated algae were inoculated on nutrient agar plates. Algal pigments were not affected by radiation. Phycocyanin appeared to be as active as chlorophyll in photosynthesis. Growth requirements of treated cultures were unaltered. Thus ultraviolet radiation was effective in obtaining and maintaining axenic algal cultures.

INTRODUCTION

Several methods have been described for obtaining axenic cultures of green and blue-green algae (1, 2). Among the methods tried were the use of antibiotics (3, 4, 5, 6), elevated temperature (7), gamma radiation (8), UV radiation (9), and chlorine water (10).

None of these methods indicate a means of maintaining axenic algal cultures. The purpose of this study, therefore, was to determine if UV radiation could eliminate bacterial growth from algal cultures and maintain them axenically for analytical experiments.

MATERIALS AND METHODS

A green alga, *Chlorella pyrenoidosa* Chick, and blue-green algae, *Anabaena variabilis* Kuetzing and *Lyngbya birgei* G. M. Smith, were used in the experiments. All cultures were grown and maintained in ASM-1 liquid medium (2) at a constant temperature (25-28 C) on a shaker at 80 cycles per min and exposed to continuous light of 3500 lux (KEN-RAD F40cw fluorescent tube, Westinghouse).

This experiment was carried out in an inoculation room fitted with an UV tube. Cultures were exposed to UV radiation under a 30-W G30T8 BI-PIN Base cool light GE (General Electric Co.) germicidal tube. Data pertaining to wavelength of the radiation were unavailable. Hence, to judge the effect of UV radiation in terms of energy emitted, the irradiation was carried out at various set distances; exposure times were also varied to establish irradiance dose levels.

A 4-W GE germicidal bulb (General Electric Co.) was also used at a set distance and again time factors were used to set irradiance dose levels. The 4-W bulb was used to minimize laboratory working space required.

Owing to differences in spore and vegetative cell sizes and the filamentous nature of algae, it was not possible to establish a reliable and accurate count of bacteria by conventional methods. Moreover, cultures were not synchronized. Growth obtained on Bactopeptone nutrient agar (11), with and without 2% sucrose, was rated as +; ++; +++; and ++++, when compared with a control, which was rated as ++++, to determine how much bacterial growth occurred after UV exposure. This method gave a relative measure of the bacteria present. As a routine sterilization procedure the inoculation room was swabbed with 3% phenol solution and irradiated with UV radiation 2-3 hours before each experiment. The respective algal cultures in 10-ml portions were placed in open, 100 × 15 mm disposable Petri dishes, 92 and 152 cm directly below the UV source. Optical density of the cultures was between 0.4 and 0.9 at 678 nm. Petri dishes were agitated frequently during the course of the experiment. After 0, 5, 10, 15, 20, 25, 30, 45, 60 and 120 min, 0.5-ml samples of the cultures were introduced into sterile ASM-1 liquid medium (Table 1). Bacterial counts were estimated by inoculating nutrient agar plates in duplicate. Algal cultures were also exposed to the 4-W germicidal bulb at 8 cm distance. The sampling schedule was as mentioned above.

Liquid cultures were grown under continuous light at 25-28 C. Nutrient agar,

with and without sucrose, was found adequate to isolate bacterial contamination. Agar plates were examined daily and liquid cultures examined after one week for bacterial growth.

RESULTS AND DISCUSSION

There was no apparent effect of UV radiation on *Chlorella pyrenoidosa* and *Lyngbya birgei. Anabaena variabilis* showed slight loss of color when exposed for 2 hours. However, cells recovered when subjected to normal continuous light.

Algal cultures exposed at 152 cm gave inconsistent results on nutrient agar at 37 C as well as 25-28 C (Table 1). Random bacterial contamination was not observed until after 64 hr incubation on nutrient agar and bacterial growth did not occur before 10 days in cultures grown in liquid medium

TABLE 1. *Growth of bacteria on nutrient agar 64 hours after UV exposure at 152 cm* [a]

		Irradiation time (min)									
Species	Temp. (C)	0 [b] (control)	5	10	15	20	25	30	45	60	120
Chlorella pyrenoidosa	37	++++ [d]	— [c]	—	+	—	—	—	+	+	—
	25-28	++++	—	+	—	—	—	—	—	—	+
Anabaena variabilis	37	++++	—	+	+	+	—	—	—	—	—
	25-28	++++	+	—	—	—	—	—	—	—	+
Lyngbya birgei	37	++++	—	—	+	+	+	+	—	—	one colony
	25-28	++++	—	—	—	—	—	—	—	—	—

[a] UV intensity 22.4 $\mu W/cm^2$.
[b] Control growth appeared within 48 hr.
[c] — = no growth.
[d] Growth rating + = 25%; ++++ = 100%.

TABLE 2. *Growth of bacteria on nutrient agar 64 hr after UV exposure at 92 cm.* [a]

		Irradiation time (min)									
Species	Temp. (C)	0 [b] (control)	5	10	15	20	25	30	45	60	120
Chlorella pyrenoidosa	37	++++ [c]	— [b]	—	—	—	—	—	—	—	—
	25-28	++++	—	—	—	—	—	—	—	—	—
Anabaena variabilis	37	++++	—	—	—	—	—	—	—	—	—
	25-28	++++	—	—	—	—	—	—	—	—	—
Lyngbya birgei	37	++++	—	—	—	—	—	—	—	—	—
	25-28	++++	—	—	—	—	—	—	—	—	—

[a] UV intensity 52 $\mu W/cm^2$.
[b] Control growth appeared within 48 hr.
[c] — = no growth.
[d] Growth rating + = 25%; ++++ = 100%.

TABLE 3. *Growth of bacteria on nutrient agar 64 hr after UV exposure at 8 cm* [a] *with 4-W bulb.*

		Irradiation time (min)									
Species	Temp. (C)	0 [b] (control)	5	10	15	20	25	30	45	60	120
Chlorella pyrenoidosa	37	++++ [d]	—	— [c]	—	—	—	—	—	—	—
	25-28	++++	—	—	—	—	—	—	—	—	—
Anabaena variabilis	37	++++	—	—	—	—	—	—	—	—	—
	25-28	++++	—	—	—	—	—	—	—	—	—
Lyngbya birgei	37	++++	—	—	—	—	—	—	—	—	—
	25-28	++++	—	—	—	—	—	—	—	—	—

[a] UV intensity 400 $\mu W/cm^2$.
[b] Control growth appeared within 48 hr.
[c] — = no growth.
[d] Growth rating + = 25%; ++++ = 100%.

in UV-treated cultures. As Table 1 shows, bacterial growth was observed in *Chlorella* cultures after 15, 45 and 60 min irradiation at 37 C and after 10 and 120 min at 25-28 C; in *Anabaena* cultures, after 10, 15, and 20 min irradiation at 37 C and after 5 and 120 min at 25-28 C, and in *Lyngbya*, after 15, 20, 25, 30 and 120 min irradiation at 37 C. Thus a distance of 152 cm and intensity of 22.4 $\mu W/cm^2$ was not adequate for destruction of bacteria.

No bacterial growth was observed on nutrient agar at 37 C as well as 25-28 C in cultures exposed to UV at 92 cm distance (Table 2). Similar observations were made when cultures were exposed to the 4-W bulb at 8 cm distance (Table 3).

Although 5 min UV exposure at 92 cm was found effective for cultures, in our judgment 30-60 min was a more adequate exposure time. This was to compensate for unsynchronized algal as well as bacterial growth.

Lockhart and Brodfuhrer-Franzgrote (12) have shown short wave-length UV radiation (200-280 nm) to be germicidal. However, most investigators (13, 14, 15) investigating the effects of UV radiation on chlorophyll have indicated no appreciable decomposition of the pigment, whether in solid state or solution. Schulze (16), using radiation of 280 nm on *Spirogyra, Cladospora, Nitella,* and *Tradescantia,* showed that pigment was not decomposed. Kluyver (15) found decomposition of pigment only after 55-60 hr of irradiation with a mercury arc-lamp.

In this study, only one species showed some effects of UV radiation. A slight loss of green color observed in *Anabaena* after 2 hr exposure was reversed when the alga was grown in ASM-1 medium under continuous light. Kelner (17) and Dulbecco (18) have also reported photoreversal of UV effects in microorganisms and bacteriophage, and suggested that this effect is apparently widespread in lower forms of both plants and animals. Tanada and Hendricks (19) reported photoreversal of UV effects on soybean leaves subjected to visible light before the dark cycle began. Cline and Salisbury (20) have also suggested the reversal of UV radiation damage by subsequent visible light. Other studies (12) have indicated that under controlled conditions UV radiation was stimulatory to plant growth. Pulich (21) has shown resistance to UV in *Chlorella sorokiniana.* Pirschle and Wettstein (22) performed a UV study under controlled conditions of a climate room and obtained, besides injury and inhibition, stimulation of plant growth. Van Baalen (23) has shown that a coccoid blue-green alga possesses a powerful photoreactivation system. The photoreactivation system is believed to be directly related to respiration and photosynthetic processes.

It is known that blue-green algae possess phycocyanin and chlorophyll as photosynthetic pigments. Phycocyanin is photosynthetically active only by virtue of transferring the light energy it absorbs to chlorophyll (23). Thus the light energy absorbed by phycocyanin activates fluorescence of chlorophyll, enhancing photosynthesis, and more energy is available to repair damage done by UV radiation. However, electron microscopic observations made on all three algae revealed no morphological or anatomical damage (Figures 1, 2, 3). Wolk (24) has shown that blue-green algae have a cell wall with 4 layers. Layers one and three are electron-transparent and two and four are electron-dense. Ultraviolet radiation, being of short wavelength, will be absorbed near the point of impact and not penetrate algae deeply so as to cause irreparable damage. However, UV radiation, having high penetrating energy, can kill bacteria in contact with the algal cell wall. By repeating the process of UV radiation treatment, we could maintain algal cultures axenically for several generations without observing any changes in their growth, morphology, temperature requirements, nutritional need, or other phenotypic characters. Shestakov (25) has shown that agents such as UV light, X-rays, hydroxylamine, Acridine Orange, and mitomycin C were poor mutagens for algae and their effect could be repaired by photoreactivation processes.

The genotypic changes in algae were not expected because algal cultures were grown with normal life regulatory factors such as nutrient medium, light source, and growth temperature, during the course of experiments.

Basic structural differences between prokaryotes and eukaryotes would make them respond in different ways to selective ex-

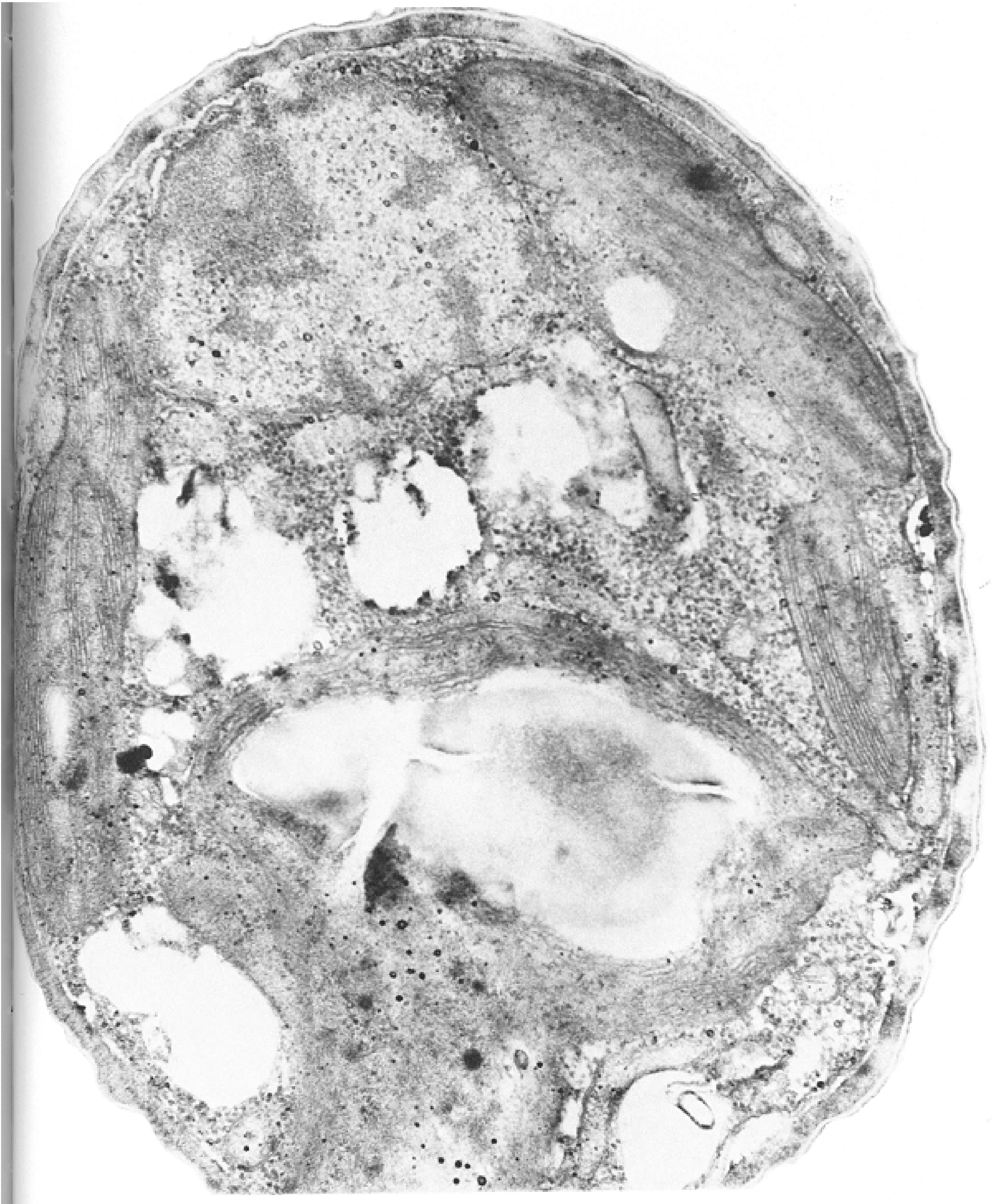

FIGURE 1. *Chlorella pyrenoidosa* after exposure to UV radiation for 2 hours. Note no damages to cell wall and no disarray of cellular organelles. $\times$ 20,000 on viewing screen.

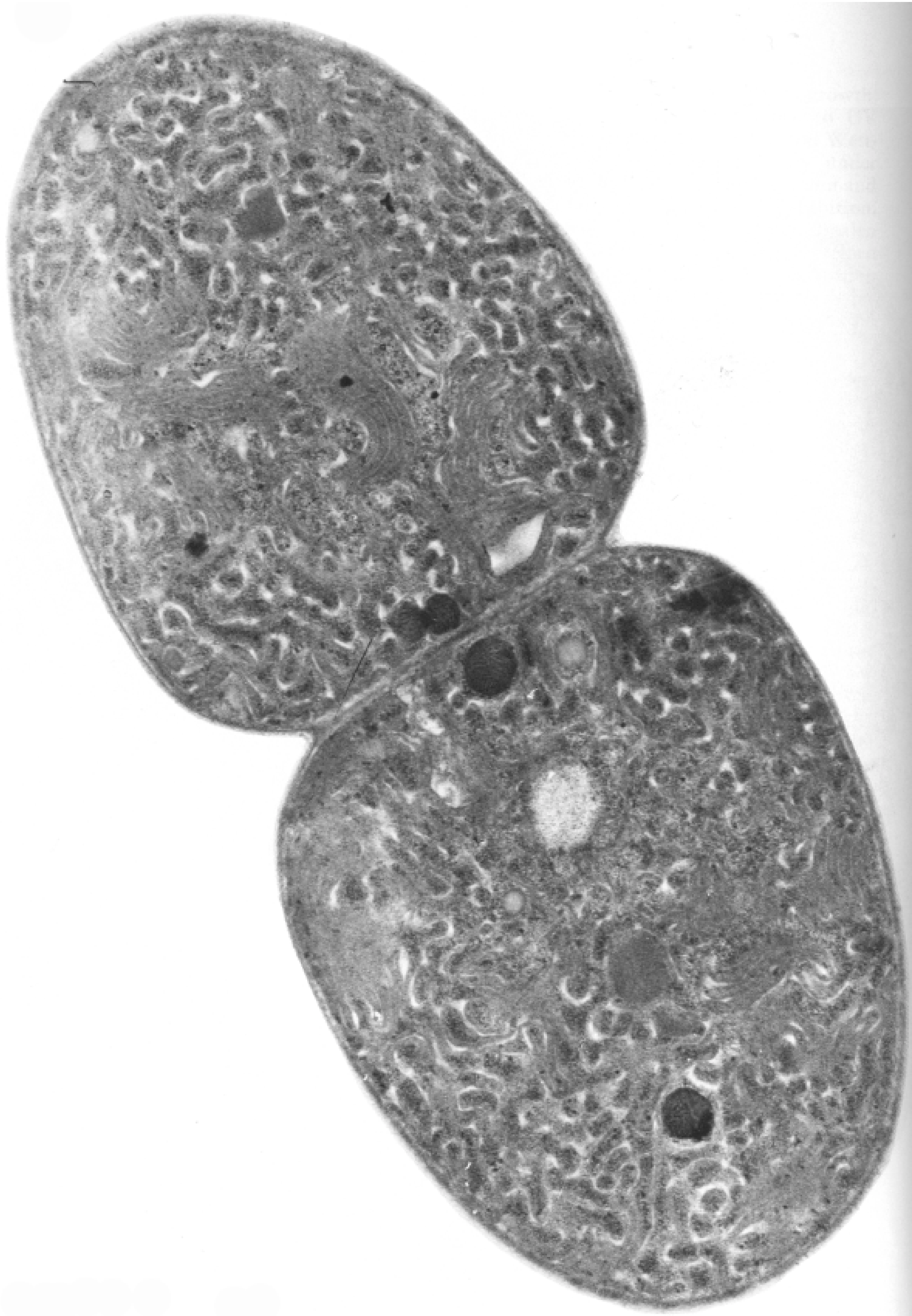

FIGURE 2. *Anabaena variabilis* after exposure to UV radiation for 2 hours. Note no damage to cell wall and no disarray of cellular organelles even during cell division process. $\times 10{,}000$ on viewing screen.

FIGURE 3. *Lyngbya birgei* after exposure to UV radiation for 2 hours. Note no damage to cell wall. Each septum shows complete preservation of cell organelles. $\times$10,000 on viewing screen.

perimental pressure and to the expression of mutational change. However, Sager (26) pointed out that there is no equivalent of the organelle DNA of eukaryotes in blue-green algae; hence, there is no possibility of non-nuclear inheritance on this basis. The suggestion by Van Baalen (23) that a photoreactivation system was responsible for repair of damage in a coccoid blue-green alga, was supported by observations of Wu, *et al.* (27). They showed that in *Plectonema boryanum,* UV-induced damage to the cells could be repaired by white, blue, red, or "black" light. We, as described earlier, have used white light to grow UV-treated algal cultures.

The purpose of this study was to eliminate bacteria by UV radiation from algal cultures without causing physiological and metabolic disturbances in algal cells. Therefore, elaboration on algal metabolism is considered to be out of context. Questions pertaining to a common link betwen respiration and photosynthesis, transfer of light energy with the pigment systems present in photosynthesizing cells, and photokinetics must be studied separately.

Effective removal of bacteria from *Chlorella, Lyngbya* and *Anabaena* cultures suggest that UV can penetrate mucilaginous layers around algae. UV treatment may therefore prove effective on other algal species. We believe the role of UV in algal growth may soon become better understood and it may prove to be the most effective way to achieve and maintain algal cultures axenically.

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