Chapter 7

INHIBITION OF GROWTH AND RESPIRATION BY VISIBLE AND NEAR-VISIBLE LIGHT

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1. Introduction

Visible light is one of the dominant factors in determining the growth, development, and reproductive patterns in most organisms. During the course of evolution one of the most consistent environmental characteristics has been the repetitive diurnal variation in light intensity. The potential information content of the light environment was high, allowing for the development of various photoreceptor systems which permitted the organism to sense impending changes in the environment and to respond accordingly.

The light environment, however, is not entirely beneficial. Reports have long been accumulating in the literature that light, particularly in the blue and neighboring near ultraviolet, is inhibitory to growth and reproduction in certain plant and animal tissues and in numerous microorganisms, both prokaryotic and eukaryotic. In this chapter are dis-

cussed recent studies on the nature of the inhibitory actions of blue light and near-ultraviolet radiation on growth and respiration in various prokaryotic and eukaryotic organisms.

2. Light and Growth: General Studies

2.1 Prokaryotes

Among the prokaryotes, visible plus near-ultraviolet radiation, primarily in the range 330–490 nm, has been reported to kill or inhibit the growth of a wide variety of bacteria, including such heterotrophic bacteria as *Streptococcus salivarius* (Buchbinder *et al.*, 1941), *Escherichia coli* (Hollaender, 1943; Kashket and Brodie, 1962; Jagger *et al.*, 1964), *Bacterium prodigiosum* (Swart-Fuchtbauer and Rippel-Baldes, 1951), *Pseudomonas aeruginosa* (Kashket and Brodie, 1962), *Mycobacterium phlei* (Kurup and Brodie, 1966), and *Hemophilus influenzae* (Jagger and Stafford, 1962) and certain carotenoidless mutants of bacteria, such as *Sarcina lutea* (Mathews and Sistrom, 1959), *Mycobacterium* sp. (Wright and Rilling, 1963), *Halobacterium salinarium* (Dudas and Larsen, 1962), and *Myxococcus xanthus* (Burchard and Dworkin, 1966; Burchard *et al.*, 1966), as well as the chemoautotrophic bacteria *Nitrosomonas europaea* (Schon and Engel, 1962; Block, 1965) and *Nitrobacter winogradskyi* (Block, 1965; Muller-Neugluck and Engel, 1961) and the denitrifying bacterium *Micrococcus denitrificans* (Mutze, 1963; Harm and Engel, 1965).

Buchbinder *et al.* (1941) found *Streptococcus* to be killed by natural daylight, direct sunlight, and even 2 hours of low intensity fluorescent light from a 15-W "daylight" fluorescent lamp. The portion of the emitted spectrum responsible for the killing was not determined. *Bacterium prodigiosum* was likewise found to be killed by sunlight (Swart-Fuchtbauer and Rippel-Baldes, 1951). In experiments using filters and sunlight, Swart-Fuchtbauer and Rippel-Baldes (1951) demonstrated that this bacterium was most sensitive to the sun's radiation in the spectral range 365–405 nm.

Mathews and Sistrom (1959) reported that the carotenoidless mutant of the nonphotosynthetic bacterium *Sarcina lutea* is also killed in an oxygen-dependent reaction by sunlight, although the carotenoid-containing wild type is not. Wright and Rilling (1963) reported similar results for a carotenoidless species of *Mycobacterium;* the active portion of the spectrum was 360–590 nm. Dudas and Larsen (1962) found that a carotenoidless mutant of the salt-water bacterium *Halobacterium salinarium* is not killed by exposure to very high intensity white light, but its growth is strongly inhibited. The portion of the spectrum responsible for the inhibition, however, was not determined.

Hollaender (1943) in early studies with starved cultures of *Escherichia coli* reported that this bacterium is killed by radiation in the near-ultraviolet and visible regions of the spectrum. In subsequent studies with actively growing cultures, Kashet and Brodie (1962) presented evidence which suggested that it is the near-ultraviolet radiation which is specifically inhibitory and that it acts on oxidative metabolism. A detailed discussion of this and subsequent works on the specific effects of near-ultraviolet radiation on *E. coli* and other bacterial systems whose oxidative metabolism has also been shown to be sensitive only to near-ultraviolet radiation, but not visible light, has been recently reviewed elsewhere (Jagger, 1972) and will not be presented here.

The chemoautotrophic bacteria *Nitrosomonas europaea* and *Nitrobacter winogradskyi* were found to be killed by high intensities of filtered sunlight (Schon, and Engel, 1962; Muller-Neugluck and Engel, 1961). Block (1965) found that very high intensities of light from an unfiltered high-pressure mercury lamp significantly inhibit NH_4^+ oxidation in *N. europaea*. He reported similar results for nitrite oxidation in *N. winogradskyi*. In both bacteria, after 24 hours of irradiation both cytochromes c and a_2 are destroyed. Mutze (1963) reported that high-intensity visible plus near-ultraviolet radiations is lethal to the denitrifying bacterium *Micrococcus denitrificans*. Harm and Engel (1965) in later studies showed that both mixotrophic and heterotrophic growth were sensitive either under aerobic or anaerobic conditions. As in the case of the autotrophic bacteria, the cytochromes were reported to be destroyed. The cytochromes were found to be more sensitive to photodestruction under anaerobic than under aerobic conditions, suggesting that the photodestruction was not due to a photooxidative-type mechanism.

Burchard and Dworkin (1966) found that dark-grown carotenoidless cells of the soil bacterium *Myxococcus xanthus* lysed when exposed to visible light while in stationary phase. The photoinduced lysis appears to be due to photooxidation, sensitized by an endogenous pigment which, apparently, accumulates only during the stationary state (Burchard and Dworkin, 1966). Burchard *et al.* (1966) report that the spectrum resembles the spectrum of a porphyrinlike pigment, the main active region of the spectrum being between 395 and 430 nm, with a less active secondary peak around 525 nm.

2.2 Eukaryotic Microorganisms

Among eukaryotic microorganisms light has been reported to be inhibitory to the green algae *Chlorella pyrenoidosa* (Sorokin and Krauss, 1959) and *Euglena gracilis* (Padilla and Cook, 1964; Cook, 1968), to the colorless algae *Prototheca zopfii* (Epel and Krauss, 1966), *Prototheca*

portoricensis (Kowallik, 1965), and *Astasia longa* (Cook, 1968), to certain colorless mutants of *Chlamydomonas* (Gross and Dugger, 1969), to the yeast *Saccharomyces cerevisiae* (Matile and Frey-Wyssling, 1962; Matile, 1962a; Epel and Krauss, 1966; Ehrenberg, 1966a,b, 1968; Sulkowski *et al.*, 1964; Guerin and Sulkowski, 1966), and to the plasmodia of the myxomycete *Physarum polycephalum* (Daniel, 1966) and to be lethal to the fungi *Dacryopirax spathularia* (Goldstrohm and Lilly, 1965) and *Sporidiobolus johnsonii* (Goldstrohm, 1964) and to the protozoan *Tetrahymena pyriformis* (Epel and Krauss, 1966).

2.2.1 Green Algae

Sorokin and Krauss (1959) showed that the onset of division of light-dark synchronized cultures of *Chlorella pyrenoidosa* is inhibited if the cells are left in the light at the end of the light period when they normally would go into the dark. From this observation, plus the fact that the light saturation level for growth of synchronized cultures is greater than that of exponential steady-state cultures, they postulated that light inhibited some process essential to division but not to photosynthetic growth. Padilla and Cook (1964) and Cook (1968) have also claimed that the division of exponentially growing cultures of *Euglena gracilis* is inhibited by light. Cook (1968) has shown that if *Euglena gracilis* cells are grown in the dark on organic medium and then exposed to an irradiance of 1000 fc of incandescent light, no inhibition is detected; if however, the cells are exposed to 300 fc of fluorescent light, an immediate inhibition of division is detected, followed by a recovery that is never complete. Cook (1968) has interpreted these results as indicating a blue light-induced inhibition of some growth or division process.

2.2.2 Colorless Algae

Cook (1968) has also reported that the division of both the naturally colorless euglenoid *Astasia longa* and a colorless mutant of *Euglena gracilis*, *E. gracilis* var. *bacillaris* SM-LI, are inhibited when transferred from the dark to 300 fc of fluorescent light. The degree of inhibition observed was greater for cells grown on salt media with acetate than for cells grown on 1% proteose peptone.

Gross and Dugger (1969) have shown that visible light inhibits the growth of a yellow and a colorless mutant of the alga *Chlamydomonas reinhardi*. They reported that room light or moderate intensities of white light severely inhibit the growth of the white form while only moderately inhibiting the growth of the yellow form. Blue light was found to stop motility and growth in the white form and to induce

clumping, whereas in the yellow form it only reduced the growth rate. In the white mutant but not the yellow, red light was also found to be inhibitory to growth but to a lesser extent than with blue light.

Epel and Krauss (1966) found the growth of the colorless alga *Prototheca zopfii* to be inhibited by white light from fluorescent lamps; the authors suggested that the inhibition was primarily related to a light-induced inhibition of cell division. Later studies by Epel and Butler (1970b) showed that the inhibition of cell division was not a primary effect (see discussion in Section 3.1).

2.2.3 Fungi Other Than Yeast

Goldstrohm and Lilly (1965) reported that unpigmented cells of the dark-grown fungi *Dacryopirax spathularia* and *Sporidiobolus johnsonii* are killed in an O_2-dependent reaction by sunlight whereas cells which had been induced to form pigment by exposure to low light intensities are not. The spectral region of the radiation responsible was not determined.

Daniel (1966) has shown that light depresses the growth and respiration in the plasmodia of the myxomycete *Physarum polycephalum*. The respiratory response is rapid and reversible with changes in the rate of oxygen uptake being detectable within 15 seconds. The inhibition of respiration has been demonstrated with isolated mitochondria as well as with the intact plasmodia.

2.2.4 Yeast

Sulkowski *et al.* (1964) have shown that the respiratory adaptation, protein synthesis, and growth of the colorless yeast *Saccharomyces cerevisiae* is inhibited by low-intensity light when anaerobically grown cells are placed in air. Fermentation, in contrast, is unaffected (Guerin and Sulkowski, 1966). The cells were sensitive only for a short period after the admission of oxygen (1 hour), and fully adapted cells were not inhibited at all (Guerin and Sulkowski, 1966). In earlier studies by Matile and Frey-Wyssling (1962) and Matile (1962a), it was demonstrated that the growth and respiration of fully adapted cells is inhibitable if they are exposed to much higher intensities of light. Under high-light conditions, using starved fully respiratory adapted cells, Matile (1962a) showed that O_2 uptake is inhibited, but CO_2 output remains constant. In addition an initial stimulation of endogenous respiration, followed by inhibition, is observed. Ehrenberg (1966a) in studies with growing cultures, showed that growth, protein synthesis, respiration, and cell propagation are all inhibited by strong visible light, the blue portion of the visible spectrum being the most inhibitory. She

reported that respiration is most inhibited during growth on ethanol, less so during growth on glucose and least during stationary phase (Ehrenberg, 1968). Epel and Butler (1969) and Ninnemann *et al.* (1970a) have studied the mechanism of these light-induced inhibitions in yeast, the results of which are presented in a later section.

2.3 Animal Tissues

Wells and Giese (1950) have shown that sea urchin sperm become immobilized when irradiated with the 435 nm mercury line. Norman and Goldberg (1959) and Norman *et al.* (1962) have reported similar results for bovine, human, and cock sperm. In a crude action spectrum for the light-induced inactivation of sperm, Norman *et al.* (1962) demonstrated that blue and yellow light were the most effective in inactivating the sperm.

The developing embryos of brook and rainbow trout from pale eggs have been shown by Perlmutter (1961) to be killed by very low-intensity light from a 40-W fluorescent lamp, the most active wavelengths being in the blue-violet region of the spectrum. Isolated rat thymocytes have also been reported to be killed upon exposure to high intensities of visible light (Myers and Dewolfe-Slade, 1963).

Klein and Edsall (1967) have claimed that the growth of cultured HeLa cells is inhibited upon continuous exposure to either near ultraviolet or green radiation for prolonged periods; it is claimed that red light reverses the detrimental effects of green, but not those of near ultraviolet radiation.

Santamaria and Primo (1964) have shown that Yoshida hepatoma ascites cells when irradiated under oxygen but not nitrogen with wavelengths greater than 320 nm show a marked decrease in both respiration and glycolysis. They found that isolated calf retina, in contrast, showed a marked decrease in respiratory activity only. The portion of the emitted spectrum responsible for the reported inhibitions was not determined.

High-intensity blue, green, or red light has been shown by Rounds and Olson (1967) to inhibit the respiration of isolated rat cerebellar cells. Although it was not experimentally demonstrated, these workers suggested that the light inhibition was the direct result of photodestruction of one or more of the respiratory cytochromes. They based this suggestion on the observation that the pulsing of a suspension of cells with 50–200 kW of power from a red laser (=6096 + 6013 Å) resulted in an inhibition in the reduction of cytochrome oxidase (Rounds and Olson, 1965). However, the observation made with the laser may not be relevant to the other situation since the extremely high intensity of

the laser pulses may have led to local heating effects; these could have resulted in the observed inhibition of the cytochrome oxidase.

2.4 Higher Plant Tissues

The respiration and growth of higher plant tissues have also been shown to be inhibited by light. Ninnemann and Epel (1968) have shown that blue-violet light is inhibitory to mitosis, but not meiosis, in synchronized tissue cultures of *Trillium* and lily microsporocytes. Near ultraviolet or green radiation is reported to slow the growth of *Parthenocissus* plant tissue culture and of *Ginkgo* pollen, and to interfere with mitosis in onion root tip meristem (Klein and Edsall, 1967; Wolff *et al.*, 1967; Klein, 1964).

Bjorn *et al.* (1963) have shown that in excised wheat roots visible light induces multiple responses. From action spectra studies, Bjorn and co-workers found that blue and red light inhibited cell elongation, with peaks in the action spectrum at 430 and 650 nm, while blue-violet light inhibited cell division, with the main action spectrum peak around 400 nm.

In studies made with isolated mitochondria from cauliflower, Matile (1962b) has reported that the oxidation of Krebs cycle intermediates, as measured by oxygen uptake, is slowed by intense visible light. Cytochrome c oxidase activity was reported to have been inhibited, the enzyme becoming completely reduced in time. Bjorn *et al.* (1963) interpreted this as being due to a direct light-induced reduction of the cytochromes of the mitochondria and concluded that the primary lesion caused by light is in the Krebs cycle. In similar studies Mosolova and Sisakyan (1964) showed that although in mitochondria isolated from etiolated pea plants the oxidation of succinate is inhibited by light, in mitochondria isolated from green pea plants oxidation proceeded normally. In neither the studies of Bjorn nor those of Mosolova and Sisakyan was it determined whether intense visible light produces similar effects *in vivo*.

3. Mechanisms of Photoinhibition

3.1 Studies with the Colorless Alga *Prototheca zopfii*

3.1.1 Growth Inhibition

In 1966 Epel and Krauss demonstrated that when growing cultures of the "colorless" alga *Prototheca zopfii* were irradiated with "white light" from "cool-white" fluorescent lamps, growth was inhibited. With

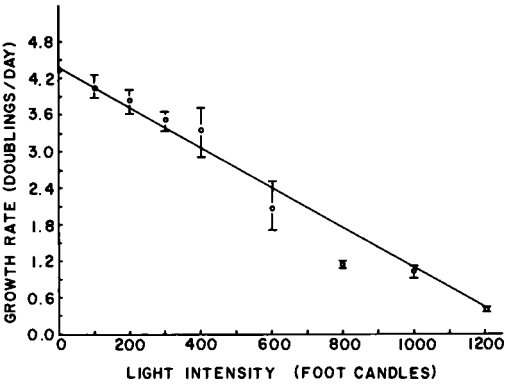

FIG. 1. Growth inhibition of *Prototheca zopfii* induced by light from "cool-white" fluorescent lamps as a function of light intensity reported in foot candles. Growth rates are in doublings per day. (After Epel and Krauss, 1966.)

increasing light intensity, the growth rate of the alga decreased linearly from about 4.5 doublings per day in the dark to less than half a doubling per day at 1200 fc (Fig. 1). The authors suggested that the inhibition of growth was primarily related to a light-induced inhibition of cell division.

Subsequent studies by Epel and Butler (1970b) showed, however, that the inhibition of cell division was a secondary effect. Measurements of the action of white light on protein synthesis, nucleic acid synthesis, respiration, and cell division performed with growing cultures of *P. zopfii* revealed no differential inhibition on any of these parameters either with respect to the onset or kinetics of the inhibition. All four physiological parameters measured showed the same degree of inhibition over the 12-hour irradiation period (Fig. 2). These experiments suggested that the primary locus of photoinhibition lay elsewhere than in cell division. From the observation that the major physiological growth parameters were equally inhibited by light, it seemed probable that the primary physiological system affected must be tightly coupled to the major synthetic and growth functions. The authors reasoned that the most likely target would prove to be the respiratory apparatus since it contains enzymes (the cytochrome and flavoproteins) that absorb light in the visible region of the spectrum. Furthermore, the action spectrum studies of Epel and Krauss (1966) for the inhibition of growth implicated a porphyrinlike substance as the light receptor.

3.1.2 Light Inhibition of Respiration

This conjecture, which was eventually shown to be correct, was reinforced from the results of studies by Epel and Butler (1970b) on

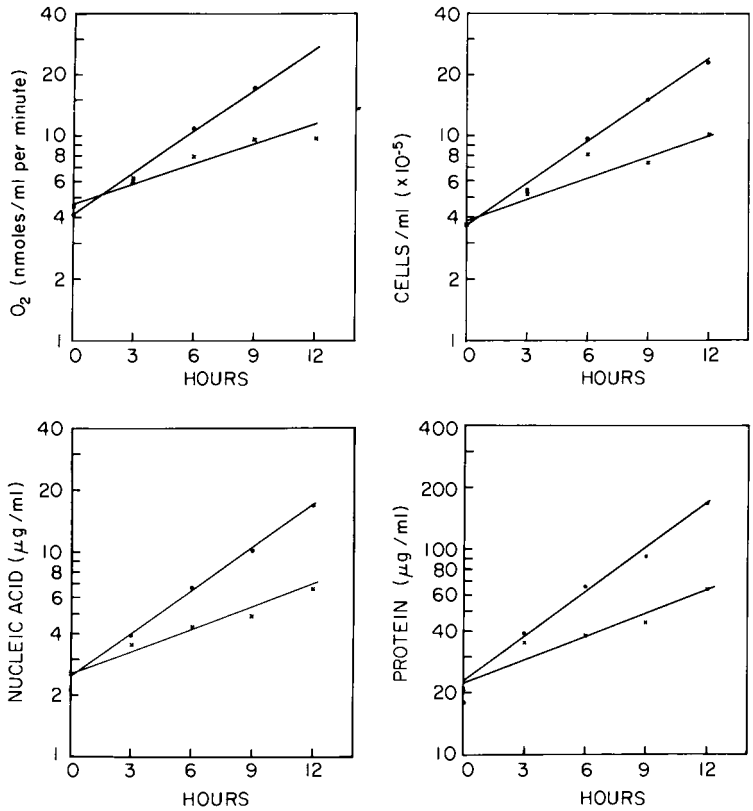

FIG. 2. Inhibition of respiration, cell division, nucleic acid synthesis, and protein synthesis in growing cultures of *Prototheca zopfii* induced by light from "cool-white" fluorescent lamps (1–2.9 × 10^4 ergs cm^{-2} sec^{-1}). Upper left: Rate of oxygen uptake per milliliter of culture versus hours of growth in light (×) and dark (○); upper right: number of cells per milliliter of culture vs hours of growth in light (×) and dark (○); lower left: concentration of nucleic acid in micrograms per milliliter of culture vs hours of growth in light (×) and dark (○); lower right: concentration of protein in micrograms per milliliter of culture versus hours of growth in light (×) and dark (○). K_D and K_L are the growth rate constants for the various parameters for dark grown and light grown cultures, respectively. (After Epel and Butler, 1970b.)

the effect of light on the respiratory capacity of a starved culture of the alga. Cultures were starved initially in the dark for 24 hours, and at various periods thereafter aliquots were removed and irradiated with moderate intensities of white light from fluorescent lamps. The respiratory capacity of the cells was then determined by measuring the rate of oxygen uptake upon the addition of substrate to a test aliquot. Figure 3 shows the results of such an experiment. Cells left in the dark for as long as 7 days showed no loss in their capacity to respire on added

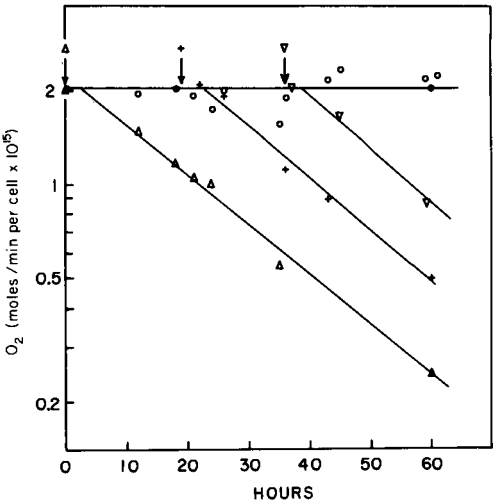

FIG. 3. Inhibition of capacity of starved cells of *Prototheca zopfii* (3×10^6 cells/ml) to respire on added substrate (0.25% ethanol) induced by white light from "cool-white" fluorescent lamps (1–2.9 × 10 ergs cm^2 sec^{-1}). Rate of oxygen uptake per cell after the addition of substrate vs hours starved. Nonirradiated control (○); cells irradiated continuously from start of starvation (□); cells irradiated after 19 hours of dark starvation (×): cells irradiated after 36 hours of dark starvation (△). Arrows indicate start of irradiation periods. (After Epel and Butler, 1970b.)

substrates. Continuous irradiation with white light from fluorescent lamps, however, was shown to result in an exponential inhibition of respiratory capacity. The degree of inhibition proved to be independent of the time of previous dark starvation. Although it could have been argued that the decrease in respiratory capacity resulted from a killing of cells, viability determinations, performed by comparing the colony-forming ability of irradiated cells to those of control cells, showed almost no loss in viability of the cells due to irradiation.

3.1.3 The Cytochrome of *P. zopfii*

The physiological and action spectra data of Epel and Butler (1970b) of Epel and Krauss (1966) strongly suggested that the target site for the photoinhibition lay in the respiratory electron transport chain. The use of special spectroscopic techniques and instrumentation in studies (Epel and Butler, 1970a) made with both intact cells and isolated mitochondria from *P. zopfii* established that the respiratory electron transport chain of the alga contained at least 7 cytochromes: two c-type cytochromes, a soluble form, c-549, and a membrane-bound form,

c-551; three b-type cytochromes, b-556, b-559, and b-564; and two a-type cytochromes, a and a_3 (cytochrome oxidase).

3.1.4 Photosensitivity of Cytochrome a_3

Of the seven cytochromes associated within the respiratory electron transport chain, three—cyt c-551, b-559, and a_3—were shown to be partially photolabile; of these, a_3 was shown to be by far the most labile. The destruction of cytochrome a_3 could be shown by a number of different spectral assays. In Fig. 4 the difference spectrum of intact cells irradiated for 2 hours with high-intensity blue light is compared with that of a dark control. Obvious qualitative differences are seen in the absorption spectra between the two samples in the spectral region 430–450 nm, the region of the Soret band of cytochrome oxidase. In addition to the large loss in absorption observed in the Soret region, there was a shift in the cytochrome oxidase α-band from 598 to 601.

Epel and Butler (1970b) were able to establish that these spectral changes were due to the destruction of the a_3 component of cytochrome oxidase (a + a_3) through a direct spectral assay for cytochrome a_3.

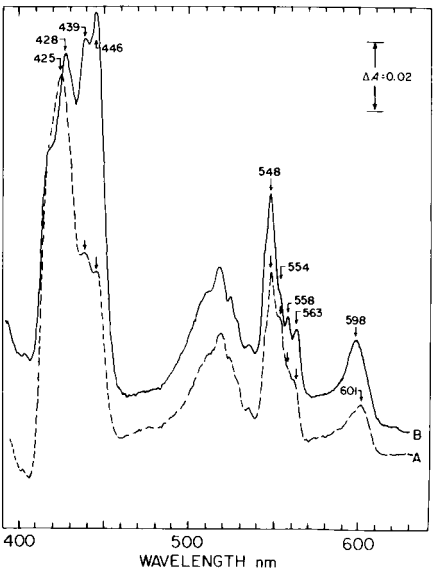

Fig. 4. Low-temperature difference spectra (dithionite versus substrate-respiring aerated cells) of cells of *Prototheca zopfii* (8.7×10^6 cells/ml) before irradiation (curve B) and after 2 hours of irradiation (curve A) (AH-6 super-pressure mercury lamp, Corning filters 5433 + 3850, $1\text{–}2.5 \times 10^5$ ergs cm^{-2} sec^{-1}) with added light-scattering agent (0.33 gm of $CaCO_3$/ml). [After B. Epel and W. L. Butler, *Science* **166**, 621 (1969). Copyright 1969 by the American Association for the Advancement of Science.]

CO, a classical inhibitor of respiration, is known to bind to the a_3 component of cytochrome oxidase and to cause a spectral shift in the absorption spectrum of the cytochrome. Furthermore, this binding is photoreversible. Chance *et al.* (1964) showed that at liquid nitrogen temperature, irradiation for a few second results in an irreversible photodissociation of the cytochrome a_3–CO complex thus allowing for the measurement of the photodissociation difference spectrum. That blue irradiation causes a destruction of the a_3 component of cytochrome oxidase was shown using this assay (Fig. 5). While the nonirradiated control cells exhibited a normal difference spectrum for the CO–cytochrome a_3 complex, the irradiated cells exhibited essentially no indication of the complex (Epel and Butler, 1970b).

The effects of high-intensity blue light on cytochrome oxidase was demonstrated even more directly through the use of a new spectral assay which directly distinguishes between cytochrome a and a_3. Epel and Butler (1970a) found that the α-bands of cytochrome a and a_3

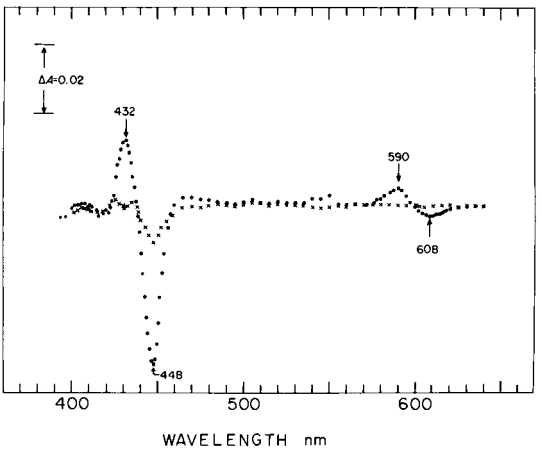

FIG. 5. Low-temperature carbon monoxide photodissociation difference spectra of nonirradiated (●) and 2-hour irradiated (AH-6 lamp, Corning filters 5433 + 3850, 1–2.5 × 10^5 ergs cm^{-2} sec^{-1}) cells of *Prototheca zopfii* (×). Starved cells (8.7 × 10^7 cell/ml) suspended in starvation medium, pH 6.9, with added scattering agent (0.33 gm of $CaCO_3$ per milliliter) were reduced with dithionite, and humidified CO was blown across the surface of the reduced suspension. Cells were frozen in both sample and reference cuvettes, and a base line was recorded. The reference cuvette was irradiated for 10 seconds with white light from a Unitron LKR lamp to dissociate the carbon monoxide–cytochrome a_3 complex, and the spectrum was recorded. The spectra presented in the figure were computed by subtracting the base line from the recorded spectra. [After B. Epel and W. L. Butler, *Science* **166**, 621 (1969). Copyright 1969 by the American Association for the Advancement of Science.]

could be resolved if the spectrum was measured in the presence of methanol, cyanide, and dithionite at low temperatures. In the absence of methanol, the absorption band of reduced cytochrome oxidase appears at low temperature (−196°C) as a single band with a maxima at 598 nm. If methanol is included with cyanide and the strong reductant dithionite, the oxidase band is split into two district bands with maxima at 595 nm and 602 nm (Fig. 6). The methanol causes a red shift of the α-band of cytochrome a to 602 nm and permits dithionite to fully reduce the cyanide–cytochrome a_3 complex. It is the reduced cytochrome a_3–cyanide complex that gives rise to the 595 nm band (Epel and Butler, 1970a). In the absence of methanol, the cyanide–cytochrome a_3 complex remains largely oxidized even in the presence of dithionite (Yonetani, 1960). This spectral assay provided a convenient assay for distinguishing effects of light on cytochromes a and a_3. With this assay, Epel and Butler (1969, 1970a) were able to show that while the a_3 component of the oxidase was nearly completely destroyed after 1 hour of irradiation, the a component was unaffected

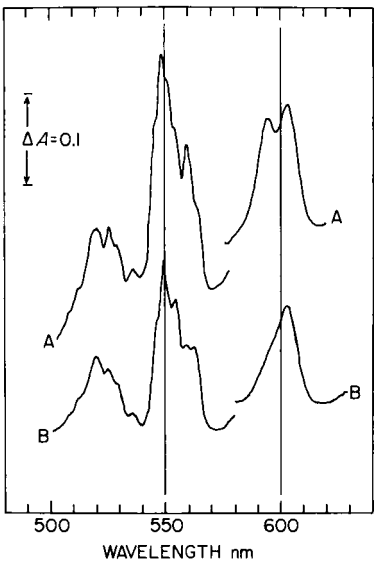

FIG. 6. Low-temperature (−196°C) absolute spectra of cells of *Prototheca zopfii* (3×10^7 cell/ml) suspended in starvation medium, pH 6.9, with added scattering agent (0.5 gm of Al_2O_3 per milliliter); cells reduced with dithionite in the presence of MeOH (2.5%) and KCN ($2.5 \times 10^{-4} M$). Curve A, nonirradiated cells; curve B, cells irradiated for 1 hour (AH-6 lamp, Corning filters 5433 + 3850, $1–2.5 \times 10^6$ ergs cm^{-2} sec^{-1}). (After B. Epel and W. L. Butler.) *Science* **166**, 621 (1969). Copyright 1969 by the American Association for the Advancement of Science.]

(Fig. 6). Cytochrome c-551 and b-559 were also shown to be photolabile as can be seen on inspection of Fig. 6, but to a much lesser degree than cytochrome a_3 (Epel and Butler, 1970b).

In most of their studies Epel and Butler irradiated the starved cells with extremely high intensities of blue light in order to shorten the irradiation period. However, irradiation with lower intensity of light for correspondingly longer irradiation periods gave identical results (Epel and Butler, 1970b).

3.1.5 Oxygen Requirement for Photodestruction

The respiratory apparatus was protected if irradiation was carried out under anaerobic conditions. The capacity to respire added substrates under aerobic conditions after an irradiation treatment under anerobic conditions was found to be essentially unaffected (7% inhibition), while after a similar irradiation treatment under aerobic conditions the exogenous respiration was almost completely inhibited (88%). A comparison of the absorption spectra of cells irradiated under aerobic and anaerobic conditions confirmed that only in the presence of oxygen were the three cytochromes photolabile (Epel and Butler, 1970b).

Cyanide, a classical inhibitor of cytochrome oxidase, is known to compete with oxygen for the active site of cytochrome a_3 (Yonetani, 1960). Epel and Butler (1970b) demonstrated that $2.5 \times 10^{-4} M$ cyanide protected cytochrome a_3 from photodestruction under aerobic conditions. These data are highly suggestive of a photooxidative type mechanism and furthermore suggest that the photooxidation probably occurs at the O_2 binding site of cytochrome a_3.

3.2 Studies with the Yeast *Saccharomyces cerevisiae*

3.2.1 Inhibition of Growth and Respiration

Although during the latter half of the 19th century and the early part of the present century numerous but often contradictory reports appeared concerning the effects of visible radiation on growth division and respiration in yeast and other fungi (Ninnemann *et al.*, 1970a), more recent studies (see Section 2.2.4) have clearly established that visible radiation exerts a marked inhibitory effect on growth, cell division, respiration and protein synthesis in yeast. Figures 7A and B shows an example of the nature of visible light effect on both exogenous and endogenous respiration on previously starved yeast cells. As was first shown by Matile and Frey-Wyssling (1962) and later confirmed by Ninnemann *et al.* (1970a), irradiation of starved yeast cells with white light inhibits the capacity of the cells to respire on added sub-

strates. Under the conditions employed in the experiments of Ninnemann *et al.* (1970a) depicted in Fig. 7A, the inhibition caused by irradiation with moderate intensities of white light was found to be approximately first order after an initial lag period. Dark control cultures in comparison showed no loss of respiratory capacity over a period of 100 hours. Under much higher light intensities, where the rate of inhibition was more rapid ($t_{½}$ = 8 minutes), Ninnemann *et al.* (1970a) reported that no lag phase was observable (Fig. 7B). As in the case of *P. zopfii*, photoinhibition requires the presence of oxygen (Ninnemann *et al.*, 1970a). Irradiation under anaerobic conditions had no effect on the capacity of the yeast cells to respire substrates when the cells were subsequently returned to aerobic conditions (Fig. 7B).

Matile and Frey-Wyssling (1962) showed that the response of the endogenous respiration of the yeast cells to irradiation is more complex and, as pointed by Ninnemann *et al.* (1970a), is a reflection of a different mechanism. During the initial part of the irradiation period, either

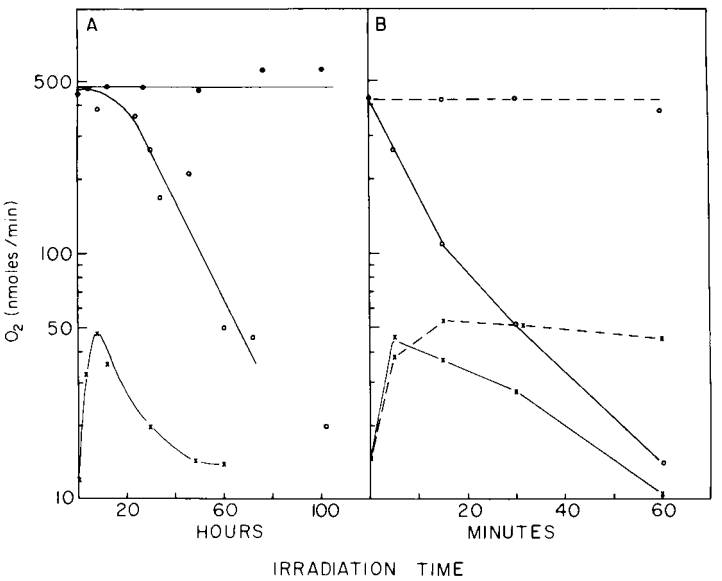

FIG. 7. Effect of light on respiration of yeast (1×10^8 cells/ml) irradiated (A) with moderate-intensity (3×10^4 ergs cm^{-2} sec^{-1}) white light from fluorescent lamps and (B) with high-intensity (1×10^8 ergs cm^{-2} sec^{-1}) blue light from high-pressure mercury lamp and Corning 5562 filter. Rate of exogenous (0.076% ethanol) respiration (○—○) and endogenous respiration (×—×) vs time of irradiation under aerobic conditions. Rate of exogenous respiration (○- - -○) and endogenous respiration (×----×) vs time of irradiation under anaerobic conditions. Exogenous respiration of nonirradiated cells vs time in dark (●). (After Ninnemann *et al.*, 1970a.)

under high- or moderate-intensity irradiation, endogenous respiration is stimulated (Matile and Frey-Wyssling, 1962; Ninnemann *et al.*, 1970a) be it under aerobic or anaerobic conditions. Only under aerobic conditions is a subsequent inhibition noted (Ninnemann *et al.*, 1970a). Examples of such complex responses are shown in Figs. 7A and 7B. The light stimulation of respiration thus appears to be a separate phenomenon unrelated to the inhibitory phenomenon. Further studies of these very interesting phenomena have not yet been pursued.

3.2.2 Photosensitivity of Cytochromes a and a_3

The nature of the light inhibition in yeast appears to be very similar to that for *P. zopfii*. Epel and Butler (1969), and Ninnemann *et al.* (1970a) in spectroscopic studies modeled after those made with *Prototheca*, established that irradiation with blue light results in the destruction of both components of cytochrome oxidase, i.e., cytochromes a and a_3, and part of cytochrome b. An example of this can be seen in Fig. 8A. In this experiment, yeast cells were irradiated with high-intensity blue light for various periods, and the cytochrome content was measured spectroscopically at low temperature (−196°C). The bands at 548, 554, and 559 nm are the reduced α-bands of cytochrome

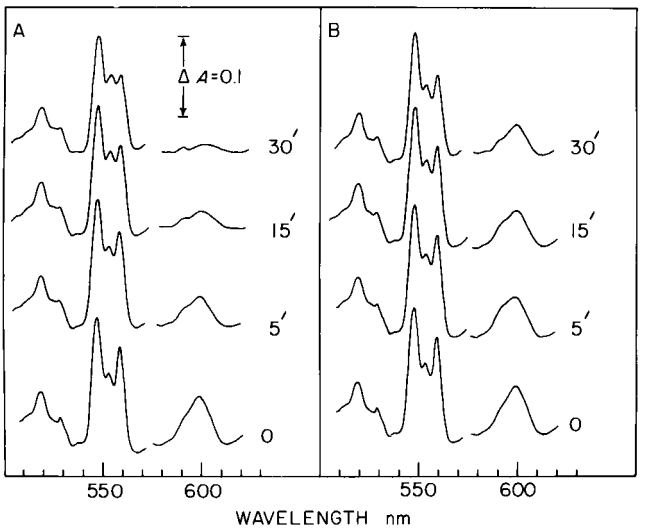

Fig. 8. Absorption spectra of yeast at 77°K after various periods (minutes) of irradiation (pH 5.5) with blue light (1.5×10^6 ergs cm^{-2} sec^{-1}). (A) In the absence of azide. (B) In the presence of 1 m*M* NaN_3. All spectra were measured in the presence of 1 m*M* NaN_3, 1 m*M* KCN, and dithionite with Al_2O_3 added as a light-scattering agent. (After Ninnemann *et al.*, 1970a.)

c, c_1, and b, respectively, while those at 590 and 603 are the reduced α-bands of the cytochrome a_3–CN complex and of cytochrome a, respectively. As can be seen in the example depicted in Fig. 8A, increasing periods of irradiation resulted in a progressive diminution in the α-bands of cytochromes a, a_3, and b. Although cytochromes a and a_3 were nearly completely destroyed within 30 minutes as determined by the disappearance of their α-bands, cytochrome b was still partially present, indicating that it is less photolabile than either cytochromes a or a_3. Ninnemann *et al.* showed that the kinetics of the inactivation of cytochrome oxidase follow very closely the inactivation of respiration, as can be seen on comparing Figs. 9A and 9B with Figs. 7A and 7B.

3.2.3 Oxygen Requirement for Photosensitivity

Epel and Butler (1969) and Ninnemann *et al.* (1970a) found that, as in the case of *P. zopfii*, oxygen is required for the photoinactivation of the yeast cytochromes. Under anaerobic conditions the cytochromes were found to be fully protected (Ninnemann *et al.*, 1970a). Furthermore

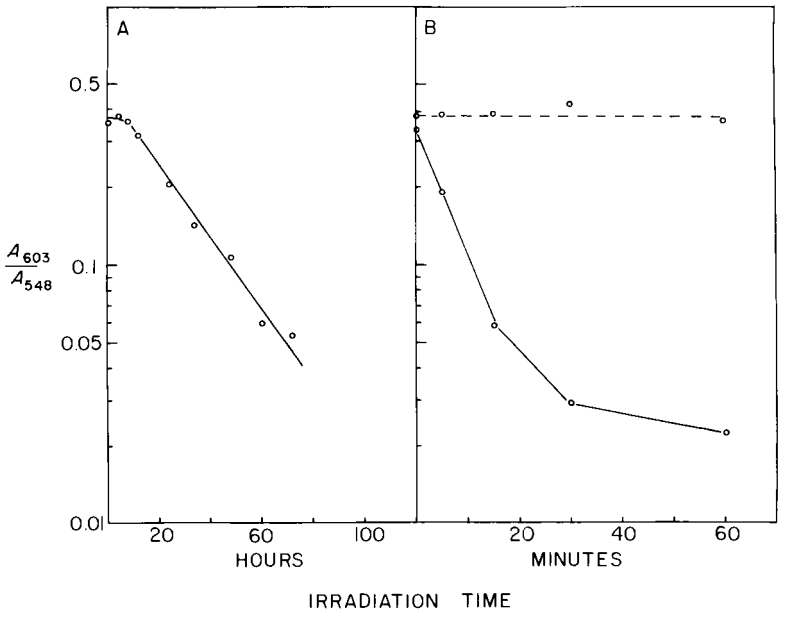

Fig. 9. Effect of light on cytochrome oxidase of yeast as measured by the ratio of the absorbances at 603 and 548 nm vs time of irradiation. Starved cultures of yeast irradiated (A) with moderate-intensity (3×10^4 ergs cm^{-2} sec^{-1}) white light from fluorescent lamps and (B) with high intensity (1×10^6 ergs cm^{-2} sec^{-1}) blue light from a high pressure mercury lamp and Corning 5562 filter. ○—○, Aerobic conditions; ○---○, anaerobic conditions. (After Ninnemann *et al.*, 1970a.)

cyanide and azide, ligands which compete with oxygen for the active site of cytochrome oxidase, were shown to protect respiration from photoinactivation. The protective action of azide can be seen in the example depicted in Fig. 8B.

3.3 Studies with Mammalian Mitochondria

As noted in Section 2.3, there have appeared in the literature a number of reports concerning the inhibitory nature of light on respiration in a number of mammalian tissues.

Epel and Butler (1969) and Ninnemann *et al.* (1970b) in light of their findings on the inhibitory nature of visible radiation in *Prototheca* and yeast reinvestigated the question of the nature of the photoinhibition in a mammalian system. Ninnemann *et al.* (1970b) found that O_2 uptake by isolated beef-heart mitochondria with succinate as substrate was inhibited by irradiation with blue light. Spectroscopic studies similar to those made with *Prototheca* and yeast established that the irradiation treatment destroyed the a_3 component of cytochrome oxidase (Fig. 10). The mammalian oxidase appeared to be of the *Prototheca* type with respect to its light sensitivity in that cytochrome a was not

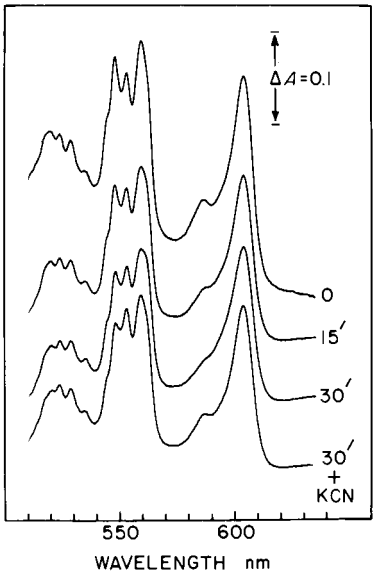

FIG. 10. Absorption spectra of beef heart mitochondria after various periods (minutes) of irradiation in the absence or presence of 1 m*M* KCN duing the irradiation as indicated. All spectra were measured in the presence of dithionite, 2.5 m*M* KCN, and 2.5% methanol at 77°K. (After Ninnemann *et al.*, 1970b.)

affected. As in the case of *Prototheca*, cytochrome a_3 was protected if the irradiation was carried out in the presence of cyanide (Fig. 10).

3.4 Studies with Purified Cytochrome Oxidase

Proof that the light acted directly on the oxidase came from studies of Ninnemann *et al.* (1970b). The effect of irradiation on the activity of purified cytochrome oxidase from beef heart measured as the rate of O_2 uptake with reduced cytochrome c as substrate is shown in Fig. 11. Irradiation of the oxidase with strong blue light under aerobic condition was shown to result in an inhibition of the oxidase activity. Irradiation under anaerobic conditions was almost without effect. Spectroscopic studies confirmed that the a_3 component was inactivated under these conditions. Although the lack of photoinhibition under anaerobic conditions is highly suggestive of a photooxidative-type mechanism, it could be argued that since the photoreceptor is a redox type pigment only the oxidized form of the pigment is photolabile and that oxygen itself is not directly required for the photoinhibition. Such a possibility was

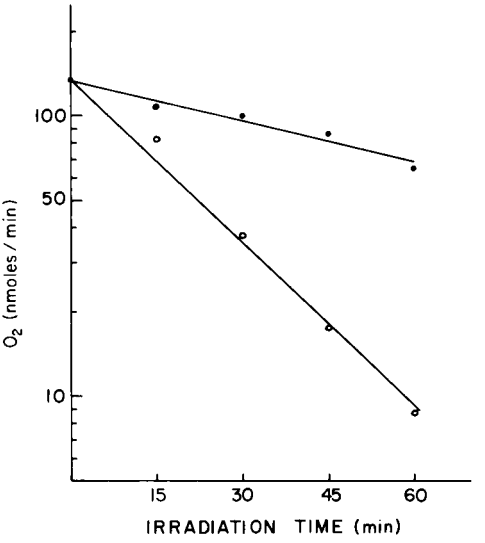

FIG. 11. Influence of oxygen on cytochrome oxidase activity as a function of the time of irradiation with light from a high-pressure mercury lamp and Corning filter 5562 ($1–2 \times 10^6$ ergs cm^{-2} sec^{-1}). Irradiation was performed at 0°C. Oxidase activity was measured as rate of oxygen uptake at 27°C with reduced cytochrome c as substrate [20 μg of oxidase protein per milliliter with 30 μM cytochrome c and 1.5 mM ascorbate; the reaction mixture contained 0.05 M Tris·HCl (pH 8.0), 1 mM histidine and 0.66 M sucrose]. ●—●, Anaerobic; ○—○, aerobic. (After Ninnemann *et al.*, 1970b.)

ruled out, however, by the observation that the purified oxidase remains in its oxidized state when made anaerobic by bubbling with argon even for as long as 2 hours. Since no inactivation of the oxidase was found under anaerobic conditions with the oxidase in its oxidized form, it was concluded that photoinactivation is oxygen dependent.

4. Concluding Remarks

Although the extent to which the photodestruction of cytochrome oxidase, and the resultant inhibition of respiration by visible light occurs in the biological world is difficult to estimate, since definitive work in intact organism has been done only with the alga *P. zopfii* and the yeast *C. cerevisiae*, the observation that the oxidase of isolated beef heart mitochondria is also sensitive argues strongly in favor of the premise that this phenomenon is probably very widespread. If it is conceded that this phenomenon is universal, it is then intuitively obvious that most organisms must process a protective or active repair mechanism since sunlight appears to be directly detrimental to few organisms. How such protective and repair mechanisms function provides an interesting area for future investigations.

Finally, it should be pointed out that inactivation of respiration by near-ultraviolet light probably reflects a different mechanism from that described here. Unpublished studies by the author comparing the action spectrum for the inactivation of respiration with the inactivation of cytochrome oxidase in intact cells show that blue light inactivated both cytochrome oxidase and respiration, while near-ultraviolet irradiation inactivated only respiration. For a recent review of studies into the nature of such near-ultraviolet induced inhibition of growth and respiration, the reader is referred to a recent review by Jagger (1972).

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