35 days of age and had no significant effect in I mice (*10*). A comparable long-lasting depression after administration at birth was described for 9,10-dimethyl-1,2-benzanthracene injected into newborn mice (*11*).

Our data are consistent with the possibility of host immunological mechanisms as regulators of malignant development, because resistance to MC oncogenesis was associated with absence of immune depression by the carcinogen in I-strain mice. The nature of the immunological mechanisms involved in the regulation of tumor development is still unknown. The immune depression induced by MC in the above experiments was caused by a decrease of the actual numbers of antibody-producing cells in the spleen. Some experiments suggest that the depression may operate primarily on the antigen-sensitive precursors (*10*). In the case of the resistant animals, no such decrease in antibody-producing cells was observed. Other nonexclusive possibilities to explain the relative resistance includes a true resistance of the target cells to the carcinogen and differences in the metabolic processing of the oncogenic agent, or both. On the other hand, since resistance was not absolute in the I strain and immune depression was not observed even in animals that developed tumors (*10*), paraimmunological and nonimmunological mechanisms should be also considered.

OSIAS STUTMAN

Department of Laboratory Medicine, College of Medical Sciences, University of Minnesota, Minneapolis

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Cytochrome a_3: Destruction by Light

Abstract. *Spectroscopic measurements on cultures of* Prototheca zopfii *irradiated with blue light revealed that inhibition of respiration was accompanied by destruction of cytochrome a_3. One of the three b-type cytochromes and one of the two c-type cytochromes of this organism were also affected. Cytochrome oxidase of yeast (not resolved into the a and a_3 components) and cytochrome a_3 of beef-heart mitochondria were also destroyed by blue light.*

Blue and near-ultraviolet radiation inhibit the growth of many plant and animal tissues and that of numerous microorganisms as well (*1*). The point of departure for our work was the report that blue light inhibited the growth of the colorless alga *Prototheca zopfii* (*2*). Studies with exponentially growing cultures of *P. zopfii* showed that a moderate intensity of white light (10 cm from a bank of four cool white fluorescent lamps) inhibited cell division, protein synthesis, nucleic acid synthesis, and respiration to approximately the same extent. The photoinhibition of respiration was assumed to be the more direct phenomenon, with the inhibitions of cell division and the synthetic processes being indirect consequences.

In order to study the inhibitory effects of light in the absence of growth and cell division, further studies were limited to the effect of light on the respiratory capacity of starved cells. In these experiments, the respiratory capacity of a portion of cells was measured with an oxygen electrode after addition of substrate (ethanol). Continuous irradiation from the cool white fluorescent lamps exponentially inhibited the respiratory capacity of the starved cells with a rate constant of 0.04 per hour (17 hours for 50 percent inhibition). Irradiation with blue light from a filtered high-pressure mercury lamp (2×10^6 erg cm^{-2} sec^{-1}) inhibited the respiratory capacity much more rapidly, 50 percent inhibition being achieved in 15 minutes. Starved cells maintained in darkness for as long as 7 days showed no loss of capacity to respire on added substrate. An action spectrum for the effect of light is not yet complete in detail, but experiments with filters have shown that blue is the most effective spectral region.

No loss of viability resulted from the irradiation treatment. Cells irradiated for 89 hours with the fluorescent lamps showed the same colony-forming ability as cells kept in darkness (80 percent for irradiated cells versus 87 percent for nonirradiated cells) even though the rate of exogenous respiration of the irradiated cells was only 3 percent of the dark control. Similar

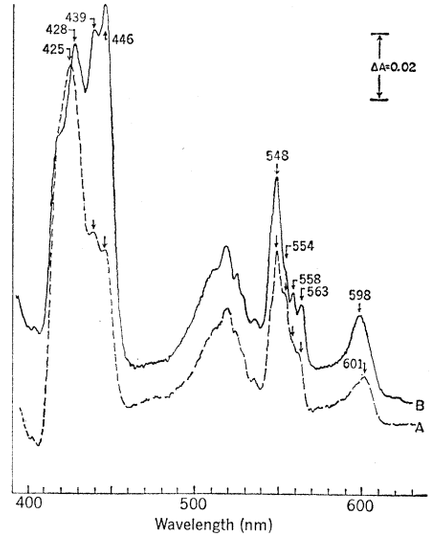

Fig. 1. Low-temperature (−196°C) difference spectra of cells (8.7×10^6 cell/ml) suspended in starvation medium, *p*H 5.5, with added scatter agent (0.33 g of $CaCO_3$ per milliliter). Curve A, cells irradiated for 2 hours (AH-6 lamp, Corning filters 5433 + 3850, $I = 2.5 \times 10^5$ erg cm^{-2} sec^{-1}); dithionite-reduced cells minus substrate-(0.25 percent ethanol) respiring aerated cells; curve B, dark control cells, dithionite-reduced cells minus substrate-respiring aerated cells.

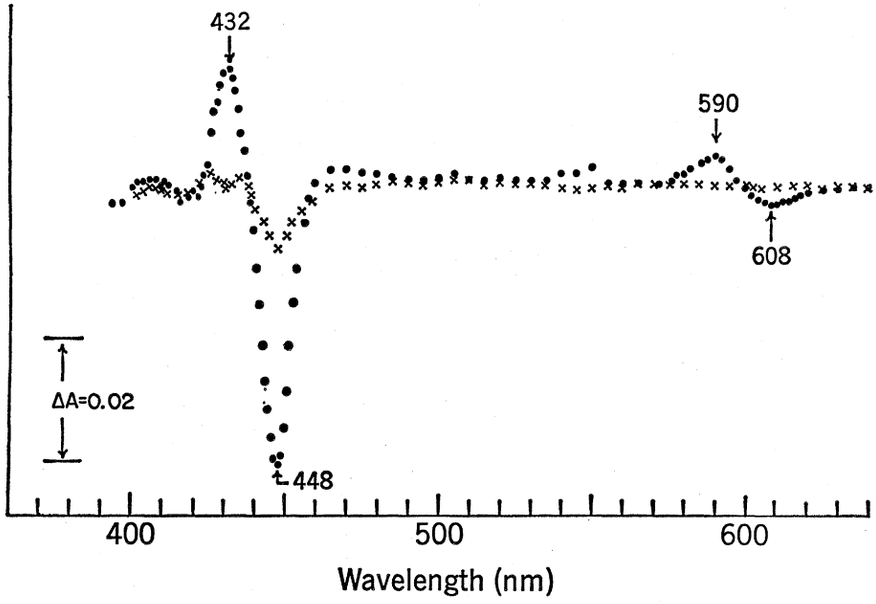

Fig. 2. Low-temperature carbon monoxide photodissociation difference spectra of nonirradiated (solid circles) and 2-hour irradiated (AH-6 lamp, Corning filters 5433 + 3850, $I = 2.5 \times 10^5$ erg cm^{-2} sec^{-1}) cells (crosses). Starved cells (8.7×10^7 cell/ml) suspended in starvation medium, *p*H 6.9, with added scattering agent (0.33 g $CaCO_3$ 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.

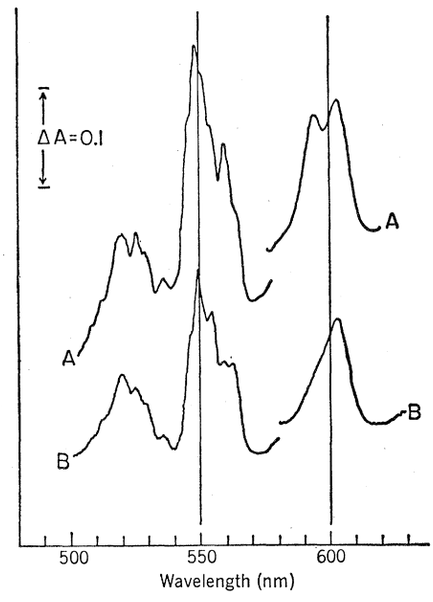

Fig. 3. Low-temperature (−196°C) absolute spectra of cells (3×10^7 cell/ml) suspended in starvation medium, *p*H 6.9, with added scattering agent (0.5 g Al_2O_3 per milliliter); cells reduced with dithionite in the presence of MeOH (2.5 percent) and KCN ($2.5 \times 10^{-4}M$). Curve A, nonirradiated cells; curve B, cells irradiated for 1 hour (AH-6 lamp, Corning filters 5433 + 3850, $I = 2.5 \times 10^6$ erg cm^{-2} sec^{-1}).

observations of photoinhibition of respiration have also been made with starved cultures of yeast cells.

Spectroscopic examinations were made on irradiated and nonirradiated cells of *P. zopfii* to determine whether light had any influence on the respiratory pigments. Spectra were measured at −196°C in a split-beam spectrophotometer (*3*) for difference spectra or in a single-beam spectrophotometer (*4*) for direct spectra. A light-scattering agent was added to the cell suspension to increase the optical path and to intensify the absorption bands (*5*). Cells irradiated for 2 hours with 2.5×10^5 erg cm^{-2} sec^{-1} of blue light had considerably less absorption in the spectral region attributed to the Soret bands of cytochrome oxidase (Fig. 1). Carbon monoxide photodissociation difference spectra further revealed that the cytochrome a_3 component of the oxidase complex was destroyed or altered by light (Fig. 2).

The effects of light on cytochrome oxidase could be seen more directly by a new assay that distinguished cytochromes a and a_3. It was found that the α-bands of cytochromes a and a_3 could be resolved if the spectra were measured in the presence of methanol, cyanide, and dithionite. In the absence of methanol, the absorption band of reduced cytochrome oxidase at low temperature appeared as a single band with a maximum of 598 nm. When methanol was included with cyanide and dithionite, the oxidase band was split into two distinct bands with maximums at 595 nm and 602 nm (Fig. 3A). Methanol red shifts the α-band of cytochrome a to 602 nm and permits dithionite to fully reduce the cyanide–cytochrome a_3 complex, which gives the 595 nm band (*6*). In the absence of methanol, the cyanide–cytochrome a_3 complex remains largely oxidized even in the presence of dithionite (*7*). This spectral assay provided a convenient means of distinguishing the effects of light on cytochromes a and a_3. The absorption spectra of the irradiated cells (Fig. 3B) in the presence of methanol, cyanide, and dithionite showed no indication of the 595 nm band attributed to the reduced cyanide–cytochrome a_3 complex, which further confirms the photodestruction or alteration of cytochrome a_3. The photodestruction of cytochrome a_3 was prevented if the irradiation was carried out under anearobic conditions or in the presence of $2.5 \times 10^{-4}M$ cyanide. A comparison of Fig. 3, A and B, also revealed that the irradiation treatment resulted in the loss of absorption attributed to cytochrome c(551), seen as a shoulder in Fig. 3A, and cytochrome b(559). The destruction of cytochromes a_3, b(559), and c(551) could be observed after irradiation with the cool white fluorescent lamps of much lower intenstiy, but much longer times of irradiation were required. Similar spectroscopic measurements on yeast and beef-heart mitochondria showed that the same irradiation treatment destroyed cytochrome oxidase of yeast and cytochrome a_3 of beef-heart mitochondria.

BERNARD EPEL
WARREN L. BUTLER
Department of Biology, Revelle College, University of California at San Diego, La Jolla 92307

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