will enhance the growth of differentiated tissue or in other terms inhibit senescence. Regeneration in lower invertebrates also has considerable rejuvenation effects. See L. Haranghy and A. Balazs, in *Perspectives in Experimental Gerontology*, N. W. Shock, Ed. (Thomas, Springfield, Ill., 1966), p. 224.

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Carcinogen-Induced Immune Depression: Absence in Mice Resistant to Chemical Oncogenesis

Abstract. *Administration of 3-methylcholanthrene 6 days before antigenic challenge depressed the immune response to sheep red cells in young adult mice (C3Hf/Bi strain) sensitive to the oncogenic effect of the drug (100 percent local tumors 240 days after carcinogen). The drug was not immunodepressant in the I strain, which is relatively resistant to its carcinogenic effect (11 percent local tumors 240 days after carcinogen).*

A relative resistance to local tumor development after subcutaneous injection of 3-methylcholanthrene (MC) in oil was described in mice of the I strain (*1*). Since MC and other carcinogenic aromatic hydrocarbons depress lymphohemopoietic tissues (*2*) and reduce immune responses (*3*) in mice, it is of interest to study the effect of MC on the immune response to sheep red cells in the relatively resistant mouse strain I and in the carcinogen-sensitive strain C3Hf/Bi.

Table 1. Incidence of local tumors in 35-day-old mice 240 days after subcutaneous injection of 0.1 mg of 3-methylcholanthrene (MC) in 0.1 ml of corn oil.

Strain	Treatment	Mice with tumors per number of treated mice
I	None	0/50
I	Oil	0/40
I	MC	10/87 (11 percent)
C3Hf/Bi	None	0/50
C3Hf/Bi	Oil	0/60
C3Hf/Bi	MC	50/50 (100 percent)

Since the demonstration of tumor-specific antigens in many experimental tumors (*4*), immunodepression has been considered a possible factor in carcinogenesis by favoring the development of antigenic tumor cells (*5*). It has been proposed that the evolutionary value of immune mechanisms (especially cell-mediated immunity of the homograft type) may be related to a general homeostatic mechanism directed toward the recognition and destruction of abnormal (malignant) cells (*6*). Malignant development may occur when the immunological surveillance is bypassed. One possible mechanism of this bypassing may be the immunodepressive effect of the oncogenic agent itself, since immunodepressive effects have been described for physical (*7*), chemical (*3*), and viral carcinogens (*8*).

I now report that no significant immunodepression was observed when MC was administered 6 days before antigenic stimulation with sheep red cells in the relatively resistant I strain. In contrast, MC was a potent immunodepressor in the carcinogen-sensitive C3Hf/Bi mice under comparable conditions. The incidence of tumors was 100 percent in the C3Hf/Bi mice and 11 percent in the I mice (Table 1). Both mouse strains are derived from the colony of the late Dr. J. J. Bittner and are highly inbred. In my experiments all the animals were 35-day-old males.

Sheep red blood cells (0.2 ml of 10 percent suspension in saline) were administered intraperitoneally 6 days after injection of MC or oil vehicle alone, and the response was measured 4 days later by a direct hemolysis plaque-forming technique (*9*). Controls also included mice injected with red cells alone. The plaque-forming technique was used to measure hemolysin production by single cells in suspension. The results are expressed as number of hemolysis plaque-forming cells (PFC) per total number of nucleated cells in spleen (PFC per spleen in Table 2) and as PFC per million nucleated spleen cells. The number of PFC in nonimmunized animals ranged from zero to 120 per total number of spleen cells (mean, 88), and the results are corrected for background counts. Neither MC nor oil alone contributed to background counts.

Methylcholanthrene had no depressive effect on the response of the I mice to sheep red cells. The magnitude of the response in the MC-treated and control groups was comparable. Analogous results were obtained in four other experiments in which the MC dose ranged from 0.2 to 1.0 mg. By contrast, MC produced a marked depression of the immune response in C3Hf/Bi mice. Comparison of oil- and MC-injected groups showed that the reduction of PFC per 10^6 nucleated cells was 85 and 88 percent when results were expressed as PFC per total number of nucleated cells in spleen. A reduction of 16 percent of the total nucleated cells per spleen was observed in the MC group. Comparable results have been obtained (*10*) for circulating hemolysin and agglutinin responses. Both responses to sheep red cells were depressed in C3Hf/Bi mice treated with MC and were not modified in the I strain. Similarly, 0.1 mg of MC at birth produced marked immune depression in C3Hf/Bi mice when the response to the red cells was tested at

Table 2. Immune response to sheep red cells in mice (35-day-old males, 12 animals per group) measured as direct hemolysis plaque-forming cells (PFC). Results are expressed as means ± standard errors. Treatment consisted of 0.1 mg of 3-methylcholanthrene (MC) in 0.1 ml of corn oil injected subcutaneously. Oil indicates oil injection alone. Six days later mice were given intraperitoneal injection of 0.2 ml of 10 percent sheep red cells in saline. Response was measured 4 days after this injection.

Strain	Treatment	10^6 nucleated cells per spleen	10^6 PFC spleen cells	10^3 PFC per spleen
I	None	200 ± 14.2	162 ± 30.1	32.4 ± 6.9
I	Oil	188 ± 21.2	110 ± 33.6	20.6 ± 8.8
I	MC	205 ± 16.9	178 ± 28.4	36.4 ± 6.1
C3Hf/Bi	None	161 ± 15.0	111 ± 21.2	17.8 ± 6.8
C3Hf/Bi	Oil	153 ± 18.2	104 ± 29.6	15.9 ± 6.8
C3Hf/Bi	MC	130 ± 10.1	16 ± 6.0	2.0 ± 0.5

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.

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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

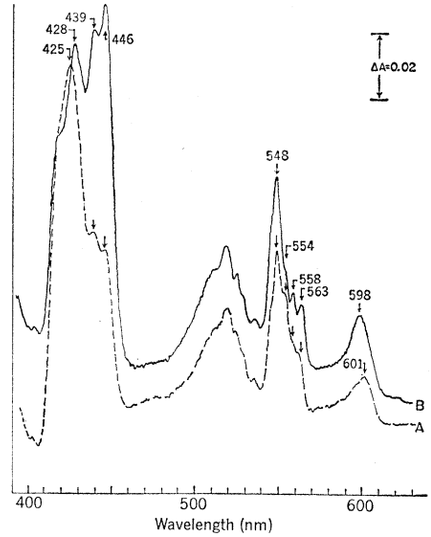

Fig. 1. Low-temperature (−196°C) difference spectra of cells (8.7×10^6 cell/ml) suspended in starvation medium, *p*H 5.5, with added scatter agent (0.33 g of $CaCO_3$ per milliliter). Curve A, cells irradiated for 2 hours (AH-6 lamp, Corning filters 5433 + 3850, $I = 2.5 \times 10^5$ erg cm^{-2} sec^{-1});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