Mutagenesis in Escherichia coli by Visible Light

Abstract. *Mutation to resistance to bacteriophage T5 in continuous cultures of* Escherichia coli *was induced by visible light (wavelength longer than 408 nanometers) and by black light (300 to 400 nanometers). Mutation rates more than 18 times greater than the spontaneous rate (no light) were obtained with moderate, nonlethal intensities of visible light. Mutation rates for both visible and black light were proportional to irradiance.*

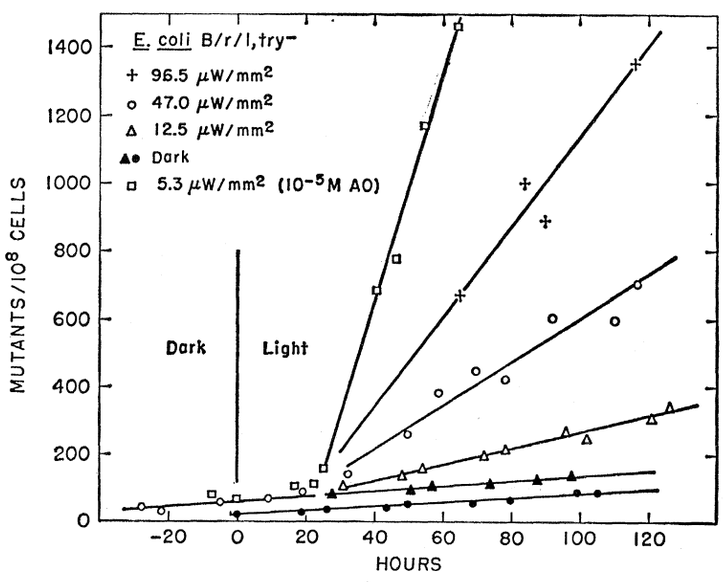
Mutation by visible light in the presence of acridine dyes has been reported in continuous cultures (chemostats) of bacteria (*1*). During subsequent studies of photodynamic mutagenesis, we have found that visible light alone (without an added dye) is mutagenic. Although mutagenesis by visible light has not been reported, inactivation by visible light has been observed in a slime bacterium (*2*) and a carotene-deficient photosynthetic bacterium (*3*). We now report initial results on the induction of mutations by visible light. Mutagenesis by long-wave ultraviolet radiant energy (black light) is included for comparative purposes. Chemostat cultures have been used in these studies because of this system's sensitivity in the detection of low mutation rates.

The basic techniques used by us have been described (*1*, *4*, *5*). Chemostat cultures of *Escherichia coli* B/r/l,try$^-$, were grown in a minimal medium (M9) containing an excess of tryptophan (8 mg/liter) and a limiting concentration of glucose (100 mg/liter with air; 300 mg/liter with N_2). These conditions maintained cell concentrations of 1 to 3×10^8 cells per milliliter in balanced growth. The mutation (to resistance to the bacteriophage T5) was assayed by plating cells in the presence of excess phage T5 on nutrient agar (Difco) supplemented with iron. All colony counts were made after 24-hour incubation at 37°C. The chemostats were placed in small, light-tight incubators designed for these experiments or were individually isolated in an incubator room; the temperature of the growth tubes was maintained at 37°C.

Radiant energy was supplied by lamps containing either (i) two 4-watt "cool white" fluorescent bulbs with less than 1-percent emission below 380 nm (Sylvania F4T5/CW), or (ii) two 4-watt "black light" fluorescent bulbs with a mean emission of about 355 nm (G.E. BLB). The irradiance (intensity) for each condition was measured with a Schwarz vacuum thermopile and a Keithley 150A microvoltmeter; the thermopile was standardized against a National Bureau of Standards lamp. Measurements of irradiance were reproducible within 2 percent. Mutation rates and their standard errors were estimated by a least-squares procedure with a digital computer.

Mutation rates significantly exceeding the spontaneous rate (no light) were produced by continuous visible-light intensities as low as 12.5 μw/mm^2 (Fig. 1). The mutation rate in the dark was 7.3 (S.E., $\pm$ 0.5) mutants per 10^9 cells per hour, whereas at an intensity of 12.5 μw/mm^2 it was 24 (S.E., $\pm$ 1) mutants per 10^9 cells per hour—more than triple the dark mutation rate. At an intensity of 96.5 μw/mm^2, the mutation rate was 135 (S.E., $\pm$ 28) mutants per 10^9 cells per hour, more than 18 times the spontaneous rate (Figs. 1 and 2). Mutation rates were proportional to irradiance over the range from 12.5 to 96.5 μw/mm^2 (Fig. 2). Black light also produced mutation rates greatly exceeding the spontaneous rate (Fig. 2); again the mutation rate was proportional to irradiance.

To eliminate the possibility that the small increment of ultraviolet energy emitted from the white fluorescent lamp was producing the mutagenic effect, a filter with a 37-percent "cut-off" at 424 nm (Corning 3389; 1-percent transmission at 408 nm) was used; the resulting mutation rate was 26 (S.E., $\pm$ 2) mutants per 10^9 cells per 10^6 erg/mm^2, which compares with a value of 29 (S.E., $\pm$ 3) mutants per 10^9 cells per 10^6 erg/mm^2 for the fluorescent lamp without the cut-off filter. Therefore virtually all the mutagenic effect of the white fluorescent lamp results from



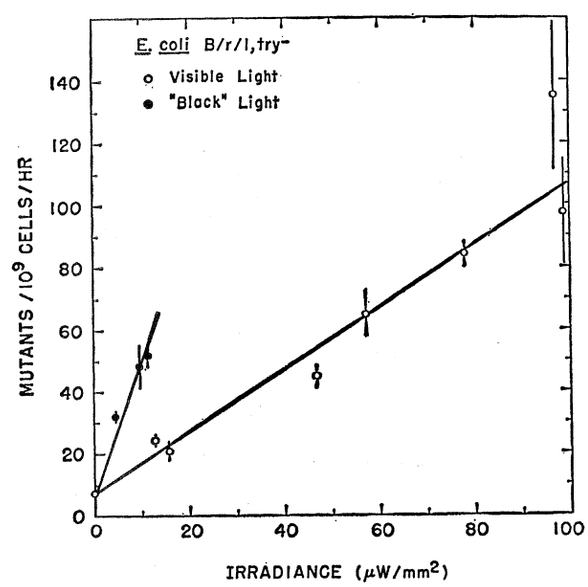


Fig. 1 (left). Visible-light and photodynamic (acridine orange, *AO*) mutation rates (resistance to phage T5) in glucose-limited chemostat cultures at several levels of irradiance by visible light. Growth rates were 0.18 to 0.22 division per hour except for the *AO* chemostat (0.10 division per hour). Fig. 2 (right). Mutation rate (resistance to phage T5) in chemostat cultures in relation to irradiance for both long-wave ultraviolet (black light) and visible light. Standard errors are shown by vertical lines.

wavelengths longer than 408 nm. Inactivation and filament formation were below detection in both visible- and black-light experiments. For comparison, photodynamic mutagenesis with acridine orange also is shown in Fig. 1; addition of an acridine dye to the culture substantially increases the mutation rate from that for light alone.

The mean delay in expression of the light-induced T5-resistant mutants was approximately four generations—a delay of about 20 hours when the cell-division time was 5 hours (Fig. 1). This value is substantially greater than the mean delay of two generations observed for photodynamic mutagenesis with acridine dyes, but less than the value of 5.2 generations observed for caffeine-induced mutagenesis (*4*).

The mutation rate under anoxic conditions (95 percent N_2, 5 percent CO_2) for chemostat cultures irradiated with white fluorescent light (with a 424-nm "cut-off" filter) at an irradiance of 57 $\mu w/mm^2$ was 3.1 (S.E., ± 0.5) mutants per 10^9 cells per hour—similar to the spontaneous mutation rate under anoxia. However, with black light at an irradiance of 11 $\mu w/mm^2$ under the same anoxic conditions, the mutation rate was 12 (S.E., ± 2) mutants per 10^9 cells per hour, a value significantly greater than the dark mutation rate and one-third the rate obtained with black light at this intensity in the presence of oxygen.

This oxygen requirement may be consistent with a photodynamic mechanism of action for mutagenesis, but no conclusions are possible at present because the amount of the chromophore(s) present may be much smaller under anoxic conditions.

Effects of long-wave ultraviolet (300 to 400 nm) on DNA (*6*) and cells (*7*) have been reported. Since DNA shows some absorption at 310 nm, at least some of the effects of black light, reported by us and by others, may involve direct absorption by DNA. However, our preliminary data suggest that the major chromophore for black-light mutagenesis is not DNA. Black-light mutagenesis has also been observed in an ultraviolet-sensitive strain of *E. coli* in chemostat cultures by Kubitschek of our division (*8*).

The nature of the chromophore(s) is of special interest because of its (their) possible association with the DNA of the cell. Several components of *E. coli* are possible candidates for the chromophore. Riboflavin, a planar three-ring molecule similar to the acridines, conceivably could bind to DNA through intercalation as Lerman has suggested for other such planar molecules (*9*). Riboflavin is photodynamically active in certain systems in vitro (*10*). However, vitamin K and any of several porphyrins also are possibilities for the chromophore.

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Paramagnetic Resonance Spectra of Methyl Radicals on Porous Glass Surfaces

Abstract. *Methyl radicals stabilized on surfaces of porous Vycor glass at 77°K show three types of paramagnetic resonance spectra. One of them represents physically trapped radicals, whereas the other two types indicate interactions with surface sites.*

Recent interest in the stabilization of free radicals on solid surfaces prompts us to report further results of our electron paramagnetic resonance studies of methyl radicals, CH_3, on surfaces of porous Vycor glass (Corning 7930) at 77°K. The experimental conditions were similar to those described in our previous report (*1*).

Methyl radicals, when generated by ultraviolet photolysis of adsorbed methyl iodide, are stabilized in quantity on porous glass surfaces over a wide range of temperatures. It would be very interesting if such radicals exhibited in their electron paramagnetic resonance spectra an additional structure due to a hyperfine interaction with atomic nuclei at the trapping sites, which would help us to understand the behavior of the solid surface.

The spectrum of CH_3 usually consists of four lines with the intensity ratio 1 : 3 : 3 : 1. In the case of a monolayer of the adsorbate, an apparent asymmetrical intensity distribution was reported (*1*, *2*). However, we have come to the conclusion, after careful measurements of line widths, that the intensity ratio is actually 1 : 3 : 3 : 1. The line widths of the component lines were 1.8, 1.2, 1.1, and 1.0 ± 0.2 gauss, in order of increasing resonance field. The difference in line widths of hyperfine components may be explained as an effect of a time correlation in randomly tumbling motion of the radical in the presence of a trapping potential (*3*). However, such an explanation does not help to reveal the nature of the surface site. The observed hyperfine constant $A_1 = 23.4 \pm 0.2$ gauss, and g-factor $g_1 = 2.0024 \pm 0.0001$ are very much the same as those for CH_3 in an inert-gas matrix (*4*); therefore, such CH_3 radicals are believed to be "physically" trapped on the surfaces.

When a very small quantity of methyl iodide (less than 10 percent of a monolayer) was adsorbed and photolyzed at 77°K, two other types of spectra were observed in addition to the spectrum due to physically trapped CH_3. These extra spectra, denoted by Me′ and X in Fujimoto *et al.* (*1*), were apparently independent, and in Fig. 1, showing a typical spectrum, a superposition of three spectra is indicated.

The spectrum X appeared most significantly on glass samples outgassed at about 450°C prior to adsorption, but almost undetectable on samples preheated at 700°C or above for a prolonged period of time. It was also recognized that the intensity of the spectrum X relative to the main quartet spectrum became smaller as the surface coverage exceeded 10 percent. The spectrum X is interpreted as being due to a CH_3 radical interacting with a nucleus of spin 3/2, which is most likely the boron-11 isotope, B^{11} (natural abundance 81.17 percent), on Vycor surfaces.

The Vycor glass used for this experiment contains B_2O_3 as a major impurity (approximately 3 percent). Boron impurities may form active sites or inactive boron oxide networks on silicate surfaces, the former being responsible for catalytic activities (*5*). A methyl radical, when trapped at such