cell tumors and cell lines (*16*), $JLSV_6$ control cultures of mixed spleen and thymus (*17*), and C_3H/HeN cultures (*18*). Even though adequate controls should correct for any effects these particles might produce, their presence may be a significant factor in many of the tissue culture, virological, and biochemical studies in which these established murine cell lines are used.

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Mutagenesis by Near-Visible Light

Abstract. *Mutants resistant to bacteriophage T5 were produced both in continuous and in stationary cultures of* Escherichia coli *by near-visible light, 320 to 400 millimicrons, at rates greatly exceeding spontaneous rates in the absence of light. Aerobic mutation rates were about twice anaerobic rates, which shows that mutations were induced in either of at least two different processes. Mutations induced by near-visible light involve different photochemical processes than those induced by ultraviolet light.*

Although mutation has been observed with ultraviolet light of wavelengths as long as 315 mμ (*1*), there has been no definitive report of mutagenesis with the wavelengths in the near-visible region, 320 to 400 mμ. Since light of these wavelengths has been observed to inactivate transforming DNA (*2*) and bacteria (*3*), the possibility of mutagenesis with near-visible light might be anticipated.

This report concerns primarily the production of mutants resistant to bacteriophage T5 in continuous, chemostat cultures of the tryptophan-requiring strain of *Escherichia coli* WP2-HCR− when these were exposed to near-visible light. These cultures were grown in M9 minimal medium in the presence of excess L-tryptophan (5 μg/ml) and limiting glucose (100 μg/ml), giving cell concentrations of 1 to 2×10^8 per milliliter. The composition of this medium and the techniques used to measure mutation rates have been described (*4*). Growth rates were controlled by the rate of addition of nutrient. Growth and irradiation of cultures was carried out in a dark room at 37°C. Two kinds of light source were used: (i) two 4-watt "black light" fluorescent bulbs (Westinghouse F4T5/BLB, at a distance of 12.5 cm), for which the emission spectrum is largely continuous (Fig. 1), and (ii) a single 100-watt "black light" mercury vapor lamp (Magnaflux CH-4, at a distance of about 25 cm), giving a much purer line spectrum (Fig. 1). Dose rates were determined with a calibrated thermopile and a Keithley 150A microvoltmeter.

Cultures were exposed to an irradiance of about 60 erg/mm² per second with the 4-watt lamps. (Light output varies from lamp to lamp.) At this irradiance, T5-resistant mutants were induced at a rate of 2.68×10^{-6} mutant per bacterium per day. There was neither detectable cell death nor detectable variation in mutation rate over the measured range of growth rates, 3.7 to 12 divisions per day. In the absence of light, mutation rates were growth-rate dependent, and had values some 10 to 20 times smaller. With the much greater irradiances available with the 100-watt lamp, mutations were induced at a rate some 40 times greater than the spontaneous rate in the absence of light. Specific mutation rates per unit irradiance were about the same (Table 1). Mutants have also been produced under similar conditions in a related strain of *E. coli* (*5*).

In addition, it is possible to induce mutation in cells in stationary cultures. Cultures were removed from chemostats for a period of time sufficient to allow for any residual cell division (about 1 hour), and were exposed at about 40 erg/mm² per sec-

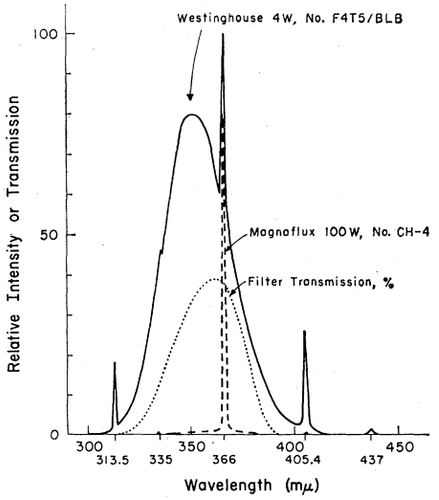

Fig. 1. Emission spectra and filter transmission, as measured with a Zeiss PMQ II spectrophotometer. Spectral intensities are given in arbitrary units and not to the same scale: solid line, for the 4-watt lamps; dashed line, for the 100-watt lamp. The dotted line represents the percentage transmission of the Corning No. 5860 filter.

Table 1. Mutation rates per unit irradiance. Mean values of the mutation rate per unit irradiance, Y, and standard errors of Y, are given for cultures grown aerobically (+) or anaerobically (−). The absolute standard errors for the 100-watt lamp are somewhat larger than those shown, since they include an error of about 20 percent in the determination of the average value of the irradiance because of the rather nonuniform spatial distribution of radiation from this lamp. Y = (mutant/bacterium per day)/(watt/mm^2).

Oxygen	Filter	Y ± S.E.
	4-watt lamp	
+	None	0.45 ± 0.03
+	No. 5680	.51 ± .02
−	None	.26 ± .02
	100-watt lamp	
+	None	.37 ± .03
+	No. 5680	.57 ± .10

ond. Mutation rates were increased by about one order of magnitude for exposures up to 1 hour, but there was significant killing after this time; about half of the cells were inactivated after an exposure of 8 hours.

To investigate the nature of mutagenesis with near-visible light, continuous cultures were grown anaerobically (5 percent CO_2, 95 percent N_2) and irradiated as before. For these, the average mutation rate was 1.55×10^{-6} mutant per bacterium per day, about 60 percent of the aerobic rate (rates from 1.9 to 13 divisions per day). This result shows that induction of mutation with near-visible light involves at least two mechanisms, one of which requires oxygen and therefore may be a photodynamic effect. The results are similar to those of Cabrera-Juarez, who found oxygen-dependent and independent inactivation in transforming DNA (*2*).

Near-visible light is very inefficient compared to ultraviolet light at 260 mμ. Under the same growth conditions, a much smaller irradiance of ultraviolet light, 0.051 erg/mm^2 per second, induced mutation to T5-resistance in a closely related strain at almost half the aerobic rate mentioned above, 1.27×10^{-6} mutant per bacterium per day (*6*). Since the well-known 260-mμ absorption peak of DNA extends to 310 mμ, this great sensitivity to ultraviolet light suggested the possibility that our light sources emitted some ultraviolet light below 320 mμ, and that this component was responsible for the induction of mutation. For this reason, exposures were made through a filter (7.5 by 7.5 cm, Corning No. 5860) that transmitted less than 1 percent of the incident irradiation at wavelengths less than 310 mμ (Fig. 1). With this filter, the irradiance was reduced to 18.6 percent of the unfiltered rate for the 4-watt lamps, and to 21 percent for the 100-watt lamp. However, the presence of the filter did not decrease the value of the mutation rate per unit irradiance (Table 1). Thus, there could have been no material fraction of mutations induced by light of those wavelengths that were severely attenuated by the filter. For this reason, with the "continuous" spectrum most of the mutants were produced by wavelengths between 330 and 380 mμ. The results with the "line" spectrum are even clearer: almost all of the mutants must have been induced with light near 365 mμ.

The photochemical reactions initiated in mutagenesis with near-visible light are quite unlikely to be identical with those initiated in mutagenesis by ultraviolet light. Absorption by the 260-mμ peak falls precipitously at wavelengths greater than 300 mμ; extrapolated values of the molar absorption coefficients are reduced from the value at 260 mμ by factors of about 10^4 at 320 mμ and 10^6 at 365 mμ. This precipitous decline is inconsistent with the mutation rates observed both with and without the filter. That is, this absorption mechanism should have led to few or no mutants with the "line" spectrum, whereas with the 100-watt lamp the observed specific mutation rate per unit irradiance was more than half of the value for the 4-watt lamps. Thus mutations induced with near-visible light do not show the wavelength dependence expected for mutations induced with ultraviolet, and other photochemical reactions must be involved.

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Eperythrozoon coccoides: Influence on Course of Infection of Plasmodium chabaudi in Mouse

Abstract. *Mice infected with* Plasmodium chabaudi *obtained from two sources were found to be contaminated with* Eperythrozoon coccoides. *At each transfer of blood parasitized with plasmodia, eperythrozoa were also passed. In the presence of these organisms, the malarial infection assumed a low-level, chronic course infrequently resulting in death of the mice. When the eperythrozoa were eliminated through treatment with oxophenarsine hydrochloride, the malarial infection took an acute course always ending in death.*

Several studies (*1*) indicate that organisms such as *Eperythrozoon coccoides* influence the course of *Plasmodium berghei* infections. These reports emphasize the need to examine for the presence of such contaminating organisms whenever unexpected host responses occur. Such responses were observed in our laboratory in mice infected with *Plasmodium chabaudi* Landau and were found to be caused by an intercurrent infection with eperythrozoa.

We followed the malarial infection in groups of CF_1 male mice (approximately 20 g) by counting, usually every other day, parasitized cells in Giemsa-stained thin blood-smears and by counting, with a hemacytometer, red blood cells diluted by the unopette method (*2*). In all cases the mice were inoculated intravenously with 1×10^6 cells parasitized with plasmodia, unless specified otherwise. We prepared the inocula by appropriately diluting heart blood from a single animal when the malarial infection was near its peak.

The low-level, chronic course of infection with *Plasmodium chabaudi* (*3*), reported by others (*4, 5*) and seen by us (Fig. 1), was characterized by extreme anemia and extensive reticulocytosis. These responses exceeded those expected as a result of the malarial parasitemia. Severe anemia was evident on the 4th day after inoculation, when