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Ultraviolet Light Inactivation and Photoreactivation in the Mycobacteria

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The kinetics of inactivation of mycobacteria by ultraviolet light were investigated. *Mycobacterium tuberculosis* and *M. marinum* were shown to be capable of photoreactivation.

Because tuberculosis is essentially an airborne infection, the ultraviolet light (UV) irradiation of the environment is recommended where droplet nuclei are likely to be found or where the manipulation of pathological specimens can originate aerosols. However, very little information is available on the quantitative aspects of inactivation of the tubercle bacilli by UV and prior to this study it was not known whether these bacteria were capable of photoreactivation (3). The purpose of the experiments described in this report was to analyze the kinetics of UV inactivation of *Mycobacterium tuberculosis* and other mycobacteria and to verify whether photoreactivation would occur.

M. tuberculosis H37Rv, two strains of *M. fortuitum*, two strains of *M. marinum*, and *M. smegmatis* ATCC 607 were used in the investigation. The *M. fortuitum* and *M. marinum* strains were isolated from clinical specimens and were submitted to this laboratory for reference purposes. The bacteria were grown at 35 to 36 C in Middlebrook and Cohn 7H-9 liquid medium (Difco) containing 0.05% Tween 80 added to the late logarithmic phase. The incubation times varied according to the species (2, 4, 8, and 15 days for *M. smegmatis*, *M. fortuitum*, *M. marinum*, and *M. tuberculosis*, respectively). The cultures were stirred in a Vortex Junior Mixer (Scientific Industries, Inc., Queens Village, N.Y.) and then poured in glass petri dishes to a depth of about 3 to 5 mm. After the lids were removed, the cells were irradiated with a 15-w General Electric G15T8 low-pressure mercury vapor germicidal lamp yielding 34.29 μw/cm^2 at a distance of 1 m. The lamp was located at a distance of 10 cm from the surface of the medium, and the intensity of irradiation at this distance measured 810 μw per sec per cm^2 (8,100 ergs of energy). We thank Karl W. Branch, Epidemiology Program, Center for Disease Control, who measured the intensity of irradiation. At regular intervals, samples were taken, diluted in sterile distilled water and plated on Middlebrook 7H-10 agar medium (Difco). All manipulations were carried out in dimmed light, and the plates to be incubated in the dark were wrapped in aluminum foil

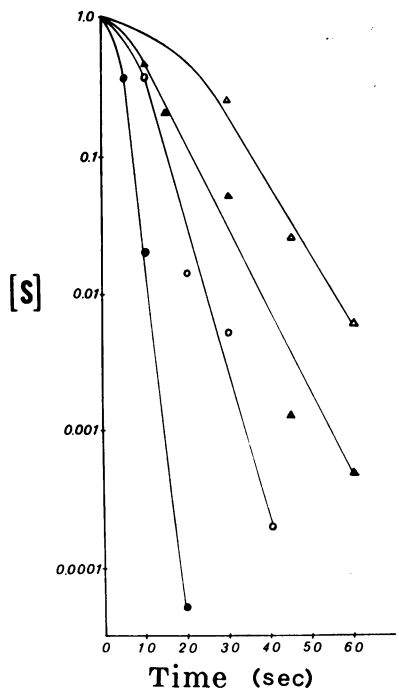

FIG. 1. *Kinetics of ultraviolet inactivation and photoreactivation of M. tuberculosis and M. marinum. The cell survival ([S] = cell density at time t/cell density at time zero) was plotted in the ordinate on semilogarithmic paper, and the time of irradiation in seconds was plotted in the abscissa. Symbols:* (●) *M. tuberculosis (dark),* (○) *M. tuberculosis (light),* (▲) *M. marinum (dark), and* (△) *M. marinum (light).*

TABLE 1. *Inactivation of mycobacterial cells by ultraviolet light irradiation*

Species	Cell densities		Irradiant energy necessary for 90% inactivation	
	Time zero	Time 30 sec	UV dose (sec)	UV (erg)
Mycobacterium tuberculosis H37Rv	3.4×10^9	1.2×10^2	7	56,700
M. fortuitum strain 1	9.0×10^{10}	3.4×10^5	4	32,400
M. fortuitum strain 2	4.0×10^{10}	1.5×10^7	10	89,100
M. marinum strain 1	9.3×10^7	1.1×10^6	22	178,200
M. marinum strain 2	5.0×10^8	8.5×10^5	21	170,010
M. smegmatis ATCC 607	2.0×10^{10}	9.0×10^7	30	243,000

TABLE 2. *Photoreactivation in Mycobacterium tuberculosis and in M. marinum*

Species	Cell survival[a]		Recovery (%)
	Dark	Light	
M. tuberculosis	3.4×10^8	1.1×10^8	56
M. marinum	9.3×10^6	4.7×10^7	40

[a] Data indicate the cell survival on the illuminated plates showing 90% inactivation when incubated in the dark.

immediately after irradiation. For photoreactivation (*M. tuberculosis* and *M. marinum*), duplicate plates were irradiated with visible light for 1 hr, with a 116-w, 120-v filament bulb located at a distance of 40 cm. The colony counts were established after incubation at 35 to 36 C for the appropriate length of time.

The kinetics of inactivation of *M. tuberculosis* and *M. marinum* are represented in Fig. 1. To inactivate 90% of the *M. tuberculosis* and *M. marinum* cells, 7 and 22 sec of irradiation were required, respectively. As indicated by Hollander (2), the product of the intensity of irradiation and the time of exposure is a constant for a certain percentage of killing. On this basis, the data (Table 1) indicated that the energy necessary to inactivate 90% of the mycobacterial cells was within the limits reported by Hollander (2) in his review of the literature (11,000 to 197,000 ergs). However, *M. smegmatis* required 243,000 ergs (Table 1), a value that was higher than the upper limit reported by Hollander (2).

M. tuberculosis and *M. marinum* were shown to be capable of photoreactivation (Fig. 1; Table 2). Fifty-six per cent of the tubercle bacilli cells and 40% of the *M. marinum* cells were photoreactivated. The phenomenon of photoreactivation, discovered by Kelner in 1949 (3), has not been described before in the mycobacteria.

Collins (1) recently reported the UV inactivation of *M. tuberculosis* Erdman and *M. bovis* BCG. According to his data, exposure for 60 sec to a UV source yielding 40 $\mu w/cm^2$ or about 24,000 ergs was required to decrease the cell viability by 90%. This amount of irradiating energy is about half of that required in our study. The difference is probably due to the techniques used.

In summary, UV light irradiation from a germicidal lamp was very effective in killing the tubercle bacilli and other mycobacteria. *M. tuberculosis* and *M. marinum* were shown to be capable of photoreactivation. Therefore, it seems justifiable to recommend the use of UV irradiation after working hours and for long periods of time in laboratories where aerosolization of tuberculous materials can occur.

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