LYSERGIC ACID DIETHYLAMIDE: PHOTODYNAMIC INACTIVATION OF REPAIR DEFICIENT E. COLI AND T1 BACTERIOPHAGE*

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SUMMARY

E. coli B derivatives differing in ability to repair DNA damages produced by ultraviolet radiation showed corresponding responses to photo effects sustained in LSD-sensitized organisms. The excision-repair system (hcr) was a most significant determinant for allowing recovery of both treated bacteria and T1 bacteriophage. Caffeine, a known inhibitor of the hcr system impaired this recovery.

Although these findings suggest an association of LSD with DNA in vivo, the relatively high dose of LSD employed failed to produce detectable DNA damage in the absence of radiant energy.

Previous experiments in this laboratory have indicated that lysergic acid diethylamide (LSD) caused a dose-dependent decrease in the growth rate of E. coli B/r and slightly enhanced the lethality of ultraviolet light to irradiated organisms (1). These effects of LSD on bacteria were almost completely reversible and, if binding to deoxyribonucleic acid (DNA) played a causative role, at best a weak association with this macromolecule would be indicated. In fact, precisely such an association of LSD with DNA has recently been observed in vitro (2).

In order to shed more light on the status of this presumptive association in vivo, we decided to study the photodynamic effects of ultraviolet radiation. Other investigators had shown that a number of dyes can sensitize bacteria and phages to inactivation by visible light (3,4,5). The photodynamic

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effects, which were primarily due to lesions sustained in DNA, were subject to dark repair by two enzyme systems which are known to operate on damage produced by ultraviolet irradiation at 253.7 mμ (i.e., hcr and rec systems). Most photosensitizing dyes can complex with DNA in rather specific ways (e.g. intercalation) (6) and are believed to produce DNA damage by transmitting absorbed light energy to the neighboring macromolecule, perhaps via resonance transfer (7).

The present paper illustrates that LSD can sensitize certain bacteria and T1 bacteriophage to the action of radiant energy emitted at the appropriate wavelength. DNA damage appears to be implicated since the lethal photodynamic effects greatly depend on the inherent repair capability (ies) of the strains employed.

MATERIALS AND METHODS

Bacterial strains employed in this study were: E. coli B derivatives* B/r (fil$^-$, exr$^+$, hcr$^+$) and Bs-1 (fil$^+$, exr$^-$, hcr$^-$) described by Dr. Ruth Hill (8); 26x (fil$^-$, exr$^-$, hcr$^-$), Bs-2/r-5 (fil$^-$, exr$^-$, hcr$^+$), Bs-1-A_{22} (fil$^+$, exr$^-$, hcr$^-$), and 26xA_2 (fil$^-$, exr$^+$, hcr$^-$) described by Dr. Evelyn Witkin (9); and E. coli K12 derivatives AB1157 (hcr$^+$) and AB 1886 (hcr$^-$) obtained through the courtesy of Dr. P. Howard-Flanders of Yale University Medical School. T1 bacteriophage was a laboratory strain originally obtained from Dr. R. M. Herriott of the Johns Hopkins University. LSD maleate, obtained from Edgewood Arsenal, was recrystallized from methanol and characterized by its ultraviolet and infrared spectra.

Bacteria were grown overnight to a limit in 0.8% nutrient broth (Difco) with 0.5% NaCl in a reciprocating incubator shaker at 37°C. Following a 25-fold dilution into fresh nutrient broth containing 1 mg LSD maleate per

*The "fil$^+$" characteristic is related to the tendency of cells to form filaments when DNA synthesis is inhibited; the "exr$^+$" characteristic is related to a factor responsible for achieving repair of breaks in the DNA chromosome caused by x-rays and other agents; the "hcr$^+$" characteristic is related to the ability of the cell to excise and repair U.V. induced thymine dimers, and to host-cell-reactivate U.V. inactivated phages.

ml, they were incubated for an additional several hours until the turbidity of the slowly growing cells had increased 5-fold. LSD is not lethal to bacteria, since when diluted they readily resume the normal growth rate and fully retain colony forming ability (1). For the irradiation experiments, bacteria were next diluted 20-fold into 0.9% NaCl containing 1 mg LSD maleate per ml; T1 bacteriophages, stored in nutrient broth at 2 x 10^{10} viable particles/ml, were diluted 50-fold into Harm's absorption medium (10) containing 1 mg LSD maleate per ml and allowed to stand at room temperature for 1 hour prior to irradiation. In our experience, T1 particles are more stable in Harm's absorption medium than in saline solutions, and their photodynamic sensitization by LSD is greatly enhanced at the lower ionic strength. Viable bacteria and phages were measured in appropriately diluted aliquots by Harm's method (5) which was modified as follows: The plating agar used for cell titers consisted of 1.5% Bacto agar; the diluting agar for phage titers consisted of 0.75% Bacto agar. All agars were supplemented with 0.8% nutrient broth (Difco) and 0.5% NaCl. The plating and diluting agars were kept liquid at 45°C until used. After hardening, assay plates were inverted and incubated at 37°C for 24 hours (phage assays) or 2-3 days (cell assays). Except for the highest inactivations, each value reported in this paper represents the calculated average from several experiments comprising at least 100 or more plaques or colonies. In experiments utilizing caffeine, plating and diluting agars were supplemented with 1.5 mg of caffeine monohydrate per ml (Merck and Co.). All manipulations subsequent to irradiation were carried out in subdued light.

Irradiation was carried out by exposing 3-ml samples in small petri dishes to a Hanovia type #30,600 lamp at a distance of 16 cm from a 2 mm filter disk (Corning #0-54). This filter, which blocks transmission below 300 mμ was introduced between the broad spectrum ultraviolet emitting source and the sample to prevent the lethal germicidal light from impinging on the organisms. However, the filter allowed more than 1/3 of the light quanta

absorbable by LSD to be transmitted. The relationships between the germicidal action spectrum, the LSD absorption spectrum and the filter characteristics are shown in Fig. 1.

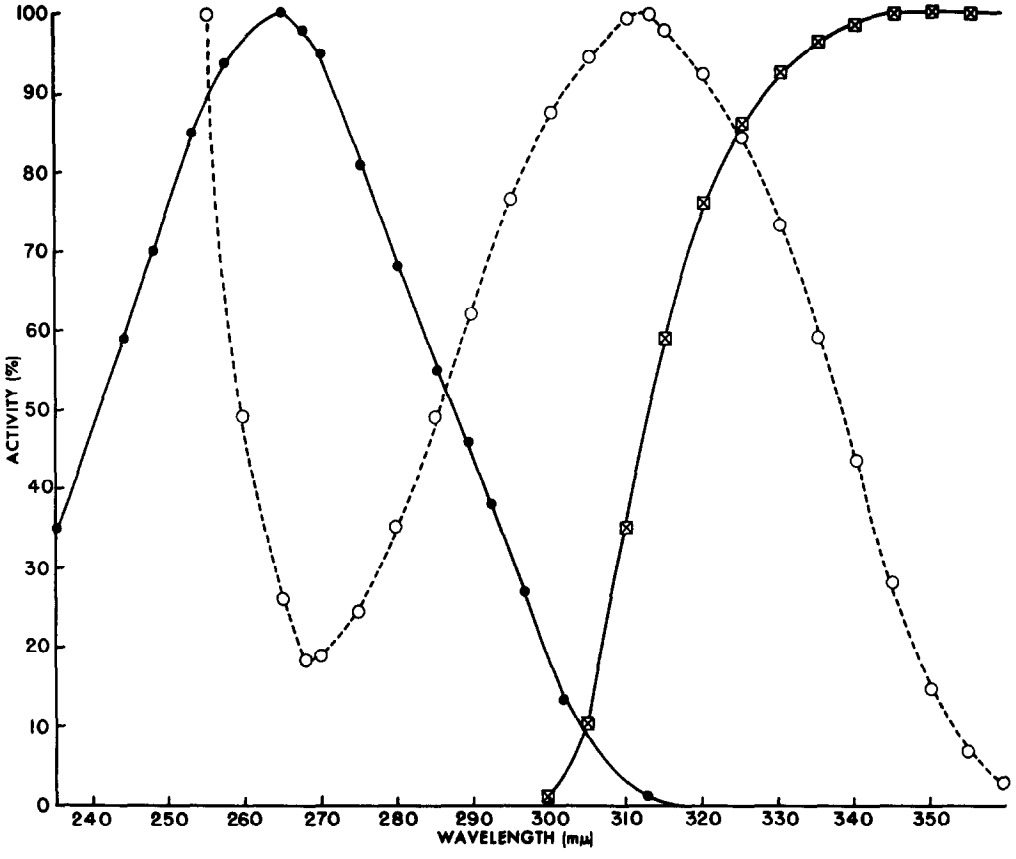

Fig. 1. Relationships between germicidal activity, light absorption by LSD and light transmission through filter in the ultraviolet region. (●) Bactercidal action spectrum according to Buttolph (16). (○) Absorption of light by LSD maleate in .15M NaCl (.50 μg/ml). (▣) Transmission of light through Corning glass filter (#0-54) as measured in a Beckman DK2 Spectrophotometer.

RESULTS AND DISCUSSION

The large differences in the sensitivities of E. coli B derivatives to U.V. light (253.7 mμ) are due to mutations at 3 loci in the bacterial chromosome. Each of these loci is responsible for a portion of the 500-fold difference between the most resistant strain, B/r (fil^-, exr^+, hcr^+) and the most sensitive strain, Bs-1 (fil^+, exr^-, hcr^-) (9). These same loci

also appear to be responsible for determining the response of cells exposed to other DNA-damaging agents (e.g., alkylating agent-induced crosslinks (11-14), photodynamic damage of acridine-sensitized DNA (5), etc). The common denominator for achieving the recovery of cells and phage is the ability to repair

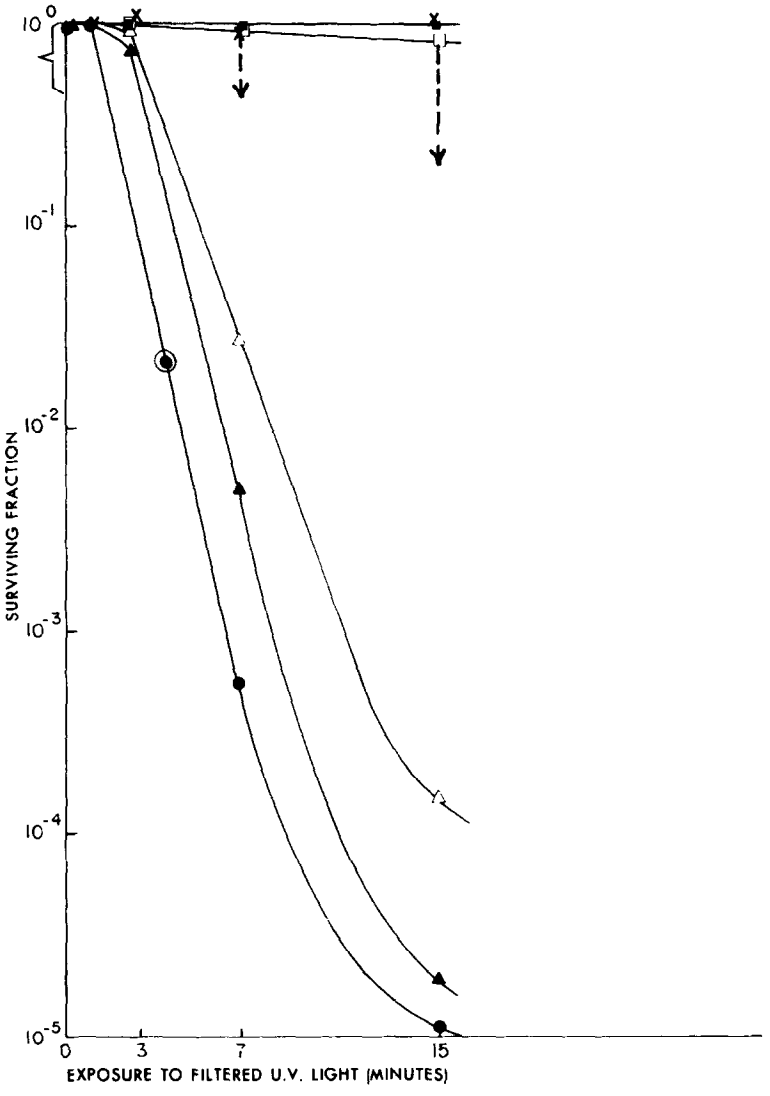

Fig. 2. Survival of E. coli B derivatives as a function exposure to non-germicidal ultraviolet light in the presence of 1 mg/ml LSD maleate: (●)Bs-1, (○)Bs-1-A_{22}, (▲)26xA_2, (△)26x, (■)B/r, (□)Bs-2/r-5. The (x) represents survival of strain Bs-1 in the absence of LSD. The bracket on the ordinate indicates the range of actual survival values for the various strains when exposed to LSD alone. All points in the figure are calculated values which are based on 100% survival at zero minutes of U.V. The arrows show the survival obtained when photodynamically treated Bs-2/r-5 are plated in the presence of 1.5 mg caffeine/ml.

many different types of potentially lethal DNA defects.

If the photodynamic action of U.V. light on LSD-sensitized organisms were also to produce DNA damage subject to dark repair, different responses of the various E. coli B derivatives might be expected. This expectation was indeed fulfilled. Figure 2 shows the survival of 6 strains of varying U.V. sensitivities after exposure to non-germicidal ultraviolet light (i.e., above 300 mμ) in the presence of LSD. The results indicate that hcr^- strains are highly sensitive to doses which have little or no effect on hcr^+ strains. The involvement of hcr-related repair is suggested by the observation that the survival of a photodynamically treated hcr^+ strain was decreased when caffeine, an inhibitor of the hcr system, was included in the plating medium (indicated by arrows in Figure 2).

The exr^- and fil^+ characteristics appear to play a lesser role in conferring sensitivity, although an exact assessment of their effect has not been made. Preliminary experiments indicate that an hcr^- E. coli K_{12} derivative (AB1886) was also more sensitive to this photodynamic inactivation than was the repair capable (hcr^+) parent strain (AB 1157). The K_{12} hcr^- strain, for reasons not presently known, required considerably higher doses for inactivation than did the hcr^- E. coli B derivatives. No assessment of the influence of the recombination system (rec) on sensitivity has been made. Control experiments showed no inactivation of any of the strains employed by filtered ultraviolet light alone (i.e., in the absence of LSD) or by LSD alone (i.e., without irradiation).

The above experiments strongly implicate DNA as the sensitive target in the photodynamic inactivation of LSD-sensitized bacteria. The following data extend this notion to bacteriophage T1. Results illustrated in Fig. 3 show that irradiation of LSD-sensitized extracellular virus caused a dose-dependent loss of viability. It should be noted that survival was considerably higher when exposed phages were plated on repair capable hosts (i.e., B/r) than on repair deficient hosts (i.e., Bs-1). The finding that the repair

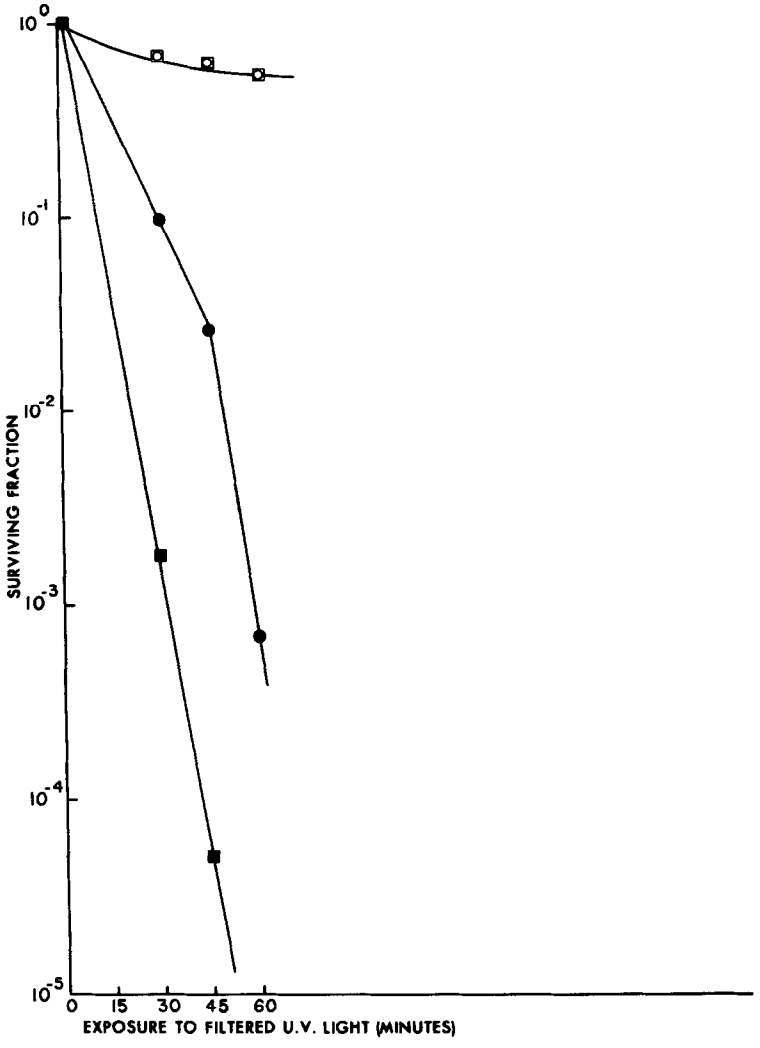

Fig. 3. Survival of phage T1 as a function of extracellular exposure to non-germicidal ultraviolet light in the presence of 1 mg/ml LSD maleate, plated on B/r (●) or Bs-1 (■). Controls (open symbols) were treated similarly as experimentals, but in the absence of LSD.

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inhibitor caffeine abolished this difference in survival (Table 1) suggested that a considerable fraction of the photodynamic lesions in DNA had been repaired by the hcr system. The small loss of titer observed in controls irradiated in the absence of LSD (Fig. 3) was not due to any apparent DNA damage (equivalent decreases were observed with hcr^+ and hcr^- hosts) and was not related to irradiation dose (similar small losses of titer were

TABLE 1. EFFECT OF CAFFEINE ON SURVIVAL OF PHOTODYNAMICALLY INACTIVATED T1 BACTERIOPHAGE

Exposure to filtered U.V. light (minutes)	Survival of T1 phage plated on: B/r		Bs-2/r-5		Bs-1		26xA2	
	-Caf	+Caf	-Caf	+Caf	-Caf	+Caf	-Caf	+Caf
0	1.0	1.0	1.0*	1.0*	1.0	1.0	1.0	1.0
45	2.8×10^{-3}	3×10^{-6}**	9.5×10^{-4}	3×10^{-6}**	3×10^{-6}**	3×10^{-6}**	3×10^{-6}	3×10^{-6}**

*The titer of unirradiated phage on this strain was only approximately 50% that obtained with other strains. Caffeine did not affect this unexplained loss.
**These values are approximations since they are calculated from plates containing only a few plaques.

Extracellular phage was exposed for 0 and 45 minutes to filtered U.V. light in the presence of 1 mg/ml LSD maleate and aliquots were plated on the indicated hosts in the presence or absence of 1.5 mg/ml caffeine.

obtained on allowing phages to stand at room temperature in the suspension medium).

Although no mechanism is specified at present to account for the photodynamic damage to LSD-sensitized DNA, the high susceptibility of bacteria to this action suggests an intimate association of LSD with genetic material *in vivo*. Recent experiments in our laboratory have demonstrated similar photodynamic effects to be quite effective in killing a cultured mammalian cell line (HeLa). It is not known if and/or how photodynamically induced DNA damage related to the reported production of chromosomal aberrations (15-19) and the teratogenic (20-23) and mutagenic (24) effects of LSD.

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