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Interactions Between UV Radiation of Different Energies in the Inactivation of Bacteria

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A strong lethal interaction was observed between various monochromatic wavelengths (254, 334, 365, and 405 nm) in the repair-proficient *E. coli* K-12 strain AB 1157, except in the case of preexposure to 405-nm radiation which resulted in a protection against the inactivation resulting from subsequent exposure to 365- or 254-nm radiations. The results may be tentatively explained by assuming two classes of DNA lesions and two classes of damage to repair (reversible and irreversible) whose proportions vary according to wavelength.

The bactericidal action of near-UV radiation at various monochromatic wavelengths is now well documented (for a recent review, see 13). Because radiation at these long wavelengths sensitizes repair-proficient bacteria to a wide spectrum of DNA-damaging agents (for a recent review of synergism and antagonism, see reference 9a), including far UV, ionizing radiation, and heat and alkylating agents, it may be predicted that lethal interactions would be observed between radiations within the near-UV range. Indirect evidence for the occurrence of such phenomena in *Salmonella typhimurium rec* mutants appeared from the work of Mackay et al. (5), and experimental control data from experiments with monochromatic wavelength radiations have provided direct evidence that such phenomena also occur in wild-type *Escherichia coli* strains (6, 13).

A study of the interactions between radiations of different wavelengths may help in understanding the mechanism of action of the individual wavelengths on bacteria. In addition, there is some evidence that there may exist an interaction between different regions of the UV spectrum in the induction of cutaneous damage (9a, 14, 15) and even for induction of skin tumors (1, 12; P. D. Forbes, Program and Abstr. 1st Annu. Meet. Am. Soc. Photobiol., 1973, p. 136), although the latter phenomena in particular cannot be said to be clearly established (P. D. Forbes, personal communication). However, we decided to test the various wavelengths of radiation which had previously shown interesting results in bacteria (see above) and to examine possible interactions in more detail. The following work illustrates that although the existence of such phenomena is in no doubt, their explanation must await a more profound knowledge of the action of the individual wavelengths.

A description of the preparation of suspensions of the repair-proficient strain *Escherichia coli* K-12 AB 1157 (described by Howard-Flanders and Boyce [2]) from minimal medium-grown exponential-phase cultures appears elsewhere (9). Viability assays, irradiations at monochromatic wavelengths, and dosimetry were also performed as previously described (9). Cell suspensions were irradiated at 0°C in a Pyrex-jacketed cuvette (334, 365, and 405 nm) or at room temperature in a petri dish (254 nm). In the sequential irradiation experiments, the interval between the irradiations was always less than 5 min. Cell populations were exposed to a dose of radiation selected from the shoulder region at that wavelength. The patterns of inactivation of cell populations at a single wavelength (control curves in each figure) remained unchanged after holding the freshly harvested cells on ice for up to 3 h (the longest irradiation time used in these experiments).

Previous studies concerning the interaction between near-UV radiation and mild heat (9) and near-UV radiation and methyl methane sulfonate (I. S. Correia and R. M. Tyrrell, Photochem., Photobiol., in press) have indicated that the dose needed to remove the large shoulder on the near-UV survival curve can be quantitatively correlated with the ability of each wavelength (334, 365, and 405 nm) to sensitize to the other two DNA-damaging agents. These results support a model for near-UV inactivation involving the accumulation of dose-dependent damage to repair systems (8, 9, 9a, 11, 13, 13a). Additional data concerning the lethal interaction between

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near-UV and other agents in repair-deficient strains support the proposed model (8, 9a, 13a). From Fig. 1, it is clear that essentially nonlethal doses of 334- and 365-nm radiation (see also control curves in Fig. 3 and 4) are able to sensitize wild-type cells to far-UV (254-nm) radiation. The 365-nm:254-nm lethal interaction has been reported previously in both B/r (11) and wild-type K-12 strains (13a). Preexposure to a dose of 405-nm radiation which eliminates more than half the shoulder from the 405-nm survival curve itself (see Fig. 5) and which strongly sensitizes cells to both mild heat and methyl methane sulfonate actually protects the cell population against the lethal effects of radiation at 254 nm. From Fig. 2, it can be seen that this protection is dose dependent and reaches a peak at a 405-nm dose of 3×10^6 J m^{-2}.

The remainder of the figures (Fig. 3–5) illustrate the lethal interactions observed when the various possible combinations of 334-, 365-, and 405-nm radiations are tested. Except where a protection is seen (Fig. 4), the final slopes of control and dually irradiated cell survival curves are always close to parallel (Fig. 3–5). These results suggest that the three wavelengths induce a common type of sublethal damage which according to the model would be damage to DNA repair systems. It should be noted that pre-irradiation of starved log-phase cells at 334 nm (6) or stationary-phase cells at 365 nm (13) led to very much larger lethal interactions with 405-nm radiation than those reported here.

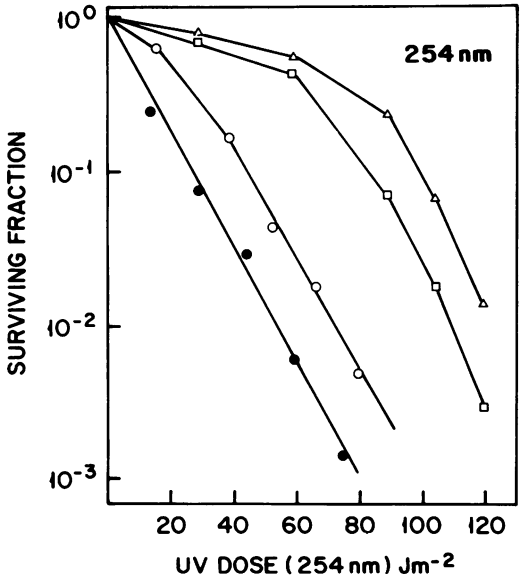

FIG. 1. *Inactivation of E. coli K-12 AB 1157 by 254-nm radiation after pretreatment with: control (no pretreatment)* (□); *2.5 × 10^6 J m^{-2} at 405 nm, 72% survival* (△); *1.5 × 10^5 J m^{-2} at 334 nm, 90% survival* (○); *1.0 × 10^6 J m^{-2} at 365 nm, 50% survival* (■).

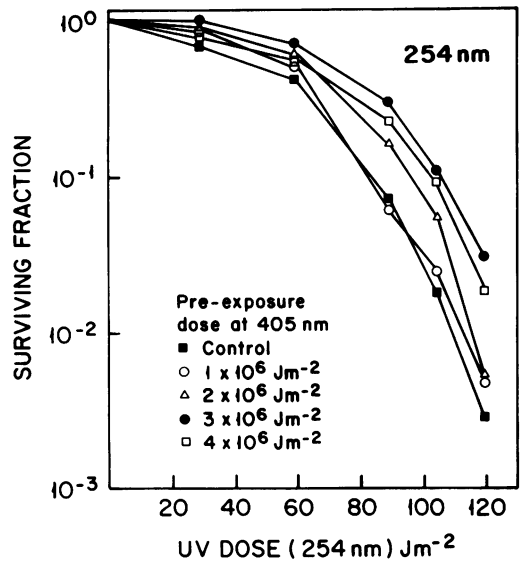

FIG. 2. *Inactivation of E. coli K-12 AB 1157 after pretreatment with a series of doses at 405 nm: control* (■); *10^6 J m^{-2}, 77% survival* (○); *2 × 10^6 J m^{-2}, 70% survival* (△); *3 × 10^6 J m^{-2}, 60% survival* (●); *4 × 10^6 J m^{-2}, 50% survival* (□).

The interpretation of the results is complicated by the observation that populations irradiated at 405 nm were protected against subsequent exposure to both 254-nm radiation (Fig. 1 and 2) and 365-nm radiation (Fig. 4). Furthermore, in the case of the 365-nm:405-nm interaction, the sequence of radiation treatment is critical because a protection (Fig. 4) or a sensitization (Fig. 5) was observed depending on whether the 405- or 365-nm radiation pretreatment was given first. However, these results are consistent with a model (8) which proposes that DNA lesions fall into two broad categories depending upon whether or not they rapidly become irreversible ("stabilized") in the absence of effective repair (an idea strongly supported by recent results in this laboratory with bacteriophage, manuscript in preparation). In addition, we suggest that the sublethal damage to repair that occurs may include a rapidly reversible component whose proportion varies with wavelength and whose level is high after irradiation at 405 nm.

The lesions induced at 254 nm (predominantly pyrimidine dimers) almost certainly fall into a class of lesions which do not rapidly become irreversible in the absence of repair. Both at 334 and 365 nm, a sufficient component of the dam-

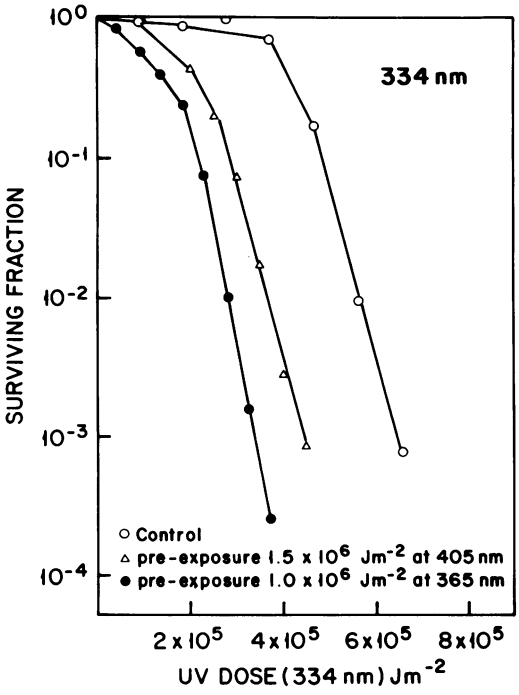

FIG. 3. *Inactivation of E. coli K-12 AB 1157 by 334-nm radiation after pretreatment with: control (○); 1.5 × 10⁶ J m⁻² at 405 nm, 80% survival (△); 1.0 × 10⁶ J m⁻² at 365 nm, 50% survival (●).*

age to repair was only slowly lost or was nonreversible so that a significant lethal interaction with 254-nm radiation was observed (Fig. 1). The effect was much less marked than the synergistic interaction between near-UV and mild heat or methyl methane sulfonate (see above). Although 405-nm radiation probably damages repair systems (9, 9a), no 405-nm sensitization to 254-nm inactivation occurred, possibly because the damage to repair is reversed before it becomes critical. Furthermore a dose-dependent photoprotective effect is superimposed on the overall interactions (Fig. 1 and 2).

Pretreatment with 405-nm radiation was not able to protect against the damage caused by 334-nm radiation (Fig. 3), suggesting that the damage induced by 334-nm radiation may be predominantly of the rapidly stabilized type so that even reversal of a considerable portion of the repair damaging effect at 405 nm does not prevent a lethal interaction.

Approximately equal numbers of both pyrimidine dimers (7) and single-strand breaks (10) were induced by radiation at a wavelength of 365 nm. However, photoprotection against dimer damage does not seem a likely explanation of the 405-nm:365-nm interaction because several other studies have indicated that the dimer is not an important lethal event in wild-type strains after irradiation at 365 nm (13). The positive interaction obtained when the treatment order is reversed (i.e., 365 nm:405 nm; Fig.

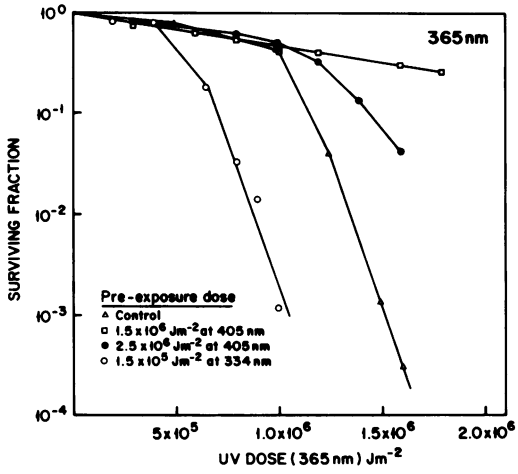

FIG. 4. *Inactivation of E. coli K-12 AB 1157 by 365-nm radiation after pretreatment with: control (△); 1.5 × 10⁶ J m⁻² at 405 nm, 75% survival (□); 2.5 × 10⁶ J m⁻² at 405 nm, 65% survival (●); 1.5 × 10⁵ J m⁻² at 334 nm, 90% survival (○).*

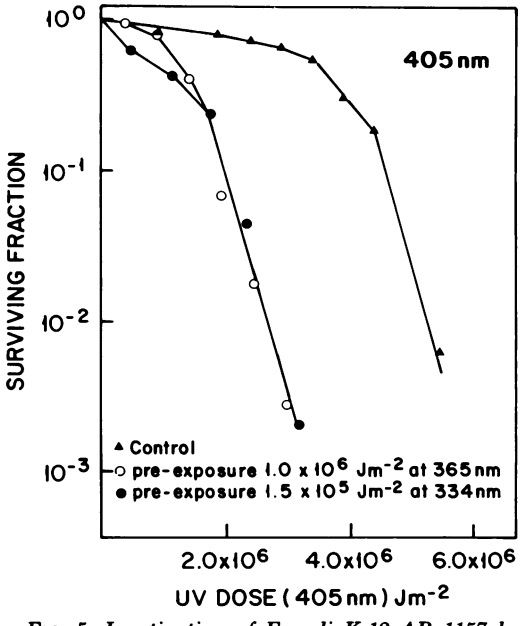

FIG. 5. *Inactivation of E. coli K-12 AB 1157 by 405-nm radiation after pretreatment with: control (▲); 1.0 × 10⁶ J m⁻² at 365 nm, 50% survival (○); 1.5 × 10⁵ J m⁻² at 334 nm, 90% survival (●).*

5) suggests that some component of the damage caused by 405-nm radiation, possibly damage to repair, is rapidly reversible, even in cold buffer, between irradiation at 405 nm and the second treatment. By the proposed model, 365-nm radiation would induce a certain proportion of as yet unrecognized lesions which only slowly become irreversible in the absence of repair.

Near-UV photoprotection is not normally observed in wild-type K-12 strains at least after treatment with 334-nm radiation (4). Furthermore, evidence from action spectra (3) indicated that although strong photoprotection against 254-nm damage in *E. coli* B was observed by pre-irradiation at 334 nm, there was negligible photoprotection by 405 nm. The photoprotection against 254-nm damage (Fig. 1 and 2) and 365-nm damage (Fig. 4) seen in the wild-type strain after pretreatment at 405 nm may be either the tail end of the action spectra for a photoprotection similar to that obtained by previous workers (because the 405-nm doses employed are extremely large) or else a newly described phenomenon, as yet not understood.

Despite the complication in interpretation caused by the photoprotective effect at 405 nm, these results appear to support previous data that indicate that wavelengths in the range of 340 to 405 nm lead to a class of sublethal damage to DNA repair systems. We further suggest that these may be subdivided into reversible and nonreversible types whose relative proportions vary with the wavelength employed. Sufficient non- or slowly reversible damage to repair may occur at all wavelengths to cause a lethal interaction when one of the radiations employed induces rapidly stabilized lesions.

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LITERATURE CITED

1. **Bain, J. A., H. P. Rusch, and B. E. Kline.** 1943. The effect of temperature upon ultraviolet carcinogenesis with wavelengths 2800–3400 Å. Cancer Res. **3**:610–612.
2. **Howard-Flanders, P., and R. P. Boyce.** 1966. DNA repair and genetic recombination: studies of mutants of *E. coli* deficient in these processes. Radiat. Res. Suppl. **6**:156–184.
3. **Jagger, J., R. S. Stafford, and J. M. Snow.** 1969. Thymine-dimer and action-spectrum evidence for indirect photo-reactivation in *Escherichia coli*. Photochem. Photobiol. **10**:383–395.
4. **Lakhaura, B. D.** 1972. Photoprotection from killing in ultraviolet-sensitive *Escherichia coli* K12 mutants: involvement of excision-resynthesis repair. Photochem. Photobiol. **16**:197–202.
5. **Mackay, D., A. Eisenstark, R. B. Webb, and M. S. Brown.** 1976. Action spectra for lethality in recombination-less strains of *Salmonella typhimurium* and *Escherichia coli*. Photochem. Photobiol. **24**:337–343.
6. **Ramabhadran, T. V., and J. Jagger.** 1975. Evidence against DNA as the target for 334 nm-induced growth delay in *Escherichia coli*. Photochem. Photobiol. **21**:227–233.
7. **Tyrrell, R. M.** 1973. Induction of pyrimidine dimers in bacterial DNA by 365 nm radiation. Photochem. Photobiol. **17**:69–73.
8. **Tyrrell, R. M.** 1976. $RecA^+$-dependent synergism between 365 nm and ionizing radiation in log-phase *Escherichia coli*: a model for oxygen-dependent near-UV inactivation by disruption of DNA repair. Photochem. Photobiol. **23**:13–20.
9. **Tyrrell, R. M.** 1976. Synergistic lethal action of ultraviolet-violet radiations and mild heat in *Escherichia coli*. Photochem. Photobiol. **24**:345–351.

9a. **Tyrell, R. M.** 1978. Radiation synergism and antagonism, p. 35–113. *In* K. C. Smith (ed.), Photochemical and photobiological reviews, Vol. 3. Plenum Publishing Corp., New York.

10. **Tyrrell, R. M., R. D. Ley, and R. B. Webb.** 1974. Induction of single strand breaks (alkali-labile bonds) in bacterial and phage DNA by near UV (365 nm) radiation. Photochem. Photobiol. **20**:395–399.
11. **Tyrrell, R. M., and R. B. Webb.** 1973. Reduced dimer excision in bacteria following near ultraviolet (365 nm) radiation. Mutat. Res. **19**:361–364.
12. **Urbach, F., J. M. Epstein, and P. D. Forbes.** 1974. Ultraviolet carcinogenesis: experimental, global and genetic aspects, p. 259–283. *In* M. A. Pathak, L. C. Harber, M. Sejii, and A. Kukita (ed.), Sunlight and man. University of Tokyo Press, Tokyo.
13. **Webb, R. B.** 1977. Lethal and mutagenic effects of near-ultraviolet radiation, p. 169–261. *In* K. C. Smith (ed.), Photochemical and photobiological reviews, vol. 2. Plenum Publishing Corp., New York.

13a. **Webb, R. B., M. S. Brown, and R. M. Tyrrell.** 1978. Synergism between 365- and 254-nm radiations for inactivation of *Escherichia coli*. Radiat. Res. **74**:298–311.

14. **Willis, I., A. Kligman, and J. M. Epstein.** 1973. Effects of long ultraviolet rays on human skin: photoprotective or photo-augmentative. J. Invest. Dermatol. **59**: 416–420.
15. **Ying, C. Y., J. A. Parrish, and M. A. Pathak.** 1974. Additive erythemogenic effects of middle (280–320 nm) and long (320–400 nm) wave ultraviolet light. J. Invest. Dermatol. **63**:273–278.