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THE SYNERGISTIC ACTION OF ULTRAVIOLET AND X RADIATION ON MUTANTS OF *ESCHERICHIA COLI* K-12

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Abstract—Prior UV irradiation increased the X-ray sensitivity of wild-type *E. coli* K-12. This synergistic effect of combined UV and X irradiation was also observed, but to a reduced extent, in *uvrA*, *uvrB*, *uvrC*, and *polA* mutants, but was absent in *exrA*, *recA*, *recB*, or *recC* mutants of *E. coli* K-12. Alkaline sucrose gradient studies demonstrated that the *rec* and *exr* gene-controlled, growth-medium-dependent (Type III) repair of X-ray-induced DNA single-strand breaks was inhibited by prior UV irradiation. This inhibition probably explains the synergistic effect of these two radiations on survival.

INTRODUCTION

For certain bacterial strains, combined UV and X radiation was found to inactivate more cells than would be expected if the effects of both radiations were additive. This synergistic interaction between UV and X radiation for lethal damage was found in *E. coli* B/r (Haynes, 1964a; Bhaumik and Bhattacharjee, 1968), *E. coli* B (Haynes, 1966), *E. coli* 15 TAU (Bhaumik and Bhattacharjee, 1968), *Serratia marcescens* (Clarkson and Dewey, 1971), spores of *Clostridium botulinum* (Durban and Grecz, 1969), interdivisional diploid yeast (Uretz, 1955), budding haploid and diploid yeast (Elkind and Sutton, 1959), *Micrococcus lysodeikticus*, *Pseudomonas fluorescens*, *Hemophilus influenzae* and *Micrococcus pyogenes* (Haynes, 1964a), but not in *E. coli* B_{s-1} (Haynes, 1964a; Smith and Ganesan, 1966) *Micrococcus radiodurans* (Moseley and Laser, 1965), interdivisional haploid yeast (Uretz, 1955) or in bacteriophage T1 (Hill, 1958).

The synergistic effect was independent of the order of the irradiations if cells of *E. coli* B/r were irradiated in buffer, indicating that processes which occur after irradiation are involved (Haynes, 1964a). If the cells were irradiated in growth medium, however, the degree of synergism was very much dependent upon the order of the two irradiations. For the same radiation doses, more cells were killed if they were first exposed to UV radiation than if they were first X irradiated (Bhaumik and Bhattacharjee, 1968; Martignoni and Smith, unpublished observations). These results suggest that post-irradiation events that occur only in growth medium markedly affect the synergistic response.

No synergism was found in *E. coli* when certain repair processes had been suppressed by the incorporation of 5-bromouracil into DNA (Baptist and Haynes, 1972), by purine starvation (Baptist and Haynes, 1972) or by mutation (e.g., *E. coli* B_{s-1}) (Haynes, 1964a). If cells of *E. coli* B/r were photoreactivated between the exposure to UV radiation and the subsequent X irradiation, the synergistic effect was reduced by about one-half (Haynes, 1964a). On the basis of these results, it has been postulated that the synergistic effects of UV and X radiation are mediated by repair processes (Haynes, 1964a, 1966; Bridges *et al.*, 1967; Baptist and Haynes, 1972).

In order to investigate which of the known repair systems might be responsible for this synergistic interaction, we have studied the response of different radiation sensitive mutants of *E. coli* K-12 to combined UV and X irradiation and the effect of prior UV irradiation on the ability of the cells to repair X-ray-induced DNA single-strand breaks.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used are listed in Table 1. All cultures were grown in supplemented minimal growth medium (SMM) containing 10 μg/ml thymine (Ganesan and Smith, 1968).

Culture conditions. Overnight stationary-phase cultures were diluted 1:50 in fresh SMM and grown at 37°C in a Gyrotory water bath (New

Brunswick Scientific) to exponential growth phase (about 1×10^8 cells/ml). For the sedimentation experiments, cells were grown in SMM containing 50 μCi/ml ^{3}H-thymine (New England Nuclear, 15–20 Ci/mmole) and 1·7 μg/ml of unlabeled thymine. The cells were harvested by Millipore filtration (0·45 μm), washed, and resuspended in SMM.

UV irradiation. Five ml samples for survival curves and 2 ml samples for gradients were irradiated in plastic Petri dishes (60 mm diameter for 5 ml samples and 35 mm for 2 ml samples) on a rotary shaker with a General Electric 8 W germicidal lamp emitting primarily at 254 nm. The exposure rate was 590 ergs mm^{-2} min^{-1} as measured by the photodecomposition of uranyl oxalate (Bowen, 1946).

To prevent photoreactivation, the irradiations and subsequent handling were carried out under yellow lights (Westinghouse Bug-a-Way, 100 W).

X irradiation. Cell suspensions (2 or 5 ml in SMM; about 10^8 cells/ml in log-phase growth) exposed to air were irradiated with X rays (50 mA and 48 mA, 0·3 mm aluminum filters) using the twin-tube 50 kVp beryllium window X-ray unit developed by Loevinger and Huisman (1965). The mean absorbed dose rate was 7·9 krads/min for the 5 ml cell suspensions in 60 mm diameter plastic Petri dishes and 8·3 krads/min for 2 ml in 35 mm plastic Petri dishes as determined by ferrous sulfate dosimetry (Fricke and Hart, 1966). All irradiations were performed at room temperature.

Survival curves. Immediately after irradiation, the cells were diluted in 0·067 *M* sodium–potassium phosphate buffer (pH 7·0) and plated on SMM (10 μg/ml thymine) agar. Colonies were counted after 2–3 days of incubation at 37°C. The data shown are the average of at least two experiments.

Alkaline sucrose gradients. A modified McGrath and Williams (1966) technique was used (Town, Smith and Kaplan, 1971a). Cell suspensions in sodium–potassium phosphate buffer (pH 7·0) were adjusted to $OD_{650} \sim 0{\cdot}03$ (Zeiss PMQ II spectrophotometer). About 40 μl samples ($\sim 10^6$ cells) were layered onto a 0·1 ml cap of 0·5% Sarkosyl (Geigy, NL30) in 0·5 *N* NaOH on top of a 4·8 ml gradient [5–20% (weight to volume) sucrose in 0·1 *N* NaOH]. The top of each gradient was gently stirred with a pin, and the gradients were allowed to stand for 1 h before they were centrifuged at 20°C for 105 min at 30,000 rev/min in an SW 50·1 rotor in a Beckman L2 or L2 65B centrifuge. After centrifugation, the bottom of each tube was pierced and about forty 5-drop fractions were collected onto paper discs (Whatman 3 MM). These were washed twice with 5% trichloroacetic acid, and once each in ethanol and acetone and assayed for radioactivity in a liquid scintillation counter.

RESULTS

Survival curves. With exposure to increasing amounts of UV radiation (254 nm), the surviving population of wild-type *E. coli* K-12 showed an increasing rate of X-ray-induced killing (Fig. 1), similar to the synergistic effect found for *E. coli* B/r (Haynes, 1964b). While the D_0 for wild-type

Table 1. *Escherichia coli* K-12 derivatives used

Designation	Mating type	Relevant genotype	Other markers
AB2487	F⁻	*recA13*	*thr leu arg his thi pro thy ara lac gal mtl xyl str^r T6^r λ^s*
AB2497	F⁻	wild-type	*thr leu arg his thi pro thy ara lac gal mtl xyl str^r T6^r λ^s*
AB2499	F⁻	*uvrB5*	*thr leu arg his thi pro thy ara lac gal mtl xyl str^r T6^r λ^s*
AB2500	F⁻	*uvrA6*	*thr leu arg his thi pro thy ara lac gal mtl xyl str^r T6^r λ^s*
AB2498	F⁻	*uvrC34*	*thr leu arg his thi pro ara lac gal mtl xyl str^r T6^r λ^s*
JC 5489	F⁻	*recC22*	*thr leu arg his thi pro ara lac gal mtl xyl str^r T6^r λ^s*
SR188	F⁻	*exrA*	*rha lacZ str^r thy*
JG138	F⁻	*polA1*	*rha lacZ str^r thy*

Abbreviations: (Demerec *et al.*, 1966; Curtiss, 1968; Taylor, 1970). The symbols *arg, his, leu pro, thi, thr, thy,* denote requirements for arginine, histidine, leucine, proline, thiamine, threonine, thymine, respectively; *ara, gal, lac, mtl, rha* and *xyl,* the inability to utilize arabinose, galactose, lactose, mannitol, rhamnose and xylose, respectively; *T6,* λ, and *str,* response to the phages *T6* and λ, and to the antibiotic, streptomycin (^{r} indicates resistance; ^{s} sensitivity); *uvr* designates genes affecting host cell reactivation and UV sensitivity, *rec* designates genes affecting genetic recombination and UV and X-ray sensitivity, *exr* designates genes affecting UV and X-ray sensitivity, *pol* designates genes affecting the synthesis of DNA polymerase I and UV and X-ray sensitivity.

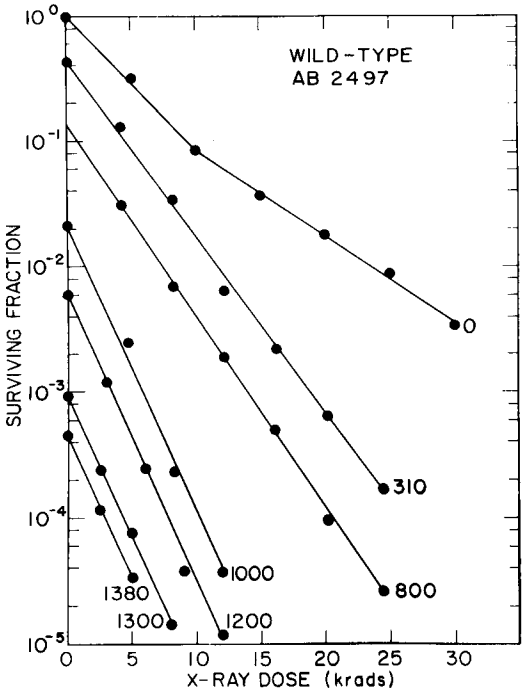

Figure 1. X-ray survival curve of *E. coli* K-12 wild-type (AB2497) after prior exposure to different amounts of UV radiation. The UV esposure in ergs/mm² is indicated beside the curves. Cells in exponential growth phase in SMM were X irradiated at room temperature (~ 23°C) in equilibrium with air immediately after UV irradiation. The cells were diluted in phosphate buffer and plated on SMM-agar.

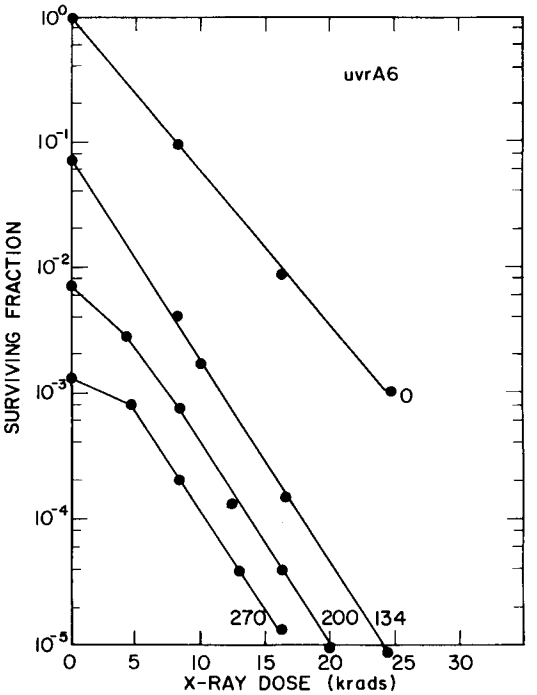

Figure 2. X-ray survival curves of *E. coli* K-12 *uvrA6* (AB2500) after prior exposure to different amounts of UV radiation. For further explanation see Fig. 1.

E. coli K-12 without prior UV treatment was 4·0 krads (initial slope), the D_0 decreased to 1·9 krads after a prior exposure to 1000 ergs/mm² of UV radiation. No further change in the X-ray sensitivity was observed with UV exposures greater than 1000 ergs/mm² (Fig. 1).

To test the hypothesis that the UV–X-ray synergism arises from the failure of a repair process (Haynes, 1964a, 1966; Bridges *et al.*, 1967), different calsses of UV-sensitive mutants were tested. The X-ray survival curves for *E. coli* K-12 *uvrA*, *uvrB* and *uvrC*, after exposure to different classes of UV-sensitive mutants were shown in Figs. 2–4. All three *uvr* mutants tested showed synergism, but somewhat less than observed in the wild-type strain. Maximum sensitization of the wild-type and *uvr* strains was obtained by a prior exposure to UV radiation which resulted in about 5% survival. However, the ultimate X-ray survival after maximum sensitization was slightly different for the various *uvr* and wild-type strains. The D_0 values after

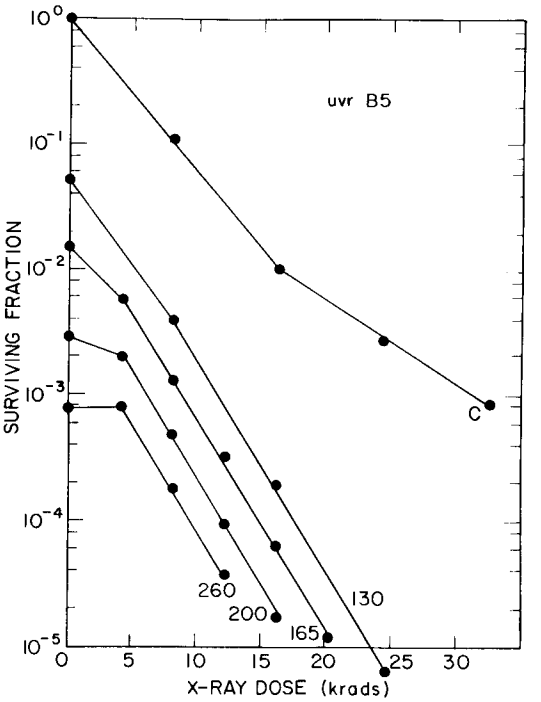

Figure 3. X-ray survival curves of *E. coli* K-12 *uvrB5* (AB2499) after prior exposure to different amounts of UV radiation. For further explanation see Fig. 1.

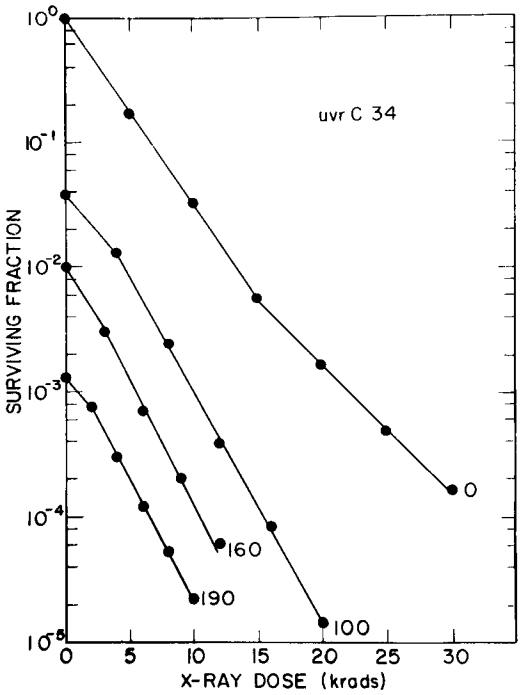

Figure 4. X-ray survival curves of *E. coli* K-12 *uvrC34* (AB2498) after prior exposure to different amounts of UV radiation. For further explanation see Fig. 1.

maximum sensitization were 2·3–2·6 krads for the *uvr* strains compared with 1·9 krads for the wild-type strain (Table 2).

Table 2. Sensitization of different derivatives of *E. coli* K-12 to X radiation by prior UV irradiation

	X-ray sensitivity		
	Without prior UV irradiation D_0 (krads)	After maximum sensitization by UV radiation D_0 (krads)	UV sensitization factor*
Wild-type	4·0† (6·3)‡	1·9	2·1 (3·3)
uvrA6	3·5	2·6	1·3
uvrB5	3·6† (6·3)‡	2·6	1·4 (2·4)
uvrC34	2·9† (5·3)‡	2·3	1·3 (2·3)
recA13	1·1	1·1	1·0
recC22	1·2	1·2	1·0
exrA	1·2	1·2	1·0
polA1	1·0	0·6	1·7

*(D_0 without prior UV irradiation)/(D_0 after maximum sensitization by UV radiation).

†Initial slope of survival curve (until ~ 10^{-2} inactivation).

‡Final slope of survival curve.

The *polA* mutant, deficient in DNA polymerase I, was also sensitized by prior UV irradiation (Fig. 5), and the amount of synergism was greater than that observed for the *uvr* mutants (Table 2) but less than that observed for the wild-type strain.

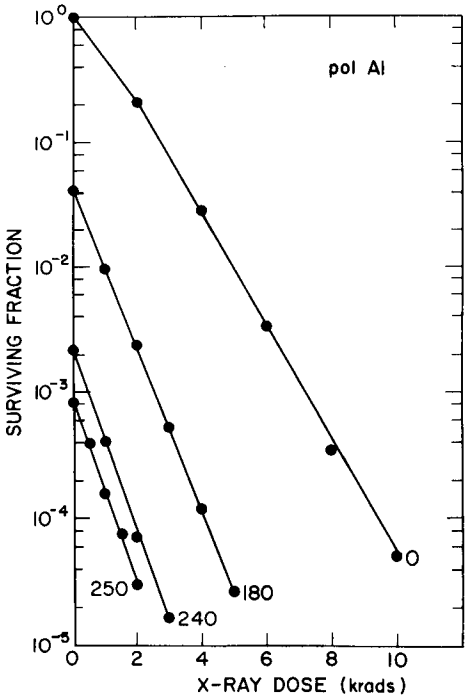

Figure 5. X-ray survival curves of *E. coli* K-12 *polA1* (JG138) after prior exposure to different amounts of UV radiation. For further explanation see Fig. 1.

In contrast to the *uvr* and *pol* mutants, prior UV irradiation did not sensitize the *recA*, *recB* (data not shown), *recC* or *exrA* mutants to X irradiation (Figs. 6–8). This suggests that functional *rec*$^+$ and *exr*$^+$ genes are necessary for UV–X-ray synergism to occur.

Production and repair of DNA single-strand breaks in E. coli *K-12.* The synergistic interaction between UV and X rays on viability could mean that prior exposure to UV radiation inhibits the repair of X-ray-induced lesions, or that X radiation interferes with the repair of UV damage, or both. In order to explore the first possibility, the effect of prior UV irradiation on the production and repair of X-ray-induced single-strand breaks was determined.

Three systems are now known for the repair of X-ray-induced DNA chain breaks. Their characteristics are summarized in Table 3. The

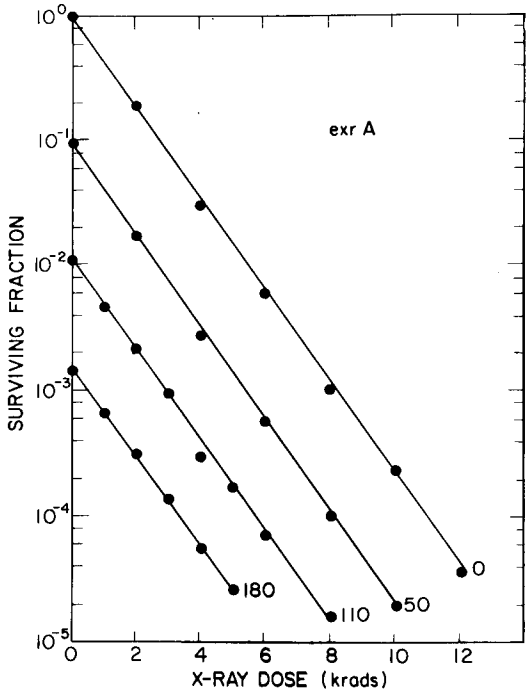

Figure 6. X-ray survival curves of *E. coli* K-12 *exrA* (SR188) after prior exposure to different amounts of UV radiation. For further explanation see Fig. 1.

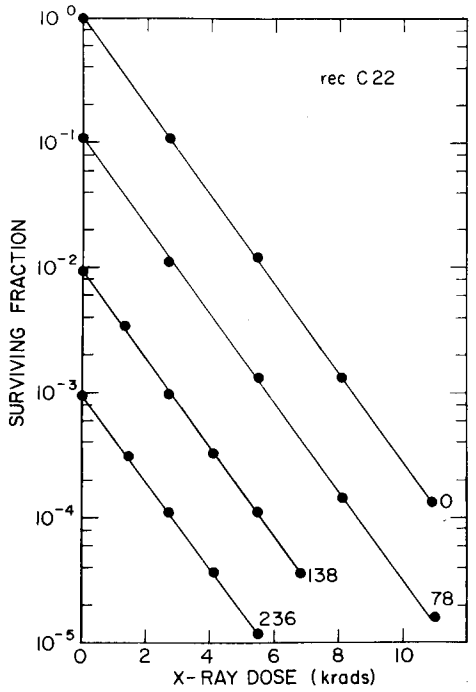

Figure 8. X-ray survival curves of *E. coli* K-12 *recC22* (JC5489) after prior exposure to different amounts of UV radiation. For further explanation see Fig. 1.

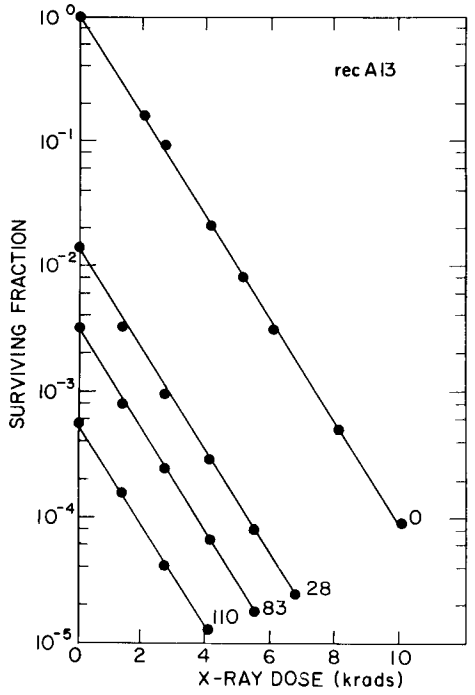

Figure 7. X-ray survival curves of *E. coli* K-12 *recA13* (AB2487) after prior exposure to different amounts of UV radiation. For further explanation see Fig. 1.

Table 3. Systems for the repair of X-ray-induced DNA single-strand breaks (Town *et al.*, 1973a)

Designation	Characteristics
Type I	Very fast (< 2 min at 0°C); occurs in buffer; does not require DNA polymerase I; repairs 75% of the X-ray-induced breaks produced under N_2 but only 25% of the breaks produced under O_2.
Type II	Fast ($T_{1/2}$ for aerobic X irradiation ~ 10 min at 0°C, ~ 1 min at 37°C); occurs in buffer; is largely deficient in strains which lack DNA polymerase I; repairs ~ 90% of the breaks presented to it whether produced under O_2 or N_2.
Type III	Slow (20–60 min at 37°C); requires complete growth medium; controlled by *rec* and *exr* genes; does not require DNA polymerase I; repairs approximately two (2) breaks per single-strand genome whether produced under O_2 or N_2.

speed of repair systems Type I and II and their lack of requirement for growth medium make it unlikely that the synergistic reaction involves these systems. It is the Type III system that is suspect. It is a slow process that requires growth

medium and functional rec^+ and exr^+ genes. Our experiments were specifically designed, therefore, to test the effect of prior UV irradiation on the efficiency of the Type III repair system.

For this study the excision-repair-dificient mutant *E. coli* K-12 *uvrB5* was used, because, in contrast to the wild-type strain, it does not show any excision-repair-induced DNA single-strand breaks after UV irradiation (K. C. Smith, unpublished observations). The present results confirmed this observation (Fig. 9b) and also indicated that incubation of the cells for 1–2 h after UV irradiation did not change the sedimentation profile (data not shown). Therefore, all of the DNA single-strand breaks appearing after combined UV and X-ray irradiation of *E. coli* K-12 *uvrB* should be caused by X irradiation alone. This can be confirmed by comparing the sedimentation profile for the sample treated with 10 krads alone with that treated with 206 ergs/mm² plus 10 krads (Figs. 9c and 9d). No significant difference in sedimentation characteristics was observed between these two samples immediately after irradiation. However, subsequent incubation of these samples had a profound and differential effect upon their sedimentation patterns. After 60 min of incubation in growth medium, nearly complete repair of the DNA single-strand breaks occurred in the cells irradiated only with X rays (Fig. 9e), but no repair was observed after 60 min for cells that had been treated both with UV and X radiation (Fig. 9f). In the latter case, even 120 min of incubation did not result in an increase in molecular weight of the irradiated DNA (data not shown).

Except for the complication of the extra DNA single-strand breaks produced by the action of the *uvr* genes, the UV-induced inhibition of the repair of X-ray-induced DNA single-strand breaks was also observed for wild-type *E. coli* K-12 (this laboratory) and for *E. coli* B/r (M. Quintiliani, personal communication).

DISCUSSION

The wild-type strain of *E. coli* K-12 was sensitized to killing by X radiation (Fig. 1) by a prior exposure to UV radiation to about the same extent (2-fold) as previously observed for *E. coli* B (Haynes, 1966). The DNA polymerase I deficient mutant, *polA* (Fig. 5), and the excision defective mutants *uvrA*, *uvrB* and *uvrC* (Figs. 2–4) were also sensitized. In contrast, the *recA*, *recB*, *recC* and *exrA* mutants showed no UV–X-ray synergism (Figs. 6–8).

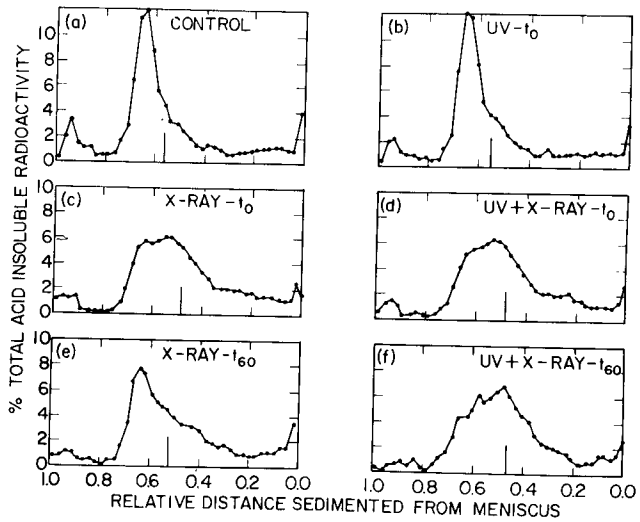

Figure 9. Repair of DNA single-strand breaks in *E. coli* K-12 *uvrB5* (AB2499) cells after combined UV–X-irradiation and X irradiation alone as seen in alkaline sucrose gradients. Cells in exponential growth phase in SMM with and without prior UV irradiation were X irradiated at room temperature (~ 23°C) in air equilibrium. (a) Unirradiated control; (b) 206 ergs/mm² UV without incubation; (c) 10 krads X radiation without incubation; (d) combined UV (206 ergs/mm²) and X radiation (10 krads) without incubation; (e) 10 krads X radiation and 60 min incubation in SMM at 37°C before lysis; (f) 206 ergs/mm² UV and 10 krads X rays and 60 min incubation in SMM at 37°C before lysis. The position of the first moment of each sedimentation profile was determined according to Town, Smith and Kaplan (1971b) and is indicated by a vertical bar.

The results for the *uvr* mutants suggest that, while the presence of the excision repair system is not required for the expression of UV–X-ray synergism, it is required in order to obtain the maximum synergistic effect. By comparing the UV sensitizing factors listed in Table 2, we may estimate that the action of the *uvr* genes accounts for about 40% of the synergism observed in wild-type strains.

The *polA* mutant shows a UV sensitization factor (Table 2) about 80% of that shown by the wild-type strain. This partial deficiency in synergism in *polA* is consistent with its apparent partial deficiency in excision repair (Boyle *et al.*, 1970; Monk *et al.*, 1971; Cooper and Hanawalt, 1972).

The lesions in DNA that induce the synergistic effect must be subject to excision repair since maximum synergism was observed after much lower exposures to UV radiation in *uvr* mutants than in the wild-type strain. In each of these strains, the maximum effect was observed after an exposure to UV radiation that yielded about 5% survival. These observations are consistent with data for the synergistic induction of mutations by combined UV and X radiation. Thus, for an *hcr*$^-$ mutant of *E. coli* B/r WP2 (deficient in excision repair), a much lower amount of UV radiation was required to obtain the same synergistic effect on the production of X-ray-induced mutants as compared to *E. coli* B/r WP2 (Davies *et al.*, 1967).

A presently unexplained feature of the results for the *uvr* mutants (Figs. 2–4) is that, after an exposure to UV radiation that leaves a surviving fraction of about 10^{-3}, the subsequent X-ray survival curve has a shoulder. Thus, the prior UV irradiation serves to protect the cells from low doses of X-rays (up to about 5 krads) but sensitizes the cells to higher doses of X radiation Prior UV irradiation also protected *E. coli* B_{s-1} (*fil*$^+$, *exr*$^-$, *hcr*$^-$) from subsequent X irradiation (Smith and Ganesan, 1966). Possible mechanisms for this protective effect, including, for example, the radiation induction of repair enzymes, are presently only conjectural.

In contrast with the results for the *uvr* and *polA* mutants, UV–X-ray synergism was not observed in *recA*, *recB*, *recC* or *exrA* mutants (Figs. 6–8). Since functional *rec* and *exr* genes are required both for the Type III repair of X-ray-induced DNA single-strand breaks (Table 3) and for the expression of synergism, it seems likely that the two processes are related.

The possible involvement of the Type III repair of X-ray-induced chain breaks in the UV–X-ray synergistic effect on survival was tested by direct experimentation. The kinetics and capacity of this repair process was followed using the alkaline sucrose gradient method of McGrath and Williams (1966). From these experiments (Fig. 9), it is clear that prior UV irradiation irreversibly inhibited the Type III repair of DNA single-strand breaks, and brought the X-ray survival curve of these treated cells close to that observed for the *recC* mutant (Table 2), which is genetically dificient in the Type III repair process (Kapp and Smith, 1970; D. A. Youngs and K. C. Smith, in preparation).

The fact that the X-ray survival curve of the UV-sensitized wild-type strain was not identical with that of the *recC* strain, even though in each case the Type III repair appeared to be completely inhibited, may be interpreted to suggest that the *recC* gene product is involved in additional processes for the repair of X-ray-induced damage other than the Type III repair of DNA single-strand breaks. Similar arguments have been put forth for the additional involvement of the *recA* gene product(Town *et al.*, 1973b) and the *exrA* gene product (Van der Schueren *et al.*, 1973) in processes for the repair of X-ray-induced damage other than the repair of DNA single-strand breaks. In this regard it is of interest to note that the D_0 for *polA* after maximal UV–X-ray synergism (Table 2) was the same (0·6 krads) as that for the double mutant, *polA exrA*, without prior UV irradiation (Youngs and Smith, 1973). Thus, the inhibition of the *exr* gene-controlled repair of X-ray-induced single-strand breaks, either by a mutation at *exrA* or by prior UV irradiation, produces a similar effect upon the survival of a *polA* strain after X irradiation.

Although our data demonstrate that prior UV irradiation exerts its synergistic effect on the survival of X-irradiated cells by inhibiting the *rec* and *exr* gene-controlled Type III repair of X-ray-induced DNA single-strand breaks, the molecular mechanism of this effect is unknown. Our studies with the various radiation sensitive mutants indicate that this UV-induced inhibition of the repair of X-ray-induced damage is due, in part, to the action of the products of the *uvr* and *pol* genes ($\sim$ 40% and $\sim$ 20%, respectively).

Although it appears logical that the residual synergism ($\sim$ 60%) observed in *uvr* mutants may be due to the *rec* and *exr* gene-controlled repair of UV-induced damage, the absence of synergism in the *rec* and *exr* mutants cannot be so easily interpreted in this manner, since these mutants

are also deficient in the X-ray repair system that is inhibitable by prior UV irradiation. The clarification of this problem must await the isolation of mutants or the discovery of chemicals that inhibit some step in the *rec* and *exr* gene-controlled repair of UV-induced damage that may not be required for the *rec* and *exr* gene-controlled repair of X-ray-induced damage.

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