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Prior Irradiation Can Sensitize or Protect Bacterial Cells From Subsequent Irradiation: A Genetic and Biochemical Study*

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PART A. SENSITIZATION INDUCED BY RADIATION

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to UV radiation than if they were first X irradiated (Bhaumik and Bhattacharjee, 1968; K.D. Martignoni and K.C. Smith, unpublished observations). These results suggest that post-irradiation events that occur only in growth medium markedly affect the synergistic response.

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 (Martignoni and Smith, 1973).

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used were *Escherichia coli* K-12 wild-type (AB2497); *uvrA6* (AB2500); *uvrB5* (AB2499); *uvrC34* (AB 2498); *polA1* (JG138); *exrA* (SR188); *recA13* (AB2487) and *recC22* (JC5489). Their nutritional requirements are summarized in Martignoni and Smith (1973). 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 x 10^8 cells/ml). For the sedimentation experiments, cells were grown in SMM containing

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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.

The conditions for UV irradiation (254 nm) and for X irradiation are described by Martignoni and Smith (1973).

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 to 3 days of incubation at 37 °C. The data shown are the average of at least 2 experiments.

Alkaline sucrose gradients. A modified McGrath and Williams (1966) technique was used (Martignoni and Smith, 1973).

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. No further change in the X-ray sensitivity was observed, however, with UV exposures greater than 1000 ergs/mm^2 (Fig. 1).

To test the hypothesis that the UV-X-ray synergism arises from the failure of a repair process, different classes of repair deficient mutants were tested. The X-ray survival curves for *E. coli* K-12 *uvrA, uvrB* and *uvrC* were determined after exposure to different initial amounts of UV radiation. All three *uvr* mutants showed synergism, but somewhat less than observed in the wild-type strain. The results for the *uvrB* strain are shown in Fig. 2. Maximum sensitization of the wild-type and *uvr* strains was obtained by a prior exposure to UV radiation which resulted in about 5% survival.

The *pol A* mutant, deficient in DNA polymerase I, was also sensitized by prior UV irradiation; the amount of synergism was greater than that observed for the *uvr* mutants but less than that observed for the wild-type strain.

In contrast to the *uvr* and *pol* mutants, prior UV irradiation did not sensitize the *recA* (Fig. 3), *recB, recC* or *exrA* mutants to X irradiation. This suggests that functional *rec*+ and *exr*+ genes are necessary for UV-X-ray synergism to occur. The survival data for all the strains tested are summarized in Table 1.

Repair of DNA single-strand breaks in E. coli K-12. We have tested the hypothesis that prior exposure to UV radiation inhibits the repair of X-ray-induced lesions by studying the effect of prior UV irradiation on the repair of X-ray-induced DNA single-strand breaks.

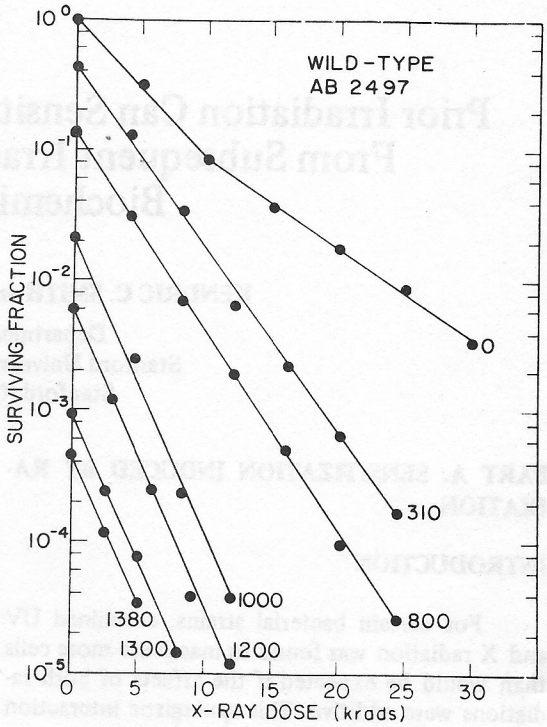

FIGURE 1

X-ray survival curve of E. coli *K-12 wild-type (AB2497) after prior exposure to different amounts of UV radiation. The UV exposure in ergs/mm^2 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 (Martignoni and Smith, 1973).*

Three systems are now known for the repair of X-ray-induced DNA chain breaks. Their characteristics are summarized in Table 2. The 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-deficient mutant *E. coli* K-12 *uvrB5* was used, because *uvrB5,* in contrast to the wild-type strain, does not show any excision repair-induced DNA single-strand breaks after UV irradiation (K.C. Smith,

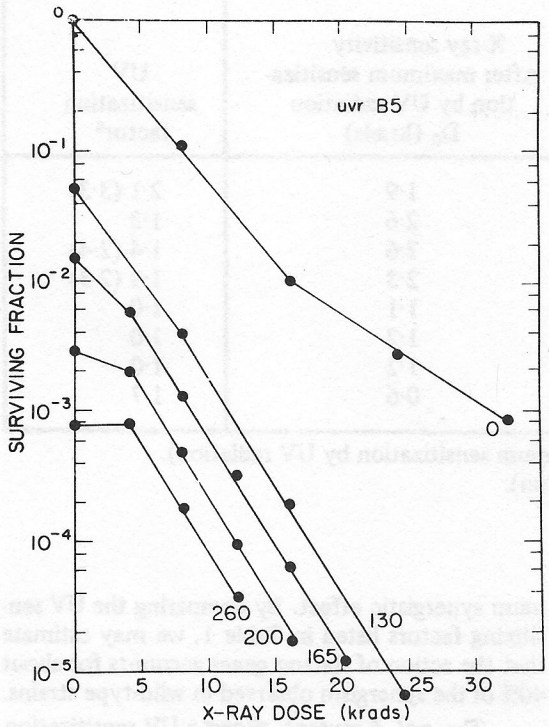

FIGURE 2

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 (Martignoni and Smith, 1973).*

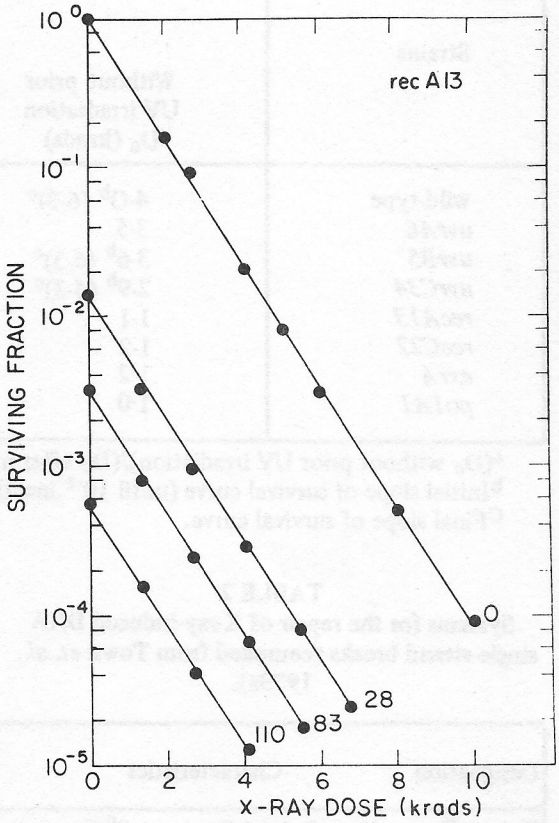

FIGURE 3

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 (Martignoni and Smith, 1973).*

unpublished observations). 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/mm^2 plus 10 krads (Figs. 4C and 4D). 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. 4E), but no repair was observed after 60 min for cells that had been treated both with UV and X radiation (Fig. 4F). 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 (data not shown).

DISCUSSION

The wild-type strain of *E. coli* K-12 as well as the excision defective mutants *uvrA, uvrB* and *uvrC,* and the DNA polymerase I deficient mutant *pol A*,were sensitized to killing by X radiation by a prior exposure to UV radiation. In contrast, the *recA, recB. recC* and *exrA* mutants showed no UV-X-ray synergism (Table 1).

The results for the *uvr* mutants suggest that, while the presence of the excision repair system is

TABLE 1
Sensitization of different derivatives of *E. coli* K-12 to X radiation by prior UV irradiation (Martignoni and Smith, 1973).

Strains	Without prior UV irradiation D_0 (krads)	X-ray sensitivity After maximum sensitization by UV radiation D_0 (krads)	UV sensitization factor[a]
wild-type	4·0[b] (6·3)[c]	1·9	2·1 (3·3)
uvrA6	3·5	2·6	1·3
uvrB5	3·6[b] (6·3)[c]	2·6	1·4 (2·4)
uvrC34	2·9[b] (5·3)[c]	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

[a](D_0 without prior UV irradiation)/(D_0 after maximum sensitization by UV radiation).
[b]Initial slope of survival curve (until 10^{-2} inactivation).
[c]Final slope of survival curve.

TABLE 2
Systems for the repair of X-ray-induced DNA single-strand breaks (compiled from 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 0_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 0_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 0_2 or N_2.

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 1, we may estimate that the action of the *uvr* genes accounts for about 40% of the synergism observed in wild-type strains.

The *pol A* mutant shows a UV sensitization factor (Table 1) about 80% of that shown by the wild-type strain. This partial deficiency in synergism in *pol A* 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 (see Fig. 2) 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)

*Now known to be due mainly to chemical restoration rather than to enzymatic repair of damage, see Roots and Smith, Int. J. Radiat. Biol. 26, 467 (1974).

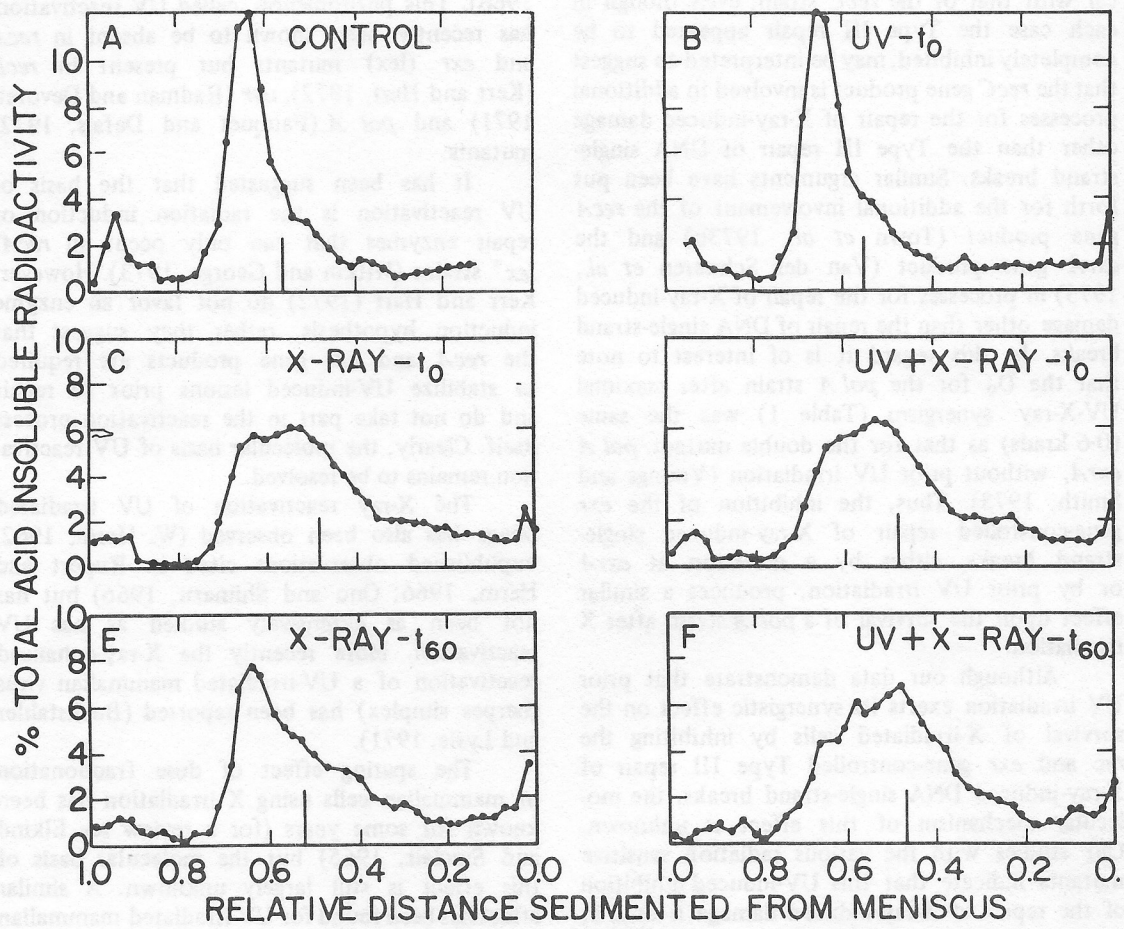

FIGURE 4

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/mm^2 UV irradiation without incubation. (C) 10 krads X irradiation without incubation. (D) combined UV (206 ergs/mm^2) and X irradiation (10 krads) without incubation. (E) 10 krads X irradiation and 60 min incubation in SMM at 37°C before lysis. (F) 206 ergs/mm^2 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 (1971) and is indicated by a vertical bar (from Martignoni and Smith, 1973).*

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). The radiation induction of resistance to subsequent irradiation will be discussed in Part B of this paper.

In contrast with the results for the *uvr* and *pol A* mutants, UV-X-ray synergism was not observed in *recA, recB, recC* or *exrA* mutants (Table 1). Since functional *rec* and *exr* genes are required both for the Type III repair of X-ray-induced DNA single-strand breaks (Table 2) 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 using the technique of alkaline sucrose gradient sedimentation. From these experiments (Fig. 4), 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 1), which is genetically deficient 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 the *pol A* strain after maximal UV-X-ray synergism (Table 1) was the same (0·6 krads) as that for the double mutant, *pol A 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 *pol A* 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 (~40% and ~20%, respectively).

Although it appears logical that the residual synergism (~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.

PART B. PROTECTION INDUCED BY RADIATION

INTRODUCTION

UV irradiated bacteriophage show a higher survival if grown on host cells that have also received a small amount of UV radiation (for a review of earlier work see Rupert and Harm, 1966). This phenomenon, called UV reactivation, has recently been shown to be absent in *recA* and *exr* (lex) mutants but present in *recB* (Kerr and Hart, 1972), *uvr* (Radman and Devoret, 1971) and *pol A* (Fauquet and Defais, 1972) mutants.

It has been suggested that the basis of UV reactivation is the radiation induction of repair enzymes that can only occur in *recA*$^+$ *lex*$^+$ strains (Witkin and George, 1973). However, Kerr and Hart (1972) do not favor an enzyme induction hypothesis, rather they suggest that the *recA* and *exr* gene products are required to stabilize UV-induced lesions prior to repair and do not take part in the reactivation process itself. Clearly, the molecular basis of UV reactivation remains to be resolved.

The X-ray reactivation of UV irradiated phage has also been observed (W. Harm, 1962, unpublished observations cited in Rupert and Harm, 1966; Ono and Shimazu, 1966) but has not been as extensively studied as has UV reactivation. More recently the X-ray-enhanced reactivation of a UV-irradiated mammalian virus (herpes simplex) has been reported (Bockstahler and Lytle, 1971).

The sparing effect of dose fractionation in mammalian cells using X irradiation has been known for some years (for a review see Elkind and Sinclair, 1965) but the molecular basis of this effect is still largely unknown. A similar effect has been found for UV-irradiated mammalian cells, but the kinetics were much slower than for fractionated doses of X radiation (Todd *et al.*, 1969). A similar split dose recovery phenomenon has been observed in yeast for both electrons and UV radiation. Energy-rich metabolites must be present in the cells during the time between the two doses of radiation for this effect to be expressed (Kiefer, 1971). Calkins (1973) has interpreted the "humped" UV and X radiation dose-response curves for protozoans (i. e., where a small dose of radiation is more lethal than a large dose) as indicating the radiation induction of repair capacity.

The present report documents, for *E. coli* K-12, the genetic control and the physiological requirements for the X-radiation-induction of resistance to a subsequent dose of UV radiation.

MATERIALS AND METHODS

Bacterial strains. In addition to certain of the strains described in Part A., the following

strains were also used in the present study: *recB21* (SR78) (Van der Schueren *et al.*, 1973) and (DY151); *exrA* (DY99) (Youngs and Smith, 1973); *recA13* (JC2926); and *recA56* (MM450) (Youngs and Smith, 1973).

Experimental conditions. Exponential phase cells grown in supplemented minimal medium (SMM) (see Part A.) were irradiated with X-rays and incubation was continued at 37°C. At various times, a sample of the cells was diluted 1:10 in phosphate buffer, pH 7 (see Part A.). One portion was plated for viable counts, and another portion was UV irradiated (254 nm) and then plated immediately on SMM agar (1·6% Difco Noble agar).

RESULTS

When *E. coli* wild-type, *uvr*B, or *pol A* strains were X irradiated and then incubated at 37°C in supplemented minimal medium they became progressively more resistant to a subsequent exposure to UV radiation. The maximum resistance to UV radiation was achieved 45-60 min after the X irradiation. The kinetic results for *pol A* are shown in Fig. 5.

The full UV survival curves for wild-type cells before and after 15 krads of X radiation are shown in Fig. 6. There appears to be both a change in shoulder and in final slope after the X-ray treatment.

The *exrA* strain also showed an increase in resistance to UV irradiation after X irradiation, but it took longer to achieve (>120 min) (Fig. 7). A *recB* strain showed little or no radiation induced resistance, however (Fig. 8).

Three *recA* strains were tested (Table 3). The strains showed no radiation-induced protection after incident doses of UV radiation of less than 7 ergs/mm^2. One strain did show protection at incident UV doses greater than 7 ergs/mm^2 (survival $\sim 3 \times 10^{-2}$).

To determine the nutritional conditions required to express this radiation induced protective effect, *exrA* cells were held in buffer (DTM minimal buffered salts solution), minimal growth medium without required amino acids, or in complete growth medium for various periods of time after X irradiation and before the UV irradiation. In all cases, the protective effect was observed (Fig. 9), however, the amount of protection observed in complete growth medium was about 3 times that observed in buffer or in growth medium devoid of amino acids. Wild-type and *uvr* cells cannot be used for this type of experiment since the post-irradiation deprivation of required amino acids kills both UV and X-irradiated cells (Ganesan and Smith, 1972).

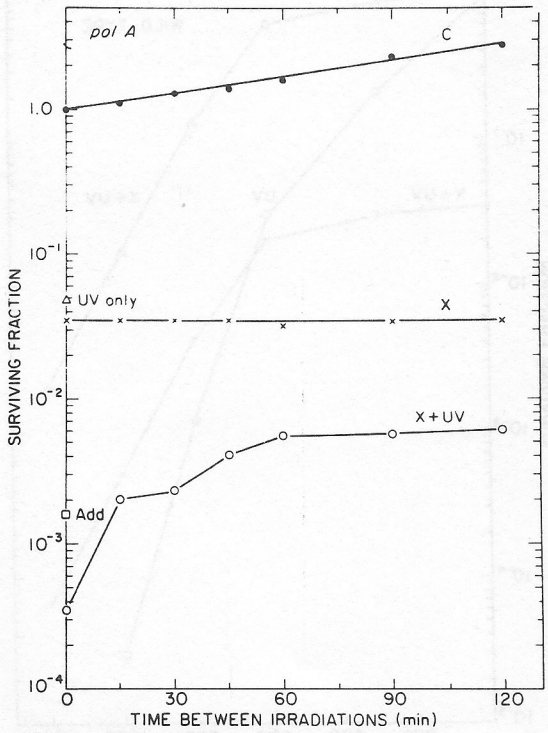

FIGURE 5

E. coli *K-12* polA *cells were X irradiated in complete growth medium with 5 krads, and then incubated for various periods of time at 37°C in complete growth medium before being diluted into buffer and irradiated with 100 ergs/mm^2 of UV radiation (254 nm) and plated. C, unirradiated control culture. X. X-irradiated culture. X + UV, culture X irradiated and then UV irradiated at the times indicated. Δ, survival after UV irradiation only. □, numerical addition of the separate UV and X-ray effect on viability.*

DISCUSSION

The incubation of X-irradiated wild-type, *uvrB*, and *pol A* cells in growth medium for 45-60 min led to their becoming more resistant to a subsequent exposure to UV radiation. Part of the initial rise in resistance in these strains is probably due to the disappearance of synergism between the two radiations (see Part A.). The final level of survival, however, is greater than that expected if the killing effects of the two individual irradiations were merely additive (i. e., without synergism).

The protective effect observed with the *uvrB* strain indicates that the *uvr* gene-dependent excision repair pathway cannot be uniquely res-

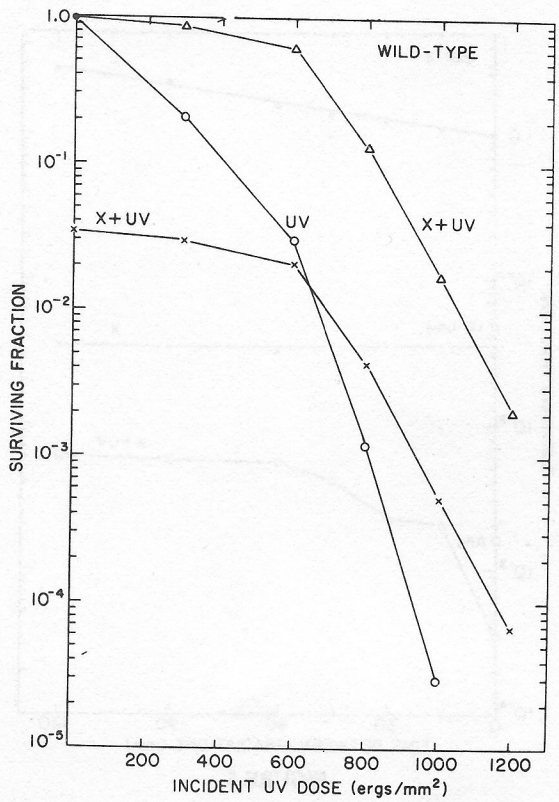

FIGURE 6

E. coli K-12 wild-type cells were X irradiated (15 krads) in complete growth medium, and then incubated at 37°C in complete growth medium for 60 min before being diluted into buffer and then exposed to various incident doses of UV radiation. o, UV irradiation only. X, X irradiation plus UV irradiation. Δ, X irradiation plus UV irradiation normalized to sample exposed only to X radiation as 1·0.

ponsible for the enhanced resistance to UV radiation resulting from prior X irradiation. A similar statement may be made about processes involving DNA polymerase I in view of the results with the *pol A* mutant.

The *exrA* strain shows no UV-X-ray synergism (see Part A.) but still showed a progressive increase in resistance to UV radiation after prior X irradiation. Thus, processes controlled by the *exrA* gene cannot be uniquely responsible for the enhanced resistance to UV radiation produced by prior X irradiation.

The nutritional requirements for the X-ray-induction of resistance to UV radiation in the *exrA* strain were determined. Maximum resistance was observed if the cells were incubated in complete growth medium. Incubation of the cells in buffer or growth medium devoid of required amino acids resulted in about one-third of the resistance induced in complete growth medium (Fig. 9). These results suggest that the major component of the radiation-induced-resistance phenomenon requires incubation of the cells in complete growth medium.

The one mutant tested that consistently showed no enhanced resistance to UV radiation after X irradiation was the *recB* strain. This suggests that the *recB* gene product, a complex nuclease (Karu *et al.*, 1973), is required in order to show the protective effect.

Two separate *recA* strains (Table 3) showed no X radiation-induced resistance to UV radiation. A third strain (AB2487), however, did show enhanced resistance to UV radiation, but only after incident doses greater than 7 ergs/mm² (survival $\sim 3 \times 10^{-2}$). The X-ray-induced enhancement of resistance in this strain to 15 ergs/mm² was not reduced if the cells were incubated

TABLE 3
Protection of different derivatives of *E. coli* K-12 against UV radiation by a prior exposure to X radiation

Strains	Average radiation protection factor*		Radiation doses	
			X-ray (krads)	UV (ergs/mm²)
wild-type (AB2497)	>10	(3)	15	500-1200
uvrB5 (AB2499)	>10	(1)	15	100
polA1 (JG138)	>10	(1)	5	100
exrA (SR188)	>10	(2)	5	120
(DY99)	>10	(3)	5	120
recA13 (AB2487)	>10+	(5)	5	15
(JC2926)	1	(1)	5	15
recA56 (MM450)	1	(2)	5	15
recB21 (SR78)	1·6	(3)	5	500-560
(DY151)	1	(1)	5	500

* The survival after X and UV irradiations with a 60-120 min delay between the two irradiations (incubation at 37°C in complete growth medium), divided by the survival with no delay between the two irradiations. These values are only offered for a qualitative comparison between strains, since the absolute values will certainly be a function of the doses of radiation used. These values also appear to vary from experiment to experiment. The values in parentheses are the number of experiments run on each strain.

\+ In a separate experiment where a full UV survival curve was run, protection was only observed at incident UV doses greater than 7 ergs/mm² (survival $\sim 3 \times 10^{-2}$). In another experiment with this strain, the amount of protection against 15 ergs/mm² was not reduced if the cells were incubated in buffer or growth medium devoid of amino acids between irradiations.

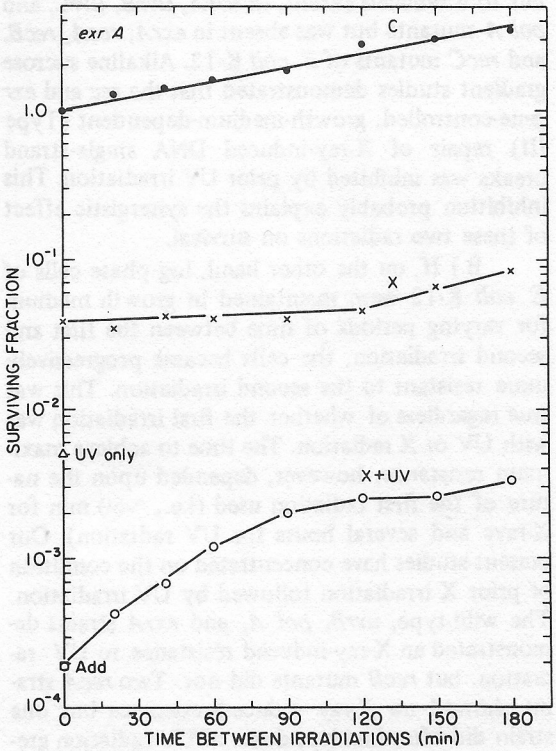

FIGURE 7

E. coli *K-12* exrA *cells were treated as described in the legend to Fig. 5 except that the incident UV dose was 120 ergs/mm^2.*

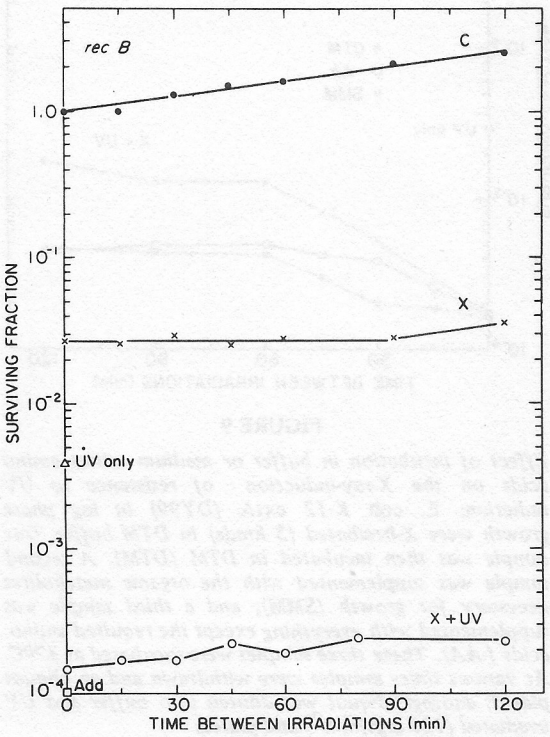

FIGURE 8

E. coli *K-12* recB *cells were treated as described in the legend to Fig. 5 except that the incident UV dose was 500 ergs/mm^2.*

between irradiations in buffer or growth medium devoid of amino acids instead of complete growth medium (data not shown).

The results in Fig. 9 demonstrate that the post-X-irradiation deprivation of required amino acids had no lethal effect on the *exrA* strain. The post-irradiation deprivation of amino acids had previously been shown to be lethal to wild-type and *uvr* strains but not to *recA* and *recB* strains of *E. coli* K-12 (Ganesan and Smith, 1972).

The portion of the X-ray-induced resistance to UV irradiation that requires the presence of complete growth medium depends upon the presence of functional *recA* and *recB* genes, but not on the presence of functional *exrA*, *pol AI* or *uvr* genes. This requirement for complete growth medium is consistent with the concept of the radiation-induction of the synthesis of new or additional repair enzymes (see references in the Introduction, Part B). An alternative hypothesis is that the cells may simply progress into a physiological state in which they are intrinsically more resistant to radiation, similar to the cell cycle dependency of radiation sensitivity observed for mammalian cells (Elkind and Sinclair, 1965).

The radiation-induced resistance to radiation that occurs in buffer or in growth medium devoid of amino acids also depends upon functional *recA* (below 7 ergs/mm^2) and *recB* genes but does not depend upon functional *exrA*, *pol AI* or *uvr* genes. Two hypotheses can be offered to explain the portion of the resistance induced in the absence of complete growth medium.

1.) As suggested above, the cells may progress into a physiological state in which they are intrinsically more resistant to radiation. 2.) The induction of resistance depends upon the function controlled by the *recB* gene—a constitutive nuclease having multiple functions *in vitro* (Clark, 1971). Its functions *in vivo*, however, are not adequately known. Radiation-induced lesions may somehow "turn on" the *recB* nuclease to perform a specific function peculiar to repair.

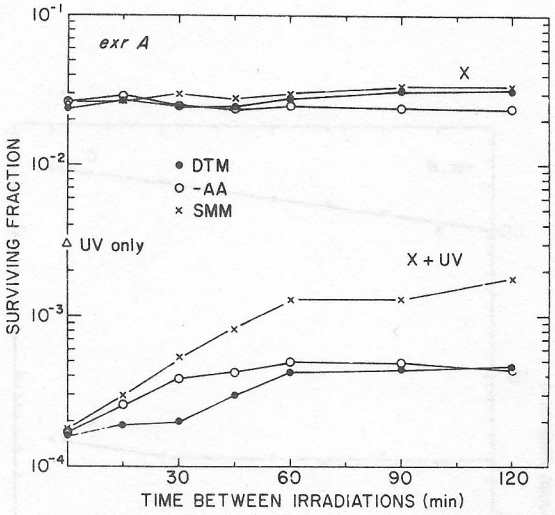

FIGURE 9

Effect of incubation in buffer or medium minus amino acids on the X-ray-induction of resistance to UV radiation. E. coli *K-12* exrA *(DY99) in log phase growth were X-irradiated (5 krads) in DTM buffer. One sample was then incubated in DTM (DTM). A second sample was supplemented with the organic metabolites necessary for growth (SMM), and a third sample was supplemented with everything except the required amino acids (-AA). These three samples were incubated at 37°C. At various times samples were withdrawn and an aliquot plated; another aliquot was diluted into buffer and UV irradiated (120 ergs/mm^2) and plated.*

This could be via an allosteric effect on the *recB* enzyme, or alternatively, products of the action of the *recB* enzyme could activate another enzyme in the repair pathway.

Since the genetic control of the UV reactivation of UV irradiated phage is known (references in the Introduction to Part B.), we plan to extend our studies to determine the genetic control and physiological requirements for the UV-induction of resistance to UV and X radiation in *E. coli* K-12.

On the basis of the present data, one might predict that the *recA* and *recB,* but not the *exrA, pol A,* or *uvr* strains, might be deficient in the X-ray-reactivation of UV irradiated phage.

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SUMMARY

A.) When wild-type cells of *E. coli* K-12 were UV irradiated just prior to X irradiation, they were rendered much more sensitive to the killing action of X-rays. This synergistic effect of combined UV and X irradiation was also observed, but to a reduced extent, in *uvrA, uvrB, uvrC,* and *pol A* mutants but was absent in *exrA, recA, recB,* and *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.

B.) If, on the other hand, log phase cells of *E. coli* K-12 were maintained in growth medium for varying periods of time between the first and second irradiation, the cells became progressively more resistant to the second irradiation. This was true regardless of whether the first irradiation was with UV or X radiation. The time to achieve maximum resistance, however, depended upon the nature of the first radiation used (i.e., ∿60 min for X-rays and several hours for UV radiation). Our present studies have concentrated on the condition of prior X irradiation followed by UV irradiation. The wild-type, *uvrB, pol A,* and *exrA* strains demonstrated an X-ray-induced resistance to UV radiation, but *recB* mutants did not. Two *recA* strains showed no X-ray induced-resistance but one strain did after incident doses of UV radiation greater than 7 ergs/mm^2. This latter *recA* strain showed the radiation induced protective effect even if the incubation between irradiations was in buffer or in growth medium devoid of required amino acids.

The nutritional requirements for the protective effect were determined with an *exrA* strain. Maximum protection required incubation of the cells in growth medium. Only about one-third of the total radiation induced protective effect was observed when the cells were incubated in buffer or in growth medium devoid of amino acids. Thus, there may be two components of the radiation induced protection phenomenon–a major one requiring complete growth medium, and a minor one that does not. Both appear to require the *recB* gene product, however. The growth medium-dependent component also appears to require the *recA* gene product.

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