JOURNAL OF BACTERIOLOGY, Aug. 1972, p. 575-585

Vol. 111, No. 2
Printed in U.S.A.

Requirement for Protein Synthesis in *rec*-Dependent Repair of Deoxyribonucleic Acid in *Escherichia coli* after Ultraviolet or X Irradiation

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Received for publication 11 May 1972

Deprivation of amino acids required for growth or treatment with chloramphenicol or puromycin after irradiation reduced the survival of Rec$^+$ cells of *Escherichia coli* K-12 which had been exposed to either ultraviolet (UV) or X radiation. In contrast, these treatments caused little or no reduction in the survival of irradiated *recA* or *recB* mutants. The effect of chloramphenicol on the survival of X-irradiated cells was correlated with an inhibition of repair of single-strand breaks in irradiated deoxyribonucleic acid (DNA), previously shown to be controlled by *recA* and *recB*. In UV-irradiated cells no effect of chloramphenicol was detected on the repair of single-strand discontinuities in DNA replicated from UV-damaged templates, a process controlled by *recA* but not by *recB*. From this we concluded that inhibiting protein synthesis in UV or X-irradiated cells may interfere with some biochemical step in repair dependent upon the *recB* gene. When irradiated Rec$^+$ cells were cultured for a sufficient period of time in minimal growth medium before chloramphenicol treatment their survival was no longer decreased by the drug. After X irradiation this occurred in less than one generation time of the unirradiated control cells. After UV irradiation it occurred more slowly and was only complete after several generation times of the unirradiated controls. These observations indicated that replication of the entire irradiated genome was probably not required for *rec*-dependent repair of X-irradiated cells, although it might be required for *rec*-dependent repair of UV-irradiated cells.

Inhibitors of protein synthesis can reduce the survival of some strains of *Escherichia coli* following ultraviolet (UV) or ionizing radiation (1, 2, 8, 14, 22, 24). We wished to determine whether strain K-12 was similarly affected, and, if so, whether the decrease in survival could be attributed to the inhibition of any of the systems responsible for repairing damage caused by UV or X radiation. We therefore examined several derivatives of *E. coli* K-12, including mutants deficient in one or more repair systems, for their response to chloramphenicol, puromycin, or deprivation of required amino acids following UV and X irradiation.

The results of our experiments indicate that, following UV or X irradiation, inhibitors of protein synthesis interfere with some step(s) in recovery controlled by the *recA* and *recB* genes.

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MATERIALS AND METHODS

Bacterial strains. Table 1 lists the derivatives of *E. coli* K-12 used. All cultures were grown at 37 C unless otherwise specified. Cultures growing exponentially in minimal medium were used for all experiments, except when otherwise indicated.

Chemicals. Chloramphenicol (Chloromycetin, 1,000 μg/mg), was obtained from Parke, Davis & Co.; puromycin dihydrochloride from Nutritional Biochemicals Corp.; thymine-*methyl*-^3H was obtained from New England Nuclear Corp. (13.6 Ci/mmole) and from Schwarz BioResearch, Inc. (15.0 Ci/mmole).

Media. The media used included a complex medium, YENB (11), and phosphate-buffered minimal media (10).

When needed, L-amino acids were incorporated at

TABLE 1. *Escherichia coli K-12 derivatives used*[a]

Designation	Mating type	Relevant genotype	Other markers	Reference
SR58	F⁻	*recA56 uvrB5*	λ[s] *thr leu arg his pro thi* (*ara lac gal mtl xyl*) Str[r] T6[r]	
SR87	F⁻	*recB21 uvrB5*	λ[s] *thr leu arg his pro thi* (*ara lac gal mtl xyl*) Str[r] T6[r]	11
SR111	F⁻	*recA13 recB21*	λ[s] *thr leu arg his pro thi thy* (*ara lac gal mtl xyl*) Str[r] T6[r]	
JG136	F⁻	*polA1 uvrA6*	λ[s] *thy rha lacZ14*	23
AB1884	F⁻	*uvrC34*	λ[s] *thr leu arg his pro thi ara lac gal mtl xyl* Str[r] T6[r]	17
AB1886	F⁻	*uvrA6*	λ[s] *thr leu arg his pro thi ara lac gal mtl xyl* Str[r] T6[r]	17
AB2497	F⁻		λ[s] *thr leu arg his pro thi thy ara lac gal mtl xyl* Str[r] T6[r]	17
AB2499	F⁻	*uvrB5*	λ[s] *thr leu arg his pro thi thy ara lac gal mtl xyl* Str[r] T6[r]	17
JC2918	F⁻		λ[s] *thr leu arg his pro thi ara lac gal mtl xyl* Str[r] T6[r]	
JC2926	F⁻	*recA13*	λ[s] *thr leu arg his pro thi ara lac gal mtl xyl* Str[r] T6[r]	
JC5743	F⁻	*recB21*	λ[s] *thr leu arg his pro thi ara lac gal mtl xyl* Str[r] T6[r]	
W3110	F⁻		λ[s]	

[a] Abbreviations (see references 4, 6, 29): *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); *rec* denotes genes affecting genetic recombination and radiation sensitivity; *uvr* designates genes affecting host cell reactivation and UV sensitivity; *polA* indicates a deficiency in DNA polymerase I. Markers in parentheses have not been tested, but are inferred from the characteristics of the parent strains.

a final concentration of 10^{-3} M; thymine, 10 μg/ml; and thiamine, 0.5 μg/ml.

Irradiations. Conditions used for UV irradiation have been described before (11). A dose rate of 750 ergs per mm² per min, as estimated by the photodecomposition of potassium ferrioxalate (16, 18), was used for doses above 100 ergs/mm²; 30 ergs per mm² per min for lower doses. Usually 10-ml volumes of cells suspended in minimal growth medium at densities between 5×10^6 and 1×10^8 cells/ml were irradiated in 10-cm petri dishes on a rotary shaker. All operations were performed in yellow light from General Electric "Bug Lites" or "Gold" fluorescent lights to prevent photoreactivation.

For X irradiation, the twin-tube 50 k*Vp* beryllium window X-ray unit developed by Loevinger and Huisman (21) was used with 0.3 mm of aluminum added filtration. Exponentially growing cultures of bacteria in minimal medium were washed and resuspended at densities between 5×10^6 and 1×10^8 cells/ml in minimal medium from which glucose, thiamine, amino acids, and thymine had been omitted (DTM [21]). Three-milliliter amounts of the suspensions, exposed to air, were irradiated in 35-mm plastic petri dishes at room temperature (approximately 23 C). The dose rate under these conditions was estimated to be 7.8 krads/min. This estimate was derived from a value of 8.8 krads/min measured by the Fricke dosimeter (13), corrected by a factor of 0.89 on the assumption that water was the principal intracellular milieu (21).

Treatment with inhibitors. A portion of the irradiated culture was transferred to minimal growth medium containing the inhibitor being tested, or to minimal medium lacking amino acids. Another portion of the irradiated culture was incubated in parallel in minimal growth medium. Unirradiated controls were treated in the same way. At designated times, samples were diluted to remove the inhibitor and plated on agar medium.

Gradients. The techniques used for alkaline sucrose gradients have been described previously (28, 30). The spheroplast mixture contained lysozyme at 50 μg/ml (Fig. 5) or 100 μg/ml (Fig. 10). Cells were labeled by growth with ^{3}H-thymine at a final concentration of 2.1 μg/ml, 15 μCi/ml (Fig. 5); or 2.8 μg/ml, 90 μCi/ml (Fig. 10).

Transduction. A clear-plaque-forming mutant of P1kc was employed for transduction. The techniques used to isolate Rec⁺ transductants have been described (10).

Reversion. Rec⁺ revertants were obtained as previously described (11), except that they were selected after plating irradiated cells on minimal instead of complex medium.

RESULTS

Experiments with X-irradiated cells. We first examined reference strains of K-12 possessing wild-type levels of resistance to X radiation. Chloramphenicol, puromycin, and deprivation of required amino acids reduced the survival of X-irradiated cells of AB2497 by a factor of at least 10 after a dose of 12 krads (Fig. 1). Similar results (not shown) were also obtained with a *uvrB5* mutant, AB2499.

The results of several control experiments indicated that the conditions used in our experiments were sufficient to produce the maximum decrease in the survival of X-irradiated cells. Figure 2 illustrates the effect of different chloramphenicol concentrations. Although 20 μg/ml appeared to be adequate for the max-

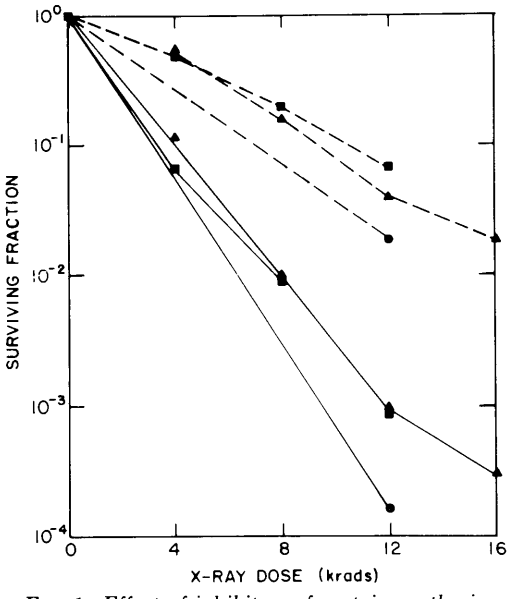

FIG. 1. *Effect of inhibitors of protein synthesis on survival of X-irradiated cells of AB2497. After irradiation, cells were transferred to minimal growth medium, minimal growth medium containing 40 μg chloramphenicol (CAP) per ml, minimal growth medium containing 2 mg of puromycin (Pur) per ml, or minimal medium containing thymine and glucose but no amino acids (−AA). They were diluted and plated on YENB immediately after transfer and again after 90 min of incubation. The surviving fraction of untreated cells is expressed as the number of colony-forming cells per milliliter of the irradiated population immediately after irradiation, divided by the number of colony-forming cells per milliliter of the unirradiated population at the same time. The surviving fraction of treated cells is expressed as the number of colony-forming cells per milliliter of the irradiated population after 90 min of treatment (CAP, Pur, or −AA) divided by the number of colony-forming cells per milliliter of the unirradiated population after 90 min of the same treatment. Survival of unirradiated cells treated for 90 min (expressed as the number of colony-forming cells per milliliter of the unirradiated population after 90 min of treatment divided by the number of colony-forming cells per milliliter of the unirradiated population at the beginning of the experiment) was 1.0 for CAP, 1.3 for Pur, and 1.4 for −AA. Solid lines indicate the response of treated cells; broken lines, the corresponding untreated controls. CAP (■); Pur (●); −AA (▲).*

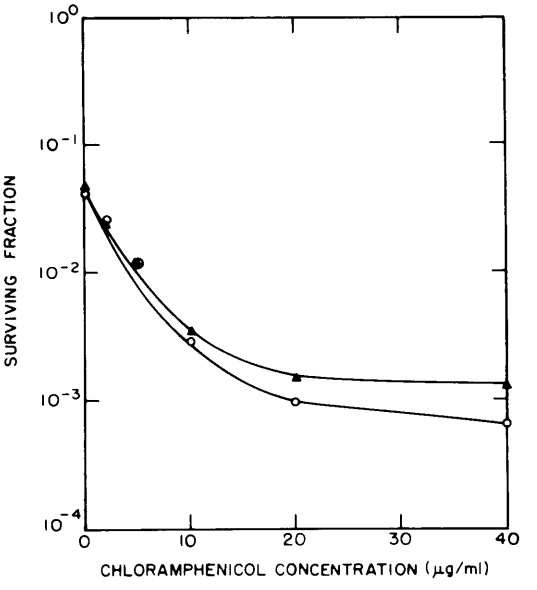

FIG. 2. *Effect of different concentrations of chloramphenicol on the survival of X-irradiated cells of AB2497. Cultures were irradiated with 12 krads and incubated for 90 min in minimal medium with several concentrations of chloramphenicol. Survival is expressed as described in the legend to Fig. 1. Cells were plated on YENB (▲) or on minimal agar medium (○). Survival of unirradiated cells treated with 40 μg of chloramphenicol per ml was 1.3 for cells plated on YENB, and 1.1 for cells plated on minimal medium.*

imal effect, a concentration of 40 μg/ml was chosen for routine use. When we examined the effect of different treatment times, we found that most of the decrease in survival occurred during the first 20 min of treatment, after which there was little further change. Figure 3 presents results obtained with cells of AB2499 (*uvrB5*) deprived of amino acids. Similar results (not shown) were obtained with chloramphenicol. From these data we judged the 90-min treatment time usually used to be ample. We also studied the response to delayed treatment. If cells were incubated in minimal growth medium following irradiation, they became resistant to treatment. After 20 min their survival was almost unaffected by deprivation of amino acids (Fig. 4) or treatment with chloramphenicol (not shown). We therefore transferred cells into the treatment medium as rapidly as possible (within a few seconds) after irradiation.

Having established that the survival of X-irradiated cells of the reference strains of K-12 could be reduced by inhibiting protein synthesis, we examined X ray-sensitive derivatives of K-12, including *recA*, *recB* (see reference 3 for review), and *polA* (5, 15) mutants, to see whether they would be similarly affected. After a dose of 8 krads, chloramphenicol treatment reduced the survival of the *polA uvrA*

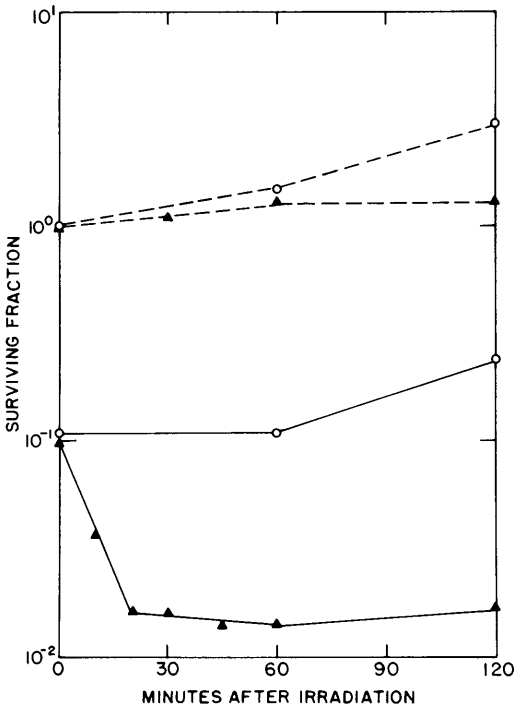

FIG. 3. *Effect of different periods of deprivation of amino acids on the survival of X-irradiated cells of AB2499 (uvrB5). After X irradiation (12 krads), cells were transferred to minimal growth medium (○) or minimal medium without amino acids (▲). At intervals, indicated on the abscissa, samples were diluted and plated on agar minimal medium. Broken lines indicate the response of unirradiated cells; solid lines, the response of irradiated cells. The surviving fraction is expressed as the number of colony-forming cells per milliliter of the irradiated population at the specified time after irradiation, divided by the number of colony-forming cells per milliliter of the culture immediately before irradiation.*

mutant more than 100-fold (Table 2). In contrast, after the same X-ray dose, the survival of the *recA* and *recB* derivatives showed only a two- to fourfold decrease following chloramphenicol treatment, and no decrease after deprivation of amino acids (Table 2). Both chloramphenicol and deprivation of amino acids had a greater effect on *rec*$^+$ cells than on *rec*$^-$ cells (Table 2). These results suggest that *recA* and *recB* control the response of X-irradiated cells to these conditions.

More direct evidence for the role of the *recA* gene was provided by the examination of Rec$^+$ derivatives of a *uvrB5 recA56* strain, SR58. Four Rec$^+$ clones were isolated after transduction of SR58 with phage P1clr grown on a *rec*$^+$ donor, W3110. Two Rec$^+$ revertants (possibly sibs) were obtained after UV irradiation of SR58. The resistance of all six of these derivatives to X radiation was higher than that of their *rec*$^-$ parent and similar to *rec*$^+$ reference strains (cf. Tables 2 and 3). The survival of all of them after X irradiation was reduced by incubation with chloramphenicol, supporting the idea that the effect of this drug on X-irradiated cells depended upon *recA* gene activity.

The *recA* mutants are defective in the repair of single-strand breaks in X-irradiated deoxyribonucleic acid (DNA) (19). We wished to determine whether chloramphenicol would inhibit repair in *rec*$^+$ cells. For this experiment, cells of AB2497 were labeled with ^{3}H-thymine before irradiation. After irradiation they were incubated for 60 min with and without chloramphenicol, and then their DNA was analyzed on alkaline sucrose gradients. The DNA from the irradiated, drug-treated cells sedimented less rapidly than DNA from irradiated cells incubated without the drug (Fig. 5), indicating that chloramphenicol did inhibit the repair of single-strand breaks. The inhibition seemed to be irreversible, since the sedimentation characteristics of the DNA from cells treated with chloramphenicol did not change appreciably when the cells were transferred to medium

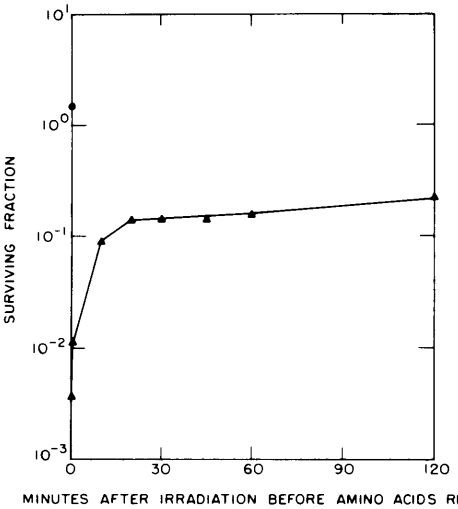

FIG. 4. *Duration of sensitivity of X-irradiated cells of AB2499 (uvrB5) to deprivation of amino acids. Cells irradiated with 12 krads were transferred to minimal growth medium. At the times indicated on the abscissa, samples were diluted into minimal medium without amino acids, incubated for 90 min, and plated on agar minimal medium (▲). Unirradiated controls (incubated for 90 min without amino acids) (●). Survival is expressed as described in the legend to Fig. 3.*

TABLE 2. *Effect of chloramphenicol and deprivation of required amino acids on the survival of some E. coli K-12 derivatives after X irradiation*[a]

Strain	Treatment	Survival		
		Untreated	Treated	Ratio (untreated/treated)
JC2918	−AA	1.1×10^{-1}	1.3×10^{-2}	8
	CAP	1.1×10^{-1}	3.6×10^{-3}	31
AB2499 (*uvrB5*)	−AA	1.3×10^{-1}	1.4×10^{-2}	9
	CAP	1.7×10^{-1}	1.1×10^{-2}	15
JC2926 (*recA13*)	−AA	7.8×10^{-4}	6.4×10^{-4}	1
		6.2×10^{-4}	5.4×10^{-4}	1
	CAP	5.8×10^{-4}	1.6×10^{-4}	4
		7.0×10^{-4}	2.1×10^{-4}	3
JC5743 (*recB21*)	−AA	1.6×10^{-3}	2.6×10^{-3}	1
		2.2×10^{-3}	1.6×10^{-3}	1
	CAP	2.6×10^{-3}	1.3×10^{-3}	2
		1.8×10^{-3}	8.8×10^{-4}	2
SR111 (*recA13 recB21*)	CAP	1.0×10^{-4}	2.8×10^{-5}	4
SR87 (*recB21 uvrB5*)	CAP	7.7×10^{-4}	1.3×10^{-3}	1
JG136 (*polA1 uvrA6*)	CAP	3.1×10^{-4}	8.5×10^{-7}	365
		2.2×10^{-4}	1.0×10^{-6}	220

[a] Cultures were irradiated with 8 krads, incubated for 90 min in minimal growth medium (no treatment), minimal growth medium containing 40 μg of chloramphenicol (CAP) per ml, or minimal medium lacking amino acids (−AA), and then plated on YENB. Survival is expressed as described in the legend to Fig. 1.

without the drug and incubated for an additional 60 min before being lysed and analyzed (Fig. 5).

Experiments with UV-irradiated cells. Most of the experiments described in the preceding section were repeated with UV-irradiated cells to compare their responses with those of X-irradiated cells. When we examined the reference strain, AB2497, which has the wild-type level of resistance to UV, we found that chloramphenicol reduced its survival after UV irradiation (Fig. 6A). Figure 6B shows the response of a *uvrC34* mutant, AB1884. After UV irradiation, *uvr* mutants show a higher survival on minimal than on complex medium (11). Treatment with chloramphenicol, plating on complex medium, or a combination of the two, resulted in approximately the same survival level (Fig. 6B). Consequently little or no decrease in survival due to chloramphenicol treatment could be detected when irradiated *uvr* cells were plated on complex medium.

Treatment with puromycin (2 mg/ml) or deprivation of required amino acids produced results (not shown) similar to those obtained with chloramphenicol.

Several control experiments were performed to verify that the conditions used for treatment were adequate for the maximal reduction in survival of UV-irradiated cells. The response of AB2497 (*uvr*$^+$) to different concentrations of chloramphenicol after UV irradiation (not shown) resembled its response after X irradiation (Fig. 2). The same was true of UV-irradiated cells of AB2499 (*uvrB5*) when they were plated on minimal medium (Fig. 7). Consistent with the results obtained with AB1884, the survival of untreated cells of AB2499 plated on complex medium was as low as that of cells treated with the highest chloramphenicol concentration, 40 μg/ml (Fig. 7). Based on the response of AB2497 and of AB2499 on minimal medium, we chose to use the same concentration (40 μg/ml) for UV-irradiated cells as we had used for X-irradiated cells. Because most of the reduction in survival caused by chloramphenicol occurred during the first 45 min of treatment (Fig. 8), treatment times of 90 and 120 min were judged long enough to permit the maximum reduction in survival to occur.

When incubated in minimal medium the UV-irradiated *uvr* cells lost their sensitivity to chloramphenicol much more slowly than did X-irradiated cells under the same conditions. For example, cells of AB1884 (*uvrC34*) irradiated with 125 ergs/mm^2 at 254 nm required 6 hr of incubation before their survival was no longer affected by chloramphenicol (Fig. 9). They needed the same length of time to lose their sensitivity to complex medium (Fig. 9).

TABLE 3. *Effect of a recA mutation on the response of X-irradiated cells to chloramphenicol*[a]

Strain	Survival		
	No CAP	CAP	Ratio (no CAP/CAP)
SR58 (*recA13 uvrB5*)	5.1×10^{-5}	2.8×10^{-5}	2
	4.3×10^{-5}	2.6×10^{-5}	2
Rec$^+$ (*uvrB5*) transductants:			
no. 1	1.1×10^{-1}	7.1×10^{-3}	16
2	9.5×10^{-2}	9.3×10^{-3}	10
3	8.1×10^{-2}	9.3×10^{-3}	9
4	7.0×10^{-2}	7.8×10^{-3}	9
Rec$^+$ (*uvrB5*) revertants:			
no. 1	9.0×10^{-2}	7.7×10^{-3}	12
2	9.9×10^{-2}	9.3×10^{-3}	11

[a] Cells were irradiated with 8 krads, treated with chloramphenicol (CAP) as described in the legend to Fig. 1, and plated on YENB. Survival is expressed as described in the legend to Fig. 1.

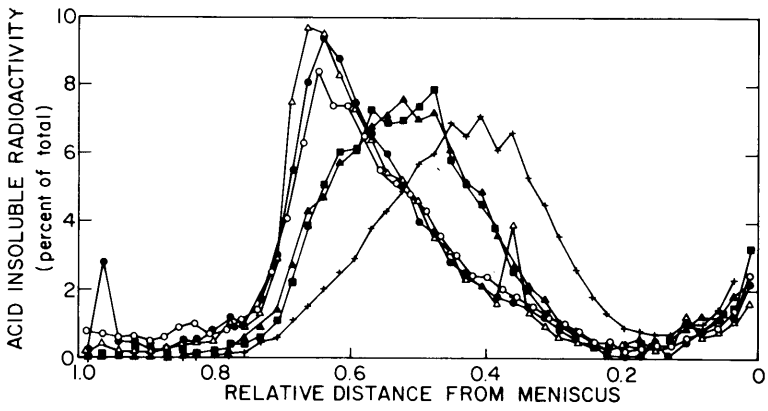

FIG. 5. *Effect of chloramphenicol on the repair of single-strand breaks in DNA after X irradiation. Cells of AB2497 were labeled with ^{3}H-thymine before irradiation. They were irradiated with 20 krads, treated as described below, and analyzed on alkaline sucrose gradients. Unirradiated control* (○); *unirradiated control incubated for 60 min in minimal medium with 40 μg of chloramphenicol per ml* (●); *irradiated cells, not reincubated* (+) (*survival = 1.2 × 10^{-2}*); *irradiated cells reincubated for 60 min without chloramphenicol* (△); *irradiated cells, reincubated for 60 min with 40 μg of chloramphenicol per ml* (▲); *irradiated cells, incubated for 60 min with chloramphenicol, and then 60 min more without chloramphenicol* (■).

Having ascertained that chloramphenicol reduced the survival of UV-irradiated cells of wild-type sensitivity, and also *uvrA*, *uvrB*, and *uvrC* mutants (Fig. 6–9; Table 4), we next examined *recA* and *recB* derivatives. Chloramphenicol did not reduce the survival of UV-irradiated *recA* cells, and only slightly decreased that of *recB* cells (Tables 4 and 5). The survival of the *recA56 uvrB5* strain, SR58, was not affected by chloramphenicol, but that of its two Rec$^+$ (*uvrB5*) revertants and four transductants was reduced by the drug (Table 5). These results supported the idea that chloramphenicol inhibited processes controlled by the *recA* and *recB* genes needed for the recovery of cells from UV irradiation.

Since the *recA* gene may control the repair of discontinuities formed in DNA replicated from UV-irradiated templates (26, 28), we wished to determine whether this function was inhibited by chloramphenicol. Cells of AB2499 (*uvrB5*) were irradiated with UV and labeled for 10 min with ^{3}H-thymine. They were then washed and reincubated for 60 min in minimal medium with or without 40 μg of chloramphenicol per ml. Their DNA was then analyzed on alkaline sucrose gradients. As expected, the labeled DNA from the irradiated cells lysed immediately after the 10-min labeling period sedimented more slowly than DNA from unirradiated controls (Fig. 10). If the cells were incubated for 60 min either with or without chloramphenicol before being lysed, the sedimentation of DNA from irradiated cells resembled that from unirradiated cells (Fig. 10). Essentially the same result was also obtained when irradiated cells were treated with chloramphenicol before (10 min) and during the labeling period, as well as after it (data not shown). These experiments revealed no inhibition by chloramphenicol of the repair of discontinuities in DNA synthesized after UV irradiation.

DISCUSSION

Each of the three inhibitors of protein synthesis that we tested, chloramphenicol, puromycin, and deprivation of required amino acids, reduced the survival of "wild-type" *E. coli* K-12 after UV and X irradiation. These results are consistent with those reported by other investigators using other *E. coli* strains (1, 2, 8, 14, 24). Although Marshall and Gillies (22) reported that chloramphenicol caused no reduction in the survival of gamma-irradiated cells of K-12, we feel that differences between their experimental conditions and ours may account for the discrepancy.

We attribute the reduction in survival in *E. coli* K-12 to the inhibition of DNA repair processes controlled by the *recA* and *recB* genes necessary for the recovery of cells from damage caused by radiation. The *recA* and *recB* mutants are more sensitive than corresponding *rec*$^+$ strains to both UV and X irradiation, implying that the *rec* genes mediate repair of damage caused by both types of radiation (for review see reference 27). Following X irradiation the survival of *recA* and *recB* mutants was

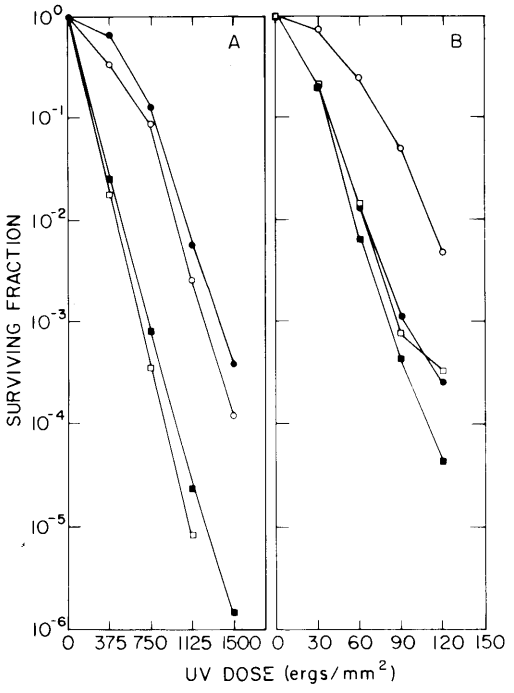

FIG. 6. *Survival of cells treated with chloramphenicol after various doses of UV. Cells were irradiated with UV, incubated for 120 min in minimal medium with 40 μg of chloramphenicol per ml, and plated on YENB (■) or on minimal medium (□). Irradiated cells were also incubated for 120 min without chloramphenicol and plated on YENB (●) or on minimal medium (○). The surviving fraction is expressed as described in the legend to Fig. 1. A, AB2497 (uvr⁺); the survival of unirradiated, treated controls was 1.1 on YENB and 1.0 on minimal medium. B, AB1884 (uvrC34); the survival of unirradiated, treated controls was 1.1 on YENB and 1.0 on minimal medium.*

not affected by amino acid deprivation and only slightly reduced by chloramphenicol, whereas the other K-12 derivatives tested, including a DNA polymerase I-deficient mutant, were clearly sensitive to both kinds of treatment (Tables 2 and 3). After UV irradiation the survival of *recA* cells was not reduced by chloramphenicol and that of *recB* cells was only slightly reduced compared to *rec*⁺ cells (Tables 4 and 5). These results indicated that conditions which prevent protein synthesis inhibit some step in the type(s) of recovery controlled by the *recA* and *recB* genes. Although no *recC* mutants were tested, we expect them to behave like *recB* mutants since the *recB* and *recC* genes jointly specify a single nuclease, and *recB* and *recC* cells show the same sensitivity to UV and X irradiation (see reference 3 for review).

We attempted to identify the step in repair affected by chloramphenicol by examining the sedimentation in alkaline sucrose of DNA from irradiated cells treated with this drug. Previous work has implicated the *recA* gene product in the repair of single-strand discontinuities after both UV and X irradiation. Following X irradiation the discontinuities appear in the irradiated DNA, whereas after UV irradiation they are formed in daughter strands replicated from the irradiated DNA. Wild-type cells incubated in minimal growth medium can repair all of the discontinuities induced after 22 krads of X radiation or 60 ergs of UV/mm² (i.e., the repaired molecules regain the sedimentation characteristics of normal DNA on alkaline sucrose gradients). In *recA* mutants both types of repair are defective and their DNA remains discontinuous (19, 28). If the decreased survival of irradiated cells

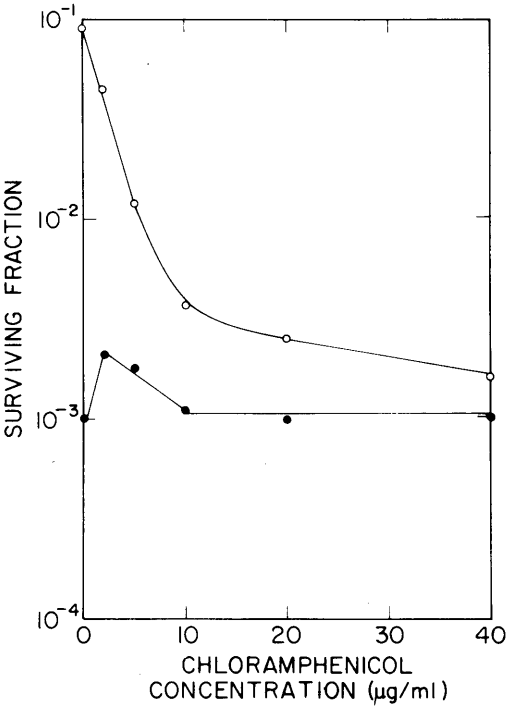

FIG. 7. *Effect of different concentrations of chloramphenicol on the survival of UV-irradiated cells of AB2499 (uvrB5). Cells were irradiated with 125 ergs/mm², incubated for 90 min in minimal medium with different concentrations of chloramphenicol and plated on YENB (●), or on minimal medium (○). The surviving fraction is expressed as described in the legend to Fig. 1. After treatment with 40 μg of chloramphenicol per ml, the survival of unirradiated, treated controls was 1.3 on YENB and 0.7 on minimal medium.*

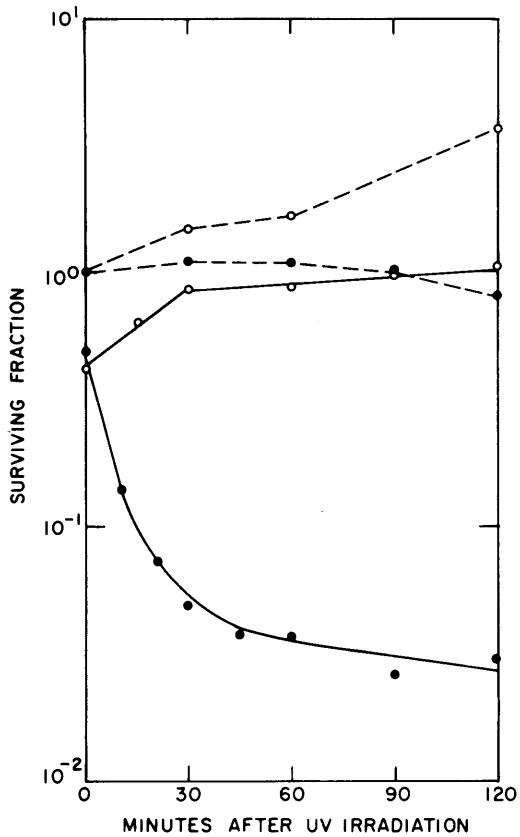

FIG. 8. *Effect of different periods of chloramphenicol treatment on the survival of UV-irradiated cells of AB1884 (uvrC34). After UV irradiation (100 ergs/mm²) cultures were incubated in minimal medium (without chloramphenicol), or minimal medium containing 40 μg of chloramphenicol per ml. At intervals, they were plated on agar minimal medium. Dotted lines indicate the response of unirradiated cells; solid lines the response of irradiated cells. The surviving fraction is expressed as described in the legend to Fig. 3. Cells incubated without chloramphenicol (○). Cells incubated with chloramphenicol (●).*

treated with chloramphenicol resulted from inhibition of repair mediated by *recA*, it should be correlated with an inhibition of the repair of single-strand discontinuities after both UV and X irradiation.

The role in repair of the *recB* and *recC* genes, and of the complex nuclease for which they code, has not been clearly established. A mutation in either gene increases sensitivity to UV and to X radiation, indicating that the nuclease participates in repairing damage caused by both of these agents. The *recB* and *recC* genes may be involved in the same repair process controlled by *recA*, since the introduction of a *recB* or *recC* mutation into *recA* cells does not strikingly increase their sensitivity to UV or X radiation (19, 31). However, in contrast to *recA* mutants, *recB* and *recC* mutants do not seem to be defective in repairing single-strand discontinuities caused by replication of UV-irradiated DNA (28). In addition, they seem to show a less pronounced deficiency in repairing single-strand breaks in X-irradiated DNA (19). If chloramphenicol interfered with the step in repair mediated by *recB* and *recC*, it might cause a partial inhibition of the repair of single-strand breaks in DNA after X irradiation, but no effect on the repair of discontinuities formed by the replication of UV-irradiated DNA.

Our results indicated that chloramphenicol

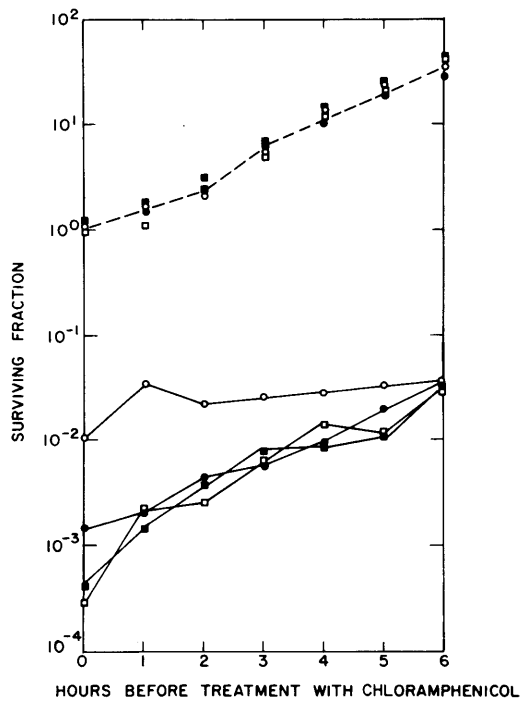

FIG. 9. *Duration of sensitivity of uvr cells to chloramphenicol after UV irradiation. AB1884 (uvrC34) was irradiated with 125 ergs/mm² and then incubated in minimal growth medium. At intervals, indicated on the abscissa, samples were removed, diluted, and plated on YENB (●) or minimal medium (○), or transferred to minimal medium containing 40 μg of chloramphenicol per ml, incubated for 90 min, diluted, and plated on YENB (■) or minimal medium (□). Broken lines indicate the response of unirradiated cells; solid lines, the response of irradiated cells. The surviving fraction is expressed as described in the legend to Fig. 3.*

TABLE 4. *Effect of chloramphenicol on the survival of some E. coli K-12 derivatives after UV irradiation*[a]

Strain	UV dose (ergs/mm²)	Survival		
		No CAP	CAP	Ratio (no CAP/CAP)
JC2918	375	1.3×10^{-1}	1.4×10^{-3}	93
		1.6×10^{-1}	1.1×10^{-3}	145
AB1884 (*uvrC34*)	63	3.1×10^{-1}	5.2×10^{-3}	60
AB1886 (*uvrA6*)	63	5.5×10^{-1}	4.6×10^{-3}	120
JC2926 (*recA13*)	63	8.8×10^{-4}	2.0×10^{-3}	<1
	60*	5.1×10^{-4}	9.3×10^{-4}	1
		5.2×10^{-4}	6.0×10^{-4}	1
JC5743 (*recB21*)	375	7.4×10^{-3}	4.1×10^{-3}	2
		4.1×10^{-4}	1.5×10^{-4}	3
SR87 (*recB21 uvrB5*)	63	5.9×10^{-5}	3.2×10^{-5}	2

[a] After UV irradiation cells were incubated in minimal medium (no CAP), or minimal medium containing 40 μg of chloramphenicol (CAP) per ml for 90 min or for 120 min (*), and then plated on agar minimal medium. Survival is expressed as described in the legend to Fig. 1.

TABLE 5. *Effect of a recA mutation on the response of UV-irradiated uvr cells to chloramphenicol*[a]

Strain	Survival		
	No CAP	CAP	Ratio (no CAP/CAP)
SR58 (*recA13 uvrB5*)	3.7×10^{-4}	5.0×10^{-4}	1
Rec⁺ (*uvrB5*) transductants:			
no. 1	2.5×10^{-3}	4.4×10^{-5}	57
	2.0×10^{-3}	1.6×10^{-4}	13
2	9.0×10^{-4}	1.1×10^{-5}	82
3	7.8×10^{-4}	5.2×10^{-5}	15
4	2.0×10^{-3}	6.7×10^{-5}	30
Rec⁺ (*uvrB5*) revertants:			
no. 1	1.8×10^{-3}	7.4×10^{-6}	242
2	2.4×10^{-3}	1.0×10^{-5}	240

[a] Cultures growing exponentially in minimal medium were irradiated, incubated for 90 min in minimal medium (no CAP) or minimal medium containing 40 μg of chloramphenicol (CAP) per ml, and then diluted and plated on agar minimal medium. Survival is expressed as described in the legend to Fig. 1. SR58 was exposed to 3 ergs/mm²; the Rec⁺ derivatives to 125 ergs/mm².

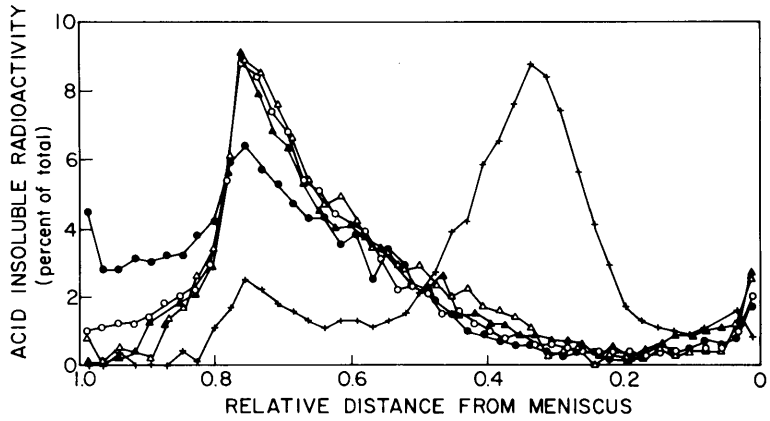

FIG. 10. *Effect of chloramphenicol on postreplication repair of DNA. Cells of AB2499 (uvrB5) were irradiated with 60 ergs/mm² (survival on agar minimal medium was 5.4 × 10^{-1}), labeled for 10 min with ³H-thymine, and then lysed immediately (+), or reincubated for 60 min in minimal medium (△), or minimal medium containing 40 μg of chloramphenicol per ml (▲). Unirradiated control cells were lysed immediately (○) or incubated for 60 min with chloramphenicol (●).*

treatment partially inhibited the repair of single-strand breaks in DNA after X irradiation (Fig. 5) and did not inhibit the repair of single-strand discontinuities in DNA synthesized after UV irradiation (Fig. 10). This observation is clearly different from the one expected if chloramphenicol affected the *recA* function. On this basis we feel it is more likely that the effect of chloramphenicol on the survival of irradiated cells is due to the inhibition of a step in repair dependent upon *recB*.

The length of time that cell survival remained sensitive to the inhibition of protein synthesis after irradiation provided an estimate of the time required for the type of recovery mediated by the *rec* genes. Although this estimate could be too small (because irradiated cells might regain resistance to the inhibition of protein synthesis before completing recovery), it should not be too large (since the irradiated cells cannot be considered to have recovered completely until they have regained

the resistance characteristic of unirradiated cells). Sensitivity to inhibitors of protein synthesis lasted much longer after UV than after X irradiation. After a dose of 125 ergs/mm^2 at 254 nm (survival was 2% without chloramphenicol treatment) *uvrC34* cells required 6 hr in minimal medium to become resistant to chloramphenicol (Fig. 9). We previously reported that *uvr* cells required several hours to recover resistance to complex medium after UV irradiation (12). For example, after 63 ergs/mm^2 (from which 50% of the cells recovered) about 5 hr was needed for this process. In the present report, we have shown that the time required for UV irradiated *uvrC34* cells to regain resistance to complex medium was the same as that required to recover resistance to chloramphenicol (Fig. 9). Both criteria, therefore, indicate that *uvr* cells require the equivalent of several generation times of unirradiated cells to recover from UV irradiation. This observation is consistent with models (25) which entail DNA replication as a prerequisite for *rec*-dependent repair of damage caused by UV.

In contrast to UV-irradiated cells, the survival of X-irradiated cells remained sensitive to the inhibition of protein synthesis for less than one generation time. After 12 krads of X radiation (survival was 20% without treatment) resistance to amino acid deprivation (Fig. 4) and to chloramphenicol returned within about 20 min. Similar results were obtained by using the loss of sensitivity to quinacrine (9) and to an impure preparation of hydroxyurea (20) to estimate the time needed for *rec*-dependent recovery of *E. coli* K-12 from X irradiation. Because recovery appeared to require the equivalent of less than one generation time of unirradiated control cells (which was about 60 min under our conditions), we concluded that, in X-irradiated cells, *rec*-dependent repair does not entail replication of the entire irradiated genome, although it may involve some repair synthesis (7).

In summary, postirradiation inhibition of protein synthesis reduced the survival of *rec*$^+$ cells of *E. coli* K-12 after UV or X irradiation. It did not similarly affect *recA* or *recB* mutants, suggesting that protein synthesis is needed for some repair function controlled by the *recA* and *recB* genes. Consistent with this idea, chloramphenicol inhibited the repair of single-strand breaks in the DNA of X-irradiated cells, a process controlled by *recA* and *recB*. However, in UV-irradiated cells chloramphenicol did not detectably interfere with the repair of single-strand discontinuities in DNA synthesized from irradiated templates. This result would not be expected if protein synthesis were needed for the step in repair controlled by *recA*. It is compatible with the possibility that protein synthesis is needed for a step controlled by *recB*.

The requirement for protein synthesis persists for a longer period of time after UV irradiation than after X irradiation.

ACKNOWLEDGMENTS

We are grateful to John Miller for excellent technical assistance, and to Eliane Suffolk for performing the alkaline sucrose gradient experiments.

This work was supported by Public Health Service Research grant CA-02896, Research Program Project grant CA-10372, and Research Career Development Award CA-03709 (to K.C.S.) from the National Cancer Institute.

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