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Recovery of Recombination Deficient Mutants of *Escherichia coli* K-12 from Ultraviolet Irradiation

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Although the recombination deficient, or *rec* mutants of *Escherichia coli* K-12 are more sensitive to ultraviolet light (UV) than their *rec*$^+$ counterparts (Clark and Margulies, 1965; Howard-Flanders and Theriot, 1966; Van de Putte et al. 1966), some of them appear to have acquired two recovery processes not observed in wild-type K-12.

Similar processes were originally described in *E. coli* B irradiated with UV. The first, called liquid holding recovery, or LHR (Castellani, Jagger, and Setlow, 1964; Harm, 1966; Jagger, 1964; Jagger, Wise, and Stafford, 1964), is characterized by an increase in the proportion of colony-forming units when irradiated cells are held in the dark in buffer and plated at intervals on a complex medium such as nutrient agar or yeast extract (Charles and Zimmerman, 1956; Hollaender and Claus, 1937; Hollaender and Curtis, 1935; Roberts and Aldous, 1949). The second, which we shall refer to as minimal medium recovery (MMR), was observed as a higher survival of irradiated cells plated on minimal medium compared to those plated on nutrient agar (Roberts and Aldous, 1949; Alper and Gillies, 1959).

Neither process is observed in most K-12 derivatives, at least under the conditions we have used to look for them. However, they do appear in certain *rec* mutants. We have used these mutants to study some of the characteristics of LHR and MMR and to try to elucidate their relationship to each other and to DNA repair processes in *E. coli*.

EXPERIMENTAL

Materials and Methods

The bacteria used were all derivatives of *E. coli* K-12 (Table 1). Cultures were incubated at 37°C.

Most of the procedures and media employed have been previously described (Ganesan and Smith, 1968).

LHR was measured by holding irradiated cells in sodium-potassium phosphate buffer, pH 7.0, 0.067 M, at 37°C and plating at intervals on yeast extract agar. Recovery is expressed as the ratio of the number of colony forming units present after holding to the number present before holding. Cultures for this purpose were usually harvested during exponential growth in minimal medium. They were washed with buffer and incubated in buffer for two hours before being exposed to UV.

MMR was measured by plating irradiated cells on yeast extract agar and on minimal medium supplemented with the growth requirements of the strain being tested. Recovery is expressed as the ratio of the number of colonies formed on minimal medium to the number on yeast extract.

The release of acid soluble material from cellular DNA was measured by irradiating cells labeled with thymine-2-^{14}C (24.4 mCi/mmole). These were held in buffer or other liquid medium after irradiation and samples pipetted at intervals into perchloric acid (0.5 N final concentration) in chilled tubes. After one hour, the samples in acid were filtered through Millipore HA membranes. The radioactivity in the filtrates was determined by counting in a scintillation counter, using a counting solution of 2,5-diphenyloxazole, 1,4-bis-2-(5-phenyloxazolyl)-benzene and naphthalene in p-dioxane and xylene. The efficiency of counting was approximately 70%.

Genetic Characteristics

Effect of rec *mutations on LHR.* Of the 18 derivatives of K-12 tested for LHR, only five showed significant recovery in buffer (Ganesan and Smith, 1968). These all contained *rec* mutations (Clark, 1967). Not all the Rec$^-$ strains tested showed LHR. Those carrying *recB21* and *recC22* (N. Willetts and D. Mount, pers. commun.) did not. But those containing *recA1*, *rec-12*, *recA13* (two derivatives) and *rec-56* showed at least a 20-fold increase in colony-forming units over an 8-hr recovery period. The largest increase observed in any of the other derivatives was a 3-fold increase, but most showed no change or even a decrease during this period.

More direct evidence that the expression of LHR depends upon the *rec* mutations was obtained by genetic analysis of AB2487, one of the strains carrying *recA13*. Rec$^+$ derivatives were obtained from it by conjugation with *rec*$^+$ *uvr*$^+$ Hfr strains, by transduction with P1*kc* phage grown on a *rec*$^+$

TABLE 1. *Escherichia coli* K-12 DERIVATIVES USED.

Designation	Mating type	Relevant genotype	Phenotype
AB1157	F⁻		Leu Arg His Pro Thr Thi Lac Ara Gal Mtl Xyl Str^r T6^r λ^s
AB2463	F⁻	*recA13*	Leu Arg His Pro Thr Thi Lac Ara Gal Mtl Xyl Str^r T6^r λ^s
AB2470	F⁻	*recB21*	Leu Arg His Pro Thr Thi Lac Ara Gal Mtl Xyl Str^r T6^r λ^s
AB2480	F⁻	*recA13 uvrA6*	Pro Gal Str^r λ^s
AB2487	F⁻	*recA13*	Leu Arg His Pro Thr Thy Thi Lac Ara Gal Mtl Xyl Str^r T6^r λ^s
AB2497	F⁻		Leu Arg His Pro Thr Thy Thi Lac Ara Gal Mtl Xyl Str^r T6^r λ^s
AB2498	F⁻	*uvrC34*	Leu Arg His Pro Thr Thy Thi Lac Ara Gal Mtl Xyl Str^r T6^r λ^s
AB2499	F⁻	*uvrB5*	Leu Arg His Pro Thr Thy Thi Lac Ara Gal Mtl Xyl Str^r T6^r λ^s
AB2500	F⁻	*uvrA6*	Leu Arg His Pro Thr Thy Thi Lac Ara Gal Mtl Xyl Str^r T6^r λ^s
JC5088	Hfr	*rec-56*	Thr Ilu Thi Spm^r
SR57	F⁻	*rec-56 uvrC34*	His Thr Leu Thi Arg Pro Lac Ara Gal Mtl Xyl T6^r λ^s Str^r
SR58	F⁻	*rec-56 uvrB5*	His Thr Leu Thi Arg Pro Lac Ara Gal Mtl Xyl T6^r λ^s Str^r
SR59	F⁻	*rec-56 uvrA6*	His Thr Leu Thi Arg Pro Lac Ara Gal Mtl Xyl T6^r λ^s Str^r

Abbreviations: Each genetic locus is designated by three lower case italicized letters (Demerec et al., 1966; Taylor and Trotter, 1967). All other symbols refer to phenotype. Arg, His, Ilu, Leu, Met, Pro, Thi, Thr, Thy, Trp, denote arginine, histidine, isoleucine and valine, leucine, methionine, proline, thiamine, threonine, thymine and tryptophan, respectively, (⁻ indicates a requirement; ⁺, no requirement); Ara, Gal, Lac, Mal, Mtl, and Xyl denote arabinose, galactose, lactose, maltose, mannitol and xylose, respectively, (⁻ indicates nonutilization; ⁺, utilization); T6, λ, Spm, Str, response to the phages T6 and λ, and to the antibiotics spectinomycin and streptomycin (^{r} indicates resistance; ^{s}, sensitivity) *rec* denotes genes affecting genetic recombination and UV sensitivity; *uvr* designates genes affecting host-cell reactivation and UV sensitivity.

donor, and by reversion. Twenty-three Rec⁺ recombinants from matings, 23 Rec⁺ transductants and 4 Rec⁺ revertants were tested for LHR. None of them showed LHR. All of the 17 Rec⁻ clones tested as controls during these experiments showed LHR.

Effect of uvr *mutations on LHR.* The three *uvr* genes of *E. coli* K-12, *uvrA*, *uvrB*, and *uvrC*, control the excision of pyrimidine dimers from cellular DNA. Mutants which are *uvr*⁻ are more sensitive to UV than the wild-type K-12 and are defective in the capacity to reactivate irradiated phage (Boyce and Howard-Flanders, 1964; Howard-Flanders et al., 1966).

The *uvr* mutants examined, containing *uvrA6*, *uvrB5* and *uvrC34*, respectively, did not show LHR (Ganesan and Smith, 1968). In addition, strains containing a *uvr* mutation together with a *rec* marker permitting expression of LHR did not show recovery in buffer. Forty of these were tested: AB2480 which contains the markers *recA13* and *uvrA6*, and 39 recombinants constructed by mating a *rec-56* Hfr with three different *uvr*⁻ strains. Seven of the recombinants contained *uvrA6* and 21 carried *uvrB5*. None of them showed any LHR. The 11 *uvrC34* recombinants examined showed some LHR, but much less than their *rec-56* parent. Table 2 illustrates the recovery of representative *uvr*⁻ *rec-56* recombinants compared to their parents.

In control experiments, fifteen Uvr⁺ Rec⁻ recombinants obtained by mating the *rec-56* Hfr to a *uvr*⁺ recipient all showed LHR. Forty-two Uvr⁺ Rec⁻ progeny obtained by mating two different *uvr*⁻ *rec-56* recombinants to *uvr*⁺ *rec*⁺ Hfr strains also all showed LHR.

Two Uvr⁻ Rec⁻ strains were tested for photoreactivation. Both showed about a tenfold recovery under our conditions before and after a 4-hr post-irradiation holding period in buffer. This indicated that detectable levels of photoreactivable lesions were present in the irradiated cells and were not repaired during the four hour period in buffer. If LHR reflects the repair of such lesions (Castellani et al., 1964; Jagger et al., 1964) the failure to observe it in the Uvr⁻ Rec⁻ cells is consistent with

TABLE 2. RECOVERY IN REPRESENTATIVE *rec-56 uvr*⁻ RECOMBINANTS COMPARED TO THEIR PARENTS

Strain	Markers affecting UV sensitivity: *rec*	*uvr*	UV dose (ergs/mm²)	Surviving fraction without recovery	Recovery: LHR (8 hrs)	MMR
JC5088	*rec-56*	+	90	2.8×10^{-4}	258.1	225.8
AB2500	+	*uvrA6*	270	1.9×10^{-3}	1.0	3.8
SR59	*rec-56*	*uvrA6*	2.5	8.7×10^{-4}	1.2	0.9
AB2499	+	*uvrB5*	135	1.4×10^{-3}	0.6	261.9
SR58	*rec-56*	*uvrB5*	2.5	9.6×10^{-4}	0.8	0.9
AB2498	+	*uvrC34*	315	4.0×10^{-4}	1.0	47.4
SR57	*rec-56*	*uvrC34*	4	1.6×10^{-4}	5.1	8.6
AB2497	+	+	2160	1.1×10^{-2}	0.6	0.3

TABLE 3. MMR IN DERIVATIVES OF *E. coli* K-12 LACKING LHR

Strain	Markers affecting UV sensitivity *rec*	*uvr*	UV dose (ergs/mm²)	Surviving fraction without recovery (× 10³)	LHR (8 hr)	MMR
AB1157	+	+	2160	3.5	0.6	0.9
AB2470	*recB21*	+	540	7.0	2.1	20.7
AB2498	+	*uvrC34*	405	0.01	1.1	27.8
AB2499	+	*uvrB5*	135	1.4	0.6	261.9

the idea that they lack the capacity for this type of repair (Harm, 1966).

Evidence that LHR is not identical to MMR. All of the *rec* mutants tested which showed LHR also showed MMR (Ganesan and Smith, 1968). In addition, the *rec-56 uvrA6* and *rec-56 uvrB5* recombinants failed to show either LHR or MMR (Table 2). In the *rec-56 uvrC34* recombinants LHR and MMR were both reduced compared to the *rec-56 uvr*$^+$ parent strain (Table 2). These results suggested that LHR and MMR might reflect the same dark repair process. However, Witkin (1968) reported that B_{s-1}, a derivative of *E. coli* B which shows very little LHR (Castellani et al., 1964; Harm, 1966), shows significantly higher survival after UV if plated on minimal medium than if plated on complex medium. We have found the same behavior in three K-12 derivatives. One of these contains a *rec* mutation; the others, *uvr* markers (Table 3). The existence of such mutants implies that MMR and LHR are not identical.

PHYSIOLOGICAL CHARACTERISTICS

Effect of yeast extract. The measurement of LHR and MMR under our conditions requires that the irradiated cells be able to recover in buffer or in minimal medium, and that their recovery be inhibited by plating on yeast extract agar. Recovery completed before the first plating or continued after plating will not be detected as recovery, but as increased resistance to UV.

Inhibition by yeast extract appears to be due, not to the physical aspects of plating, but to some component of the yeast extract. Our yeast extract plates contain 7.5 mg/ml of Difco Bacto-yeast extract. Adding this concentration of yeast extract to cells undergoing recovery in buffer prevented further recovery. A dialysate of yeast extract was also active in this context. Figure 1 illustrates the response of irradiated cells of a *recA13* strain, AB2487, to a dialysate obtained from 0.75 mg/ml of yeast extract. The same dialysate incorporated into minimal agar medium prevented MMR in the *recB21* strain, AB2470.

Other complex media, including nutrient broth, peptone and Difco Antibiotic Medium No. 3, also inhibited recovery.

If cells of AB2487 that had been in contact with yeast extract for only a short time were removed to buffer without yeast extract, the inhibition could be reversed. With increased exposure to yeast extract up to one hour, the inhibition became progressively less reversible (Fig. 2).

Figure 3 illustrates the response of cells of AB2487 to yeast extract and chloramphenicol. These cells had been deprived of amino acids for two hours before irradiation. The addition of

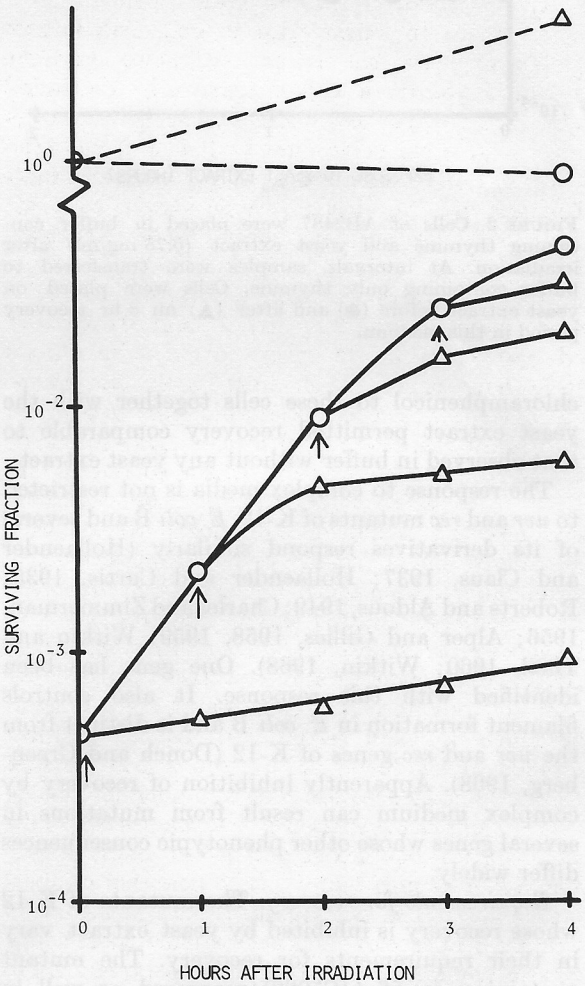

FIGURE 1. Inhibition of LHR in AB2487 by a dialysate of yeast extract (0.75 mg/ml). Cells were held in buffer (○) after irradiation. The dialysate (△) was added at the times indicated by arrows. Solid lines indicate irradiated cells; dotted lines, unirradiated controls.

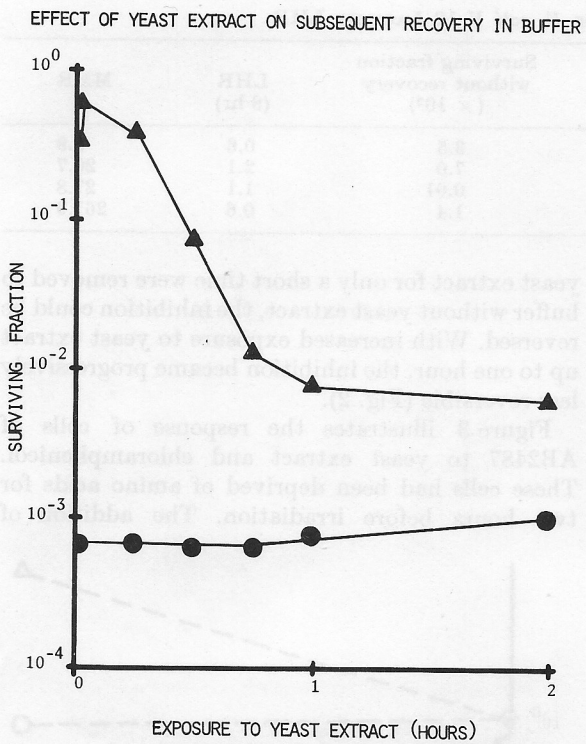

FIGURE 2. Cells of AB2487 were placed in buffer containing thymine and yeast extract (0.75 mg/ml) after irradiation. At intervals, samples were transferred to buffer containing only thymine. Cells were plated on yeast extract before (●) and after (▲) an 8 hr recovery period in this medium.

chloramphenicol to these cells together with the yeast extract permitted recovery comparable to that observed in buffer without any yeast extract.

The response to complex media is not restricted to *uvr* and *rec* mutants of K-12. *E. coli* B and several of its derivatives respond similarly (Hollaender and Claus, 1937; Hollaender and Curtis, 1935; Roberts and Aldous, 1949; Charles and Zimmerman, 1956; Alper and Gillies, 1958, 1959; Witkin and Theil, 1960; Witkin, 1968). One gene has been identified with this response. It also controls filament formation in *E. coli* B and is distinct from the *uvr* and *rec* genes of K-12 (Donch and Greenberg, 1968). Apparently inhibition of recovery by complex medium can result from mutations in several genes whose other phenotypic consequences differ widely.

Requirements for recovery. The mutants of K-12 whose recovery is inhibited by yeast extract vary in their requirements for recovery. The mutant containing *rec-56* (JC5088) recovered as well in buffer as on minimal medium (Table 2). The strains carrying *recA13*, however, recovered better on minimal medium than in buffer (Ganesan and Smith, 1968). The *recB21* mutant AB2470 showed MMR, but did not recover in buffer (Table 3). Both the *recA13* and *recB21* derivatives recovered as well in minimal medium lacking amino acids as on minimal agar containing those required for growth. The *uvrB5* mutant, AB2499, did not recover either in buffer or in minimal medium lacking amino acids. It appeared to require amino acids for recovery as well as for growth. Since *uvrB5* has been shown to inactivate the excision of pyrimidine dimers from DNA (Howard-Flanders et al., 1966), this type of MMR should be independent of the excision process.

Release of acid soluble material from the DNA of irradiated cells. Both *recA1* and *recA13* strains have been shown to release abnormally large amounts of their DNA as acid soluble material (Clark et al., 1966; Howard-Flanders and Theriot, 1966). Not only is the spontaneous rate of release higher than in *rec*$^+$ cells, but it is increased by UV irradiation. It has been suggested that, unlike the excision of pyrimidine dimers, this process reflects DNA degradation rather than repair (Clark et al., 1966; Howard-Flanders and Theriot, 1966).

Both *recA1* and *recA13* recover in buffer and in minimal medium. We felt that their recovery might depend upon preventing DNA degradation

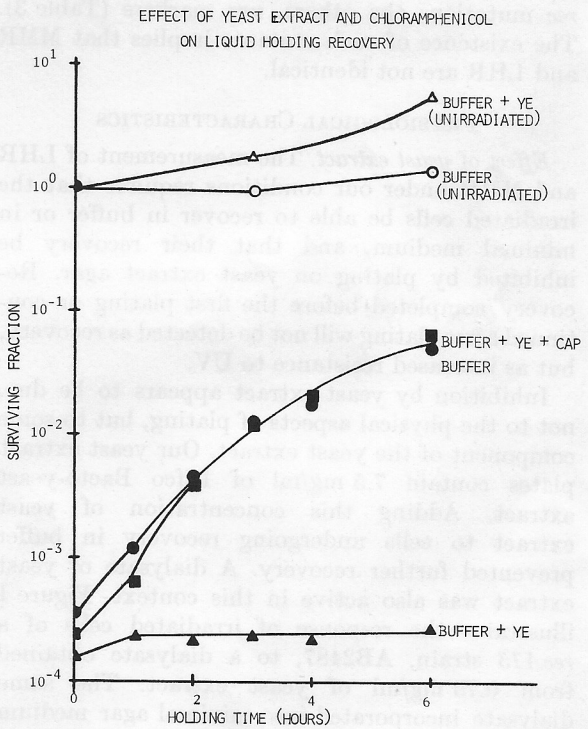

FIGURE 3. Cells of AB2487 were deprived of amino acids for 2 hr before irradiation. They were held in buffer (●), buffer containing 7.5 mg/ml yeast extract (▲) or buffer containing yeast extract and 40 μg/ml chloramphenicol (■) after irradiation. Unirradiated controls were held in buffer (○) or buffer containing yeast extract (△).

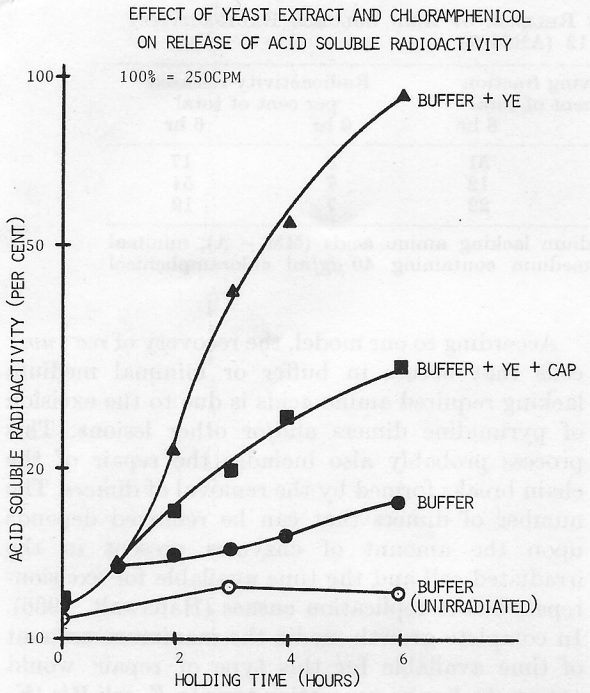

FIGURE 4. The release of acid soluble radioactivity was determined for the same cell suspensions whose recovery is shown in Fig. 3.

after irradiation. If this were true, one would expect to find very little recovery under conditions favoring the release of acid soluble material from DNA. To test this prediction, we measured the release of radioactivity from the DNA of a *recA13* strain (AB2487) labeled by growth with thymine-2-^{14}C before irradiation. Release of ^{14}C as cold acid soluble material was measured at various times after irradiation and compared with the amount of recovery shown by the same cultures.

We found some striking qualitative correlations between increases in the amount of radioactivity released and reductions in recovery. However, there were also some quantitative discrepancies, as illustrated by the following examples.

A comparison of Figs. 3 and 4 shows that cells deprived of one or more required amino acids before irradiation, and held in buffer after irradiation, released little ^{14}C and recovered efficiently. Adding yeast extract to the buffer increased the rate of ^{14}C released and reduced the amount of recovery. The further addition of chloramphenicol (40 μg/ml) restored recovery to its original level, and reduced the amount of radioactivity released. These results agree qualitatively with the idea that a high rate of release is incompatible with good recovery. However, chloramphenicol in the presence of yeast extract completely restored recovery, but did not reduce the release of ^{14}C to its original rate. The amount of recovery was thus greater than would have been predicted from the rate of ^{14}C released.

The response of cells held in minimal growth medium after irradiation was similar. In agreement with Howard-Flanders and Theriot (1966) we found that cells of AB2487 irradiated in minimal medium without amino acids released a large proportion of their incorporated radioactivity after a dose of 60 ergs/mm^2. However, if they had been incubated in medium without amino acids for two hours before irradiation, less ^{14}C was released and recovery improved (Table 4). The addition of required amino acids to these cells after irradiation resulted in the release of virtually all of the ^{14}C. Although this treatment reduced the amount of recovery observed, it did not completely inhibit it. It appeared that these conditions might provide a situation in which the proportion of radioactivity released exceeded the proportion of cells which did not recover. However, when the dose of UV was adjusted to 30 ergs/mm^2 to permit 10% or more of the cells to survive after recovery, the amount of ^{14}C released was diminished to an amount easily accounted for by the dead cells in the population (Table 5). Thus our data did not rule out the hypothesis that the release of ^{14}C reflects DNA degradation, and in fact were in qualitative agreement with it. The largest amounts of release were observed under conditions permitting growth and DNA replication of the unirradiated controls, i.e., in the presence of yeast extract and in minimal growth medium.

TABLE 4. EFFECT OF PRE-IRRADIATION MEDIUM ON RELEASE OF ACID SOLUBLE RADIOACTIVITY from *E. coli* K-12 (AB8248)

Pre-irradiation medium	Post irradiation medium	UV dose (ergs/mm^2)	Surviving fraction per cent of total 0 hr	Surviving fraction per cent of total 6 hr	Radioactivity released per cent of total 0 hr	Radioactivity released per cent of total 6 hr
MM + A	MM + A	60	0.02	0.63	16	100
MM + A	MM − A	60	0.02	0.48	16	85
MM − A	MM − A	60	0.02	33.	12	37

Cell suspensions were irradiated immediately after removal from minimal growth medium (MM + A) or after 2 hours incubation in minimal medium lacking amino acids (MM − A). After irradiation they were held in minimal growth medium (MM + A) or in minimal medium lacking amino acids (MM − A).

TABLE 5. EFFECT OF REQUIRED AMINO ACIDS ON RELEASE OF ACID SOLUBLE RADIOACTIVITY FROM *E. coli* K-12 (AB2487)

Postirradiation medium	UV dose (ergs/mm²)	Surviving fraction per cent of total		Radioactivity released per cent of total	
		0 hr	6 hr	0 hr	6 hr
MM − A	30	0.38	51	8	17
MM + A	30	0.37	12	7	51
MM + A + CAP	30	0.50	22	7	19

Cells were held after irradiation in minimal medium lacking amino acids (MM − A), minimal growth medium (MM + A) or minimal growth medium containing 40 μg/ml chloramphenicol (MM + A + CAP).

DISCUSSION

Our data can be explained by the following model: the *rec* and *uvr* genes of *E. coli* K-12 mediate two dark repair processes which differ with respect to the type of lesion they repair. The *uvr* genes mediate the excision-repair of damaged bases in DNA while the *rec* genes mediate the repair of gaps left in the daughter strand when DNA synthesis proceeds past damaged bases.

Irradiation of cells with UV causes the production of pyrimidine dimers in the DNA of the cells (Wacker, Dellweg, and Weinblum, 1960). The dimers *per se* are not necessarily lethal. Replication along a template containing dimers, however, results in gaps in the daughter DNA strand (Howard-Flanders et al., this volume; Rupp and Howard-Flanders, 1968). Replication, or attempted replication, of these gaps should produce incomplete strands (double chain breaks) and result in reproductive death.

The *uvr* mutations prevent the efficient removal of pyrimidine dimers from the DNA of irradiated cells. This results in an increased sensitivity to UV, but not to X-irradiation which does not produce dimers (Boyce and Howard-Flanders, 1964; Howard-Flanders et al., 1966).

The *rec* mutations, at least *recA* and *recB* mutations, reduce the efficiency with which gaps in cellular DNA are healed. This results in greater sensitivity to X-irradiation (Howard-Flanders and Theriot, 1966) which produces both single and double strand breaks in DNA (Freifelder, 1965; Kaplan, 1966; McGrath and Williams, 1966). The *rec* mutations also result in a decreased capacity for recombination requiring joining of the recipient chromosomes to incoming DNA fragments (Oppenheim and Riley, 1966). It does not affect the cellular incorporation of DNA fragments which do not require insertion into bacterial chromosomes (Clark and Margulies, 1965).

Both *rec*$^+$ and *uvr*$^+$ alleles are required for the wild-type level of UV resistance. Inactivation of either gene increases the UV sensitivity of the cells presumably by reducing the total number of lesions per cell that can be repaired.

According to our model, the recovery of *rec*$^-$ *uvr*$^+$ cells that occurs in buffer or minimal medium lacking required amino acids is due to the excision of pyrimidine dimers and/or other lesions. This process probably also includes the repair of the chain breaks formed by the removal of dimers. The number of dimers that can be removed depends upon the amount of enzymes present in the irradiated cell and the time available for excision-repair before replication ensues (Hanawalt, 1966). In complete growth media the maximum amount of time available for this type of repair would appear to be one generation time in *E. coli* B/r (K. C. Smith 1968, manuscript in preparation). If a cell receives a dose of UV sufficient to produce more dimers than it can excise and repair in this allotted time, under conditions conducive to replication, it will then make DNA containing gaps by synthesizing past dimers. If one assumes that these gaps are not efficiently repaired in *rec*$^-$ cells, the probability would be high that the progeny of such cells would not be viable. The higher resistance to UV of the *recB* mutant compared to the *recA* mutants suggests that the "Rec$^+$ mode" of repair is more completely blocked by *recA* mutations. The observation that a *recA13 recB21* recombinant has the UV sensitivity characteristic of *recA13* (N. Willets, pers. commun.) supports the idea that *recA* and *recB* mutations affect the same repair process.

Recovery of the *uvrB* and *uvrC* mutants in minimal growth medium may reflect repair determined by *rec*$^+$ genes of gaps produced by DNA synthesis past dimers and/or other lesions. In these cells dimers are not removed and the newly synthesized daughter strands of DNA are found to contain gaps which are slowly repaired (Rupp and Howard-Flanders, 1968). If unrepaired gaps are lethal, we may infer from our viability studies that growth in complex medium reduces the number of gaps that can be repaired efficiently, either by directly inhibiting the repair process or by enhancing the rate of replication relative to repair (Alper and Gillies, 1960; Hanawalt, 1966; Jagger et al., 1964).

In the *rec*$^-$ *uvr*$^-$ strains, both the excision-repair and the gap-repairing processes have been

damaged. Placing irradiated cells in buffer or in minimal medium should, therefore, not affect the survival of cells which completely lack the capacity for repair. The *rec-56 uvrA6*, *rec-56 uvrB5* and *recA13 uvrA6* strains appear to be of this type. The slight amount of recovery observed in the *rec-56 uvrC34* recombinants (Table 2) suggests that the *uvrC34* mutation does not completely inactivate excision-repair.

According to our model, the survival of UV irradiated cells is a function of two systems both of which help prevent cells from succumbing to the effects of pyrimidine dimers and/or other lesions. The system controlled by *uvr* genes acts directly upon damaged bases and removes them from the irradiated DNA. This process can occur in buffer. The system controlled by *rec* genes does not act directly upon damaged bases. Instead this process facilitates repair of gaps formed when lesion-containing DNA is replicated. It is postulated that this latter mode of repair cannot occur until replication has resulted in the production of gaps (opposite the damaged bases), and therefore cannot take place while the irradiated cells are held in buffer or minimal medium lacking required amino acids.

It is hoped that this model can be tested directly by measuring the size of newly synthesized DNA in a $rec^- uvr^-$ mutant as a function of time after UV irradiation. This model predicts that the short pieces of newly synthesized DNA would not be joined together in a $rec^- uvr^-$ strain as they are in a $rec^+ uvr^-$ strain (Rupp and Howard-Flanders, 1968).

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Note Added in Proof:

Horii and Suzuki [Photochem. Photobiol. *8:* 93 (1968)] have also found that amino acid starvation prevents release of acid soluble radioactivity from the DNA of a *recA1* mutant.

Howard-Flanders [Ann. Rev. Biochem. *37:* 175 (1968)] has reported that W. D. Rupp and R. B. Setlow have obtained data supporting the idea that *uvrC* mutations do not completely inactivate the excision process.

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