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Dark Recovery Processes in *Escherichia coli* Irradiated with Ultraviolet Light

I. Effect of *rec*⁻ Mutations on Liquid Holding Recovery[1]

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We have examined various derivatives of *Escherichia coli* K-12 for liquid holding recovery, a type of recovery originally observed in *E. coli* B irradiated with ultraviolet light. Although most of the K-12 derivatives tested showed relatively little or no recovery under our conditions, four of the six independent *rec*⁻ mutants examined, those carrying *recA1*, *rec-12*, *recA13*, and *rec-56*, respectively, displayed marked recovery. These mutants are distinguished from *rec*⁺ strains by their increased sensitivity to ultraviolet radiation and decreased ability to undergo genetic recombination. Two of them have also been reported to release large amounts of their deoxyribonucleic acid as acid-soluble material, especially after irradiation. None of the three *uvr*⁻ mutants examined, containing *uvrA6*, *uvrB5*, or *uvrC34*, showed comparable liquid holding recovery. The one *rec*⁻ *uvr*⁻ derivative tested, carrying *recA13* and *uvrA6*, did not appear to undergo liquid holding recovery, although *recA13 uvr*⁺ strains did. Genetic analysis of one strain, a *recA13* mutant, indicated that all the *rec*⁺ derivatives obtained from it by conjugation, transduction and reversion, had lost the property of showing liquid holding recovery. From these results, we conclude that in *E. coli* K-12 the expression of liquid holding recovery depends upon certain *rec*⁻ mutations.

When cells of *Escherichia coli* B irradiated with ultraviolet light (UV) are held in the dark in buffer or saline, a gradual increase occurs in the number of cells able to form colonies on a complex medium (9, 17, 18, 29). This response has been called "liquid holding recovery" or LHR by subsequent authors (8, 15, 22, 23).

The experiments to be described were designed to obtain genetic data which would elucidate the relationship of LHR to other recovery processes in *E. coli*. Since *E. coli* K-12 is more amenable to genetic analysis than *E. coli* B, we examined various derivatives of K-12 for LHR. Among those tested were several UV-sensitive mutants carrying *rec* or *uvr* mutations. In addition to UV sensitivity, the *uvr* genes control host-cell reactivation of UV-irradiated phage and excision of pyrimidine dimers (7), and the *rec* genes affect genetic recombination (10, 12). Three of the derivatives tested contained *recA* mutations and had previously been characterized as "reckless" because they release large proportions of their deoxyribonucleic acid (DNA) as acid-soluble material (10, 31). This release is enhanced by UV irradiation (11, 21). Two of the derivatives carried *recB* and *recC* mutations (N. Willetts and D. Mount, *personal communication*). These derivatives were of the "cautious" variety; i.e., they did not release abnormally large amounts of DNA even after irradiation (14, 19).

Five of the K-12 derivatives tested displayed significant amounts of LHR. All were *rec*⁻ strains, representing four different *rec* mutations. Conjugation, transduction, and reversion studies were performed on one of these mutants to determine whether the *rec*⁻ allele might be necessary for the expression of this type of recovery.

The K-12 derivatives were also tested for survival on minimal agar medium after UV irradiation. *E. coli* B shows better survival on minimal agar than on complex medium under these conditions (3, 29). It has been suggested that the higher survival on minimal medium and LHR both result from inhibiting or delaying the growth of irradiated cells until repair of UV-induced damage has occurred (2, 3, 23). However, Witkin (32) indicated that B_{s-1}, a derivative of *E. coli* B in which very little LHR

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can be demonstrated (8, 15), showed higher survival levels on minimal medium than on complex medium. We examined the K-12 derivatives to see whether the two types of recovery, LHR and recovery on minimal medium, could be dissociated in any of them.

MATERIALS AND METHODS

Bacterial strains. The derivatives of *E. coli* used are listed in Table 1. We are grateful to E. Lederberg and J. Lederberg for W2252, W3110, and W4099; to R. P. Boyce for AB2480, AB2487, AB2497, AB2498, AB2499, and AB2500; to A. J. Clark for AB2470, JC5410, and KL-16; to John Folls for JC5088; and to Antonio Siccardi for AB1157, AB2462, AB2463, JC1557, and JC1569.

The origins and directions of chromosomal transfer of the Hfr strains used are given in Fig. 1.

Bacterial cultures were routinely incubated at 37 C.

Media. The following minimal growth media were used: a liquid containing 4×10^{-2} M K_2HPO_4, 1.5×10^{-2} M KH_2PO_4, 4.1×10^{-4} M $MgSO_4$, 7.6×10^{-3} M $(NH_4)_2SO_4$, 1.4×10^{-3} M sodium citrate, 3.4×10^{-5} M $CaCl_2$, 9.0×10^{-7} M $FeSO_4$, and 0.4% glucose (24); and a solid medium containing 4×10^{-2} M K_2HPO_4, 1.5×10^{-2} M KH_2PO_4, 8.3×10^{-4} M $MgSO_4$, 7.6×10^{-3} M $(NH_4)_2SO_4$, 0.4% glucose, and 0.9% Agar-agar No. 3 (Oxo, Ltd.).

L-Amino acids were used at a concentration of 10^{-4} M, thymine at 10 μg/ml, thiamine at 0.5 μg/ml, and dihydrostreptomycin sulfate at 200 μg/ml.

Complex media included: Penassay broth (Difco Antibiotic Medium 3); yeast extract-agar (0.75% Difco yeast extract, 2.3% Difco Nutrient Agar); and L broth and L broth agar (26).

A sodium-potassium phosphate buffer, *p*H 7.0, 0.067 M, was employed (8). For experiments on thymine-requiring strains this was supplemented with 10 μg of thymine per ml.

Bacterial mating. Overnight cultures of the parental strains in Penassay broth were diluted 1:50 in fresh broth. After 2 hr of growth in a shaking water bath, 0.5 ml of the Hfr culture was added to 5 ml of the F^-, and the mixture maintained at 37 C for 2 hr longer. Samples of the mating mixture were then spread on plates of selective media. For time of entry experiments (31, 33), samples were removed at intervals, agitated for 1 min on a Vortex Junior Mixer (model K500-J3, Scientific Industries, Inc., Hempstead, N. Y.), and spread on plates of selective media. To select *rec*$^+$ recombinants, samples of mating mixtures were spread on yeast extract-agar containing streptomycin, incubated for 30 min, and then irradiated with 200 ergs/mm^2 of UV.

Recombinants were purified by at least one single colony isolation on yeast extract-agar before being tested for genetic markers.

TABLE 1. *Escherichia coli derivatives used*[a]

Designation	Mating type	Genotype	Reference
E. coli B		"wild type"	
E. coli K-12	F^+	"wild type"	
W2252	Hfr_1	*lam*^{s} *met*	
W3110	F^-	*lam*^{s}	
W4099	Hfr_6	*lam*^{s}	—[b]
KL-16	Hfr	*lam*^{s}	10, 31
JC1557	F^-	*leu arg his met lac gal xyl mtl str*^{r} *lam*^{s}	11
JC1569	F^-	*recA1 leu arg his met lac gal xyl mtl str*^{r} *lam*^{s}	10, 11, 31, —[c]
JC5088	Hfr	*rec-56 thr ilv thi spm*^{r}	10
JC5410	F^-	*recC22 leu arg his pro thr trp thi lac ara gal mtl xyl str*^{r} *tsx*^{r} *lam*^{s}	14, 31, —[c,d]
AB1157	F^-	*leu arg his pro thr thi lac ara gal mtl xyl str*^{r} *tsx*^{r} *lam*^{s}	10, 21
AB2462	F^-	*rec-12 leu arg his pro thr thi lac ara gal mtl xyl str*^{r} *tsx*^{r} *lam*^{s}	10, 21
AB2463	F^-	*recA13 leu arg his pro thr thi lac ara gal mtl xyl str*^{r} *tsx*^{r} *lam*^{s}	19, 21, —[c]
AB2470	F^-	*recB21 leu arg his pro thr thi lac ara gal mtl xyl str*^{r} *tsx*^{r} *lam*^{s}	19, —[c, d]
AB2480	F^-	*recA13 uvrA6 pro gal str*^{r} *lam*^{s}	19, —[c]
AB2487	F^-	*recA13 leu arg his pro thr thy thi lac ara gal mtl xyl str*^{r} *tsx*^{r} *lam*^{s}	21, —[c]
AB2497	F^-	*leu arg his pro thr thy thi lac ara gal mtl xyl str*^{r} *tsx*^{r} *lam*^{s}	20
AB2498	F^-	*uvrC34 leu arg his pro thr thy thi lac ara gal mtl xyl str*^{r} *tsx*^{r} *lam*^{s}	20
AB2499	F^-	*uvrB5 leu arg his pro thr thy thi lac ara gal mtl xyl str*^{r} *tsx*^{r} *lam*^{s}	20
AB2500	F^-	*uvrA6 leu arg his pro thr thy thi lac ara gal mtl xyl str*^{r} *tsx*^{r} *lam*^{s}	20

[a] Abbreviations (13, 31): *arg, his, ilv, leu, met, pro, thi, thr, thy, trp,* denote genes determining biosynthesis of arginine, histidine, isoleucine and valine, leucine, methionine, proline, thiamine, threonine, thymine, and tryptophan, respectively; *ara, gal, lac, mal, mtl,* and *xyl,* utilization of arabinose, galactose, lactose, maltose, mannitol, and xylose, respectively; *ton, tsx,* and *lam,* response to the phages T1, T6 and λ, respectively; *str* and *spm,* response to streptomycin and spectinomycin, respectively; *rec* denotes genes affecting genetic recombination and UV sensitivity; *uvr* denotes genes affecting host-cell reactivation and UV sensitivity.

[b] A. A. Richter, Ph.D. Thesis, Univ. of Wisconsin, Madison, 1959.

[c] A. J. Clark, *personal communication.*

[d] A. Willetts and D. Mount, *personal communication.*

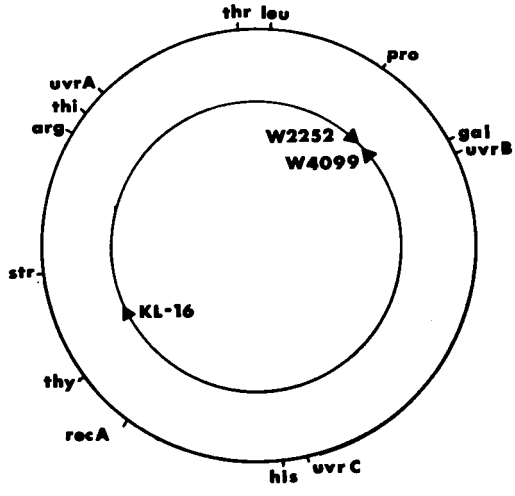

FIG. 1. *Diagram of the E. coli K-12 linkage map (10, 19, 31). Positions of various genetic markers are indicated on the outer circle. The arrowheads on the inner circle indicate the origin and direction of transfer of the Hfr strains used (10, 31; A. A. Richter, Ph.D. Thesis, University of Wisconsin, Madison, 1959).*

Reversion. After UV treatment of a *rec*⁻ strain, Rec⁺ revertants were obtained. Cultures of the *rec*⁻ mutant in Penassay broth, inoculated from single colonies on yeast extract-agar, were grown to stationary phase. Samples were then spread on yeast extract-agar and irradiated with a dose of UV (60 ergs/mm²) sufficient to leave approximately 200 colony-forming cells per plate. After incubation for 2 days, each plate was replica-plated (25) to two plates of yeast extract-agar. These were irradiated with doses of 600 or 900 ergs/mm² and then were incubated for 18 to 24 hr. Colonies which appeared UV-resistant from their response on the irradiated replicas were picked from the original plates, purified by at least two single-colony isolations, and tested for UV resistance. Only revertants obtained from different clones of the original *rec*⁻ strain were considered to be of independent origin.

Phage lysates and transduction. Phage P1*kc* was grown and titered as described by Lennox (26), with W3110 as the host.

Transduction was performed according to the procedure described by Luria, Adams, and Ting (28). For selecting Rec⁺ transductants from Rec⁻ recipients, the method devised by Hertman and Luria (16) was employed.

Phage T1 was grown and titered on W3110 as described by Adams (1).

Irradiations. Cells were irradiated at room temperature (23 C). Usually, 10 ml of cells suspended in buffer was irradiated in 10-cm petri dishes on a rotary shaker. In some cases, smaller volumes were irradiated in smaller containers, or bacteria were inoculated onto the surface of agar plates and then irradiated.

Two filtered short-wave UV lamps from Ultra-Violet Products, Inc., San Gabriel, Calif., were used: a model C-81 at a distance of 26 cm from the surface of the shaker platform for doses above 60 ergs/mm²; and a Mineralite model SL 2537 at a distance of 72 cm for lower doses. At these distances, the dose rate of the former lamp was 540 ergs per mm² per min, and of the latter 30 ergs per mm² per min as measured by the photodecomposition of uranyl oxalate (6). The output of both lamps was primarily at 253.7 nm.

All operations were performed in yellow light from General Electric "Bug Lites" or "Gold" fluorescent lights to prevent photoreactivation.

LHR: quantitative method. Cells were harvested by centrifugation at room temperature, washed, and resuspended in buffer at densities between 10^5 and 10^8 cells/ml. For thymine-requiring strains, buffer containing thymine was used. The cell suspensions were incubated for 2 hr at 37 C prior to irradiation. They were irradiated in buffer and held in buffer at 37 C during the recovery period. Recovery was measured by spreading samples of the cell suspensions on yeast extract plates at intervals throughout the recovery period, and counting the number of colonies formed after 24 to 48 hr of incubation.

The results are expressed either as the surviving fraction $N_r(t)/N_0(t = 0)$, or as the ratio of the number of survivors after recovery to the number before recovery, $N_r(t)/N_r(t = 0)$, where N_r is the number of colony-forming units per milliliter of cell suspension in the irradiated population, N_0 is the number in an unirradiated control, and (t) is the time (usually in hours) after exposure of the irradiated population to UV.

LHR: qualitative method. For cultures to be tested for the presence or absence of LHR, rather than for the amount of recovery, a more economical procedure was devised. Cells grown overnight in Penassay broth were harvested by centrifugation, washed, and resuspended in buffer to a density of approximately 10^8 cells per ml. After a 2-hr period at 37 C, 50-μliter amounts of each culture to be tested were transferred by means of disposable micropipettes (Drummond Microcaps) to 0.5-ml volumes of buffer contained in disposable plastic trays (diSPo trays, Scientific Products, Evanston, Ill.). These were irradiated with doses of UV sufficient to leave less than 10% of the cells able to form colonies if plated immediately on yeast extract-agar. Three serial dilutions of 50 μliters per 0.5 ml of buffer were made from each culture. Three separate 50-μliter drops from each dilution were placed on yeast extract-agar plates immediately after irradiation and again 4 hr later. The plates were scored after 24 hr of incubation by comparing the number of colonies formed in corresponding drops plated before and after the recovery period. Figure 2 demonstrates the response of a strain which shows LHR and one which does not.

Host-cell reactivation. The capacity of cells to reactivate UV-irradiated phage was tested by inoculating them onto yeast extract-agar spread with 2×10^6 T1 phage which had been previously irradiated with 630 ergs/mm² of UV (20). After 18 hr of incubation,

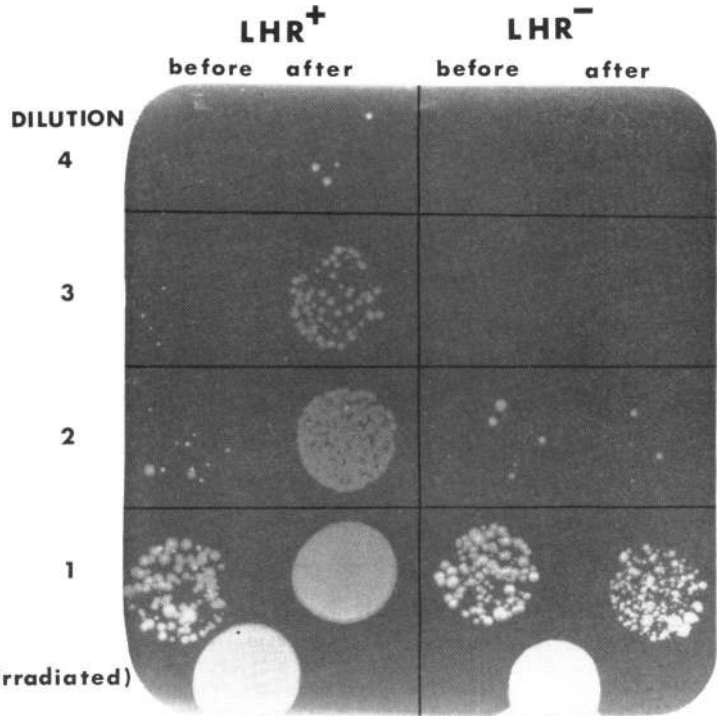

FIG. 2. *Results of a qualitative test for LHR. The left half of the plate demonstrates the response of an LHR+ strain (JC5088), the right half that of an LHR− strain (AB2499). Four dilutions of irradiated cells of each strain were plated as 50-μliter drops before and after a 4-hr recovery period in buffer. At the bottom center of each half of the plate is a drop of dilution 1 before irradiation.*

confluent growth was observed only in areas inoculated with cells which could not reactivate the phage (*uvr*⁻ or *hcr*⁻). Areas inoculated with cells which could reactivate the phage showed no growth or only a few isolated colonies.

Recombination. Strains were tested for the ability to undergo genetic recombination by inoculating them together with a suitable donor strain (usually W2252, *see* Fig. 1) on a medium selective for recombinants (usually Pro⁺ Str^r) (12).

RESULTS

Control experiments. Of the various conditions used to test for LHR, the following gave the most satisfactory results for *E. coli* B (see Materials and Methods). Cultures were grown in minimal medium, since such cultures generally showed better recovery than those grown in Penassay broth. Cells were harvested during exponential growth, washed, and resuspended in buffer. Before being irradiated, they were incubated for 2 hr to allow them to complete any residual divisions of which they might be capable (30). This treatment also increased the amount of recovery observed after irradiation (Table 2). Maximal viability was reached after about 8 hr of recovery (Fig. 3), a slightly longer period than other workers have reported (8, 15, 23). The amount of recovery obtained under these conditions varied from experiment to experiment, but was usually of the order of a 10-fold increase in survivors (Fig. 3). As reported by Roberts and Aldous (29) and Jagger et al. (23), the amount of recovery did not appear to depend upon the density of the cell suspensions. LHR was clearly detectable at survival levels between

TABLE 2. *Effect on LHR of incubating E. coli B in buffer for 2 hr before irradiation*

Time of recovery	Surviving fraction[a] $[N_r(t = 0)/N_0(t = 0)]$	
	Without 2-hr period	With 2-hr period
hr		
0	3.4×10^{-3}	5.6×10^{-4}
4	1.4×10^{-3}	4.6×10^{-3}
8	1.8×10^{-4}	6.0×10^{-3}

[a] The legend to Fig. 3 defines the terms used in this expression.

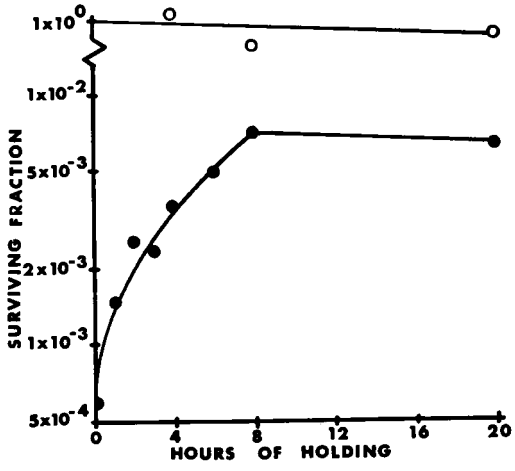

FIG. 3. *LHR in E. coli B:* (●), *survival of irradiated cells* $[N_r(t)/N_0(t = 0)]$; (○), *survival of unirradiated controls* $[N_0(t)/N_0(t = 0)]$. N_r *is the number of colony-forming units per ml of cell suspension in the irradiated population,* N_0 *is the number in an unirradiated control, and t is the time (usually in hours) after exposure of the irradiated population to UV.*

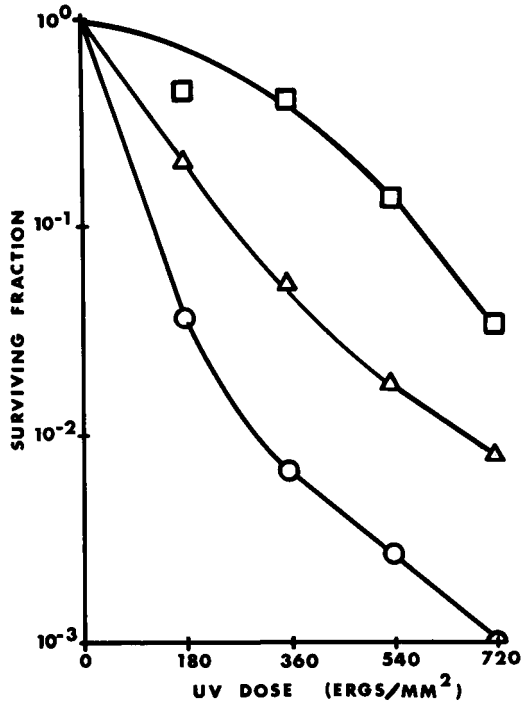

FIG. 4. *LHR in E. coli B after different doses of UV. Cells were plated after no recovery* (○), *4 hr of recovery* (△), *or 8 hr of recovery* (□).

5×10^{-2} and 10^{-4}, although the amount of recovery varied somewhat with survival level (Fig. 4). Recovery could be inhibited by the addition of 0.075% Difco yeast extract to the buffer in which the irradiated cells were held (Fig. 5).

LHR in derivatives of E. coli K-12. Various derivatives of *E. coli* K-12 were examined for their ability to undergo LHR in conditions found to be satisfactory for *E. coli* B. The strains were cultured in minimal liquid medium containing supplements required for their growth. Thymine was added to the buffer used in all of these experiments to preclude any effects arising from thymine deprivation of thymine-requiring strains, even though it was not anticipated that thymineless death would occur (5). Doses of UV were chosen to leave less than 5% of the original population capable of forming colonies on yeast extract-agar when plated immediately after irradiation. Platings were made on yeast extract-agar immediately after irradiation (t = 0 hr), and again after 4 and 8 hr of recovery in buffer. Platings were also made on minimal agar medium at t = 0 hr.

Five of the strains tested showed LHR equivalent to a 10-fold or greater increase in survivors over an 8-hr recovery period (Table 3). All of them contained *rec*⁻ markers. Four different mutations were represented, *recA1*, *rec-12*, *recA13*, and *rec-56*. None of the 10 *rec*⁺ strains tested showed as much LHR, and some showed none at all. Two *rec*⁻ derivatives, carrying *recB21* or *recC22*, showed no significant LHR. The one *rec*⁻ *uvr*⁻ strain tested, AB2480, did not appear to undergo LHR, although the corresponding *rec*⁻ *uvr*⁺ strains, AB2463 and AB2487, did.

The amount of LHR observed in the *rec*⁻ strain, AB2487, depended upon the milieu in which the cells were incubated for the 2 hr immediately preceding irradiation. Recovery was enhanced if, instead of being incubated in buffer, they were incubated in minimal medium lacking amino acids required for growth (Table 4). We do not yet know whether this is a general property of *rec*⁻ LHR⁺ strains. This procedure did not increase the amount of LHR obtained in related *rec*⁺ and *uvr*⁻ strains (Table 4).

All of the strains which showed LHR gave higher survival levels on minimal agar than on yeast extract-agar (Table 3). The *uvrB* and *uvrC* mutants tested and the *rec*⁻ mutant AB2470 also survived better on minimal agar, although they did not display significant amounts of LHR.

Relationship between rec⁻ *and LHR.* The observation that certain *rec*⁻ mutants showed LHR while closely related *rec*⁺ strains did not suggested that the expression of this type of recovery might depend upon the *rec* mutations.

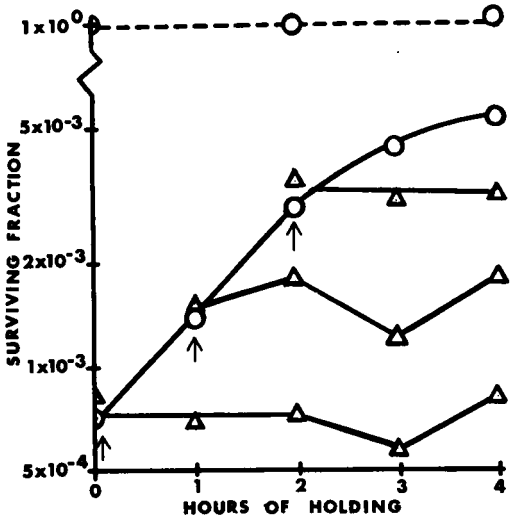

FIG. 5. *Inhibition of LHR in E. coli B by yeast extract. Cells were held in buffer* (○). *Yeast extract* (△), *0.075% (final concentration), was added at the times indicated by the arrows. Solid lines indicate unirradiated cells* $[N_r(t)/N_0(t = 0)]$; *dotted lines, the unirradiated controls* $[N_0(t)/N_0(t = 0)]$. *(The legend to Fig. 3 defines the terms in these expressions.) Unirradiated cells held in buffer containing yeast extract (not shown) exhibited a 30-fold increase in colony-orming units over the 4-hr holding period.*

To test this idea, one strain, AB2487, carrying *recA13* (10, 19, 21) was chosen, and Rec$^+$ derivatives were obtained from it by conjugation, by transduction, and by reversion. These were then examined for LHR.

In the first series of experiments, AB2487 was mated with *rec*$^+$ Hfr strains. The locus of *recA13* has been reported to lie between *his* and *thy* (10, 31). Results of time of entry experiments which we performed with AB2487 as the recipient were consistent with this location.

Two Hfr strains, W4099 and KL-16, with different points of origin and directions of chromosome transfer (Fig. 1), were used to determine the effect on LHR of introducing the *rec*$^+$ allele by conjugation. Neither Hfr showed significant amounts of LHR (Table 3). Recombinants were selected for His$^+$ Str^r and tested for various nutritional markers, UV sensitivity, recombination, and LHR.

The first Hfr used was W4099. Forty His$^+$ Str^r recombinants were selected from the mating of W4099 with AB2487. All forty retained the *thr*$^-$ *leu*$^-$ *thi*$^-$ *pro*$^-$ *arg*$^-$ markers of the recipient parent, AB2487. Of the forty, 11 had obtained the *thy*$^+$ marker of the donor, W4099, and 25 of the 40 recombinants were UV-resistant, like the donor. From the group of 40, 10 UV-sensitive and 10 UV-resistant isolates were chosen to be tested for recombination and LHR. All of the UV-resistant isolates were found to be *rec*$^+$ and to have lost the LHR property, whereas all the UV-sensitive isolates were *rec*$^-$ and LHR$^+$.

The second Hfr used was KL-16. Of the 40 His$^+$ Str^r recombinants isolated from matings of this Hfr with AB2487, all retained the *thr*$^-$, *leu*$^-$, *pro*$^-$, *arg*$^-$, *thi*$^-$, and *thy*$^-$ markers of the recipient. The low frequency of *thy*$^+$ recombinants in these experiments may be due to the transfer of *thy*$^+$ very shortly after the beginning of conjugation (27). Thirty-one of the recombinants had become UV-resistant. Thirteen of these resistant isolates were tested, and all proved to be *rec*$^+$ and phenotypically LHR$^-$. The seven sensitive recombinants examined were still *rec*$^-$ and retained the LHR$^+$ property.

In the next series of experiments phage P1*kc*, grown on W3110, was used to infect AB2487. Twenty-three UV-resistant transductants were isolated and tested for recombination and LHR. All of them were *rec*$^+$ and LHR$^-$.

Among the colonies obtained after exposing the phage-infected cells to UV were some which were UV-sensitive (16). Seven of these were isolated and examined as controls. All had retained the *rec*$^-$ and LHR$^+$ characteristics of AB2487.

In addition to recombinants and transductants, four independent Rec$^+$ revertants were obtained after exposing AB2487 to UV. These had all lost the LHR$^+$ property.

DISCUSSION

The results of our experiments suggest that the expression of LHR in *E. coli* K-12 depends upon certain *rec* mutations, including *recA1*, *rec-12*, *recA13*, and *rec-56*. The derivatives of K-12 examined which did not contain one of these mutations showed little or no LHR.

To observe LHR by our procedure, two conditions must be met: (i) the irradiated cells must be able to repair, or at least to initiate repair of, UV-induced damage in buffer, without exogenous carbon, nitrogen, or sulfur sources; and (ii) the repair process must be inhibited by plating the cells on yeast extract-agar. The amount of recovery measured depends upon the degree to which these two conditions are met.

The UV resistance of wild-type K-12, compared to its *rec*$^-$ derivatives, suggests that damage incurred from exposure to UV is efficiently repaired in these cells. Conversely, the sensitivity of the *rec*$^-$ mutants implies that they contain some alteration which results in a decreased efficiency of repair. This alteration is correlated

TABLE 3. *LHR and survival on minimal medium in various derivatives of E. coli K-12*

Strain	Markers affecting UV sensitivity		UV dose (ergs/mm²)	Surviving fraction without recovery[a] $[N_r(t=0)/N_0(t=0)]$	Recovery[a] $[N_r(t)/N_r(t=0)]$		Relative survival on minimal medium[b]
	rec	*uvr*			4 hr	8 hr	
E. coli B	+	+	540	7.3×10^{-4}	5.8	11.4	5.0
K-12	+	+	1,350	8.6×10^{-3}	0.8	0.2	5.6[c]
W3110	+	+	2,160	6.4×10^{-5}	1.1	0.6	0.1
KL-16	+	+	2,160	4.0×10^{-3}	1.5	2.3	Not tested
W4099	+	+	2,160	4.7×10^{-4}	<0.1	<0.1	1.2
JC1557	+	+	2,160	5.4×10^{-3}	0.3	0.2	1.3
JC1569	*recA1*	+	75	2.1×10^{-3}	169.5	223.7	239.8
JC5088	*rec-56*	+	90	2.4×10^{-3}	13.9	29.8	34.7
JC5410	*recC22*	+	810	1.2×10^{-2}	1.8	1.9	2.8
AB1157	+	+	2,160	3.5×10^{-3}	0.7	0.6	0.9
AB2462	*rec-12*	+	120	1.3×10^{-3}	11.7	27.5	70.8
AB2463	*recA13*	+	90	2.3×10^{-5}	11.8	28.7	681.5
AB2470	*recB21*	+	540	7.0×10^{-3}	1.9	2.1	20.7
AB2480	*recA13*	*uvrA6*	3	4.2×10^{-4}	0.9	0.9	1.2
AB2487	*recA13*	+	45	7.8×10^{-4}	11.6	47.2	102.6
AB2497	+	+	2,160	9.2×10^{-3}	0.9	0.8	0.6
AB2498	+	*uvrC34*	270	9.5×10^{-4}	3.0	2.8	36.6
AB2499	+	*uvrB5*	90	6.7×10^{-3}	0.6	0.5	242.6
AB2500	+	*uvrA6*	270	1.9×10^{-3}	1.1	1.0	3.8

[a] The legend to Fig. 3 defines the terms in this expression.

[b] Relative survival on minimal medium is expressed as the ratio between survivors on minimal medium and survivors on yeast extract-agar at $t = 0$.

[c] Since wild-type K-12 is lysogenic for λ, its survival on minimal medium may reflect a lower efficiency of prophage induction.

TABLE 4. *Effect on LHR of incubation in minimal medium without amino acids for 2 hr immediately preceding UV irradiation*

Strain	Markers affecting UV sensitivity		Preirradiation treatment[a]	UV dose (ergs/mm²)	Surviving fraction without recovery $[N_r(t=0)/N_0(t=0)]$	Recovery[b] $[N_r(t)/N_0(t=0)]$	
	rec	*uvr*				4 hr	8 hr
AB2487	*recA13*	+	Buffer	45	9.6×10^{-3}	13.8	33.1
			MM	45	2.4×10^{-3}	177.5	305.8
AB2497	+	+	Buffer	2,160	9.2×10^{-3}	0.9	0.8
			MM	2,160	3.2×10^{-3}	1.5	1.2
AB2498	+	*uvrC34*	Buffer	270	2.5×10^{-3}	1.2	1.1
			MM	270	3.7×10^{-3}	2.0	2.2
AB2480	*recA13*	*uvrA6*	Buffer	3	3.0×10^{-4}	1.0	0.9
			MM	3	4.1×10^{-4}	1.2	1.4

[a] Cultures growing in minimal liquid medium were transferred to minimal medium from which amino acids had been omitted (MM), or to buffer containing thymine (Buffer), and were incubated for 2 hr at 37 C. They were then washed and resuspended in buffer containing thymine for irradiation and recovery.

[b] The legend to Fig. 3 defines the terms in this expression.

not only with greater sensitivity to UV but also, in certain mutants, with the appearance of LHR. Thus, LHR may be the expression of an inefficient repair process which occurs slowly in buffer, over a period of several hours, and which can be inhibited by plating irradiated cells on yeast extract-agar. Relatively little LHR is seen in wild-type *E. coli* K-12, perhaps because in these cells, even though recovery occurs, condition (ii) is not met, and LHR cannot be detected under our conditions.

We have found that the recovery of *rec*⁻ mutants of *E. coli* K-12 in buffer, like that of *E. coli* B, can be inhibited by the addition of in-

gredients of complex media to the buffer. This suggests that the inhibition depends not on the mechanical aspects of transferring the irradiated cells to an agar medium but rather on some component of complex media. This component has not yet been identified, but may be the same as that present in the dialyzable material from peptone which was reported by Alper and Gillies (2, 4) to reduce the survival of UV-irradiated *E. coli* B. Minimal agar medium, which lacks this component, should permit LHR to continue. Accordingly, the survival of irradiated cells of an LHR$^+$ strain should be higher on minimal agar than on yeast extract-agar. The behavior of *E. coli* B (3, 29) and of the LHR$^+$ *rec*$^-$ derivatives of K-12 (Table 3) is consistent with this expectation.

Two *uvr*$^-$ mutants (AB2498 and AB2499) and one of the LHR$^-$ *rec*$^-$ derivatives (AB2470) of K-12 also showed better survival on minimal agar than on yeast extract-agar (Table 3). This observation implies that they are able to recover on minimal agar, and that this recovery, like that of the LHR$^+$ *rec*$^-$ mutants, is inhibited by components of complex medium. Unlike the LHR$^+$ *rec*$^-$ mutants, however, the LHR$^-$ *rec*$^-$ and the *uvr*$^-$ cells lack the capacity for recovery in buffer (LHR).

Although our data suggest that certain *rec* mutations permit the expression of LHR in *E. coli* K-12, they do not indicate whether this is a direct effect or an indirect one. If the effect were direct, the *rec* genes might specify or regulate enzymes involved in LHR. According to this hypothesis, certain mutations at the *rec* loci might result in a decrease in the efficiency or in the amount of the enzymes necessary for LHR. Recovery might then require a longer period of time for completion or become more sensitive to inhibition by components of complex media. It would thus become observable under the conditions used to measure LHR. If the effect of the *rec* genes were indirect, they might determine enzymes necessary to a repair process different from and more efficient than LHR. According to this idea, LHR could not be observed in the presence of the more efficient system, and detection of LHR would depend upon the inactivation of the other system by *rec*$^-$ mutations.

If the *rec* genes do not directly determine the enzymes involved in LHR, it should be possible to locate other genes which do. There is some evidence that the *uvr* genes might perform this function. Harm (15) has proposed that genes controlling host-cell reactivation of phage also determine LHR. The *uvr* genes control host-cell reactivation in *E. coli* K-12. If LHR depends upon this system, *uvr*$^-$ mutants should be unable to undergo LHR. To test this hypothesis, it will be necessary to determine the effect of *uvr* mutations on strains which manifest LHR, such as those containing suitable *rec* mutations. One such strain, carrying the *recA13* and *uvrA6* markers, has been tested (Table 3). It did not appear to undergo recovery. However, it would be premature to conclude from the results of a single strain that the failure to detect LHR in this case was due to the *uvr* mutation. Further studies designed to determine whether *uvr* genes affect LHR are in progress.

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