R Factors Improving Survival of *Escherichia coli* K-12 After Ultraviolet Irradiation

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Two R factors which have the capacity to improve survival of some strains of *Escherichia coli* K-12 by approximately 60% after ultraviolet light have been identified and characterized. Both are *fi*<sup>+</sup>- but neither produce colicins. The ability to enhance survival can be separated from all other identified R-factor functions. Improved survival does not result from improved excisional capacity, but does require an intact host capacity for genetic recombination. No effects on host cell growth or postirradiation lag were observed. A proposed mechanism of action is described.

Resistance factors (R factors) are episomes capable of conferring on host enterobacteria resistance to a variety of antibacterial drugs, bacteriophages, colicins (Col), and salts of heavy metals (22). Investigations by others have suggested for these elements molecular weights of between 25 × 10<sup>4</sup> (16) and 65 × 10<sup>4</sup> (14) daltons, which represents sufficient deoxyribonucleic acid (DNA) for several dozen genes. To date, approximately 15 of these genes have been identified; they have in common the property of conferring resistance to potentially harmful agents which might have been present in the bacterium’s environment. We have, therefore, examined the possibility that R factors may affect bacterial resistance to yet another environmental hazard, ultraviolet (UV) light. Such an effect has been briefly reported to occur in *Salmonella typhimurium* (6). Our data extend the observations to *Escherichia coli* and provide evidence concerning a possible mechanism by which the effect is mediated.

The effects of UV light on bacteria (reviewed by J. K. Setlow, 18) are principally the result of damage to DNA, most of which results from condensation (or dimerization) of certain adjacent nucleotides. Dimer formation reduces the available sites for interstrand hydrogen bonding, and thereby causes distortion of the usual α-helical configuration of the DNA. One consequence of UV damage is that replication of a new copy of the chromosome for a daughter cell is halted in some strains or significantly slowed in others.

With a given dose of radiation, all members of a particular bacterial strain incur equal damage (21), that is, equal numbers of dimer photoproducts. That substrains show differences in survival implies the presence of repair systems or the ability to bypass the damage produced. Through the use of UV-sensitive and resistant strains (10), specific steps in the repair of UV lesions have been identified. These mechanisms involve (i) photoreactivation, the process by which light in the visible spectrum activates the photoproduct, thus allowing enzymatic monomerization of the involved bases in situ with subsequent restoration of strand integrity, and (ii) a light-independent (dark-repair) process.

Operationally, the steps in the dark-repair process can be designated as: (i) recognition of damage, (ii) chain incision, (iii) excision of the damaged area forming a single-strand gap, (iv) repair replication, with the normal strand opposite the gap serving as template, and (v) ligation of the apposed free ends. Two principal groups of mutants of *E. coli* K-12 which show deficiencies in this pathway have been identified. The Uvr<sup>-</sup> mutants are deficient in steps (i) or (ii), or both; further, as they fail to incise the chain, they are phenotypically unable to excise and release oligonucleotides containing photoproducts, as do normal cells. Genetically, Uvr<sup>-</sup> strains have mutations at one of three distinct chromosomal loci (*uvrA*, *uvrB*, and *uvrC*), but have virtually identical phenotypes (11). Another group of mutants, designated Rec<sup>-</sup> because of their inability to form stable recombinants with newly introduced chromosomal genetic material (12), are deficient in one or more of the last three steps, and show quantitative aberrations of photoproduct release (10).

A comparison of UV dosage, photoproduct
yields, and survival curves allows certain conclusions regarding the repair pathway outlined above. If every step in repair is completely dependent upon the step preceding it, then an interruption at any point in the chain will render the pathway completely ineffective. Thus, at a similar UV dose, survival of a strain deficient in any one step would be equal to that of a strain deficient in any other single step, and a combination of these deficiencies as, for example, in a double mutant, would lead to no additional impairment of survival.

Available observations suggest that such a situation does not persist. Lowest survival rates are observed in the (Uvr− Rec−) double mutant (10) in which an average of approximately 1.3 photoproducts per genome are lethal. The fact that single mutants of either type (Uvr− Rec+ or Uvr+ Rec−) show significantly higher survival than the double mutant indicates that they are able to repair or "bypass" between 20 and 60 photoproducts per genome (10). This further suggests that the sequential nature of the dark-repair pathway is not absolute, and that other capabilities are available.

Recently, evidence has been provided (17) that such a mechanism may take the form of post-replication sister-strand exchange in the Uvr− (incision-deficient) strains. This model proposes that the DNA polymerase copies the entire chromosome, except the photoproducts, with the result that the newly formed strand contains gaps. For a viable (uninterrupted) chromosome to result, sister strands align after replication, after which reciprocal crossovers take place between a new and an old strand of similar polarity. By such a mechanism, photoproducts and gaps may be confined to two of the four (two new and two old) strands, yielding one complete strand of each polarity for a daughter cell. This new cell is thus capable of colony formation, whereas the damaged strands are segregated into a nonviable daughter cell which is lost.

With this background, we have investigated the question of whether R factors are capable of increasing host cell survival following UV irradiation, and if so, what mechanism may be involved. We have identified a specific R-factor effect which increases survival without affecting photoproduct excision. The most plausible explanation of the effect appears to be through enhancement of sister-strand exchange.

**MATERIALS AND METHODS**

**Bacterial strains.** R factors were obtained from bacteria isolated in the clinical bacteriological laboratory of the Children's Hospital Medical Center, Boston, Mass. They were transferred by conjugation into strains of *E. coli* K-12 kindly supplied by Paul Howard-Flanders. Some were altered by spontaneous mutation or by transduction with phage Plkc (Table 1).

**Media.** Tryptic Digest Broth (Difco) and Trypticase Soy Broth (BBL) were the enriched media used. Minimal medium was made by the addition of glucose (0.2%) and thiamine (0.1 μg/ml) to the "A medium" of Davis and Mingioli (5), which had been modified by the omission of citrate. This minimal medium was further enriched by the addition of 0.2% Casamino Acids (Difco), yielding "supplemented minimal medium," or by the addition of specific amino acids to a concentration of 20 μg/ml, except histidine (10 μg/ml), yielding "specifically supplemented minimal medium." In experiments using thymine-requiring (Thy+) strains, media were further supplemented with 20 μg/ml thymidine (Nutritional Biochemicals, Chagrin Falls, Ohio). Buffer used in these experiments consisted of minimal medium with (NH₄)₂SO₄ omitted. Medium for the λ-phage experiments was made using 10 g of Tryptone (Difco), 5 g of NaCl, and 50 μg of thiamine in 500 ml of water.

For the identification of drug resistance were made by the addition of antibiotics to autoclaved Levine lactose eosin-methylene blue (EMB) agar (BBL) to result in the following concentrations per ml: streptomycin (Sm), 1,000 μg; ampicillin (Amp), 100 μg; tetracycline (Tc), 20 μg; spectinomycin (Spc), 100 μg; chloramphenicol (Cm), 50 μg; and nalidixic acid (Nal), 100 μg. Resistance to mercuric chloride (Hg) was identified by growth at 24 hr on tryptic digest agar containing 10⁻⁴ M HgCl₂. Highest reliability for identification of sulfadiazine (Su) resistance was with the use of supplemented A medium containing sulfadiazine (900 μg/ml).

Viable cell counts were determined by using buffer dilutions and by surface-spreading of samples onto nutrient agar. To determine growth rates, cells grown in A medium with specific amino acid supplements were diluted repeatedly to keep them in exponential growth. Turbidimetric measurements (23) were made after the cells had been in logarithmic growth for at least 15 to 20 generations.

**Bacterial conjugations.** The following technique was used for selection of conjugants. A donor cell carrying the R factor under study was characterized for R-mediated drug resistances. The cell which was to serve as recipient for the R factor was chosen (or suitably modified) so as to differ from the donor in certain attributes (resistance to NaI or high levels of Sm, or metabolic characteristics) known to be determined by chromosomal, and not episomal, genes. Media were then constructed to allow outgrowth only of organisms which possessed two attributes: the chromosomal characteristic specific for the recipient, and a drug resistance for the R factor.

To obtain conjugants, overnight stationary-phase, broth cultures of both the R+ strain and a suitable recipient were diluted 1:10 with broth and mixed in a ratio of 1:10 (donor:recipient). After 4 hr at 37°C, the mixed suspension was spread onto plates which would allow outgrowth only of cells with chromosomal characteristics of the recipient as well.
as a drug resistance found on the R factor. The resulting clones were streaked to isolated colonies at least twice more before being used for further study. The selective plates used for various matings were as follows.

Wild type bacteria crossed with AB1884 (uvrC): EMB plates containing Sm + Tc were used when R factors mediated Tc resistance, and Sm + Amp were used for R factors lacking Tc but carrying Amp resistances.

AB1884 crossed with AB1353 (F'-lac): Recombinants were selected as Lac+ clones on EMB plates containing 1,000 μg of Sm per ml. 

E. coli 1 crossed with AB1884: One clinical isolate, E. coli 1, had a high rate of spontaneous mutation to high-level Sm resistance (15), making this antibiotic unreliable for selection against the donor. Therefore, AB1884A, a Nal-Sm doubly resistant mutant, was used as the recipient. Recombinants were selected on EMB plates containing Sm, Nal, and either Tc, Spc, or Amp.

Of the clones isolated from this mating, several were found to exhibit increased UV resistance. These also were characterized by Amp resistance and restriction of λ-phage. In contrast, some clones resistant to Amp or restrictive for λ were found which had no UV effect. We wished to use, for further study, an R factor as nearly specific for UV resistance as possible. Therefore, clone #290, which mediated the fewest (Amp, Su, λ, UV) resistances (Table 2), was selected.

AB1884/R crossed with AB1885-6 (uvrB, Rec+), AB1886-6 (uvrA, Rec+), AB1157 (uvr+, Rec+), and AB2464 (Uvr+, recA): Conjunctural transfer of certain R factors into other hosts was performed with the indicated strains as recipients (Table 1) and AB1884/R as donor. Selection of recombinants was performed by plating washed cells from the mating mixtures onto specifically supplemented medium containing either Tc (2.5 μg/ml) or Amp (100 μg/ml). 

Isolation of segregants. Elimination of the R factor from R+ host cells to obtain R- segregants was achieved by the method of Tomoeda et al. (24). Overnight cultures in broth with 10% sodium dodecyl sulfate (SDS; Matheson Coleman and Bell, Cincinnati, Ohio) were diluted, plated on nonselective plates, and replicated to Tc and Amp plates to permit identification of any sensitive clones.

Colicin production. Colicin production was assessed by the method of Fredericq (7). AB1884, AB1884/RE13, and AB1884A/RE1-290 were stabbed into nonselective plates, and after 6 hr of incubation at 37°C, an overnight culture of E. coli B was diluted 10-5 into melted, soft agar and overlaid on the original plate. After 12 to 18 hr, clear zones characteristically surround colicin-producing strains. Those showing no zones were classified Col-

**UV survival studies.** A low pressure, 15-w, General Electric, germicidal lamp was used for all experiments. Dose intensities were monitored with a model #IL254 Germicidal Photometer (International
TABLE 2. Selected-characteristics of effective R factors and AB1884 (mutC)*

<table>
<thead>
<tr>
<th>Clone</th>
<th>Amp</th>
<th>Tc</th>
<th>Spc</th>
<th>Su</th>
<th>Hg</th>
<th>Apparent λ titer</th>
<th>UV survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB1884/RE13</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2 × 10^4</td>
<td>1 × 10^{-3}</td>
</tr>
<tr>
<td>AB1884/X.RE1-290</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>1.3 × 10^4</td>
<td>2.9 × 10^{-3}</td>
</tr>
<tr>
<td>AB1884</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.6 × 10^4</td>
<td>1.3 × 10^{-2}</td>
</tr>
</tbody>
</table>

* Characteristics were determined as described in Materials and Methods.

** Titters for λ were performed using similar amounts of the same phage stock to infect the different hosts. The differences in plaque number yield differences in "apparent titer." The difference between titer in the R⁻ host and in the R⁺ hosts is interpreted as being due to restriction of the phage by the R⁺ hosts.

† Survival following 80 ergs/mm².

Light Co., Newburyport, Mass.), calibrated at 254 nm.

Initial surveys of R-factor effects were performed using cells grown overnight in a small quantity of enriched medium. These suspensions were diluted in buffer, and a 0.1-mI amount was spread in duplicate on two pairs of nutrient agar plates. One set of plates was exposed to 80 ergs/mm² and then both sets were immediately incubated in the dark at 37 C. Colonies which resulted from the survivors were counted at 18 to 24 hr; the surviving fraction (N/N₀) was then calculated. Variation between experiments using the same strain rarely exceeded a factor of three, whereas effective R factors usually increased UV resistance 10-fold. Therefore, "enhancement of survival" was considered to be present if the R factor increased host-strain survival by fivefold or more.

Extreme reduction of visible light was employed in early experiments, but it was subsequently found that identical results were obtained with more modest precautions. Therefore, exposure to ambient artificial light was minimized but not eliminated.

Although it was found that 80 ergs/mm² was a convenient dose when studying AB1884, this dose was not appropriate for all strains studied. Consequently, the UV dose was adjusted to yield a survival of approximately the same magnitude (10⁻³ to 10⁻⁴) in each host cell. This was approximately 700 ergs/mm² when the wild-type strain was used, and 27 ergs/mm² for experiments involving the Rec⁻ strain.

More precise survival data was obtained for strains harboring one of the two R factors which increased survival after UV irradiation. For this purpose, cells grown as before were diluted in buffer, and 10 ml of various dilutions in a 90-mm petri dish were exposed to UV light and stirred with magnetic stirring. After the initial exposure, samples for viable counts were spread onto groups of six plates, and each dish was reexposed for an appropriate incremental dose.

Survival curve parameters were determined as follows. Slopes were determined by eye fit of the linear portion of curves. D₀ was estimated as the increment in dose which produced 37% (1/e) decrement of survival on this linear slope. Dₙ₀ was the actual observed dose yielding 37% survival; the extrapolation number (n) was the value of the point at which the linear slope intersected the vertical axis at dose 0.

Host cell reactivation of irradiated lambda phage. Lambda-phage stock (supplied by J. Beckwith) was diluted to 2.7 × 10⁷ plaque-forming units per ml of buffer; 10-ml samples were irradiated with constant stirring for periods of up to 120 sec (320 ergs/mm²). Dilutions of these irradiated samples were made in 0.01% gelatin. A 0.1-ml amount of this diluted sample was mixed with 0.1 ml of a 10⁻⁴ dilution of an overnight culture of the test bacteria which had been grown and then diluted in peptone broth. After a 15-min period for attachment, 3 ml of soft, peptone-agar was added and the mixture was overlaid on peptone plates. Plaques were counted at 18 hr.

Mutagenesis. Exponentially growing cells were sedimented and suspended in citrate buffer (0.05 M, pH 6.0) and exposed to 100 µg of N-methyl-N'-nitro-N-nitrosoguanidine per ml (NTG; Aldrich Chemical Co., Milwaukee, Wis.) for 15 min, after which a sample was inoculated into broth and incubated overnight (1). The treated culture was then diluted and plated on nonselective plates; after 24 hr, colonies were replicated to drug-containing plates to allow selection of colonies sensitive to Amp but resistant to Tc.

Respreading experiments. Since our survival experiments measured the ability of irradiated cells to form colonies on solid media, it was essential to measure any possible growth delay under these same conditions. The respreading method of Witkin (25) was utilized for this purpose. The question to be answered was the following. When does a cell, planted on a plate and irradiated, recover sufficiently to divide and produce viable daughter cells? Under usual circumstances, the colony count does not change when this division occurs because the original cell and its progeny lie together. An increase in cell population on a plate will be appreciated only if an originally planted cell can be separated from its daughter, thus causing separate colonies to form. Consequently, if one spreads each of a group of plates with the same number of bacteria, disturbing the cells at intervals so that newly formed pairs will be separated, one can estimate by the increase in colony count the time at which division first begins to yield two viable progeny.

To evaluate this question, we diluted overnight broth cultures, plated samples on nutrient agar plates, and exposed all plates to 20 ergs/mm². All were incubated; at intervals, subgroups of plates were moistened with additional buffer and respread with a glass spreader.

Measurement of repair capability. Assessment of
the ability of the R factor to influence dark repair in the Uvr" cell was made utilizing Thy" mutants and a method similar to that of Boyce and Howard-Flam- 

ders (3). Cells were incubated for several generations of 
exponential growth in supplemented A medium 

containing 0.5 μg of thymidine/ml. At a cell density of 

approximately 10^9/ml, 20 μc of H\(^3\)-thymidine 

(New England Nuclear Corp., Boston, Mass., 1 

mc/ml) was added per ml of culture. After 2 hr of 

further incubation (approximately 3 to 3.5 genera-

tions), the bacteria were sedimented, washed with 

buffer, and resuspended with A medium identical to 

the growth medium, but lacking Casamino Acids and 

radioactive thymidine. After 90 min of incubation in 

this medium, cell suspensions were adjusted to an 

optical density corresponding to a cell density of 

about 2 × 10^9 cells/ml and divided into two approxi-

mately equal volumes. One, transferred directly to a 

dark red flask (Ray-Sorb, Kimble Glass Co., Toledo, 

Ohio), constituted the unirradiated controls. The 

other was exposed to 1,000 ergs/mm\(^2\) in an open 

petri dish while being stirred continuously. Imme-

diately after irradiation, this culture was transferred 

to a similar dark flask, and all flasks were incubated 

at 37°C.

Immediately after transfer of the irradiated cells to 

a red flask, the time 0 samples were taken for viable 

counts and isotopic studies. For the latter, samples 

were pipetted into an equal volume of iced 10% tri-

chloroacetic acid (Fisher Scientific Co., Fair Lawn, 

N.J.), and after at least 30 min were centrifuged at 

0 to 5°C. A sample of the supernatant fluid was re-

moved and filtered through a membrane filter (0.45 

nm, pore size; Millipore Corp., Bedford, Mass.). 

The filtrates were collected; a 0.1-ml amount was 

transferred to scintillation vials and then counted in 

10 ml of Bray's solution (4) in a Packard Tri-Carb 

counter, model #3375 (Packard Instrument Co., 

Inc., Downers Grove, Ill.). All samples were counted 

to a standard error of 1% or less.

The trichloroacetic acid precipitate was collected, 

washed with 5 ml of iced 5% trichloroacetic acid, 

dried, and hydrolyzed in sealed tubes at 165°C with 

0.5 ml of trifluoroacetic acid (Eastman Organic Chem-

icals, Rochester, N.Y.). The hydrolysates were dried, 

suspended with 0.05 ml of 0.1 N HCl, and chromato-

graphed (3). The developed chromatograms were cut 

into strips and counted in a Packard model #7201 

Radiochromatogram scanner. Areas of interest, 

including known dimer, nondimer, and thymine 

areas, were cut out, transferred to scintillation vials 

containing toluene scintillant (23), and counted as 

above. Background for photoproduct counts was 

determined by averaging a paper area of an equal 

size adjacent to, but not including, the photoproducts.

This average was subtracted from the counts in the 

photoproduct areas; the difference was regarded as 

counts directly attributable to photoproducts. Thymi-

necounts, calculated as the total counts within the 

peak indicated by the strip counter, were counted in 

the scintillation counter. The sum of these counts 

was used as denominator in calculation of the relative 

amount of photoproduct present.

RESULTS

The results of these studies fall into four major 
groups: (i) identification of R factors which are 
capable of enhancing host survival after UV ir-

radiation, and investigation of whether these ef-

fects are independent of, or modified by, host 

characteristics; (ii) evidence indicating that the 
effects are specific for certain R factors, and are 

not due to nonspecific effects on the host cell; 

(iii) characteristics of those R factors which 
demonstrate enhancement of UV resistance; (iv) 

measurement of the excision repair capacity of 
cells with and without the R factor.

Identification of R factors mediating UV resistance 

and the importance of host phenotypes for this 
effect. Initial surveys for R factors capable of 

enhancing postirradiation survival was done with 
an excision-deficient host, AB1884 (uvrC), for two 

reasons. First, R factors might enhance resistance 

only in cells with specific defects. This might be 

expected if R-factor genes were originally chro-

mosomal; also, their function might be identifiable 

only in hosts lacking that specific locus they carry. 

Furthermore, they might affect the three Uvr" 

loci differently. Second, if such specificity were 

found, isolation of an active product, such as an 

enzyme, would be facilitated if the host itself 

were incapable of producing this substance. A 

representative screening experiment involving 

AB1884 alone and with R factors is shown in 

Fig. 1. In experiments of this type, 50 R factors 

were evaluated, and 48 had no effect on the sur-

vival of AB1884. However, two (RE13 and 

RE1-290) were found significantly to enhance the 

survival (Fig. 2). The types of bacteria which 

originally harbored the R factors were screened are 

shown in Table 3. Both effective R factors were

Fig. 1. Survival of E. coli uvrC with and without R factors after UV irradiation.
originally found in E. coli. Among 14 R factors derived from other species, none were identified which increased survival.

These two R factors were then transferred into hosts deficient in the other Uvr loci; Fig. 3 shows that RE1-290 and RE13 affect the UV resistance of AB1885-6 (uvrB) and AB1886-6 (uvrA), as they affected the resistance of AB1884. These experiments established two characteristics. First, the R factors which mediate the enhanced UV resistance are transferable, thus confirming that the elements under study remained autonomous. Second, UV resistance is consistently enhanced in all three Uvr\(^{-}\) hosts.

The identity of the R-factor effect in all three Uvr\(^{-}\) strains suggested that the effect might be nonspecific and that similar results might be found with other related hosts. Consequently, RE13 was transferred into wild-type and Rec\(^{-}\) members of the same strain. The effect of RE13 in the wild type (AB1157) was identical to that in Uvr\(^{-}\) (Fig. 4). In contrast to all previous results, however, the R factor was ineffective in Rec\(^{-}\) (AB-2464, Fig. 5). Furthermore, subsequent experiments revealed that the effect observed in the Uvr\(^{-}\) host was not seen in a Uvr\(^{-}\) Rec\(^{-}\) double mutant.

Data from these experiments (Table 4) confirmed that the R-factor effect in the wild-type and Uvr\(^{-}\) host was identical, whereas the presence of the Rec mutation rendered the R factor ineffective. Enhancement of survival was, therefore, dependent upon normal function at the recA locus.

Specificity of R factor effects. The specificity of the effects we have observed was confirmed by several additional considerations: (i) requirement for the continued presence of the R factor in the host cell, (ii) lack of effect on UV-irradiated bacteriophage, (iii) ineffectiveness of other epi-

<table>
<thead>
<tr>
<th>Original host</th>
<th>No. examined</th>
<th>No. enhancing UV resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>36</td>
<td>2</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Citrobacter</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Salmonella</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Shigella</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

**FIG. 2. Survival of E. coli uvrC with and without RE13 and RE1-290 after UV irradiation.**

**FIG. 3. Survival of E. coli uvrA and uvrB with and without RE13 and RE1-290 after UV irradiation.**

**FIG. 4. Detailed survival of E. coli uvrC and wild type with and without RE13 after UV irradiation.** The rise of R\(^{+}\) wild type to survival values > 1 is an artifact, and the value of 1 is within confidence limits. The slight decline of slope of R\(^{+}\) wild type was neglected in favor of fitting to a straight line.
somes. (iv) absence of nonspecific R-factor effects, and (v) failure to show dependence of the UV effect on other known R-factor genes.

Loss of the R factor from the host was associated with complete loss of UV resistance. AB 1884/RE13 “cured” of the R factor by the SDS method showed reversion to their original UV-sensitivity pattern in conjunction with loss of drug resistance determinants. The R-factor effects were not, therefore, mediated through any permanent effects, as, for example, in the host genome.

As an indication of whether R-factor effects were confined to the host bacterium, the effect of RE13 and REI-290 on host cell reactivation was investigated. Certain bacteriophages lack a mechanism of their own to repair UV damage; they utilize, for this purpose, the repair mechanism of the host cell. Hosts capable of performing this repair are said to show host cell reactivation (Hcr+). Uvr- cells are known also to be Hcr- (18).

The experiment was complicated by the fact that strains containing RE13 and REI-290 were approximately 10 times more resistant to λ-phage infection than were their R+ parents. Nonetheless, relative effects could be determined by plotting surviving fractions. Superimposed curves were obtained for R- and R+ hosts (Fig. 6). Furthermore, segregants of the RE13 which lost their capacity to restrict λ-phage, but retained their capacity to enhance UV survival (see below), showed identical results as well. This final test indicated that restriction was not obscuring a low

![Diagram of survival curves](image)

**Fig. 5.** Survival of E. coli recA with and without RE13 after UV irradiation. The tail in both curves is interpreted (P. Howard-Flanders, personal communication) as representing a minority population of cells which, because of delay in onset of postirradiation division, show greater UV resistance than the majority population.

**Fig. 6.** Effect of RE13 and REI-290 on ability of E. coli uvrC to reactivate irradiated phage.

### Table 4. Attributes of survival curves

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pertinent genotype</th>
<th>n</th>
<th>Do</th>
<th>Dn</th>
<th>D₀R- / D₀R+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1157-6</td>
<td>uvr+ rec+</td>
<td>37</td>
<td>80</td>
<td>370</td>
<td>0.64</td>
</tr>
<tr>
<td>1157-6/RE13</td>
<td>uvr+ rec+</td>
<td>12</td>
<td>125</td>
<td>420</td>
<td></td>
</tr>
<tr>
<td>1884</td>
<td>uvrC rec+</td>
<td>1</td>
<td>17</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>1884/RE13</td>
<td>uvrC rec+</td>
<td>1</td>
<td>30</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>2464</td>
<td>uvr+ recA</td>
<td>1</td>
<td>3.8</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>2464/RE13</td>
<td>uvr+ recA</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3114</td>
<td>uvrA recA</td>
<td>1</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>3114/RE13</td>
<td>uvrA recA</td>
<td>1</td>
<td>0.23</td>
<td>0.23</td>
<td>0</td>
</tr>
</tbody>
</table>

* These data were derived as described in Materials and Methods from the plots in Fig. 4 and 5 and from a similar experiment with AB3114 and AB3114/RE13.
rate of host cell reactivation. Phage survival was not, therefore, improved by the presence of UV-resistant R factors. Thus, the R factors which enhance UV resistance of bacteria do not affect host cell reactivation, indicating that the R-factor effect is limited to the DNA of the bacterial host.

It was of interest to know whether the R factors which we had found to be effective had a specific capacity, or whether their effects were nonspecific, being attributable only to the presence of an episome. The fact that enhanced UV resistance was associated with only 2 of 50 R factors examined suggested that they mediated a specific effect. Nonetheless, there remained a possibility that the two R factors in question were, perhaps, unusually large; they might, therefore, have an effect due nonspecifically to the presence of a large amount of extrachromosomal DNA. We, therefore, employed another episome, F'-lac, whose molecular weight of $55 \times 10^6$ (8) makes it approximately equal in size to the largest R factors which have been reported. Even this large increment in DNA contributed by the F'-lac was ineffective in enhancing UV survival (Fig. 7); this provided additional evidence that the R factors were acting in some specific way.

Another episome, the Col I factor, has been shown by Howarth (13) to be capable of enhancing survival of Salmonella typhimurium LT2 following UV irradiation. Although the contours of our survival curves differ from those she reported, we investigated the colicin-producing capacity of the R factors we identified. Neither produced colicin. These results, as well as those of the F'-lac experiments, confirm that the ability of certain R factors to enhance survival is due to a specific R-factor effect.

The metabolic condition of bacteria, especially following irradiation, is known to affect post-UV survival (9). If an R-factor effect on survival were mediated through some effect such as slowing of the growth rate or increased postirradiation delay, these effects might be demonstrable in an effect on doubling times or resumption of cell division after UV treatment.

We determined growth rates of R+ and R- strains under conditions which required of the bacteria maximal synthetic activities. It was felt that this would serve to exaggerate any R-factor effects which might be observed if growth were studied in enriched medium. Therefore, strains with and without effective R factors were grown with a minimal amount of required nutrients through 18 to 20 divisions of exponential growth. Turbidimetric measurements were made for 3 hr at the end of this period, at which time cell densities increased from approximately $5 \times 10^6$ to $5 \times 10^8$/ml. These data allowed calculation of the following exponential doubling times: AB1884, 49.7 min; AB1884/RE13, 49.0 min; AB1884/RE1-290, 49.1 min. The R factor had no apparent effect on host cell growth rate.

Although this indicated that the R factor had no effect on host cell growth in exponential phase, there remained the possibility that the R factor might delay resumption of growth following irradiation, thus allowing for greater repair, similar to a liquid-holding effect. To investigate this possibility, we employed the resspreading technique of Wiktin (25).

Figure 8 illustrates a representative experiment. AB1157, known to have the capability for dark repair, begins to double approximately 2 to 3 hr after UV exposure. AB1884 and AB1884/RE13
do not resume cell division until 3 to 4 hr after UV. However, there is no significant difference between these two strains themselves, and what difference does exist indicates that the R-containing cell resumes growth earlier than the uninfected host. This finding is the reverse of that expected if the R factor retarded growth resumption and indicates that the R-factor effect is not attributable to a prolongation of the postirradiation lag. It does not, therefore, act by allowing additional time for repair by an inefficient system, as is postulated to occur during liquid-holding in E. coli B.

Characteristics of resistance-enhancing R factors. Both R factors found to enhance UV resistance are R, mediate resistance to Amp, and restrict λ-phage (Table 2). To evaluate further the coincidence of Amp resistance, three other R factors which mediated Amp resistance, but which had previously yielded a negative result on the original screening for UV effects, were reexamined; none enhanced UV resistance. Previous work in this laboratory had shown that of the other R factors tested for UV resistance, three restrict λ-phage, but none of these affected UV resistance.

It was, nonetheless, of interest to examine whether an R factor which did have the UV effect required the loci for λ restriction or Amp resistance, or both, for the effect on UV resistance. Two Amp-sensitive mutant clones of AB1884/RE13 arising from a single NTG mutagenic treatment were identified and investigated for λ restriction and UV resistance. Both had lost their λ-restricting ability. The capacity to enhance UV survival of AB1884 remained unaltered (Fig. 9). Thus, it appears that the locus responsible for the R-factor-mediated enhancement of UV resistance is independent of those loci mediating λ restriction and ampicillin resistance; it also appears that the locus shows no association with any other R-factor attributes tested.

Evaluation of the photoproduct excision system. Figure 10 illustrates chromatographic findings of a representative experiment using the Thy- equivalents of AB1157, AB1886, and AB1886/RE13. When the three strains are exposed to UV light, similar amounts of photoproducts are produced in all strains (Fig. 10, A–C). These peaks can be identified by the studies of Boyce and Howard-Flanders (3) and Setlow and Carrier (20) as being cytosine-thymine (Rf 0.19 to 0.22) and thymine-thymine (Rf 0.27 to 0.31) dimers. As indicated in the numbers above the curves, the ratios of dimer to thymine were approximately the same in all three irradiated cultures. That these peaks were photoproducts formed by UV exposure is verified by similar data for control (nonirradiated, labeled) cells in Table 5, where comparable areas of the chromatogram showed no increase over background.

The results obtained when the three irradiated cultures were incubated for 2 hr after UV exposure are shown in panels D, E, and F of Fig. 10. The tracings show clearly that the configuration of the Uvr- strain did not change with incubation, and that the presence of the R factor had no effect on this persistence of photoproduct in the trichloroacetic acid precipitate. In contrast, the profile of the wild-type precipitate changed markedly. The dimer disappeared, and a different peak now appeared (at Rf 0.34). This change of configuration, with persistence of a peak in this area, may be seen in other published reports (3), but remains unexplained (P. Howard-Flanders, personal communication). Strips were cut (as indicated by the shadings in Fig. 10D) to insure centering of this new peak, rather than to correspond to the dimer Rf, when strip counting showed that the dimer area was flat. The ratio of radioactivity in the new peak to that in the thymine peak was not markedly different from the ratios of dimer to thymine in Fig. 10A immediately after irradiation. The significance of this observation remains unexplained.

This residual peak in the excision-competent cell raised some question as to whether photoproducts were, in fact, being excised. Measurements of label in the trichloroacetic acid-soluble

![Figure 9](https://example.com/figure9.png)

**Fig. 9.** Survival of E. coli uvrC with and without RE13 and two Amp- R-factor mutants. Confidence limits (+/− 2 sd), omitted from this plot for clarity, show overlapping of the three upper lines and no overlapping of these with AB1884.
Fig. 10. Tracings from scanning of chromatograms of trichloroacetic-acid precipitate of 3H-thymidine-labeled DNA. AB2497, wild type; AB2500, uvrA. Shading indicates areas selected to be cut out and counted with scintillant. Numerical values for each peak are indicated above or, in the case of the thymine peak (R<sub>r</sub> 0.58 to 0.66), to the right. Values for counts per minute (CPM) have been multiplied by 10<sup>3</sup>. D/T indicates the ratio of counts in the dimer peak/counts in the thymine peak. Data in panel D relates to the shaded areas, which show a change in location.

Table 5. Control data for photoproduct chromatograph experiment<sup>a</sup>

<table>
<thead>
<tr>
<th>Clone</th>
<th>0 Hr</th>
<th>2 Hr</th>
<th>0 Hr</th>
<th>2 Hr</th>
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<tr>
<td></td>
<td>RF</td>
<td>cpm × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>D/T</td>
<td>RF</td>
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<tr>
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<td>.20</td>
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<td>.30</td>
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<td>.30</td>
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<td>.62</td>
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<td>.59</td>
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<td>.09</td>
<td>.20</td>
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<td>.30</td>
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<td></td>
<td>.61</td>
<td>6,891</td>
<td>.63</td>
<td>8,521</td>
</tr>
<tr>
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<td>549</td>
<td>.09</td>
<td>.20</td>
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<tr>
<td></td>
<td>.58</td>
<td>5,614</td>
<td>.60</td>
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</table>

* These data were obtained identically to those in Fig. 10, except that, as cells were not irradiated, there were no detectable photoproduct peaks. For comparison with irradiated cells, therefore, paper areas of equal size and of the appropriate RF (as determined from the irradiated cells) were counted.

Fraction of these samples confirmed, however, that the wild-type strain was capable of release of DNA-associated label during the period when this shift in trichloroacetic acid-precipitated counts took place (Fig. 11). Also, as previously noted (3, 21), amounts of label released by the Uvr<sup>-</sup> strain were relatively very small as evidenced by the criteria of persistence of photoproduct in the DNA or their liberation into the medium presence. By both criteria the R-containing Uvr<sup>-</sup> strain was indistinguishable from the uninfected Uvr<sup>-</sup> host. Thus, the R factor has no effect on the excision of photoproducts, and its enhancement of host survival can not be explained on the basis of enhancement of the excisional repair mechanism.

DISCUSSION

These findings identify two R factors which have the capacity for conferring a dose-reduction factor of approximately 1.6 on certain K-12 strains of E. coli. The characteristics of this capacity, thus far elucidated, are the following.

(i) It appears to require an intact recA gene, since it enhances survival equally in strains with Uvr<sup>+</sup> Rec<sup>+</sup> (wild type) and Uvr<sup>-</sup> Rec<sup>+</sup>, but is ineffective in strains of genotype Uvr<sup>+</sup> Rec<sup>-</sup> and Uvr<sup>-</sup> Rec<sup>-</sup>.

(ii) There is no requisite association on the R factor between UV resistance and other specific markers. Although associated at the time of isolation with λ restriction and ampicillin resistance,
the capacity for UV resistance has been separated from these loci by mutation.

(iii) The effect is principally on the slope, and not the shoulder, of the survival curves. Howarth's (13) curves show that Col I increases the shoulder of the curve without changing the slope. The report of R-factor effects by Drabble and Stocker (6) states that they also seemed to affect shoulder and not slope. Our R factors affect the slopes almost exclusively, thus implying an enhanced repair capability rather than an increased tolerance for initially accumulating photoproducts. The effect we have observed thus appears to differ from epoque-mediated post-UV effects previously reported.

(iv) Enhancement of UV resistance does not depend on, or result in, permanent alteration of the host genome. Segregation of the entire R factor led to return of the host's previous level of UV sensitivity.

(v) The capacity of the R factor to enhance survival of the irradiated Uvr- host does not appear to be due to partial restoration of the function missing from these mutants. This may be inferred from the observations that (a) the R factor mediates an effect which is quantitatively similar in both the excision-competent, wild-type host and the Uvr- cells, and (b) neither of the deficiencies identified in these Uvr- mutants, namely, loss of host cell reactivation and loss of photoproduct excision capability, is restored by the presence of the R factor.

(vi) Measurements of doubling times in liquid medium and of postirradiation cell division, as determined by respreading, revealed no difference in these respects between R+ and R- cells. It therefore seems improbable that the R factor effect on UV survival is a nonspecific, secondary result of primary effects on these cell functions.

(vii) Photoreactivation plays no role. The fact that results in darkness were identical to those obtained in low levels of ambient light demonstrates that this is not the explanation. Furthermore, no decline in photoproducts in the trichloroacetic acid precipitate is evident in Fig. 10E, as would be expected were photoproducts monomerized in situ.

Our findings indicate, therefore, that the capacity of R factors to enhance survival of E. coli K-12 bacteria has three basic characteristics which must be accounted for in any proposal to explain the mechanisms. (i) It is independent of the normal excision mechanism, in that it is independent of the presence of the uvrA, -B, and -C, loci. (ii) It depends upon normal function of the recombination pathway, requiring the presence of a normal recA gene. (iii) It is quantitatively similar in both wild-type and Uvr- cells.

The only mechanism of repair described thus far which approximates these criteria is that of sister-strand exchange. Studies of this phenomenon have been done only in excision-defective cells, so that no data regarding its operation in wild-type cells are available. Nonetheless, with regard to the first two characteristics, sister-strand exchange is qualitatively identical to the R-factor effect. Furthermore, we have been unable to demonstrate any qualitatively new ability in the R+ cell. Therefore, the R factor probably acts quantitatively by improving the efficiency of a pre-existing host cell ability.

Application of this conclusion to our data leads to the inference that wild-type cells engage in postirradiation sister-strand exchange. Such exchange has been described only in Uvr- (incision-less) cells because only in these cells can the detected single-strand breaks be attributed to an incision-independent process (such as replicative gap formation). Although demonstration of these gaps is fundamental to a theory which postulates sister-strand exchange to close the gaps, attempts to identify in a wild-type cell gaps due to replicative omissions would be extremely difficult for the following reason. Whereas 37% survival in a Uvr- cell indicates the ability to circumvent 60 photoproducts per chromosome by gap formation and recombination, a wild-type cell survives equally in the presence of 3,700 photoproducts (10). In the event that an equivalent number (i.e., 60) of photoproducts yielded replicative gaps in the wild type, there would still remain a balance of more than 3,600 photoproducts which would be repaired through the excision system. A single-strand incision is presumed to be a pre-
requisite for every photoprotein repaired by excision. Thus, approximately 3,600 single-strand gaps must occur during excisional repair of this quantity of UV damage. Therefore, if single-strand gaps were measured under these conditions, 60/3,700, or less than 2%, would be attributable to replicative omissions. A small, but indeterminate, increase in percentage might result from the introduction of two refinements into the calculation: (i) the fact that all incisional events presumably do not occur simultaneously, thus reducing the total to be found at a given point in time from the maximum of 3,600; (ii) sampling of the irradiated cells after allowing time to elapse for replication, with the expectation that at this point repair gaps should be closed and those gaps dependent for appearance on the replicative events themselves would have appeared. The hazards of this second assumption have been clearly illustrated by Witkin's demonstration of the asynchrony of division of an irradiated bacterial population (26).

Our data provide strong circumstantial evidence for sister-strand exchange in the wild-type cell, thus circumventing the difficulties of direct demonstration of gaps. The quantitative identity of the R-factor effect in wild-type and UvrA+ hosts suggests an identity of mechanisms. As noted above, the R-factor effect in the UvrA- cell appears to occur through enhancement of a preexisting host cell ability. It thus seems highly likely that such an ability is also normally present in the wild type, where it is obscured by the very efficient excisional system.

How might the R factor act to enhance sister-strand exchange? Since we have been unable to show any effect on a postirradiation replication, direct influences on DNA synthesis appear unlikely. A more probable effect of the R factor would be to influence recombination. Such an effect would manifest itself as more successful repair and, therefore, a higher survival. Investigations of possible mechanisms of this type are currently in progress.

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LITERATURE CITED