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Effect of R Factors and Other Plasmids on Ultraviolet Susceptibility and Host Cell Reactivation Property of *Escherichia coli*

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Some R factors, like some colicin factors, confer partial protection against the bactericidal effect of ultraviolet (UV) irradiation. Of 31 plasmids (17 R, 3 *col*, and 11 R-*col* factors) tested in *Escherichia coli* K-12, 15 protected, 11 had little or no effect, and 5 caused increased UV susceptibility. The effect of representative plasmids was qualitatively the same in K-12 of wild-type UV sensitivity, λ-lysogenic or non-lysogenic, and in UV-sensitive mutants of classes *uvrA*, *uvrB*, *uvrC*, and *recA* (except that a sensitizing factor did not increase the sensitivity of two *recA* hosts). It is inferred that the UV-protecting effect of some plasmids does not result from their specifying enzymes similar to those deficient in such mutants. UV killing of multiply auxotrophic K-12, of wild-type sensitivity or *recA* or *uvrC*, was reduced by deprivation of required amino acids for 2 hr before irradiation, and further reduced if "starvation" was continued for 2 hr after irradiation. The plasmids tested in these conditions produced qualitatively the same effects as in nonstarved cells—except that in K-12 of wild-type UV sensitivity the effect of protecting plasmids was reversed (i.e. they caused decreased survival) when the cells were starved after irradiation. Two UV-protecting R factors reduced the ability of HCR$^+$ K-12 to support growth of irradiated phage T1.

R factors and colicin factors are transmissible extrachromosomal genetic agents or plasmids defined by properties they confer on their bacterial hosts—respectively, resistance to one or more antibacterial drugs and ability to produce a colicin (6, 14, 15). Some colicin factors confer partial protection against the bactericidal effect of ultraviolet irradiation, as also do some R factors (5, 12). This paper reports the effects of various plasmids on the UV sensitivity of *Escherichia coli* K-12. The plasmids tested were R factors previously untested in respect of UV-protecting ability, and, for comparison, three colicin factors whose ability to protect *Salmonella typhimurium* had been tested. Howarth (12) suggested that the UV-protecting effect of a colicin factor might result from the presence in it of a gene affecting the ability of its host to repair UV-damaged DNA. Representative plasmids were therefore tested in UV-sensitive mutants of K-12, namely *uvrA*, *uvrB*, and *uvrC*, deficient in the excision-repair mechanism (9), and *recA*, deficient in ability to recombine (2, 10, 11).

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The survival of UV-irradiated bacteria is affected by their physiological state at the time of irradiation and by the environment provided immediately after irradiation. The effect of plasmids on UV sensitivity was therefore tested in cultures treated in different ways before and after irradiation; in one instance, altered conditions resulted in reversal of the protecting effect of certain plasmids.

The ability of strains of wild-type UV sensitivity to support plaque formation by irradiated phage ("host-cell reactivation") depends upon their possession of a normal excision-repair mechanism. Plasmids were therefore tested for ability to modify this property in wild-type and in UV-sensitive hosts.

MATERIALS AND METHODS

Media. Nutrient Broth (Difco) or Nutrient Broth no. 2 (Oxoid), and the corresponding nutrient agars were used. Cultures were incubated at 37 C, without aeration in the case of liquid cultures.

Strains and plasmids. The *E. coli* strains used (Table 1), all derivatives of strain K-12, included one representative each of the *uvrA*, *uvrB*, and *urvC* classes, two *rec* mutants, now both assigned to *recA*,

TABLE 1. *Strains used*

Strain	Genotype[a]	Reference
RC709	*met pro* (λ) F⁻	(3)
58-161/sp	*met lam-s*, defective F	(3, 7)
AB2497	*thr leu thi pro his arg thy tlr lac gal ara xyl mtl str T6-r lam-s* F⁻	(9)
AB2487	As AB2497 but *rec-13*	(10)
AB2498	As AB2497 but *uvrC34*	(9)
AB2499	As AB2497 but *uvrB5*	(9)
AB2500	As AB2497 but *uvrA6*	(9)
JC1557	*leu his arg met lac gal ara xyl mtl str Tl-r T6-r lam-s* F⁻	(2)
JC1569	As JC1557 but *rec-1*	(2)

[a] All strains are sublines of *E. coli* K-12.

and their parent strains, of wild-type UV resistance. The 28 R factors tested were originally detected in England in strains of *Salmonella* sp. by Naomi Datta (13), and had been transferred by conjugation to an *E. coli* K-12 derivative. Eleven of the original *Salmonella typhimurium* hosts, and all strains (except perhaps the *rec*⁻ strains, *see* below) which acquired drug resistance from them by conjugation, were colicinogenic, producing colicin I of type Ib (17). The factors they carry will be termed R-*col* factors, although there is no proof that only a single factor rather than two factors are carried by each strain. Three colicin factors, two *colIb* and one *colIa*, were received from Sheila Howarth-Thompson as colicinogenic derivatives of *S. typhimurium* LT2 *trpD1*. The drug-resistance traits and other properties of the 10 plasmids used in most experiments are listed in Table 2.

Transfer of plasmids. The plasmids were transferred from their original host (*E. coli* K-12 line RC709 or 58-161/*sp* for the R and R-*col* factors, LT2 *trpD1* for the *col* factors) to various other hosts by conjugation. Broth inoculated with both donor and recipient was incubated at 37 C overnight; trypsin was added when the donor was colicinogenic. All the recipient strains were resistant to high concentrations of streptomycin. Recipient cells which had acquired the R factor of the donor were selected by streaking the mixed culture on nutrient agar with streptomycin (500 μg/ml), to kill the donor cells, and either tetracycline (30 μg/ml) or chloramphenicol (30 μg/ml). For transfer of *colI* factors, the mixed culture was streaked on streptomycin nutrient agar, and colonies were tested for colicin production. No transfer of any of the *colI* factors to the two *rec*⁻ recipients was detected in several experiments; perhaps transfer occurred but was not detectable because the *rec*⁻ character of the recipient prevented spontaneous induction and so colicin release (8). The *rec*⁻ strains given R-*col* factors, as inferred from acquisition of tetracycline resistance, did not give unequivocal evidence of colicin production, presumably for the same reason. Transfer of the *colIa* factor *c*8 to *uvrC* and of the *colIb* factor *c*1 to *uvrA* was not achieved.

Irradiation. A G.E.C. germicidal lamp, tube G875, set 57 cm from the samples, delivered about 115 ergs per mm² per sec; for UV-sensitive strains a perforated metal grill was interposed, reducing the incident energy to about 12 ergs per mm² per sec. Broth cultures, incubated at 37 C without aeration for about 18 hr, were diluted 100-fold into fresh broth, incubated at 37 C for 1 hr, washed twice in saline and resuspended at about 10^7 cells/ml in Davis minimal medium for irradiation. The irradiated and control (unirradiated) samples were diluted in saline, and standard drops (volume, 0.01 ml) were delivered to the surface of nutrient agar plates; colonies were counted after overnight incubation at 37 C.

TABLE 2. *Plasmids used*

Plasmid[a]	Original host[b]	Traits[c]									
R1	BB7268	*fi*⁺	—	Cm	Sm	Su	Pn	Km	*col*⁻	*res*⁻	*UVP*ˢˢ
R2	tm8964	*fi*⁺	Tc	—	Sm	Su	—	—	*col*⁻	*res*⁺	*UVP*⁺
R42	2M929	*fi*⁻	Tc	—	—	—	—	—	*col*⁻	NT	*UVP*⁺
R132	3M4419	*fi*⁻	Tc	—	Sm	Su	Pn	—	*col*⁻	NT	*UVP*°
R142	4M83	*fi*⁻	Tc	—	Sm	Su	—	—	*col*⁻	*res*⁺	*UVP*ˢˢ
Rc144	4M91	*fi*⁻	Tc	—	—	—	—	Km	*colIb*	*res*⁺	*UVP*⁺
Rc145	4M92	*fi*⁻	Tc	—	—	—	—	Km	*colIb*	*res*⁺	*UVP*ˢˢ
*c*1	1M304	NT							*colIb*	*res*⁺	*UVP*⁺
*c*2	1M646	NT							*colIb*	*res*⁺	*UVP*°
*c*8	M6825	NT							*colIa*	NT	*UVP*⁺

[a] As numbered in collection of N. Datta (13).

[b] All *S. typhimurium*, except BB7268 which is *S. paratyphi B.*

[c] As determined in laboratory of N. Datta, except for UVP character, determined in this investigation (and previously in *S. typhimurium* by S. Howarth-Thompson, for the three colicin factors). Symbols: Tc, Cm, Sm, Su, Pn, Km, conferring resistance to tetracycline, chloramphenicol, streptomycin, sulphonamides, penicillin, kanamycin; *res*⁺, *res*⁻, conferring or not conferring immunity to phages W31 or BF23 or both; *UVP*⁺, *UVP*ˢˢ, *UVP*°, causing increased, diminished, or unaltered UV-resistance; —, negative; NT, not tested.

For "starvation" experiments overnight broth cultures were diluted 100-fold in minimal medium with glucose (0.2%), supplemented with vitamin B_1 (100 ng/ml) and thymine (10 μg/ml) where appropriate, but lacking either all the amino acids required by the strain or at least some of them, usually proline, histidine, leucine, and threonine. The cultures were incubated in this amino acid-deficient medium for 2 hr at 37 C before irradiation. In some experiments the irradiated samples were then immediately diluted for counts of viable particles, a procedure termed "pre-UV starvation." In other experiments, the irradiated and control pre-UV-starved cells were held in the amino-acid-deficient medium for a further 2 hr at 37 C after irradiation, then diluted for viable count; this treatment is termed "post-UV-starvation." These operations were performed in a dim light, to prevent photoreactivation.

Tests of HCR property. Phage T1 grown on strain AB2497 was diluted in saline to about 2×10^8 plaque-forming units/ml and samples were irradiated for 30 and 60 sec. The control and irradiated samples were titrated by delivering drops (volume, 0.01 ml) of dilutions onto nutrient agar plates surface-inoculated with broth cultures of the strains under test.

RESULTS

Effect of plasmids on K-12 of wild-type UV sensitivity. The effect of 17 R factors and 11 R-*col* factors was tested by irradiation of derivatives of strain RC709, an *E. coli* K-12 line of wild-type UV sensitivity, lysogenic for phage λ. Three of the 17 derivative strains carrying R factors and 7 of the 11 with R-*col* factors gave survival curves which differed little if at all from that of the parent strain. Ten R and three R-*col* derivatives were more resistant than their parent, their dose to log-survival curves showing either an increased "shoulder" or a diminished slope throughout. Five derivatives, four carrying R factors and one an R-*col* factor, were more sensitive to irradiation than the parent strain. Plasmids which protected, sensitized, or had no conspicuous effect will be termed, respectively, *UVP*$^+$, *UVP*^{ss}, and *UVP*^{0}. Representative survival curves are shown in Fig. 1. As a discriminant test, the UV dose for 0.005 survival of the parent strain was estimated from the survival curve; if the similarly estimated survival of the plasmid-bearing derivative for this dose was >0.05, the plasmid was considered *UVP*$^+$, if <0.0005 as *UVP*^{ss}, if between 0.0005 and 0.05 as *UVP*^{0}. Since induction of prophage λ contributes to the bactericidal effect of UV irradiation of λ-lysogenic *E. coli* K-12, representative plasmids were next tested in a K-12 line nonlysogenic for λ. Five R factors (two *UVP*$^+$, one *UVP*^{0}, two *UVP*^{ss}) and two R-*col* factors (one *UVP*$^+$, one *UVP*^{ss}) were transferred by conjugation to a nonlysogenic K-12 line, AB2497. Two colicin factors, one of type *collb* and one of type *colla*, known to confer UV protection in *S. typhimurium* strain LT2, and one of type *collb* known to lack the protecting property were transferred from LT2 *trpD1* to strain AB2497. These 10 plasmids (Table 2) had the same effects on the UV susceptibility of AB2497 as they had produced in the λ-lysogenic host RC709 or in *S. typhimurium* strain LT2 *trpD1*$_2$. Thus the UV-protecting or -sensitizing effects are not due only to an effect on UV induction of prophage λ, nor are they peculiar to strains RC709 and LT2 *trpD1*.

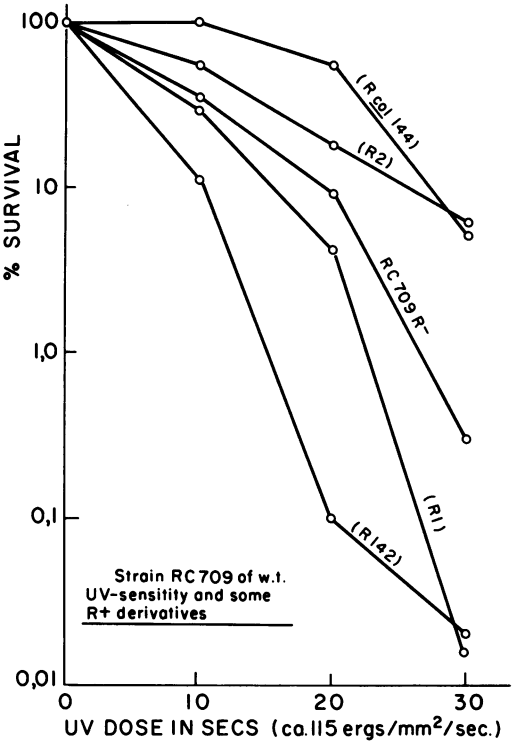


FIG. 1. *Dose to log-survival curves for strain RC709, of wild-type UV sensitivity, and its derivatives given UVP*$^+$ *plasmids, R-col 144 or R2, or UVP*^{ss} *plasmids, R1 or R142.*

Effect of plasmids on UV-sensitive mutants. Mutations at loci termed *uvrA*, *uvrB*, and *uvrC* render *E. coli* K-12 hypersensitive to killing by UV irradiation, apparently by interfering with a normal mechanism for the excision of UV-induced thymine dimers from chromosomal DNA (9, 11). Other mutations, at loci termed *rec*, prevent genetic recombination and also cause increased UV susceptibility (2, 10). Mutants *uvrA6*, *uvrB5*, *uvrC34*, and *rec-13*, all derived from the above-mentioned nonlysogenic K-12 line AB2497, and mutant *rec-1*, derived

from another nonlysogenic K-12 line, JC1557, proved as expected hypersensitive to UV irradiation (Fig. 2). The ten plasmids previously tested in the nonlysogenic parent line AB2497 were so far as possible transferred by conjugation to these five UV-sensitive mutants, and to the second parent strain (of wild-type UV sensitivity), JC1557. Tests on all 28 obtained combinations of 10 plasmids, and 3 *uvr* mutants showed that all the plasmids had qualitatively the same effect, protecting, sensitizing, or null, as in hosts of wild-type UV sensitivity. Fig. 3a illustrates the effects of a UVP^+ and of a UVP^{ss} factor on the survival curve for mutant *uvrC34*. To obtain convenient survival values, much lower UV doses had to be used than in irradiation

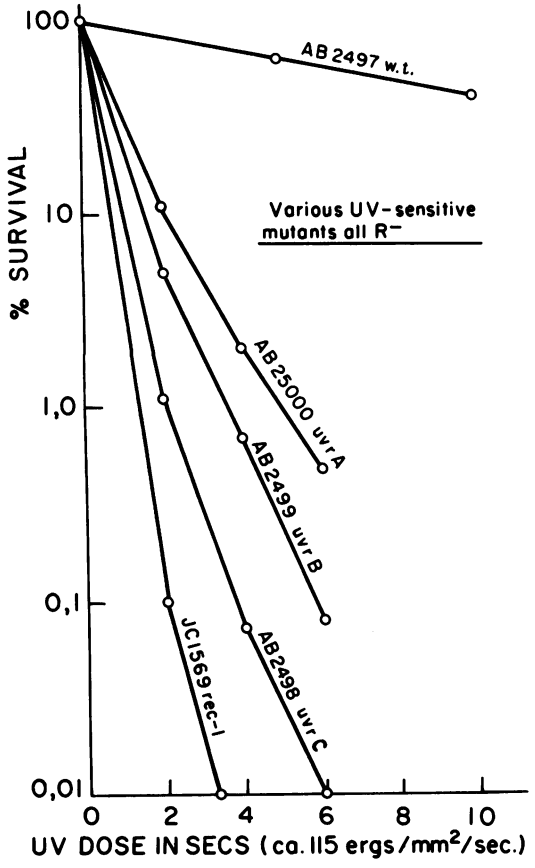
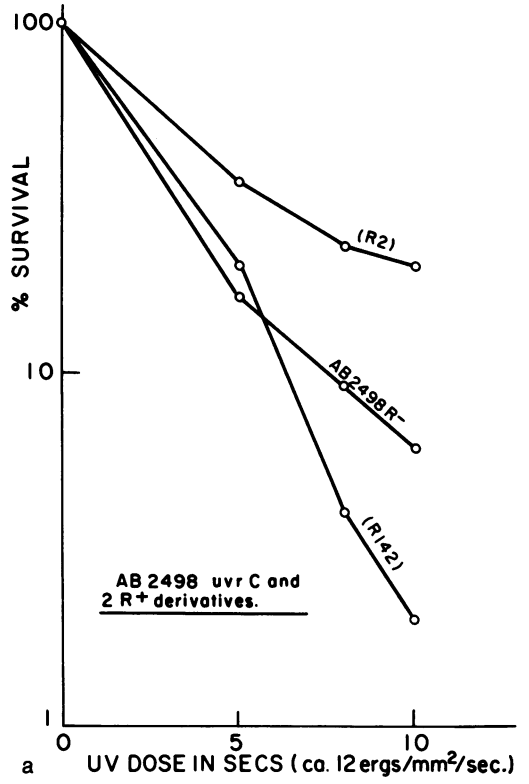


FIG. 2. *Dose to log-survival curves for AB2497, of wild-type UV-sensitivity, and for sensitive mutants uvrA, uvrB, uvrC, and recA1.*



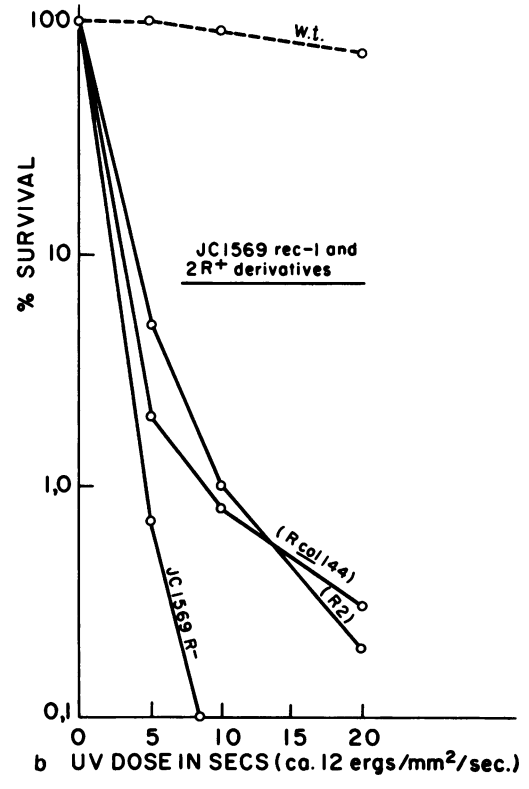


FIG. 3. *(a) Dose to log-survival curves for strain uvrC and its derivatives given the UVP^+ plasmid R2 or the UVP^{ss} plasmid R142. (b) Dose to log-survival curves for strain rec-1 and its derivatives given the UVP^+ plasmids, R2 or R-col 144, and, for comparison, survival of a strain of wild-type UV sensitivity.*

of K-12 strains of wild-type sensitivity. The dose to log-survival curves for the UV-sensitive mutants and their plasmid-bearing derivatives never showed the initial shoulder, seen in the curves of strains of wild-type sensitivity. Although the *UVP*+ factors showed their expected protecting effect in the three *uvr* mutants, none of them restored any of the three to the wild-type level of resistance—the result which might have been expected if the protecting effect of a given plasmid in a wild-type host was due to its specification of a UV-repair enzyme functionally similar to that deficient in a given *uvr* mutant.

Only the seven R and R-*col* factors could be tested in the *rec* mutants. The three *UVP*+ factors gave considerable protection (Fig. 3b) but did not restore wild-type resistance. The *UVP*0 factor, R132, produced no conspicuous effect, as in other hosts. The three *UVP*ss factors gave variable results in *rec-1* and *rec-13*. The plasmid-bearing derivatives were never more sensitive than their parent strains, instead, in some but not all experiments they were rather less sensitive.

Effect of amino-acid deprivation on survival of irradiated cells. Cultures of five nonplasmid-bearing strains were irradiated after 2 hr of incubation in medium lacking required amino acids but otherwise complete. The irradiated suspensions were either at once diluted for viable count, or incubated for a further 2 hr in the deficient medium before viable count, procedures here termed, respectively, "pre-UV starvation" and "post-UV starvation." The five hosts comprised two strains of wild-type UV sensitivity, RC709 and AB2497, and three UV-sensitive mutants, *rec-1*, *rec-13*, and *uvrC*. For all five strains the survival of irradiated pre-UV-starved cells was higher than that of irradiated nonstarved cells, and the survival of post-UV-starved irradiated cells was even greater. The effect was greatest in the two *rec*$^-$ strains (Fig. 4).

The effect of pre-UV starvation was also tested for the derivatives of the same five hosts carrying R2 or R*c*144, both *UVP*+, or the *UVP*ss factor R142, or the *UVP*0 factor R132. For these derivative strains also, the survival of pre-UV-starved cells was greater than that of nonstarved cells. Comparison of the survival curves for the plasmid-bearing strains tested with pre-UV starvation with those for their non-plasmid-bearing parents tested under the same conditions showed that the plasmids produced qualitatively the same effects in pre-UV-starved as in nonstarved cells. Thus the two *UVP*+ factors conferred as great

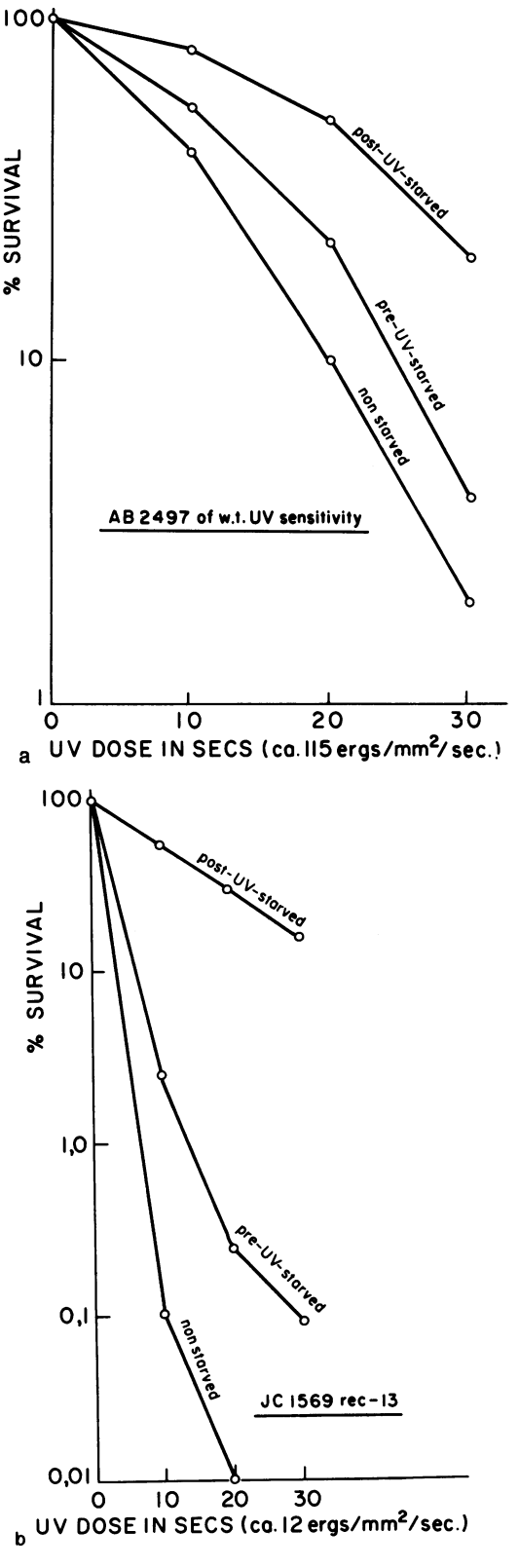


FIG. 4. *Dose to log-survival curves under nonstarvation, pre-UV starvation, and post-UV starvation conditions of nonplasmid-bearing strains, uvr*+ *rec*+ *(Fig. 4a) and rec-13 (Fig. 4b).*

or greater protection as in nonstarved cells, the *UVP*^{0} factor had no effect on survival, and the effect of the *UVP*^{ss} factor differed according to the host genotype. Its sensitizing effect in the *uvr*$^+$ *rec*$^+$ and *uvrC* strains was as great or greater for pre-UV-starved cells as for nonstarved cells, but in the *rec* hosts it had no conspicuous effect. Thus, in all tested combinations of hosts, of wild-type UV sensitivity or *rec*$^-$ or *uvr*$^-$, carrying no plasmid or carrying plasmids of type *UVP*$^+$, *UVP*^{ss}, or *UVP*^{0}, the survival of irradiated pre-UV-starved cells was greater than that of irradiated nonstarved cells, and the effect on survival of the presence of any plasmid was the same as in nonstarved cells.

Survivals under post-UV-starvation conditions were measured for the same combinations of five hosts (RC709 and AB2497, both *uvr*$^+$ *rec*$^+$; *rec-1*; *rec-13*; and *uvrC*) with four plasmids, the UVP$^+$ factors R2, R42, and Rc144 and the *UVP*^{0} factor R132. For the combinations involving the three UV-sensitive mutants the results were unsurprising (Fig. 5a): survivals were better for the post-UV-starved cultures than for pre-UV-starved, and the four plasmids had the same effects (protection in the case of the *UVP*$^+$ factors, no effect for the *UVP*^{0} factor) in the post-UV-starved hosts as in nonstarved hosts.

The presence of the *UVP*^{0} factor likewise had no effect on the survival of the *uvr*$^+$ *rec*$^+$ hosts tested under post-UV-starvation conditions. However, unexpected results were obtained in tests on the survival of post-UV-starved cultures of the six combinations of *uvr*$^+$ *rec*$^+$ host with *UVP*$^+$ plasmid. For all six combinations the survival of cells starved after, as well as before, irradiation was very much less than that of cells starved only before irradiation, and less even than that of nonstarved cells (Fig. 5b). Thus, in strains of wild-type UV-sensitivity-carrying *UVP*$^+$ plasmids, starvation of irradiated cells greatly diminished the proportion able to form colonies, instead of increasing it, as it did in all other combinations tested. The survival under post-UV-starvation conditions of the *rec*$^+$ *uvr*$^+$ strains carrying *UVP*$^+$ factors was so low as to be in some instances much less than that of their parent strains, not carrying any plasmids, tested in the same conditions (Fig. 5b). Thus, factors R2, R42, and Rc144 greatly increased the UV sensitivity of wild-type hosts if the irradiated cells were deprived of required amino acids for 2 hr after irradiation, even though these factors conferred resistance in all other situations tested—i.e., in *rec*$^+$ *uvr*$^+$ hosts tested without starvation or pre-UV-starved, and in *rec*$^-$ or *uvr*$^-$ hosts whether nonstarved, pre-UV-starved, or post-UV-starved.

Effect of plasmids on host-cell reactivation of

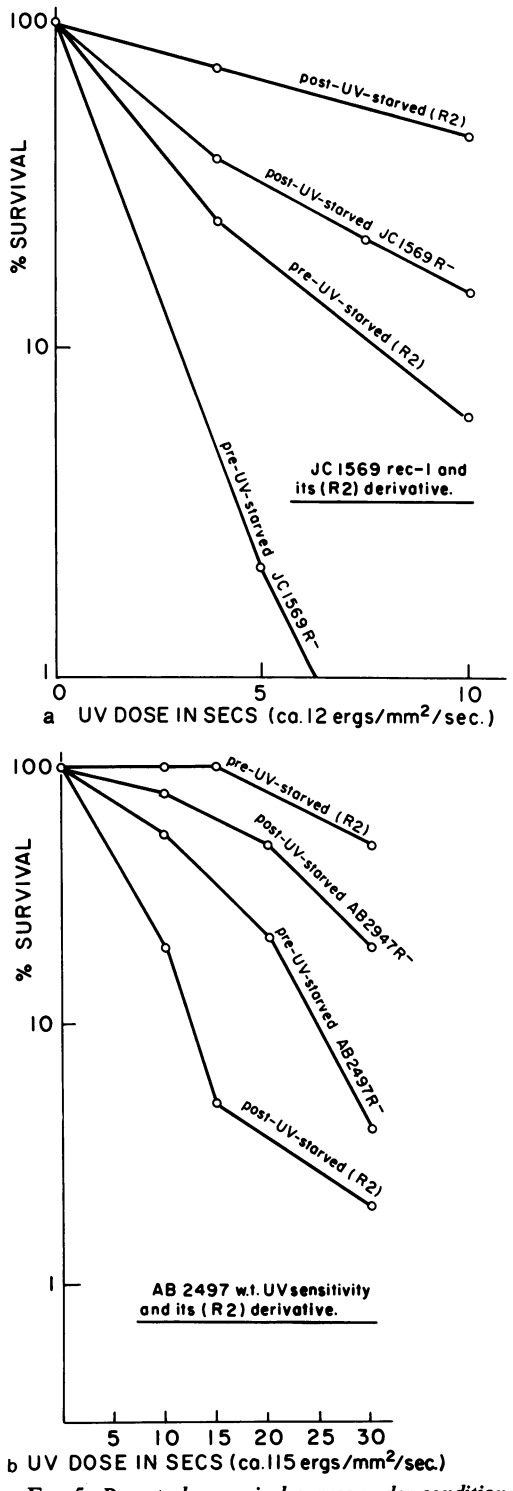


FIG. 5. *Dose to log-survival curves under conditions of pre-UV and post-UV starvation for hosts not carrying plasmids or carrying the UVP*$^+$ *factor R2: strain rec-1 (Fig. 5a); strain AB2497, rec*$^+$ *uvr*$^+$ *(Fig. 5b).*

phage T1. Some R factors, like other plasmids, confer immunity to certain phages, and 6 of the 10 plasmids used were known to confer immunity to phages W31 or BF23 or both (Table 2). Nine of the 10 plasmids had no effect on the sensitivity of any of the hosts to nonirradiated phage T1. Factor R142 conferred immunity to T1, no plaques being produced by phage T1, irradiated or nonirradiated, applied to any host carrying this plasmid (efficiency of plating $< 10^{-8}$). UV-irradiated T1 gives lower plaque counts on *uvr* mutants than on K-12 wild-type—it is believed because such mutants, called HCR-negative, fail to effect the normal "host-cell reactivation" of UV-damaged phage DNA. By contrast *rec* mutants effect normal host-cell reactivation. As expected irradiated phage T1 plated as efficiently on the HCR-positive *rec-1* and *rec-13* mutants as on the wild-type (*rec*$^+$ *uvr*$^+$) parent strains. The 9 plasmids not conferring immunity to T1 were tested for possible effect on the HCR phenotype of wild-type strains (AB2497, for all ten plasmids, and also RC709, for the seven R and R-*col* plasmids). Several plasmids were similarly tested in the HCR-positive mutants, *rec-1* and *rec-13*. Seven of the nine plasmids had little or no effect on the number of plaques produced by irradiated phage applied to any of the HCR-positive hosts. Two plasmids, Rc144 and *c*1, both *UVP*$^+$, produced an unexpected result, for their presence in the HCR-positive hosts reduced the efficiency of plating of irradiated phage. That is, they determined a more or less HCR-negative phenotype. This effect was striking in the *uvr*$^+$ *rec*$^+$ hosts: the phage count of irradiated phage on their derivatives carrying Rc144 or *c*1 was even lower than on the HCR-negative *uvr*$^-$ mutants (Fig. 6).

The HCR-negative mutants, *uvrA6*, *uvrB5* and *uvrC34*, gave as expected low plaque counts when tested with irradiated T1 (Fig. 6). So far as they were tested (Table 3) the plasmids produced qualitatively the same effects in these hosts as in HCR-positive hosts. Thus factor R142 conferred immunity, and factors R2, R42 and *c*8 did not alter the efficiency of plating of irradiated phage. The two "HCR-reducing" plasmids, Rc144 (tested in *uvrA*, *uvrB* and *uvrC*) and *c*1 (tested in *uvrB* and *uvrC*), still further reduced the already poor ability of these HCR-negative hosts to support plaque formation by irradiated phage.

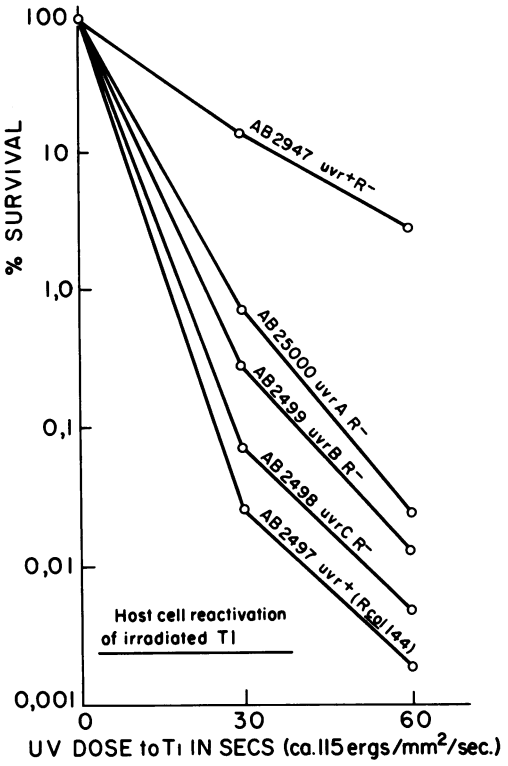


FIG. 6. *Host-cell reactivation of irradiated phage T1 by strain AB2497, of wild-type UV sensitivity, its HCR-negative mutants uvrA, uvrB, and uvrC, and its derivative carrying R-col 144, whose presence reduces HCR.*

DISCUSSION

The experiments described above confirm earlier reports (5, 12) that some plasmids, here termed *UVP*$^+$, confer partial protection against killing by UV irradiation, whereas others, here termed *UVP*^{0}, have no effect on UV sensitivity; they show also that other R and R-*col* factors, called *UVP*^{ss}, increase the UV sensitivity of their hosts. The way in which *UVP*$^+$ and *UVP*^{ss} factors produce these effects is not known. The killing of *E. coli* by UV is caused by photochemical lesions in its DNA, and in wild-type cells most potentially lethal lesions are cured by the DNA-repair mechanisms of the cell, and it is a plausible assumption that the plasmids act by altering the extent of repair of UV-damaged chromosomal DNA. Such an alteration might be produced directly, if a plasmid specified an enzyme which could take part in or modify some DNA repair process, or indirectly, if the presence of the plasmid modified the metabolism of irradiated cells in such a way as to alter the extent of repair, for instance by delaying the moment at which unrepaired lesions become irreparable, and therefore lethal. To test one hypothesis of a direct effect of plasmids on DNA repair, representative *UVP*$^+$, *UVP*^{ss}, and *UVP*^{0} factors were tested in *E. coli* UV-sensitive mutants of classes *uvrA*, *uvrB*, *uvrC*, and *recA* mutants (Fig. 2, 3, Table 3). The *UVP*^{ss} factors did not increase the

TABLE 3. *Effect of plasmids on survival of irradiated cells of different genotypes, not starved or post-UV starved, and on HCR of irradiated phage T1*

Plasmid	Host genotype									Effect on HCR of phage T1[c]
	rec$^+$ *uvr*$^+$			*recA*$^-$ *uvr*$^+$			*rec*$^+$ *uvr*$^-$			
	Strain	Effect in cells[a]		Strain	Effect in cells		Strain	Effect in cells		
		Not starved[b]	Post-UV starved		Not starved	Post-UV starved		Not starved	Post-UV starved	
R1	RC709	ss		*rec-1*	0		*uvrA*	ss		0
	AB2497	ss		*rec-13*	0		*uvrB*	ss		
	JC1557	ss					*uvrC*	ss		
R2	RC709	+	ss	*rec-1*	+	+	*uvrA*	+		0
	AB2497	+	ss	*rec-13*	+	+	*uvrB*	+		
	JC1557	+					*uvrC*	+	+	
R42	RC709	+	ss	*rec-1*	+	+	*uvrA*	+		0
	AB2497	+	ss	*rec-13*	+	+	*uvrB*	+		
	JC1557	+					*uvrC*	+	+	
R132	RC709	0	0	*rec-1*	0	0	*uvrA*	0		0
	AB2497	0	0	*rec-13*	0	0	*uvrB*	0		
	JC1557	0					*uvrC*	0	0	
R142	RC709	ss		*rec-1*	0		*uvrA*	ss		res
	AB2497	ss		*rec-13*	0		*uvrB*	ss		
	JC1557	ss					*uvrC*	ss		
Rc144	RC709	+	ss	*rec-1*	+	+	*uvrA*	+		ss
	AB2497	+	ss	*rec-13*	+	+	*uvrB*	+		
	JC1557	+					*uvrC*	+	+	
Rc145	RC709	ss		*rec-1*	0		*uvrA*	ss		0
	AB2497	ss		*rec-13*	0		*uvrB*	ss		
	JC1557	ss					*uvrC*	ss		
c1	AB2497	+					*uvrB*	+		ss
	LT2 *trpD1*	+					*uvrC*	+		
c2	AB2497	0					*uvrA*	0		0
	LT2 *trpD1*	0					*uvrB*	0		
							uvrC	0		
c8	AB2497	+					*uvrA*	+		0
	LT2 *trpD1*	+					*uvrB*	+		

[a] Symbols: +, survival of irradiated cells much greater; ss, survival much less; 0, survival about the same: as survival for same host without plasmid, tested under same condition (not starved or post-UV starved). No entry indicates not tested.

[b] All the combinations of four plasmids and five hosts tested with post-UV starvation were also tested with pre-UV starvation; in every case the effect of the plasmid, +, ss, or 0, was the same as in nonstarved cells.

[c] Symbols: 0, efficiency of plating of irradiated phage about the same as in host without plasmid; ss, efficiency of plating of irradiated phage much less than in host without plasmid; *res*, phage T1, even unirradiated, had efficiency of plating $<10^3$ on hosts carrying this plasmid. Effect on HCR was tested in AB2497, for all ten plasmids, and in RC709, for the seven R and Rc factors; R2, R42, R142, and Rc144 were tested also in *uvrA*, *uvrB*, *uvrC*, *rec-1*, and *rec-13*, *c1* also in *uvrB* and *uvrC*, and *c8* also in *uvrA* and *uvrB*.

UV sensitivity of the *rec* mutants, but with this exception all the plasmids produced essentially the same effects in the UV-sensitive mutants as in hosts of wild-type UV sensitivity, both in cultures not deprived of required amino acids and in those deprived for 2 hr before irradiation (Fig. 3 and 5; Table 3). No one class of sensitive mutant was protected to an exceptional extent by any of the *UVP*$^+$ factors tested, which indicates that no one of these plasmids protects by specifying a repair enzyme functionally similar to any of the enzymes which are (presumably) specified at these four loci. However, other enzymes are certainly involved in the various mechanisms of repair of UV-damaged DNA, so that it is still possible that the protecting effects of some plasmids result from their specification of repair components. A plasmid-determined

DNA endonuclease acting to an optimal extent might permit repair of UV lesions which would otherwise remain unrepaired, so that the presence of the plasmid would cause increased survival. The same endonuclease, produced in greater amount by another plasmid or acting in a cell in which the repair mechanism was hindered, might degrade host DNA to an extent beyond the repair capacity of the cell, and so cause diminished survival of irradiated cells. This might explain the sensitizing effect of UVP^{ss} factors under ordinary conditions, and that of UVP^{+} factors when tested in cells of wild-type UV sensitivity deprived of required amino acids both before and after irradiation (Fig. 5b, Table 3). Many R and colicin factors confer resistance to various phages, it is surmised, and in one instance proven by in vitro tests (18), by determining nuclease(s) able to degrade the phage DNA. It is, therefore, perhaps not implausible to postulate that UVP^{+} and UVP^{ss} plasmids determine nucleases able to act on host DNA, at least when it has been damaged by UV. However, the UVP^{+} and UVP^{ss} (versus UVP^{0}) property of plasmids is not obviously correlated with their ability to confer resistance to phages (Table 2 and *unpublished data*). Furthermore, if plasmid-determined nucleases accounted for the protecting and sensitizing properties of some plasmids, these plasmids would be expected to alter the ability of their host to reactivate irradiated phage T1. Of five UVP^{+} and two UVP^{ss} factors tested in both UV-sensitive and wild-type hosts, only two, both UVP^{+}, had any such effect, both of them greatly reducing the efficiency of plating of irradiated phage T1 in all hosts tested, either HCR^{+} or HCR^{-}.

Consider now how plasmids might indirectly affect repair of UV-damaged chromosomal DNA. Although the mechanisms which regulate the replication of plasmids are not well understood (6, 16), probably one factor is negative control, effected by a plasmid repressor substance determined by a plasmid gene. Inactivation of such a repressor by irradiation or mitomycin C, or its absence soon after infection or during amino acid starvation, may account for the disproportionate replication of colicin-factor DNA observed under such conditions (1, 4). If replication of R factors is subject to control by a specific repressor substance, most of the phenomena described above might be explained as follows. (i) UV irradiation, by destroying repressor or preventing its synthesis, causes disproportionate replication of (the DNA of) UVP^{+} and UVP^{ss} plasmids; (ii) excess plasmid DNA replication somehow prevents or retards chromosomal replication; and (iii) in the case of UVP^{+} factors the synthesis of plasmid repressor is after a time resumed, and disproportionate plasmid replication then ceases. The *UV*-protecting effect of UVP^{+} plasmids would result from the transient arrest of chromosomal replication caused by their derepressed replication, since it would permit repair of otherwise lethal chromosomal lesions, just as do treatments known to delay reinitiation of chromosomal replication, e.g., chloramphenicol exposure. Grossly excessive or too prolonged derepressed plasmid replication might cause irreversible inhibition of chromosome replication, accounting for the UVP^{ss} property of some R factors. Plasmids whose excess replication was not inducible by irradiation would have the UVP^{0} character. This model is compatible with the reversed effect of UVP^{+} factors on the survival of irradiated bacteria of wild-type UV sensitivity observed when the irradiated cells are deprived of amino acids both before and after irradiation (Fig. 5b, Table 3). Such post-UV deprivation increases the survival of irradiated cells not carrying any plasmid, probably because it temporarily prevents protein synthesis, and therefore reinitiation of chromosome replication, so that more time is available for the action of the repair mechanisms. If the UV-induced derepressed replication of UVP^{+} (though not of UVP^{ss}) plasmids is normally terminated by renewal of synthesis of plasmid repressor, post-UV amino acid deprivation by preventing synthesis of repressor would cause continued excess plasmid replication, which would be lethal. On this hypothesis UVP^{+} and UVP^{ss} plasmids behave alike in irradiated cells up to the time at which, in the case of UVP^{+} factors only, synthesis of a plasmid repressor protein is resumed.

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