Radiosensitivity in *Escherichia coli*

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(Received 29 April 1963)

SUMMARY

A radiosensitive mutant of *Escherichia coli* strain $\kappa 12$, w3747 was found to be similar to *E. coli* strains B and s with respect to survival following ultraviolet irradiation, plating-medium recovery, ability to 'reactivate' irradiated T1 phage, and in its cross-resistance pattern with radiomimetic chemicals. In all these respects the radiosensitive *E. coli* strain $\kappa 12$, AB1186, isolated by Howard-Flanders, was found to resemble the Hill radiation hypersensitive strain B_{s-1} , and, except in the ability to reactivate irradiated T1 phage, strain B_{s-2} . Strains w3747_s and AB1186 acquired sensitivity to radiomimetic chemicals concomitantly with radiation sensitivity. Strains B_{s-1} and B_{s-2} on becoming hypersensitive to radiation did not become correspondingly hypersensitive to radiomimetic chemicals. In fact, they acquired a small degree of resistance to these chemicals, relative to strain B, and a substantial increase in resistance to 1-methyl-3-nitro-1-nitrosoguanidine and proflavine. These results are discussed in terms of known genetic loci implicated in radiation sensitivity and resistance.

INTRODUCTION

Escherichia coli strains B and s are naturally sensitive to ultraviolet (u.v.) radiation and X-radiation but sensitivity can be demonstrated only when they are plated, following irradiation, on complex media (Roberts & Aldous, 1949; Alper & Gillies, 1960; Hill & Simson, 1961; Woody, Mandell & Greenberg, 1961). Many different radiation-resistant mutants of *E. coli* strains B and s have been isolated (Witkin, 1947; Alper & Gillies, 1960; Greenberg, Mandell & Woody, 1961*a*) and these exhibit concomitant resistance to a variety of chemical agents such as nitrogen mustard (Bryson, 1948; Woody *et al.* 1961), nitrofurazone (Szybalski & Nelson, 1954; Hill & Simson, 1961; Woody-Karrer & Greenberg, 1963), Mitomycin C (Greenberg, Mandell & Woody, 1961*b*), nitrosoguanidines (Mandell, Woody & Greenberg, 1961), azaserine (Greenberg *et al.* 1961*a*). These radioresistant mutants have been differentiated on the basis of their cross-resistance patterns with radiomimetic agents. Mutants of *E. coli* strain B have also been isolated which are even more sensitive to radiation than is *E. coli* strain B itself. These include *E. coli* strain B_{s-1} (Hill, 1958), strain B_{s-2} (Hill & Simson, 1961) and strain B_{III} (Rörsch, Edelman, van der Kamp & Cohen, 1962). Unlike *E. coli* strain B, strains B_{s-1} and B_{s-2} do not form long filaments when treated with u.v. radiation. Furthermore, strains B_{s-1} and B_{III} exhibit a peculiar property when used as hosts for u.v.-irradiated coliphage T1: when irradiated T1 phage is plated on *E. coli* strains B_{s-1} and B_{III} , it is significantly more sensitive to the lethal effects of irradiation than when it is

plated on parent *E. coli* strain B. This has been taken to mean that *E. coli* strains B_{s-1} and B_{III} lack a mechanism, present in other strains of *E. coli*, which repairs the damage caused by u.v. radiation to T1 phage. On the other hand, *E. coli* strain B_{s-2}, like its parent, strain B, 'reactivates' irradiated T1 phage.

It would be of considerable significance if genetically controlled variations in radiosensitivity were demonstrable in *Escherichia coli* strain K12, if only because more is known about strain K12 than about strain B as a genetic tool. Most derivatives of *E. coli* strain K12 are radioresistant (Adler & Copeland, 1962; Howard-Flanders & Theriot, 1962) but strain K12 AB531 was reported by Adler & Copeland (1962) to be relatively less resistant than other K12 strains tested in their laboratory. However, the ultraviolet dose, about 600 ergs/mm.², reported to give one decade of kill in strain AB531 is in the same range as that reported for the most frequently occurring radioresistant mutants of *E. coli* strain S (Greenberg & Woody-Karrer, 1963). Howard-Flanders & Theriot (1962) isolated a mutant of strain K12 AB1157 (designated AB1886 by Howard-Flanders, Boyce, Simson & Theriot, 1962), which was very sensitive to u.v. radiation and which lacked the 'reactivating' mechanism for irradiated T1. Another radiation-sensitive mutant of strain K12 was isolated in our laboratory following treatment of strain K12 F'₁₃ (w3747, Hirota & Sneath, 1961) with acridine orange (Cook & Greenberg, unpublished observations). This radiosensitive strain, w3747_s, can still transmit together, and at high frequency, the F factor and the ability to ferment lactose. A further description of this mutant was one of the purposes of the present work.

Since increased resistance to radiomimetic chemicals is an invariable concomitant of a mutation to radioresistance in *Escherichia coli* strains B and S (Greenberg & Woody-Karrer, 1963), another purpose of these experiments was to see whether mutations from resistance to sensitivity in *E. coli* strain K12 and from sensitivity to hypersensitivity in strain B were accompanied by a decrease in resistance to radiomimetic agents. Strains B, B/r, B_{s-1}, B_{s-2}, AB1886, AB1157, w3747 and w3747_s were compared for the following properties: ultraviolet survival curves, plating-medium responses, 'reactivation' of irradiated T1 phage and cross-resistance patterns with radiomimetic chemicals. The results will show that in all these properties strains AB1157 and w3747 resembled strain B/r; strain AB1886 resembled strain B_{s-1} (but not B_{s-2}); and strain w3747_s resembled strain B. There are, therefore, two kinds of mutation to radiosensitivity in *E. coli* strain K12. Both of these, significantly, are accompanied by increased sensitivity to radiomimetic chemicals, but the hyper-radiosensitive mutants of *E. coli* strain B (B_{s-1}, B_{s-2}) show a decreased sensitivity to most of the radiomimetic chemicals.

METHODS

Strains of Escherichia coli used. The *E. coli* strains used, their derivation, relative radiosensitivities assayed on tryptone agar, and source are listed in Table 1.

Chemical compounds used. The chemicals used were: 1-methyl-3-nitro-1-nitrosoguanidine (which will be referred to as nitrosoguanidine, bought from the Aldrich Chemical Co., Milwaukee, Wisconsin, U.S.A., and recrystallized from ethanol); proflavine hydrochloride (obtained from the Allied Chemical and Dye Corp., New York, N.Y.); nitrogen mustard (a gift from Merck Sharp and Dohme, Rahway,

N.J.); Mitomycin C (supplied by the Cancer Chemotherapy National Service Centre, Bethesda, Maryland, U.S.A.); nitrofurazone (5-nitro-2-furaldehyde semicarbazone; a gift from Eaton Laboratories, Division of the Norwich Pharmacal Company, Norwich, N.Y.). All compounds were prepared in sterile distilled water immediately before use.

Table 1. *Summary of strains*

Strain of <i>E. coli</i>	Derivation	Relative radiosensitivity on tryptone agar	Source
B		Sensitive	Dr R. Hill
B _s -1	Strain B	Hypersensitive	Dr R. Hill
B _s -2	Strain B	Hypersensitive	Dr R. Hill
B/r (CSH*)	Strain B	Resistant	Dr H. I. Adler
S	Strain B	Sensitive	Dr A. D. Hershey
S/Mc 1b (R ₄)	Strain S	Resistant	
AB1157	Strain K12	Resistant	Dr E. Adelberg
AB1886	Strain AB1157	Sensitive	Dr P. Howard-Flanders
w3747	Strain K12	Resistant	Dr J. Lederberg
w3747 _s	Strain w3747	Sensitive	

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Culture media. The media used contained per litre of distilled water:

(1) Tryptone agar: tryptone, 10.0 g.; glucose, 1.0 g.; sodium citrate, 2.0 g.; sodium chloride, 8.0 g.; agar, 12.0 g. (BBL, Baltimore Biological Lab., Inc.); adjusted to pH 7.0 or 7.8 with sodium hydroxide or to pH 5.5 with hydrochloric acid.

(2) Tryptone semi-solid agar: tryptone, glucose, sodium chloride, and sodium citrate at the same concentrations as in tryptone agar; agar, 6.0 g.; adjusted to pH 7.0 with sodium hydroxide.

(3) M9 agar: dibasic sodium phosphate, 5.8 g.; monobasic potassium phosphate, 3.0 g.; ammonium chloride, 1.0 g.; sodium chloride, 0.5 g.; glucose, 2.0 g.; magnesium sulphate (0.7H₂O), 250 mg.; calcium chloride 14 mg.; 1% gelatin solution, 10 ml.; agar, 8.0 g. (Ionagar, Oxo Ltd., London).

(4) Peptone broth: Bacto-Peptone, 10.0 g. (Difco Laboratories, Detroit, Michigan, U.S.A.); Difco Beef Extract, 3.0 g.; glucose, 1.0 g.; sodium chloride, 5.0 g.

Phosphate-buffered saline was 1% sodium chloride in 0.02M-phosphate buffer (pH 6.8).

Survival of u.v.-irradiated T1 phage. The u.v. radiation source was a single 15 W. General Electric germicidal lamp with a maximum output at 2537 Å. Calibrated with bacteriophage T2 according to the method of Latarjet, Morenne & Berger (1953), this lamp delivered 15.4 ergs/mm.²/sec. at a distance of 51.5 cm. from the source. T1 phage, 5 × 10⁶ particles/ml., was irradiated with a dose of 616 ergs/mm.² and 0.1 ml. was mixed with 2.5 ml. of warm (48°) semi-solid tryptone agar containing one drop of the overnight culture to be tested. The semi-solid mixtures were then overlaid on to fresh tryptone agar plates and the number of plaques counted after overnight incubation at 37°. This is essentially the rapid method described by Rörsch *et al.* (1962) for differentiating between mutants capable of 'reactivating'

irradiated T1 phage and those unable to do so. When strains able to reactivate irradiated T1 phage were used as hosts, the plaques were confluent. When those unable to 'reactivate' irradiated T1 phage were used as hosts, a countable number of plaques were seen.

Survival of u.v.-irradiated bacteria. Cultures grown overnight in peptone broth were washed twice with buffered saline (pH 6.8) and exposed with gentle agitation in 50 mm. diameter Petri dishes containing 1 ml. of bacterial suspension (2×10^6 bacteria/ml.). Appropriate dilutions in cold phosphate-buffered saline were plated in duplicate on tryptone agar (pH 7.0) and on M9 agar, incubated at 37° for 24 and 48 hr. respectively, and counted. All manipulations subsequent to irradiation were done in subdued light to minimize photoreactivation. Sensitivity to u.v. radiation was calculated as the dose to give 10 % survivors.

Measurement of resistance to chemical agents. Cultures grown overnight in peptone broth were adjusted with a model 9 Nepho-colorimeter (Coleman Instruments Inc.) to 3.5×10^8 bacteria/ml. and streaked on gradient plates according to the method of Szybalski & Bryson (1952). Gradient plates were made with tryptone agar (pH 5.5) except that pH 7.8 was used in tests with proflavine; M9 agar was used in tests with azaserine. The minimum inhibitory concentration (M.I.C.) was determined as follows: (length of solid growth/total length of streak) $\times$ maximum concentration ($\mu\text{g./ml.}$) of test compound.

RESULTS

Reactivation of u.v.-irradiated T1 phage. With the following hosts the plaques were confluent: *Escherichia coli* strains S, B, B_{s-2}, w3747 and w3747_s. Plates with *E. coli* strain B_{s-1} had 177 plaques; with strain AB1886, 145 plaques. To obtain numbers of plaques in this range for *E. coli* strains S, B, B_{s-2}, w3747 and w3747_s, the irradiated phage suspension had to be diluted 1/50. When this was done there were no significant differences in the number of plaques produced by each strain in this group.

Response to u.v. radiation. Figures 1, 2 and 3 show the survival curves following ultraviolet irradiation of *Escherichia coli* strains B, B_{s-1}, B_{s-2}, S, w3747, w3747_s, AB1157 and AB1886 when plated on tryptone agar and on M9 agar. The survival curves of the radioresistant parent strains AB1157 and w3747 (Fig. 3) indicate significant resistance, independent of plating medium, 860 ergs/mm.² and 462 ergs/mm.² respectively, resulting in 10 % survival.

Escherichia coli strains B_{s-1} and AB1886 (Fig. 1) were similar in that: (1) they were significantly more sensitive to u.v. irradiation than the other strains; (2) their survival curves were exponential to more than three decades of kill; (3) both showed a limited plating-medium response: there were more survivors at any dose when the bacteria were plated on M9 medium than when plated on tryptone medium, but the survivors on M9 medium were considerably fewer than those of strains B, B/r, AB1157 or w3747. On the other hand, *E. coli* strains w3747_s, B, and S (Fig. 2) were related in that: (1) their survival curves were identical when plated on complete medium; (2) they exhibited a marked plating-medium response: strain w3747_s could not be distinguished from its parent strain w3747 when both strains were plated on M9 medium after u.v. irradiation.

Fig. 1

Fig. 2

Fig. 3

Fig. 1. Survival of radiation-sensitive *Escherichia coli* strains B_{s-1} , B_{s-2} , and $\kappa 12$ AB1886 exposed to ultraviolet radiation and plated on tryptone agar, pH 7.0 ($\oplus$, $\odot$, $\ominus$) and m9 agar, pH 6.8 ($\triangle$, $\triangle$, $\triangle$). Each point represents an average of 3 independent determinations for strains B_{s-1} and B_{s-2} and 6 for strain AB1886. The insert in the upper right-hand corner indicates the plating-medium response of strain B_{s-1} on a more magnified scale.

Fig. 2. Survival of radiation-sensitive *Escherichia coli* strains B , S , and $\kappa 12$ w3747_s exposed to ultraviolet radiation and plated on tryptone agar, pH 7.0 ($\odot$, $\otimes$, $\oplus$) and m9 agar, pH 6.8 ($\triangle$, $\triangle$, $\triangle$). Each point represents an average of 5 independent determinations for each strain.

Fig. 3. Survival of radiation-resistant *Escherichia coli* strain $\kappa 12$ AB1157 and strain $\kappa 12$ w3747 exposed to ultraviolet radiation and plated on tryptone agar, pH 7.0 ($\oplus$, $\oplus$) and m9 agar, pH 6.8 ($\triangle$, $\triangle$). Each point represents an average of 2 to 4 independent determinations.

The survival curve for *Escherichia coli* strain B_{s-2} (Fig. 1) was exponential for more than three decades of kill, but it showed greater sensitivity than strain B only at doses higher than 154 ergs/mm.² (The survival curves for strains B_{s-2} and B varied somewhat from those reported for these strains by Hill & Simson in 1961, perhaps due to a difference in experimental procedures or to a genetic drift in the bacterial populations; Greenberg & Woody-Karrer, in press.) Strain B_{s-2}, like strain B_{s-1}, exhibited a limited plating-medium recovery when plated on M9 medium.

Table 2. *Cross-resistance patterns of radiation-sensitive and radiation-resistant mutants of Escherichia coli*

Strain of <i>E. coli</i>	Test compound					
	NG*	NM	MC	NF	PF	AS
	Minimum inhibitory concentration (M.I.C.) for <i>Escherichia coli</i> strains B and S (μg./ml.)†					
	0.08 ± 0.01	16 ± 1	0.04 ± 0.01	0.15 ± 0.01	1.2 ± 0.2	0.004 ± 0.0005
	Fold increase in M.I.C.					
B	1	1	1	1	1	1
S	1	1	1	1	1	1
w3747 _s	2.5	1.2	1.4	1.5	1.7	—
B _{s-1}	8	1.5	1	1.3	2.5	1
B _{s-2}	8	2.5	1	3.4	2.5	2.6
AB1886	17	0.5	1	1.5	1.7	—
B/r	45	20	23	19	3.8	15
S/mc 1b (R ₄)	35	16	16	14	2.5	15
w3747	35	16	11	14	2.5	—
AB1157	35	2.4	6	5.8	2.5	—

* The following abbreviations will be used: 1-methyl-3-nitro-1-nitrosoguanidine, NG; nitrogen mustard, NM; mitomycin C, MC; nitrofurazone (5-nitro-2-furaldehyde semicarbazone), NF; proflavine, PF; azaserine, AS.

† μg./ml. estimated from gradient plates (Szybalski & Bryson, 1952).

Response to radiomimetic chemicals. Table 2 shows the minimum inhibitory concentrations of five radiomimetic chemicals for each of the strains studied relative to *Escherichia coli* strains B and S. The following points should be noted: (1) The cross-resistance patterns of strains w3747, AB1157, and S/mc 1b, a prototype of the most frequently occurring radioresistant class of mutants (R₄) isolated from *E. coli* strain S, were similar to that of strain B/r. All four strains were very resistant to all the agents tested. (2) The cross-resistance pattern of strain AB1886, indicating sensitivity as compared with that of strain AB1157, was very similar to those of strains B_{s-1} and B_{s-2}, the most significant feature being resistance to nitrosoguanidine and proflavine. (3) The cross-resistance pattern of strain w3747_s, indicating sensitivity as compared with that of strain w3747, most resembled those of *E. coli* strains B and S, being relatively sensitive to all the radiomimetic agents tested.

DISCUSSION

The evidence suggests that there are at least two different kinds of radiosensitive strains of *Escherichia coli* strain K12, one of which, strain w3747_s, is analogous to *E. coli* strains B and s, the other, strain AB1886, analogous to *E. coli* strain B_{s-1} (and probably to strain B_{III}, Rörsch *et al.* 1962). The u.v. survival curve of strain w3747_s is identical with those of *E. coli* strains B and s when plated on tryptone medium, and all three strains exhibited a similar plating-medium response. Strains w3747_s, B and s were similar in sensitivity to the five radiomimetic agents tested, whereas strain w3747 was about as resistant to these compounds as the B/r and R₄ mutants of strain s. Finally, all three strains exhibited no measurable loss of ability to 'reactivate' irradiated T1 phage.

Escherichia coli strain AB1886, on the other hand, is very similar in many of its properties to *E. coli* strain B_{s-1}. Following u.v. radiation, the survival curves of strain AB1886 are similar to those of strain B_{s-1} in general shape, sensitivity, and plating-medium recovery. Strain AB1886 was sensitive, as compared with its radioresistant parent, to all of the radiomimetic agents studied, but was markedly less sensitive to nitrosoguanidine and somewhat less sensitive to proflavine than were *E. coli* strains B, s, or w3747_s. It is significant that *E. coli* strain B_{s-1}, even though more sensitive to u.v. radiation than its parent, strain B, was no more sensitive than strain B to most of the radiomimetic agents tested and was significantly more resistant to nitrosoguanidine and proflavine than *E. coli* strain B. Finally strains B_{s-1} and AB1886, in corroboration of reports by Ellison, Feiner & Hill (1960) and Howard-Flanders & Theriot (1962), did not reactivate u.v.-damaged T1 phage.

The locus which determines radiosensitivity in *Escherichia coli* strain B_{s-1} has not been mapped. However, Howard-Flanders *et al.* (1962) showed that the radiosensitivity of *E. coli* strain AB1886 is the result of a mutation (UV^R to UV^S) at a locus 0.37 the distance between the loci for arginine synthesis and arabinose fermentation; UV^R controls a mechanism, absent from UV^S mutants, which reactivates damage done by ultraviolet radiation.

The radiosensitive mutant strain B_{III}, of *Escherichia coli* strain B, isolated by Rörsch *et al.* (1962), is similar to *E. coli* strains B_{s-1} and AB1886 in its sensitivity to u.v. radiation and in its inability to reactivate u.v.-irradiated T1 phage. These investigators have shown that the radiosensitivity of mutant strain B_{III} is the result of a mutation (syn^+ to syn^-) at a locus between xylose fermentation and streptomycin resistance and closely linked to the former (Rörsch, Edelman & Cohen, 1963). The *syn* and *UV* loci, while on the same quadrant of the circular chromosome of *E. coli* as depicted by Jacob & Wollman (1961), do not map at the same place. It is possible that mutations at either of two different loci result in similar phenotypes, or that the linear arrangements of genes is not identical in at least this segment of the chromosome of *E. coli* strains B and K12.

Cook & Greenberg (unpublished observations) have demonstrated in crosses between radioresistant *Escherichia coli* strain K12 (Hfr 1 and 2) and *E. coli* strain s that there is a locus (which will be referred to as *RA* to distinguish it from *UV*) for radioresistance closely linked to the loci for lactose fermentation and resistance to phage T6. The nature of the sensitivity (RA^S) or resistance (RA^R) conferred by the gene at this locus is under further investigation. The evidence as yet unpublished

suggests that the sensitivity of strain w3747_s is a result of a mutation at or near this locus. Its parent strain w3747 can transmit radiation resistance along with the ability to ferment lactose by F-duction to *E. coli* strain s1 (F⁻ Lac⁻). *Escherichia coli* strain κ12 w3747_s, while able to transmit together and at a high frequency its Lac⁺ and F⁺ characteristics, does not transmit radiation resistance to strain s1. However, the F' episome of strains w3747 and w3747_s is extremely unstable in *E. coli* strain s, rendering quantitative analysis difficult. A more promising approach to the genetics of the *RA* locus would appear to be to 'cure' strain w3747_s of its episome, making it available as an F⁻ strain for recombination studies with donor strain κ12s.

The fact that when *Escherichia coli* strain κ12 becomes radiosensitive either by a mutation at the *UV* or the *RA* locus it also becomes sensitive to Mitomycin C, nitrogen mustard and nitrofurazone, can be interpreted to mean that some of the damage to the cell caused by these agents is similar to that caused by u.v. radiation. If the *UV* locus controls a repair mechanism this same mechanism might also repair damage done by radiomimetic chemicals. Howard-Flanders *et al.* (1962) suggested that *UV*^R reactivation acts on certain photoproducts associated with thymine in DNA. There is as yet no evidence that radiomimetic agents produce any changes in DNA thymine comparable to the thymine dimerization produced by u.v. radiation (Beukers & Berends, 1961). Nor is there any evidence that the damage done by radiomimetic agents can be photoreactivated as can thymine dimers (Setlow & Setlow, 1961; Wulff & Rupert, 1962). It is possible that the *UV*^R mechanism repairs other kinds of damage to DNA as well as thymine photoproducts. It is clear that the *UV*^R mechanism does not significantly repair damage done by nitrosoguanidine or proflavine since *UV*^S organisms are relatively resistant to these chemicals. In fact, in the mutation from *E. coli* strain B to strain B_{s-1}, in which sensitivity to ultra-violet radiation increases and sensitivity to Mitomycin C remains essentially unchanged, sensitivity to nitrosoguanidine and proflavine decreases. This is difficult to reconcile with the observation that in radioresistant mutants of *E. coli* strain B, resistance to all the radiomimetic agents increases along with increased resistance to radiation, both u.v. and X-ray (Greenberg & Woody-Karrer, 1963). It is also difficult at present to classify *E. coli* strain B_{s-2}, which resembles *E. coli* strain B_{s-1} in all its properties including a decreased sensitivity to nitrosoguanidine and proflavine, but is still able to reactivate u.v.-damaged phage. Strain B_{s-2} may represent another class of radiosensitive mutants.

The mechanisms responsible for the type of radiosensitivity observed in *Escherichia coli* strains B, s, and w3747_s may result from the lack or loss of an enzyme able to repair kinds of damage different from that repaired by the *UV* locus. Apparently, for strain w3747 to exhibit the degree of radioresistance observed the *UV* and *RA* loci must both be functional. It is not yet clear what the genetic changes are which account for radiation resistance in the various radioresistant mutants of *E. coli* strains B and s (Greenberg & Woody-Karrer, 1963). The fact that there are many different radioresistant mutants of strains B and s would suggest that either mutations in a number of different modifiers of the *RA* locus or a variety of changes within the *RA* locus result in different radioresistant phenotypes.

This work was carried out under U.S. Public Health Service Grant No. CY-5687. The technical assistance of Miss C. R. Woody is gratefully acknowledged.

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