A LOCUS FOR RADIATION RESISTANCE IN ESCHERICHIA COLI[1]

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MUTANTS of *Escherichia coli* B, or its derivatives, can be selected which are either more resistant to ultraviolet radiation (WITKIN 1947; GREENBERG, MANDELL and WOODY 1961; GREENBERG and WOODY-KARRER 1963; RÖRSCH, EDELMAN, VAN DER KAMP and COHEN 1962; ALPER and GILLIES 1960) or more sensitive to ultraviolet radiation (HILL 1958; HILL and SIMSON 1961) than the parent strains. Mutants resistant to radiation acquire concomitantly an increased resistance to a number of radiomimetic chemicals and to X rays. Many different radioresistant strains can be distinguished on the basis of their cross-resistance patterns, i.e. their different degrees of resistance to various radiomimetic chemicals and to radiation.

For genetic studies K-12 is the strain of choice. However, most derivatives of strain K-12 are approximately as resistant to radiation and to radiomimetic chemicals as radioresistant mutants of *E. coli* B (ADLER and COPELAND 1962; HOWARD-FLANDERS, BOYCE, SIMSON and THERIOT 1962; GREENBERG and WOODY-KARRER 1963). And it has not been possible to select mutants significantly more resistant to radiation than the parent strain. It has been possible to select or discover radiosensitive mutants of strain K-12 (HOWARD-FLANDERS *et al.* 1962; ADLER 1963; GREENBERG and WOODY-KARRER 1963), some of which have been shown to be sensitive to radiomimetic chemicals (GREENBERG and WOODY-KARRER 1963), but none of these radiosensitive mutants were available when the experiments to be described were begun.

E. coli B is self-sterile (CALEF and CAVALLI-SFORZA 1955; DE HAAN 1954) and does not produce efficient males when infected with fertility factors from strain K-12. Therefore, the recombination experiments described here employed strain K-12 males as chromosome donors, and strain B derivatives as recipients. The experiments were designed to determine whether radioresistance could be transferred, by sexual recombination, from K-12 to B, and whether resistance to the radiomimetic agent 1-methyl-3-nitro-1-nitrosoguanidine was transferred concomitantly with resistance to radiation.

Data from such experiments will show that (1) radioresistance can be transferred from K-12 to B by sexual recombination, (2) a locus controlling radioresistance in *E. coli* is closely linked to the locus determining resistance to the bacteriophage T6, and (3) resistance to 1-methyl-3-nitro-1-nitrosoguanidine is transferred concomitantly with resistance to ultraviolet radiation.

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MATERIALS AND METHODS

Bacterial strains: The strains used and some of their properties are given in Tables 1 and 2. The K-12 derivatives were obtained from DR. JOSHUA LEDERBERG; strain B, from DR. RUTH HILL. The B derivatives were induced and selected in this laboratory.

Media: The media used contained the following (per liter of distilled water). *Tryptone agar:* tryptone, 10.0 g; glucose, 1.0 g; sodium citrate, 2.0 g; sodium chloride, 8.0 g; and agar 12.0 g (Baltimore Biological Laboratories); the medium was adjusted to pH 7.0 with sodium hydroxide or pH 5.5 with hydrochloric acid. *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 sulfate (7 H_2O) 250 mg; calcium chloride, 14 mg; 1 percent gelatin solution, 10 ml; and agar, 8.0 g (Ionagar, Oxo Ltd., London). M9 lactose, M9 arabinose, etc. were made in the same way except that the desired sugar was substituted for glucose. Penassay agar was a commercial preparation (Difco Bacto-Penassay Antibiotic medium 2). Penassay tetrazolium sugar agar was made by adding to 30 ml penassay agar, 3 ml of a 10 percent solution of the test sugar and 0.5 ml of an 0.3 percent solution of triphenyl tetrazolium chloride. *Peptone broth*: Peptone, 10.0 g; beef extract, 3.0 g; glucose, 1.0 g; and sodium chloride 5.0 g. Phosphate-buffered saline consisted of 1 percent sodium chloride with 0.02 M phosphate buffer (pH 6.8).

TABLE 1

Characteristics of strains used

Strain	Sex	*met*	*thi*	*lac*	*ara*	*mal**	*xyl*	*gal*	*T6*	*T1*	*val**	*str*	*UV*
K-12 W1895	Hfr_1†	—	+	+	+	+	+	+	s	s	s	s	r
K-12 W4531	Hfr_2‡	+	—	+	+	+	+	+	s	s	s	s	r
B PAM 3	F⁻	+	+	—	—	—	+	+	s	s	r	s	s
B PAM 4	F⁻	+	+	—	—	—	+	+	r	s	r	s	s
B PAM 5	F⁻	+	+	—	—	—	+	+	r	r	r	s	s
B PAM 6	F⁻	+	+	—	—	—	—	+	r	r	r	s	s
B PAM 7	F⁻	+	+	—	—	—	—	—	r	r	r	r	s

* The B strains are naturally *mal*⁻ and *val*ʳ. The fermentation deficient mutants were UV induced; the phage resistant and streptomycin resistant mutants were selected from among those which occurred spontaneously.

† Hfr_1 (CAVALLI-SFORZA 1950). Order of transmission of markers is: *T6 lac T1 ara* . . . .

‡Hfr_2 (HAYES 1953). Order of transmission of markers is: *ara T1 lac T6*.

Abbreviations used: *met*—methionine; *thi*—thiamine; *lac*—lactose; *ara*—L-arabinose; *mal*—maltose; *xyl*—D-xylose; *gal*—galactose; *T6*—coliphage T6; *T1*—coliphage T1; *val*—L-valine; *str*—streptomycin; *UV*—ultraviolet light; Hfr—high frequency male or donor; F⁻—recipient, female. For *met* and *thi*, a minus indicates inability to synthesize; for sugars, minus indicates inability to ferment. For *T6*, *T1*, *val*, *str* and *UV*, s indicates sensitivity and r indicates resistance.

TABLE 2

Cross-resistance relationships among strains of Escherichia coli *B and K-12 used in recombination experiments*

Strain	Test compound			
	NG*	MC	NF	PF
Minimum concentration inhibitory to *E. coli* B PAM 7 (μg/ml)	0.08	0.04	0.15	1.2
Resistance factor				
B PAM 7	1	1	1	1
W1895	35	16	14	7
W4531	39	14	21	2.5

* Abbreviations used: NG—1-methyl-3-nitro-1-nitrosoguanidine; MC—Mitomycin C; NF—nitrofurazone; PF—proflavine.

When required streptomycin was added to basal medium at a concentration of 200 μg/ml; valine at 200 μg/ml; methionine, at 10 μg/ml; and thiamine at 0.001 μg/ml.

Chemical agents used: The chemical agents used were 1-methyl-3-nitro-1-nitrosoguanidine, purchased from Aldrich Chemical Co., Milwaukee, Wisconsin; Mitomycin C was a gift from the Cancer Chemotherapy National Service Center, Bethesda, Maryland; nitrofurazone was a gift from Eaton Laboratories, Division of the Norwich Pharmacal Co., Norwich, N.Y.; proflavine was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio.

Measurement of resistance to chemical agents: Test cultures were grown overnight in peptone broth at 37°C, adjusted with a model 9 Nepho-colorimeter to 3.5×10^8 cells/ml and streaked on gradient plates according to the method of SZYBALSKI and BRYSON (1952). Gradient plates were made with tryptone agar adjusted to pH 5.5 for testing 1-methyl-3-nitro-1-nitrosoguanidine, Mitomycin C and nitrofurazone, and to pH 7.8 for proflavine. Penicillin was tested on gradients made with M9 agar. The cross-resistance patterns of the strains used are shown in Table 2.

Sensitivity to ultraviolet radiation (UV): The UV source was a single 15w General Electric germicidal lamp with maximal output at 2537 A. Estimated with coliphage T2 according to the method of LATARJET, MORENNE and BERGER (1953), this lamp delivered 15.4 ergs/mm²/sec at the target distance used, 51.5 cm from the source of radiation. Cultures grown overnight in peptone broth were washed twice with buffered-saline and exposed with gentle agitation in 50 mm petri dishes containing 2×10^6 bacteria/ml. Dilutions in cold buffered-saline were plated

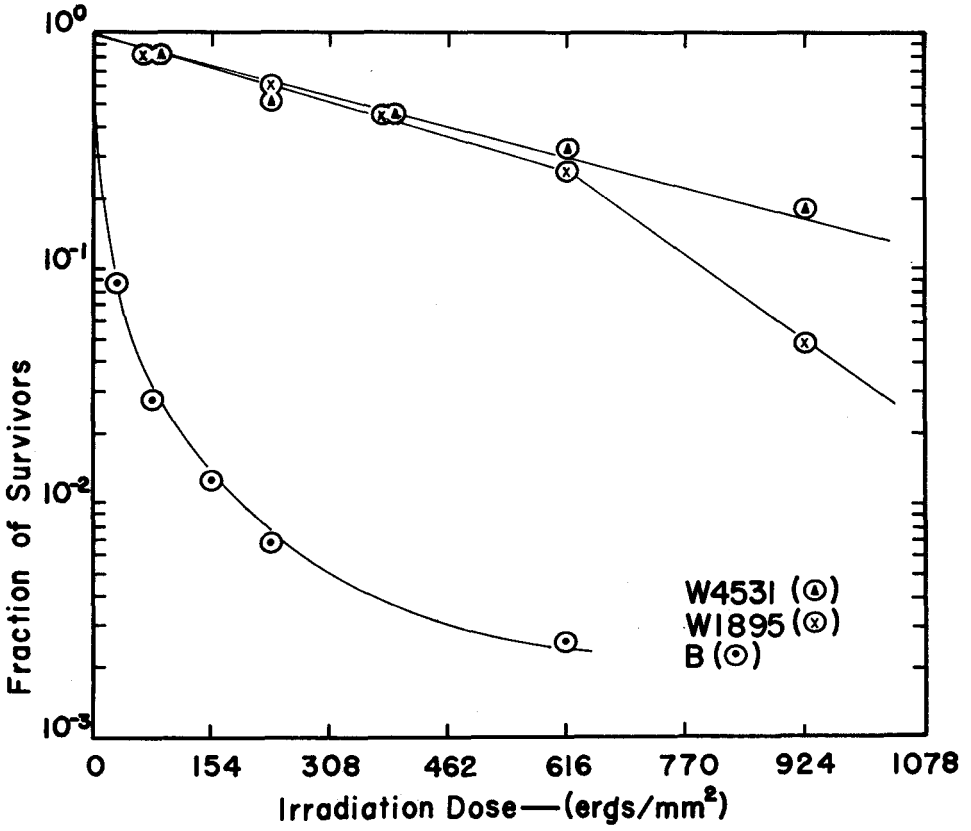

FIGURE 1.—Survival of *E. coli* strains W4531, W1895 and B exposed to UV and plated on tryptone medium.

in duplicate on both tryptone agar (pH 7.0) and M9 agar, supplemented as required. Colonies were counted after incubation at 37°C for 24 and 48 hours respectively. All manipulations subsequent to irradiation were carried out in subdued light. The ultraviolet survival curves of the parent strains used are shown in Figure 1. When plated on M9 agar survival curves for W1895 and W4531 were unchanged, while that of B coincided approximately with that of W1895.

To test UV sensitivity of recombinants a rapid method was used: Overnight peptone broth cultures were streaked with capillary tubes on square plastic plates of tryptone agar (pH 7.0). It was possible to test 50 cultures on one plate. Each plate was exposed to 154 ergs/mm^2 of UV, incubated 1 hr at 37°C, reexposed to 308 ergs/mm^2 radiation, and incubated overnight at 37°C. Streaks with isolated colonies indicated sensitivity equivalent to the B parent; those with heavy confluent growth indicated resistance equivalent to the K-12 parent. Periodic checks by the conventional method described earlier revealed no discrepancies in interpretation of the streaks.

Genetic analysis: For recombination studies, 0.2 ml of an overnight, 37°C peptone broth culture of the K-12 parent was added to 5 ml of fresh peptone broth in a 50 ml flask and shaken 130 strokes/min in a 37°C water bath. After 1 hr, 0.5 ml of an overnight, peptone broth culture of the B parent was added and the mixture shaken for 2 hr at 60 strokes/min. Viable cell counts or Nepholometer readings of both parents were made at the time the cultures were mixed. Dilutions of the mixed culture were spread on selective medium and incubated 48 hours.

Isolated colonies picked from the selective medium were streaked on fresh plates of the same medium. Single, isolated colonies were picked from the streakings to peptone broth. The cultures were grown overnight at 37°C, and tested on the appropriate medium for unselected markers. The fermentation of sugar was tested either by the ability of the test culture to grow when streaked on M9 agar containing the test sugar instead of glucose, or by its color on penassay-tetrazolium medium containing the appropriate sugar. Bacteriophage resistance was scored by cross-streaking test cultures through the phage deposited on tryptone agar plates.

RESULTS

Crosses of W1895 × B PAM 7: The frequency with which radioresistance appeared as an unselected marker in crosses of W1895 × B PAM 7 when progeny were selected for various other markers is shown in Table 3. Also shown are the frequencies of other unselected markers in the same crosses. The frequency of appearance of radiation resistance was highest among recombinants selected for

TABLE 3

Frequency of occurrence of donor markers among recombinants from the cross W1895 × B PAM 7

Parents:	W1895	Hfr	*UV^r*	*T6^s*	*lac+*	*T1^s*	*ara+*	*mal+*	*met−*	*val^s*	*xyl+*	*str^s*
	B PAM 7	F−	*UV^s*	*T6^r*	*lac−*	*T1^r*	*ara−*	*mal−*	*met+*	*val^r*	*xyl−*	*str^r*

Recombinants selected	Number examined	Frequency of donor alleles among selected recombinants (percent)									
		UV^r	*T6^s*	*lac+*	*T1^s*	*ara+*	*mal+*	*met−*	*val^s*	*xyl+*	*str^s*
lac+ str^r	100	86	87	100	72	63	22	8	4	3	. .
ara+ str^r	100	50	50	57	65	100	15	12	6	1	. .
mal+ str^r	100	57	51	62	61	76	100	62	46	29	. .
xyl+ str^r	100	26	27	27	20	25	23	38	53	100	. .
lac+ met+	100	81	77	100	48	40	14	0	NT	4	NT
lac+ val^r	100	71	77	100	54	46	13	5	0	3	1

Abbreviations as in Table 1. NT means not tested.

lac$^+$. Furthermore, the *T6*^{s} marker appeared with approximately the same frequency as *UV*^{r} among recombinants selected for *lac*$^+$. As the *lac* marker is closest, of those selected, to the point of entry of W1895, the gradient of frequencies of unselected markers is taken to reflect the order of these markers on the chromosome (JACOB and WOLLMAN 1958); this is the order in which they are arranged in the table. When male markers distal to the point of origin were selected, markers between the point of origin and the selected male markers appeared at approximately equal frequencies, whereas those distal to the selected marker exhibited a gradient of frequencies. Exceptions to this observation were the high frequencies with which *met*$^-$ and *val*^{s} appeared among recombinants selected for *mal*$^+$ *str*^{r}; and those with which *met*$^-$ appeared among *xyl*$^+$ *str*^{r} recombinants. These exceptions probably indicate linkage between the markers involved.

One hundred each of the following recombinants were also selected and tested for unselected markers: *ara*$^+$ *met*$^+$; *ara*$^+$ *val*^{r}; *mal*$^+$ *met*$^+$; *mal*$^+$ *val*^{r}; *xyl*$^+$ *met*$^+$ and *xyl*$^+$ *val*^{r}. The results of these tests confirmed the order of markers established in Table 3.

The gradient of transmission of the male markers suggested that the *UV* locus was close to the point of entry of the male chromosome and more closely linked to *T6* and *lac* than to the other markers. In crosses between W1895 and B PAM 7 selected for *lac*$^+$, the frequency of crossing over between *UV* and *T6* was 7 percent in the 600 recombinants examined.

To obtain a more conclusive demonstration of the order of the three markers, *lac*, *T6* and *UV*, crosses were done in which the outside distal marker *mal*$^+$ was selected from the Hfr parent, and the progeny examined for quadruple crossover types. If the order of the markers were *lac T6 UV*, a quadruple crossover would result in progeny of the genotype *lac*$^+$ *T6*^{r} *UV*^{r} or *lac*$^-$ *T6*^{s} *UV*^{s}. If the order were *lac UV T6*, the quadruple crossover types would be *lac*$^+$ *UV*^{s} *T6*^{s} and *lac*$^-$ *UV*^{r} *T6*^{r}. If *UV lac T6* were the correct order, the corresponding types would be *UV*^{s} *lac*$^+$ *T6*^{r} and *UV*^{r} *lac*$^-$ *T6*^{s}. It can be seen from Table 4 that the most likely order is *lac T6 UV* because the quadruple crossover type predicted for this order occurred least frequently.

Crosses of W4531 × B: In Table 5 is shown the analysis of recombinants resulting from crosses between W4531 and various derivatives of strain B. When the proximal marker *ara*$^+$ was selected, the gradient of frequencies of unselected

TABLE 4

Frequency of UV, T6, lac phenotypes among recombinants between W1895 × B PAM 7 selected for distal markers (met$^+$, mal$^+$)

Possible order of markers:	*lac*	*T6*	*UV*	*lac*	*UV*	*T6*	*UV*	*lac*	*T6*
Quadruple crossover types	+	r	r	+	s	s	s	+	r
		and			and			and	
	—	s	s	—	r	r	r	—	s
Number observed among 400 selected *mal*+ *met*+ recombinants:	40	(10%)		80	(20%)		140	(35%)	

TABLE 5

Frequency of occurrence of donor markers among recombinants between W4531 and B

W4531 × Female parent	Recombinants selected	Frequency of donor markers among recombinants (percent)							
		ara+	*T1*^{s}	*lac*+	*T6*^{s}	*UV*^{r}	*gal*+	*xyl*+	*mal*+
B PAM 3	*ara*+ *thi*+	100	. .	57	. .	53	.	.	0
B PAM 4	*ara*+ *thi*+	100	. .	73	61	66	.	.	0
B PAM 5	*ara*+ *thi*+	100	75	60	53	53	.	.	0
B PAM 6	*ara*+ *thi*+	100	69	56	59	49	.	0	0
B PAM 7	*ara*+ *thi*+	100	78	63	58	59	1	0	0
B PAM 5	*lac*+ *thi*+	69	62	100	78	73	.	.	.
B PAM 6	*lac*+ *thi*+	69	68	100	77	82	.	.	.
B PAM 7	*lac*+ *thi*+	60	52	100	74	79	.	.	.

markers established that the order of markers was as expected for this Hfr, *ara T1 lac T6*. The frequency of the *UV*^{r} marker was slightly less than that of the *lac*+ marker and approximately the same as the *T6*^{s} marker, thus confirming the close linkage between *T6* and *UV* loci without, however, clarifying the position of *T6* and *UV* relative to *lac*. Only one *gal*+ recombinant was obtained so it was not possible to analyze recombination frequencies among *lac T6* and *UV* in recombinants selected for outside distal markers.

Transfer of resistance to 1-methyl-3-nitro-1-nitrosoguanidine: One hundred recombinants of a cross between W1895 and B PAM 7, selected for *ara*+ *met*+, were analyzed for UV resistance and, by the gradient plate technique, for resistance to 1-methyl-3-nitro-1-nitrosoguanidine. Only two classes were observed: 55 which were radioresistant and as resistant to the nitrosoguanidine as W1895; and 45 which were radiosensitive and as sensitive to the nitrosoguanidine as B PAM 7. There were no recombinants observed which were intermediate in resistance to the nitrosoguanidine, none were significantly more resistant to the chemical than W1895, nor were any more sensitive than B PAM 7.

One hundred recombinants of a cross between W4531 and B PAM 7, selected for *ara*+ *thi*+, were analyzed for resistance to UV and to the nitrosoguanidine. Again only two classes were observed: 46 which were radioresistant and as resistant to the chemical as W4531 and 54 which were radiosensitive and as sensitive to the chemical as B PAM 7.

DISCUSSION

It is clear that there is a locus for radiation resistance in *E. coli* closely linked to *T6* and *lac*, and closer to *T6* than to *lac*. The position of the *UV* locus relative to *T6* and *lac* has not been unequivocally determined but the evidence seems to favor the arrangement *lac T6 UV*. A marker more closely linked to *T6* and *UV* than is *lac* should provide conclusive evidence on this point.

It is also clear that resistance to at least one radiomimetic agent is transferred concomitantly with resistance to ultraviolet radiation. Because all radioresisant

mutants of *E. coli* B (GREENBERG and WOODY-KARRER 1963) and *E. coli* S (WOODY-KARRER and GREENBERG 1963) exhibit an increase in resistance to radiomimetic chemicals and because radiosensitive mutants of K-12 display increased sensitivity to radiomimetic chemicals, it is more likely that one locus determines resistance to both kinds of agents than that there are two closely linked loci involved.

It has been possible to obtain mutants of *E. coli* B more sensitive to radiation than the parent (HILL 1958; HILL and SIMSON 1961; RÖRSCH, EDELMAN, VAN DER KAMP and COHEN 1962). There have, in fact, been described two distinct types of radiohypersensitive mutants of *E. coli* B. One type, including strains B_{s-1} (HILL 1958) and B_{III} (RÖRSCH *et al.* 1962), is characterized by an inability to allow the recovery of UV irradiated T1 and other coliphages. In the second type, exemplified by strain B_{s-2} (HILL and SIMSON 1961), recovery of irradiated T1 phage occurs as it does in *E. coli* B and K-12. The radiosensitivity of strain B_{III} has been attributed by RÖRSCH, EDELMAN and COHEN (1963) to a gene (*syn*) located between *str*^{r} and *xyl* (Figure 2). The locus for radiation sensitivity in strain B_{s-1} has been investigated in our laboratory and has not been found in any part of the chromosome of *E. coli* extending from *str* to *T6*. The locus for radiosensitivity of strain B_{s-2}, on the other hand, is very closely linked to the *mal* locus of strain B. HOWARD-FLANDERS *et al.* (1962) obtained a radiosensitive mutant of K-12 by enrichment of the population of bacteria in which irradiated T1 phage failed to recover. This strain, AB1886, which has many properties in common with B_{s-1} (GREENBERG and WOODY-KARRER 1963) was shown by HOWARD-FLANDERS *et al.* (1962) to be the result of a mutation in a gene (*UV*) located between *ara* and a locus for arginine synthesis (*arg*). Therefore, mutations at three different loci, the *UV* locus of HOWARD-FLANDERS *et al.* (1962) the *syn* locus of RÖRSCH *et al.* (1963) and that of strain B_{s-1} produce similar radiosensitive phenotypes. The locus in strain B_{s-2} appears to differ from any of these. The *UV* locus described in this report is still different, but may be the same as the one described by ADLER (1963) closely linked to *T6*. There are, then, at least five different loci controlling UV resistance in *E. coli*. It would appear that *all* of these loci must have a resistance allele in order for the phenotype to express maximum radiation resistance as in W1895 or B/r.

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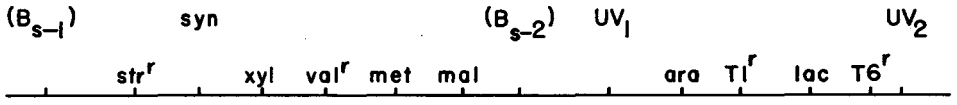

FIGURE 2.—Loci of UV resistance markers in *E. coli*. Distance between markers is arbitrary. The exact location of markers in parentheses has not been determined. *syn:* (RÖRSCH, *et al.* 1962, RÖRSCH, *et al.* 1963); UV_1: (HOWARD-FLANDERS, *et al.* 1962: subscript added to distinguish from UV_2); UV_2: (this report); (B_{s-1}) and (B_{s-2}) signify the loci controlling UV resistance in these two strains (in preparation).

SUMMARY

Radiation resistance has been transferred by genetic recombination from *Escherichia coli* strain K-12 radioresistant donors to strain B radiosensitive recipients. The locus for radioresistance in K-12 is closely linked to the locus for resistance to phage T6. Resistance to the radiomimetic agent 1-methyl-3-nitro-1-nitrosoguanidine is transferred concomitantly with resistance to ultraviolet radiation.

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