Genet. Res., Camb. (1968), **12**, *pp.* 55–63
With 4 text-figures
Printed in Great Britain

Genetic studies of strain Bs8 of *Escherichia coli*

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(*Received* 19 *February* 1968)

1. INTRODUCTION

Strain Bs8 of *Escherichia coli*, an ultraviolet-radiation(u.v.)-sensitive mutant of strain B, is unable to reactivate irradiated phage (HCR), cannot be induced by u.v. to form filaments (Hill & Feiner, 1964), and is not as sensitive to thymineless death as strain B (Cummings & Mondale, 1967). Its u.v. sensitivity is the result of a mutation in a gene (*uvr8*) linked to *gal*.* It differs from other HCR u.v.-sensitive mutants of strain B, e.g. Bs3 (*uvr3*) and Bs12 (*uvr12*) in its failure to form filaments after irradiation; it is less sensitive to u.v. than Bs12 but similar to Bs3. When the *uvr8* was co-transduced by phage P1 with *gal*$^+$ into u.v.-resistant strain B/r, the transductants were no more resistant to u.v. than Bs8. In this respect Bs8 differed from Bs3; when *uvr3* was cotransduced with *his*$^+$ into strain B/r, the transductants were much more resistant to u.v. than Bs3 (Donch & Greenberg, 1968*a*).

Strain B is itself sensitive to u.v. when compared with most wild-type strains of *E. coli*. Its sensitivity to u.v. is the result of a gene linked to T6 (Greenberg, 1964) and cotransducible with *proC* into strain K-12 (Donch & Greenberg, 1968*a*). These transductants were not only filament-inducible but also mucoid.† The phenotype produced by the *lon* mutation in strain B is similar to that of the *lon* mutants isolated by Howard-Flanders, Simson & Theriot (1964) and the *capR* mutants of Markovitz (1964) and Markovitz & Baker (1967). In strain B one of the phenotypic expressions of *lon*, namely mucoidy, is suppressed, presumably by a mutation elsewhere which limits the synthesis of mucoid polysaccharide.

The mutation from B to B/r not only reduces sensitivity to u.v. but also turns off u.v.-induced filaments (Witkin, 1947) and reduces sensitivity to thymineless death (Cummings & Mondale, 1967). Bs8 might be expected to behave as it does with respect to filament induction, u.v. sensitivity, and reduced sensitivity to thymineless death if it were, in fact, a double mutant, i.e. if it were *uvr8* in a B/r background.

This report will show that when *uvr8* was cotransduced with *gal*$^+$ into a *gal lon*

* Abbreviations, unless otherwise stated, are those recommended in Demerec, Adelberg, Clark & Hartman (1966).

† Most strains of *E. coli* are mucoid, i.e. colonies are large, spreading and watery when grown for 3 or more days on minimal medium at 30 °C. At higher temperatures and on complex media they tend to form glistening colonies, which, however, are not spreading or watery. As used in this report, mucoid strains produce large, spreading watery colonies even at 37 °C on minimal medium, and some mucoid strains form such colonies on complex media.

K-12 derivative the transductants were significantly more sensitive to u.v. than Bs8 and were filament inducible. Nevertheless, strain Bs8 contains a *lon* gene which is transducible with *proC*+ producing in a K-12 recipient a phenotype which is u.v.-sensitive, filament-inducible, and mucoid.

2. MATERIALS AND METHODS

Bacteria. Bacterial strains used are described in Table 1.

Phage. P1 *kc* was supplied to us by Dr C. Yanofsky and from this we isolated a virulent mutant, P1 *vir*, which overcomes immunity of P1 lysogenic strains and produces large, clear plaques.

P1 *vir* has a low efficiency of plating on strain B. Therefore, it was grown by the overlay method on strain B. After two cycles of plating on strain B, a high-titre preparation for strain B was obtained, and this grew efficiently on K-12 strains. This strain was used in all experiments, so we shall refer to it as P1. P1 was grown on donor strains for at least two cycles and harvested as described in Adams (1959).

Table 1. *Characteristics of bacterial strains used*

(Relevant markers only.)

Strain	*gal*	*lacZ*	*proC*	*purE*	*tsx*	*str*	*Fil*	*Mu*	Source
B251	+	+	+	+	+	+	+	−	W. Arber
Bs8	+	+	+	+	+	+	−	−	R. Hill
PAM 2011	−	+	+	+	+	−	+	+	Recombinant HfrH × AB1899
PAM 503	−	+	+	+	+	−	−	−	Recombinant HfrH × AB1157
χ478	+	−	−	−	−	−	−	−	R. Curtiss III
HB 45	−	−	+	+	−	−	−	−	H. Boyer
AB 1899	−	−	+	+	−	−	+	+	P. Howard-Flanders
AB 1157	−	−	+	+	−	−	−	−	E. A. Adelberg

Abbreviations are as recommended by Demerec, Adelberg, Clark & Hartman (1966). *Fil*+ means forms long filaments following u.v. irradition, *Mu*+ means colonies produce large amounts of mucoid material at 37 °C on minimal medium. For *tsx* and *str* + is sensitive and − is resistant.

Media. The minimal medium used for selection in transduction experiments was Davis Minimal (DM) Broth (Difco) to which Noble Agar (Difco) was added at a final concentration of 2 %, and glucose, lactose or galactose at a concentration of 0·5 %, and streptomycin to prevent contamination, at 200 μg/ml. Amino acids were used at a concentration of 50 μg/ml, vitamin B_1 at a concentration of 0·17 μg/ml. The purine requirement was satisfied interchangeably by adenine, adenosine, guanine or guanosine at a concentration of 5 μg/ml.

Complete DK broth consisted of tryptone 5 g, NaCl 5 g, yeast extract 5 g and glucose 1 g per litre of deionized water. Viable counts, survival curves and filament formation studies were done on this medium, without glucose, solidified with 1·5 % Bacto Agar (Difco).

Transductions. Transductions were performed using bacteria grown overnight

with aeration in DK broth supplemented with $2{\cdot}5 \times 10^{-3}$ M-$CaCl_2$, diluted 1:10 into fresh broth of the same composition and incubated for 2 h with aeration (about 8×10^8 cells/ml). All incubations were at 37 °C. At this time P1 was added to give a multiplicity of infection of 0·05–0·1. Adsorption was allowed to proceed without shaking for 30 min, after which the adsorption mixture was centrifuged at approximately 5000 rev./min for 15 min. The supernatant was decanted and used to determine the percentage of phages adsorbed. The pellet was resuspended in water, appropriately diluted, and 0·1 ml amounts spread on to selective media.

Table 2. *Linkage relationship among* lacZ$^+$proC$^+$purE$^+$ *and gene controlling u.v. sensitivity and mucoidy*

Selected marker†	Number examined	Number tested	Frequency of unselected donor markers (%)*					
			lacZ	*proC*$^+$	*tsx*$^+$	u.v.‡	*Mu*	*purE*$^+$
lacZ$^+$	1345	300	100	100	9	0	0	0
proC$^+$	1605	.	9	100	17	4	4	0
Non-mucoid	.	300	9	100	13	0	0	0
Mucoid	.	51	0	100	100	100	100	0
purE$^+$	1758	.	0	0	0	0·1	0·1	100
Non-mucoid	.	300	0	0	0	0	0	100
Mucoid	.	3	0	0	0	100	100	100

* Data represents the average of four experiments.
† P1 donor was Bs8 and the recipient was χ478.
‡ U.v. means sensitive to ultraviolet light.

As controls, P1 lysates were spotted on DK agar to test for bacterial sterility, and to test for reversions an aliquot of recipient cells was treated as experimental cells but without P1. Bacterial survival was determined by plating on complete medium appropriate dilutions of cells before adding P1 and also at the end of the adsorption period. All selection plates were incubated from 2–5 days. Transduction to prototrophy or to Lac$^+$ were followed by purification at least once on selective medium.

Filament formation. Filaments were induced by first growing cells overnight with shaking at 37 °C in DM broth plus supplements required by the strain. After overnight growth the cultures were diluted in supplemented DM broth, 1:50 or 1:100, depending upon growth rate, and incubated with shaking until a titre of approximately $1–4 \times 10^8$ cells/ml was reached; 1·0 ml amounts were placed in 60 mm Petri plates and exposed to 75 ergs/mm^2 of ultraviolet light from a Westinghouse germicidal lamp producing 15·4 ergs/mm^2/s at the distance of 51·5 cm. Appropriate dilutions were spotted on to ringed slides (Perma-slides, Progressive Laboratory Specialties, Inc.) set in a 150 mm Petri plate. Slides were held above the bottom of the Petri plate by applicator sticks. A moist filter paper on the bottom of the Petri plate prevented drying of the spots. The entire assembly was covered by the Petri cover and incubated for 2 h. The slides were then examined under × 100 magnification for the presence of elongated cells. Under these conditions filamentous cells were at least 4 to 5 times normal cell length.

Alternatively diluted, irradiated (75 ergs/mm²) cells were spotted on to DK agar, incubated for 3 h, and then examined under × 100 magnification. Under these conditions filamentous cells were from 10 to 50 times normal cell length.

Initial classification of filamentous strains produced by transduction was done coincidentally with testing for ultraviolet sensitivity. The rapid streak method described in Greenberg (1964) was used. U.v.-irradiated streak plates were incubated overnight and filament formation was determined by microscopic examination of areas within the streaks. Under these conditions filamentous cells were 3–4 times normal cell length.

Ultraviolet survival curves. Ultraviolet survival curves were performed by the methods described in Greenberg (1967) with the exception that log phase cells were used instead of stationary cultures.

Irradiation of approximately 1·5 ergs/mm²/s was achieved by use of a voltage regulator coupled with a rheostat and a dial set to achieve this dosage.

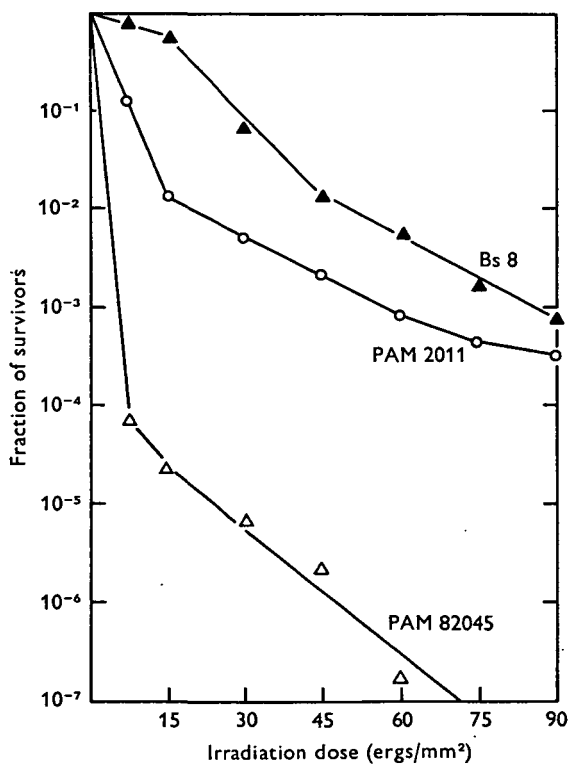

Fig. 1. U.v. survival curve of a transductant, PAM 82045, of PAM 2011 by P1·Bs8, selecting *gal*⁺, u.v. sensitivity, HCR being an unselected marker. U.v. survival curves of parental strains are shown.

3. RESULTS

(i) *Phenotype of* uvr8 lon *genotype*

The question was asked: Is Bs8 a *lon* strain like parental B, keeping in mind that some of the expression of the Lon phenotype can be suppressed by mutations elsewhere in the chromosome (Donch & Greenberg, 1968*b*). For instance, *exr* mutations turn off induction of filaments by u.v. (Hill & Feiner, 1964; Donch & Greenberg, 1968*a*); strain B/r, which is u.v.-resistant and non-filamentous, is nevertheless *lon*, the Lon phenotype being suppressed by a mutation in the gene *rad* (Donch, Chung & Greenberg, 1968).

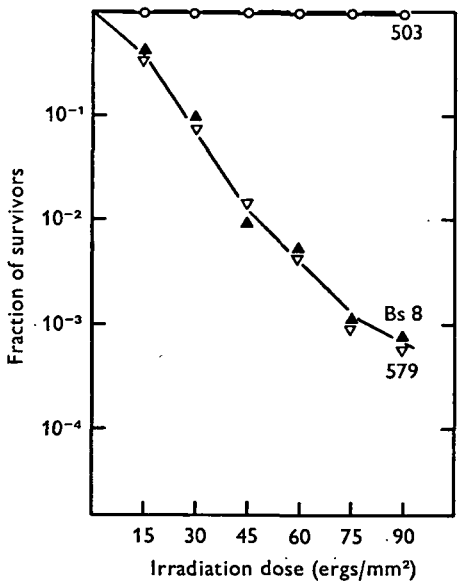

Fig. 2

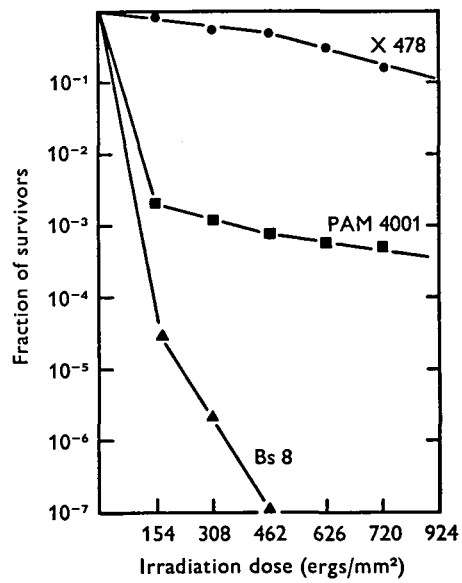

Fig. 3

Fig. 2. U.v. survival curve of transductant, PAM 579, of PAM 503 by P1·Bs8, selecting *gal*+. Survival curves of parental strains shown.

Fig. 3. U.v. survival curve of a transductant, PAM 4001, of χ478 by P1·Bs8 selecting *proC*+, u.v. sensitivity being an unselected marker. Survival curves of parental strains are shown.

In order to test the properties of *uvr8* in a background of a strain known to be *lon*, P1·Bs8 was used to transduce *gal*+ to strain PAM 2011. This K-12 derivative was used because we have not been able to isolate a non-leaky *gal* derivative of strain B. Strain PAM 2011 is a *lac*+ recombinant of strain AB 1899, a *lon* strain isolated by Howard-Flanders, Simson & Theriot (1964). Phenotypically it is u.v.-sensitive (Fig. 1), mucoid, and becomes filamentous on irradition with u.v. One hundred *gal*+ transductants were examined for u.v.-sensitivity by the rapid streak method using 75 and 225 ergs/mm²/s of u.v. Eighteen per cent were found to be more sensitive to u.v. than either Bs8 or PAM 2011. Definitive survival curves were performed on three of these u.v.-hypersensitive transductants, one of

which, PAM 82045, is shown in Fig. 1. They were observed to be more sensitive to u.v. than either parent strain. They were also HCR, mucoid, and formed filaments after irradition with u.v.

P1·Bs8 was also used to transduce *gal*$^+$ into PAM 503, a recombinant of AB 1157, the u.v.-resistant *lon*$^+$ parent of AB 1899. Fifteen per cent of the *gal*$^+$ transductants were more sensitive to u.v. than PAM 503, were HCR, and were considered to have acquired *uvr8*. These, however, could not be distinguished from Bs8 itself in u.v. sensitivity (Fig. 2), nor could they be distinguished from derivatives of strain B/r (HB 45) into which *uvr8* had been transduced (see Fig. 3; Donch & Greenberg, 1968*a*). Bs8 behaved as though it contained a *uvr8* gene in either a *lon*$^+$ or *rad* background.

(ii) *The* lon *gene in Bs8*

Filament induction in Bs8 might be suppressed, if it were genetically *lon*$^+$. The *lon* locus is cotransducible with *proC* (Donch & Greenberg, 1968*b*). Therefore, P1·Bs8 was used to transduce *proC*$^+$, as well as *lacZ*$^+$ and *purE*$^+$, into χ478 (Table 2). The cotransduction frequency of *lacZ*$^+$ and *proC*$^+$, the lack of reciprocity in cotransduction between these markers, and the frequency of cotransduction of

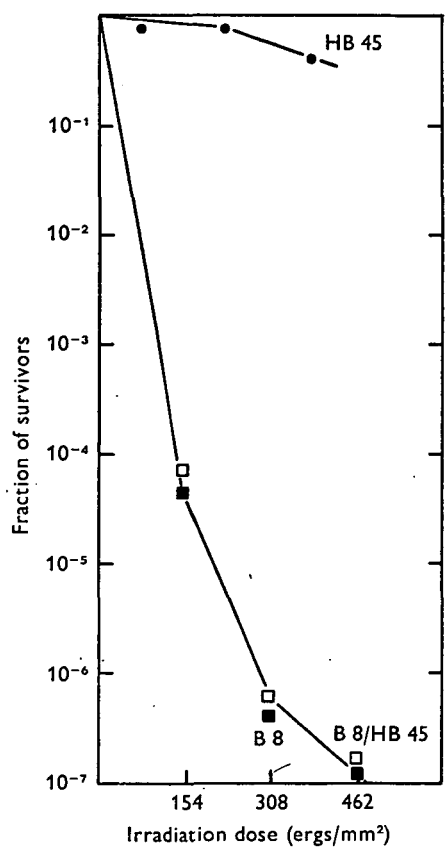

Fig. 4. Figure redrawn from data previously published in Donch & Greenberg (1968*b*).

proC$^+$ and *tsx* are similar to previous results in which strain B was donor and χ478 recipient (Donch & Greenberg, 1968*b*). The significant observation is that 4 % of the *proC*$^+$ transductants and 0·1 % of *purE*$^+$ transductants were mucoid. All the mucoid transductants tested, a typical example being PAM 4001 (Fig. 4), were about as sensitive to u.v. as PAM 2011. They were also induced to form filaments by u.v. irradiation. Strain Bs8 was therefore *lon*.

To test whether the *lon* mutations of Bs8 and B were in the same cistron, P1·Bs8 was used to transduce *proC*$^+$ to PAM 43, a derivative of χ478 in which the *lon* locus of strain B had been cotransduced with *purE*$^+$ *proC* being conserved (Donch & Greenberg, 1968*a*). All of 1100 *proC*$^+$ transductants of PAM 43 examined on the primary selection plates were mucoid. Two hundred isolated clones tested for u.v. sensitivity were as sensitive as PAM 43. There were no non-mucoid, u.v.-resistant transductants. Reciprocal experiments were performed using P1·B251 and PAM 4500, a derivative of χ478 in which the *lon* locus of Bs8 had been co-transduced with *purE*$^+$ and *proC* conserved, as a recipient. No non-mucoid, u.v.-resistant transductants were found. It was concluded that the *lon* mutation, of Bs8 and B were probably the same.

(iii) *The* exr$^+$ *locus in strain Bs8*

A mutation at the *exr*$^+$ locus, linked to *metA* and *malB*, suppresses filament formation and increases u.v. sensitivity in strain B (Hill & Feiner, 1964; Donch & Greenberg, 1968*a*). P1·Bs8 was used to transduce PAM 2011 (*metA*) to Met$^+$. As expected from previous experience (Chung & Greenberg, 1968), 11 % of the transductants were *mal*. However, none of the transductants were u.v.-sensitive. Since *exr* is linked to *metA* at a frequency of about 5 % and to *malB* at a frequency of $\geqslant$ 70 %, Bs8 does not carry a *malB*-linked *exr* mutation.

4. DISCUSSION

We conclude from these observations that Bs8 is a double mutant of strain B. One of the mutations, *uvr8*, linked to *gal*, is possibly the same as *uvrB* (Howard-Flanders, Boyce & Theriot, 1966), and determines the u.v. sensitivity and HCR properties. The other mutation is in a gene not yet located. This second mutation suppresses certain Lon properties such as the u.v. induction of filaments and u.v. sensitivity. The *uvr8* gene itself in the Lon background of PAM 2011 did not suppress the Lon phenotype: the *lon uvr8* transductants were filamentous, and much more sensitive to u.v. than the corresponding *lon*$^+$*uvr8* transductants of PAM 503 or Bs8 itself. The phenotypes of the *uvr8* transductants of PAM 503, B/r (HB 45) and Bs8 itself are almost indistinguishable in u.v. sensitivity and non-inducibility of filaments. Nevertheless, the genotype of Bs8 is *lon uvr8*, since the *lon* mutation could be cotransduced with *proC*$^+$ to χ478, producing a typical Lon phenotype: u.v.-sensitive, filament-inducible and mucoid.

Of the possible mutations by which the expression of the Lon phenotype can be suppressed, we have excluded a mutation at the *exr* locus. The Lon phenotype can

also be suppressed by a mutation such as that in B/r. This strain is also *lon*, its Lon properties being suppressed by a mutation in a gene, *rad*, which has been approximately located between *lac* and *ara* (Donch, Chung & Greenberg, 1968). Bs8 might well be mutant in the *rad*$^+$ locus and would then be the equivalent to strain B/r containing *uvr8*. This would be the least complicated explanation of the Bs8 phenotype, though we have not excluded the possibility that filament inducibility may be suppressed in Bs8 by a mutation at another as yet unidentified locus.

Because Bs8 is a double mutant, one mutation suppressing certain of the Lon properties (u.v. sensitivity and filament formation), it is appropriate to comment on the observation of Cummings & Mondale (1967) on the lack of another expression of the Lon phenotype in strain Bs8. They observed that strain B was more sensitive to thymineless death than strain B/r. They also observed that most of the u.v.-sensitive mutants of strain B (Hill & Simson, 1961; Hill & Feiner, 1964) were less sensitive to thymineless death than strain B itself. Bs2, 4, 5, 6, 7, 9 and 10, all HCR$^+$ and not induced to form filaments after u.v., are *exr* mutants (Donch & Greenberg, 1968*a*). It can be concluded that mutations at this locus produce not only increased u.v. sensitivity but also suppress the Lon properties: filament induction and hypersensitivity to thymineless death. Of the five HCR mutants of strain B, three, namely Bs1, 3 and 8, are less sensitive to thymineless death than strain B. It is probably the *exr* mutation in Bs1 (Donch & Greenberg, 1968*a*; Chung & Greenberg, 1968) which accounts for its relative insensitivity to thymineless death. It seems likely that the mutation in Bs8 which suppresses other Lon properties also suppresses its sensitivity to thymineless death. Sensitivity to thymineless death would then be a function of the *lon* mutation, and mutations which suppress the tendency to form filaments on u.v. irradiation also suppress sensitivity to thymineless death. The HCR mutations *uvr12*, linked to *mal B*, or the *uvr8* locus, linked to *gal*, do not suppress filament formation in a Lon background. According to Cummings & Mondale (1967), sensitivity to thymineless death is not suppressed in Bs12. We would predict it would not be suppressed by the *uvr8* mutation alone and, in fact, have evidence that this is so. This leaves unexplained strain Bs3, in which filaments can be induced by u.v. but which is not hypersensitive to thymineless death. Strain Bs3 is the only example of a dissociation of sensitivity to thymineless death from filament formation. Its HCR phentoype is the product of the mutation *uvr3*, linked to *his* (*uvrC*?) (Donch & Greenberg, 1968*b*). It is possible that Bs3 contains another mutation which suppresses sensitivity to thymineless death or that the dissociation is a property of the *uvr3* gene itself.

SUMMARY

Strain Bs8 is a u.v.-sensitive derivative of strain B. It is unable to reactivate irradiated phage (HCR) and it cannot be induced to form filaments. The HCR properties are attributable to a gene, *uvr8*, cotransducible with *gal*$^+$. When *uvr8* was transduced into radiation-resistant strain B/r the resulting phenotype was indistinguishable from Bs8. When transduced into a *lon* K-12 strain the phenotype

was more sensitive than Bs8, filament-inducible and mucoid. When P1·Bs8 was used to transduce *proC*$^+$ into a *proC* K-12 strain, 4% of the transductants were *lon*, i.e. about as u.v.-sensitive as *lon* K-12, filament-inducible and mucoid. Radiation resistance could not be transduced from strain Bs8 with *proC*$^+$ into a *proC lon* K-12 derivative. Nor did Bs8 show any evidence of being *exr*. Bs8 is a double mutant of strain B, behaving as though it were HCR in a B/r background.

This work was carried out under Public Health Service Grant CA 05687-06. J. Greenberg is the recipient of a Public Health Service Career Development Award.

REFERENCES

ADAMS, M. H. (1959). *Bacteriophages*. New York: Interscience Publishers, Inc.

CHUNG, Y. S. & GREENBERG, J. (1968). Genes affecting sensitivity to ultra-violet light in the *malB* region of the chromosome of *Escherichia coli*. *Genetics* (in the Press).

CUMMINGS, D. J. & MONDALE, L. (1967). Thymineless death in *Escherichia coli*: strain specificity. *J. Bact.* **93**, 1917–1924.

DEMEREC, M., ADELBERG, E. A., CLARK, A. J. & HARTMAN, P. E. (1966). A proposal for a uniform nomenclature in bacterial genetics. *Genetics* **54**, 61–76.

DONCH, J., CHUNG, Y. S. & GREENBERG, J. (1968). Locus for radiation resistance in *Escherichia coli* strain B/r (in the Press)

DONCH, J. & GREENBERG, J. (1968*a*). Loci of radiation sensitivity in Bs strains of *Escherichia coli*. *Genet. Res.* (in the Press).

DONCH, J. & GREENBERG, J. (1968*b*). The UV sensitivity gene of *Escherichia coli* strain B. *J. Bact.* (in the Press).

GREENBERG, J. (1964). A locus for radiation resistance in *Escherichia coli*. *Genetics* **49**, 771–778.

GREENBERG, J. (1967). Loci for radiation sensitivity in *Escherichia coli* strain Bs – 1. *Genetics* **55**, 193–201.

HILL, R. F. & FEINER, R. R. (1964). Further studies of ultraviolet-sensitive mutants of *Escherichia coli* strain B. *J. gen. Microbiol.* **35**, 105–114.

HILL, R. F. & SIMSON, E. (1961). A study of radiosensitive and radio-resistant mutants of *Escherichia coli* strain B. *J. gen. Microbiol.* **24**, 1–14.

HOWARD-FLANDERS, P., BOYCE, R. P. & THERIOT, L. (1966). Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. *Genetics*, **53**, 1119–1136.

HOWARD-FLANDERS, P., SIMSON, E. & THERIOT, L. (1964). A locus that controls filament formation and sensitivity to radiation in *Escherichia coli* K-12. *Genetics* **49**, 237–246.

MARKOVITZ, A. (1964). Regulatory mechanisms for synthesis of capsular polysaccharide in mucoid mutants of *Escherichia coli* K 12. *Proc. Natn. Acad. Sci. U.S.* **51**, 239–246.

MARKOVITZ, A. & BAKER, B. (1967). Suppression of radiation sensitivity and capsular polysaccharide synthesis in *Escherichia coli* K 12 by ochre suppressors. *J. Bact.* **94**, 388–395.

WITKIN, E. M. (1947). Genetics of resistance to radiation in *Escherichia coli*. *Genetics* **32**, 221–248.