Genet. Res., Camb. (1968), **11**, *pp.* 183–191
With 1 *text-figure*
Printed in Great Britain

Loci of radiation sensitivity in Bs strains of *Escherichia coli**

By JOHN DONCH and JOSEPH GREENBERG†
Palo Alto Medical Research Foundation, 860 *Bryant Street*,
Palo Alto, California 94301

(*Received* 20 *November* 1967)

1. INTRODUCTION

Hill & Simson (1961) isolated a series of mutants of *Escherichia coli* strain B more sensitive to ultraviolet light than their parent. Four of these, Bs1, 3, 8 and 12, were unable to reactivate u.v.-irradiated coliphage and were therefore classified as host cell reactivation negative (HCR). Bs3 and 12 became filamentous on irradiation, Bs1 and Bs8 did not. The other mutants were HCR+, differing among themselves in the precise shape of their u.v. survival curves and in their sensitivity to crystal violet. Some of the mutants, particularly Bs1 and Bs2, have been widely used in experiments on the mechanism of u.v. resistance. We therefore undertook to map the genes in these mutants responsible for u.v. sensitivity.

It has already been shown that u.v. sensitivity of strain Bs1 is the result of two mutations, one at a gene linked to the *gal*‡ locus, which also confers the HCR property; the other linked to the *malB* region (Mattern, Zwenk & Rörsch, 1966; Greenberg, 1967). The gene for Bs2 is also linked to *malB* (Greenberg, 1964) and is probably isogenic with one of the genes of Bs1.

In this report we shall show that the genes responsible for u.v. sensitivity in Bs4, 5, 6, 7, 9 and 10 (all HCR+ mutants) are linked to the *malB* region, as is that of Bs12 (HCR); while that of Bs3 is linked to *his*; and that of Bs8 to *gal*. The gene of Bs11 has not been mapped, because it was not possible to transduce *malB*+ into this strain or to obtain *malB*+ recombinants of it. In these respects it acts as a recombination-deficient (*rec*) strain.

2. MATERIALS AND METHODS

(i) *Strains of* Escherichia coli

B251, obtained from Dr W. Arber, is a Mal+λ^s derivative of strain B (Arber & Lataste-Dorolle, 1961). Ultraviolet (u.v.)-sensitive mutants of strain B, Bs2 to Bs12, were obtained from Dr Ruth Hill. Their properties are described in Hill & Simson (1961) and Hill & Feiner (1964). They are all *mal B* λ^r. For purposes of this report we shall refer to the gene responsible for u.v. sensitivity in strain Bs2 as

* This work was carried out under Public Health Service Grant CA 05687-06 from the National Cancer Institute.

† Recipient of a Public Health Service Career Development Award.

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

uvr2, in Bs3 as *uvr3*, etc. HB 45 is a polyauxotrophic derivative of strain B/r (Witkin, 1946, 1947) obtained from Dr Herbert Boyer (1966), which for the purposes of this report requires histidine and is unable to ferment galactose. PAM 206 is a Mal$^+\lambda^s$ transductant of strain Bs2 and is as sensitive to u.v. as strain Bs2. AB 1886, obtained from Dr P. Howard-Flanders, is a polyauxotrophic K-12 mutant whose pertinent marker is *uvrA* (Howard-Flanders, Boyce & Theriot, 1966), which makes the strain u.v.-sensitive and unable to reactivate certain phages after irradiation (HCR) or delete radiation-induced thymine dimers. AB 1911, obtained from Dr E. Adelberg, is a polyauxotrophic derivative of K-12, its relevant markers being *arg metA*. PAM 207 is an *arg*$^+$ transductant of AB 1911, in which *metA* was conserved. W 1895, obtained from Dr J. Lederberg, is a donor strain, HfrC, all the others listed being F$^-$ and *metB*.

(ii) *Phage stocks*

P1*kc* was obtained from Dr C. Yanofsky. From P1*kc* a virulent mutant was isolated in this laboratory. This mutant is able to overcome the immunity of P1 lysogens and produces large, clear plaques. Its ability to transduce and its absorption is similar to parental P1kc. In experiments using AB 1886 as donor, P1kc and P1*vir* were used interchangeably.

The efficiency of plating of P1*kc* or P1*vir* on strain B and derivatives, except B/r, on which it is 10^{-2}, is $\leqslant 10^{-7}$. To obtain high titres of P1 grown on B derivatives, the following procedure was used. About 10^9 plaque-forming units of P1*vir* were plated on B 251. One of the few plaques which developed was picked into 1 ml of broth and its titre estimated on B 251. Plates with confluent or nearly confluent lysis were harvested by scraping the top soft agar overlay as described by Adams (1959). After centrifugation to remove debris and the addition of chloroform to kill residual bacteria, the phage was regrown on B 251. This procedure was repeated twice more, by which time preparations of high titre ($\geqslant 10^{10}$) in strain B derivatives were obtained. The P1 obtained in this way now plated with almost equal efficiency on B derivatives and K-12 derivatives.

λ*vir* was obtained from Dr D. Kaiser and was modified to grow on strain B and derivatives by passage through B 251.

(iii) *Transduction*

Transductions were performed substantially as described by Lennox (1955). Recipient bacteria were grown overnight in tryptone-glucose-$CaCl_2$ broth (tryptone 1 %, NaCl 0·5 %, glucose 0·1 %, $CaCl_2$ $2{\cdot}5 \times 10^{-3}$ M) in a shaking water-bath. All incubations were done at 37 °C. The cultures were diluted 1:10 in fresh broth and grown with shaking to late log. phase or early stationary phase (about 2 h), the viable cell count being about 8×10^8 to 1×10^9/ml. P1 was added at a multiplicity of 0·05–0·1. The preparation was shaken gently in a water-bath for 30 min, centrifuged at 5000 rev/min, and the supernatant decanted and assayed for unabsorbed phage. The pellet was resuspended in water to 10 times original concentration of bacteria; 0·1 ml of this, or appropriate dilutions of it in water, were

plated on selective medium and incubated 2–5 days. Control cultures with no P1 added were treated the same way to determine frequency of revertants. In addition, 0·1 ml of the P1-transducing lysate was spotted on to a plate of complete medium. Viable counts of P1-treated cultures were also made on tryptone agar.

Transductants were streaked on the primary selecting medium, single clones were isolated, grown in tryptone-glucose broth, and tested for unselected markers of the donor.

The selecting medium for transduction to prototrophy was DM (Difco). Mal$^+$ selection was done on minimal medium (DM) in which the carbon source was maltose (Nutritional Biochemical Corp.) instead of glucose at 0·3 %. Auxotrophic properties of unselected markers were tested by spotting overnight cultures on minimal medium deficient in the nutritional requirement or by replication on to such medium. *malB* as an unselected marker was tested by spotting overnight cultures on BTB agar (Brom Thymol Blue broth, Difco) to which was added 1 % maltose and 1·5 % agar, or replication on to such agar (Lederberg & Lederberg, 1952).

(iv) *Ultraviolet sensitivity*

Methods for testing ultraviolet sensitivity of the transductants have been described in detail (Greenberg, 1964). The term u.v. resistance or sensitivity, though it can be and is described quantitatively, is used generically in terms of the relative resistance or sensitivity to the recipient. For example, when a sensitive mutant of B is transduced to u.v. resistance its survival curve resembles that of parental strain B.

(v) *Recombination*

Recombination experiments were performed by conventional methods and, as done in our laboratory, have been described (Greenberg, 1964).

3. RESULTS

(i) *Co-transduction of* mal *and u.v. resistance*

P1 grown on B 251 was used to transduce *malB*$^+$ to each of the Bs strains except Bs11. Repeated attempts to transduce *malB*$^+$ to Bs11 failed. Furthermore, it was not possible to obtain *malB*$^+$ recombinants of Bs11 str^r using W 1895 as donor. Adsorption of P1 to Bs11 was $\geqslant$ approximately 80 %, similar to adsorption of P1 to all Bs strains. However, there were no plaques found on Bs11 when infected with P1·B 251.

In strains where transduction of *malB*$^+$ was observed, 200 or more transductants were purified on selective medium and a single colony of each was transferred to broth and tested for u.v. resistance by the streak method. They were also tested for λ sensitivity by cross-streaking with $\lambda vir{\cdot}B$. All *malB*$^+$ transductants were λ-sensitive. The results of the u.v. sensitivity test are shown in Table 1*a*. No u.v.-resistant transductants were observed when Bs3 or Bs8 were recipients. All the other Bs strains exhibited a frequency of co-transduction of u.v.-resistance and *malB*$^+$ ranging from 52 % for Bs9 to 96 % for Bs4. The latter strain is an auxotroph

Table 1. *Frequency* (%) *of co-transduction of u.v. resistance with* malB+

Donor	No. Mal+ transduc-tants exa-mined	Recipient										
		Bs2 HCR+	Bs3 HCR	Bs4 HCR+	Bs5 HCR+	Bs6 HCR+	Bs7 HCR+	Bs8 HCR	Bs9 HCR+	Bs10 HCR+	Bs11 HCR+	Bs12 HCR
(*a*) B 251	200	86	0	96	94	91	81	0	52	90	—	81
(*b*) AB 1886	200	25	0	40	30	30	29	0	31	26	—	1
(*c*) Bs2 (PAM 206)	400	—	0	0·25	0	0	0	0	0	0	—	0·25

Phage P1 was used to transduce *malB*+ to each Bs strain. Transductants were tested by the streak method for u.v. resistance (231 and 462 ergs/mm²) and were considered resistant if the irradiated streaks were comparable to those of strain B.

requiring proline, with which all Mal minimal plates were supplemented. No Pro$^+$ colonies were observed among the *malB*$^+$ transductants. From these results it was concluded that in only one of the HCR mutants, Bs12, was u.v. resistance co-transducible with *malB*$^+$, whereas each of the u.v.-sensitivity genes of all the HCR$^+$ mutants was co-transducible with *malB*$^+$.

It appeared likely that the u.v.-sensitivity locus of Bs12 was isogenic with *uvrA*, found by Howard-Flanders *et al.* (1966) to be linked with *metA*, and by Schwartz (1966) to be co-transducible with *malB*. To determine if recombination for u.v. resistance were possible between *uvrA* and the Bs *malB*-linked genes, P1·AB 1886 was used to transduce *malB*$^+$ to *E. coli* B and Bs mutants. The results are shown in Table 1*b*. U.v. sensitivity (*uvrA*) was co-transduced with *malB*$^+$ to *E. coli* B at a frequency of 50 %, supporting the observations of Schwartz (1966). With all the HCR$^+$ u.v.-sensitive mutants (except Bs11) 25–40 % of the *malB* transductants were u.v.-resistant; i.e. they had survival properties after u.v. irradiation indistinguishable from parental strain B. No u.v.-resistant *malB*$^+$ transductants were observed with Bs3 or Bs8 and only 1 of 200 *malB*$^+$ transductants of Bs12 was u.v.-resistant. From these results it was concluded that the HCR$^+$ u.v.-sensitivity mutations of the Bs strains were probably not in *uvrA*, but that *uvr12* probably was.

To determine the relationship among the HCR$^+$ Bs strains, P1 grown in a Mal$^+$, but still u.v.-sensitive, transductant of Bs2 (PAM 206) was used to transduce *malB*$^+$ to the other HCR$^+$ Bs mutants. From Table 1*c* it can be seen that none of 400 *malB*$^+$ transductants examined when Bs5, 6, 7, 9 or 10 was recipient were u.v.-resistant; 1 of 400 of Bs4 and 1 of 400 Bs12 were u.v.-resistant. From this it was concluded that *uvr2* was probably a mutation in the same gene as that of *uvr4*, *5*, *6*, *7*, *9* and *10*. However, the failure to observe u.v.-resistant transductants with Bs12 reduced the certainty of the conclusion. Transductants had either Bs2 (HCR$^+$) or Bs12 (HCR) phenotypes at a ratio of approximately 1 : 1. Data in Table 1*b* suggested that *uvrA* and Bs HCR$^+$ u.v.-sensitivity genes were not isogenic, but that *uvr12* and *uvrA* were. This would mean that *uvr12* and *uvr2* were not isogenic; yet no u.v.-resistant clones were detected among Mal$^+$ transductants of Bs12 with Bs2 as donor. To explain this we invoked the possibility that the HCR$^+$ *uvr* gene was more closely linked to *malB* than the HCR *uvr* gene, for which there was evidence in Table 1*a*. With Bs2 as donor and Bs12 as recipient an additional crossover would be needed to obtain u.v.-resistant transductants.

If *uvrA* and *uvr12* are isogenic, then Bs12 as donor should behave like AB 1886 in P1 transduction of *malB*$^+$. When a Mal$^+$ derivative of Bs12, still u.v.-sensitive, was used as donor to transduce *malB*$^+$ to Bs2, 22 % of the Mal$^+$ transductants were u.v.-resistant. The *uvr12* locus is therefore separable by recombinational events from the *uvr2* gene under these conditions. *uvr12* and *uvrA* are phenotypically and genetically similar and therefore are probably isogenic.

According to Schwartz the order of markers in the *malB* region is *metA malB uvrA*. To determine the position of the *uvr* genes relative to *malB* and *metA* we performed a three-point test, transducing *metA*$^+$ to a *metA* recipient (PAM 207), and

examined the transductants for the unselected markers *uvr* and *malB*. Donors were Bs mutants (except Bs3, 8 and 11). Results are shown in Table 2. U.v. sensitivity was co-transducible with *metA*$^+$ in every instance, the frequency ranging from 3·4 to 5·8 %. *malB* was also co-transduced in every instance, the frequency ranging from 4·8 to 9·0 %.

In every series, except where Bs9 was used as donor, the *mal* marker transduced more frequently than *uvr*, establishing the order *metA malB uvr*. Results with Bs9 differed from the others in three respects: the frequency of *uvr* was greater than *mal*; the crossover class *malB*$^+$ *uvr* was larger, the class *malB uvr*$^+$ smaller. The order of markers with Bs9 as donor would appear to be *metA uvr9 malB*.

Table 2. *Frequency of unselected markers*, malB *and u.v. sensitivity among* metA$^+$ *transductants of PAM 207*

Donor	No. Met$^+$ transductants examined	U.v. sensitivity	Frequency (%) of donor markers				
			malB	*mal*$^+$*uvr*$^+$	*mal*$^+$*uvr*$^+$	*maluvr*$^+$	*maluvr*
Bs2	963	3·9	6·7	93·4	0·0	2·7	3·9
Bs4	1110	4·5	9·0	91·5	0·45	4·0	4·1
Bs5	400	4·7	7·0	92·9	0·21	2·4	4·5
Bs6	1000	5·5	8·5	91·1	0·40	3·4	5·1
Bs7	1123	4·7	7·0	93·1	0·0	2·2	4·7
Bs9	931	5·8	4·8	93·9	1·4	0·75	4·4
Bs10	929	5·4	8·6	92·1	0·43	3·5	5·0
Bs12	1281	3·4	6·5	93·3	0·08	3·1	3·3

(ii) *The locus of* uvr3 *and* uvr8

We assumed *uvr3* and *uvr8* might be close to *his* or *gal*, to both of which loci HCR u.v.-sensitivity genes have been found linked (Howard-Flanders *et al.* 1966; Greenberg, 1967). Using P1·Bs3 or P1·Bs8 *his*$^+$ and *gal*$^+$ were each transduced to HB 45. The transductants were examined for the unselected marker, u.v. sensitivity. With Bs3 as donor u.v. sensitivity was found linked to *his* at a frequency of 8 %, but not to *gal*. With Bs8 as donor u.v. sensitivity was not linked to *his* but was co-transducible with *gal* at a frequency of 17 %.

The u.v. survival curves of sensitive transductants of HB 45 were examined. HB 45 transduced with *uvr3* was less sensitive to u.v. than Bs3 (Fig. 1); that is, the *uvr3* gene in a B/r background produced less sensitivity than in a B background. On the other hand *uvr8* in HB 45 (Fig. 1) was no less sensitive than Bs8.

4. DISCUSSION

These data demonstrate that *uvr2*, *4*, *5*, *6*, *7* and *10* are mutants of the same cistron, closely linked to and to the right of *malB* relative to *metA*. The strains in which they occur (Bs2, 4, etc.) are phenotypically similar: u.v.-sensitive, HCR$^+$ and non-filament-forming on irradiation (Hill & Feiner, 1964).

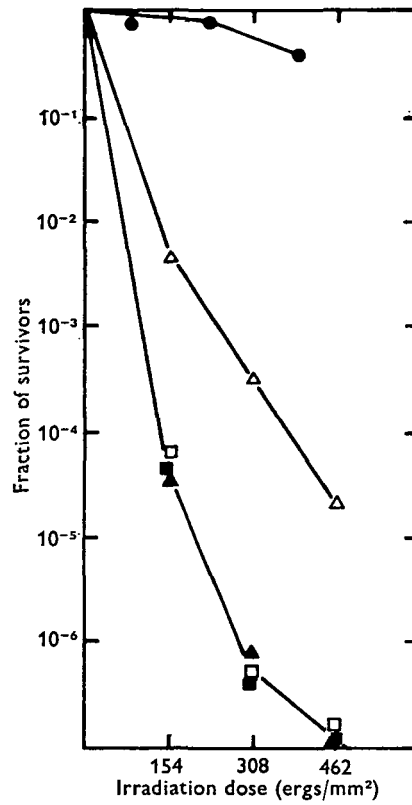

Fig. 1. Survival following u.v. irradiation of strains of *E. coli* Bs3 (▲), Bs8 (■) and HB45 (●); of a *his*$^+$ u.v.-sensitive P1 transductant from Bs3 into HB 45 (△); and of a *gal*$^+$ u.v.-sensitive P1 transductant from Bs8 into HB 45 (□).

Also mutant in this cistron is strain Bs1 (Greenberg, 1967; Mattern *et al.* 1966). Because mutants in this cistron are sensitive to X-rays, Rörsch *et al.* have called it *exr*, which is probably the equivalent of the *lex* cistron of Howard-Flanders & Boyce (1966). To reduce the confusion in nomenclature we shall refer to the cistron of *uvr2*, *4*, etc., as *exr*.

uvr12 is mutant in a cistron also linked to *malB* and to its right. This cistron is probably *uvrA* (Howard-Flanders *et al.* 1966), which is linked to *malB* (Schwartz, 1966, and confirmed here). Mutants in this cistron are u.v.-sensitive, HCR, and, in strain B, retain the property of forming filaments on irradiation (Hill & Feiner, 1964).

The observation that, with P1·AB 1886 or P1·Bs12 (*uvrA*) as donor, u.v. resistance could be co-transduced with *malB*$^+$ at a frequency of about 30 % is taken to mean that *uvrA* and *exr* are not the same. The fact that the reciprocal transduction, *malB*$^+$, transduced via P1·Bs2 (Mal$^+$) into Bs12 yielded no u.v.-resistant transductants indicates that the order of markers is probably *malB exr uvrA*, since with this order an extra crossover would be required to produce u.v.-resistant transductants.

Cartological considerations tempt us into suggesting an operon of the two cistrons involved in u.v. resistance. It is accepted now that u.v. resistance is largely a matter of repair of u.v.-damaged DNA. One step in the repair process is the excision of thymine dimers (Setlow & Carrier, 1964; Boyce & Howard-Flanders, 1964) under the control of three widely separated genes (Howard-Flanders *et al.* 1966). The excised regions might be repaired by a replication repair mechanism described by Pettijohn & Hanawalt (1964). Mutants in the *exr* cistron can excise thymine dimers but may lack a system for replicative repair on some other step subsequent to dimer excision.

On the other hand the *exr* cistron may be related functionally to the gene, probably the same as the *lon* gene of Howard-Flanders, Simson & Theriot (1964) or the *fil* gene of Van de Putte, Westenbroek & Rörsch (1963), responsible for u.v. sensitivity in *E. coli* strain B. All the *exr* mutants of the Bs series turn off u.v.-induced filaments of strain B. Furthermore, these mutants exhibit less sensitivity to thymineless death than strain B (Cummings & Mondale, 1967). And, finally, the *lon* and *exr* mutations are not additive in their effect on u.v. sensitivity (Greenberg, 1964). This would suggest that mutations in *exr* are epistatic to mutations in *lon*, which in turn suggests a sequential action of the two wild-type genes.

SUMMARY

The genes responsible for u.v. sensitivity in ten sensitive mutants of *E. coli* strain B (Bs strains of R. Hill) have been mapped by transduction. The *uvr* genes of all the mutants able to reactivate u.v.-irradiated phage (HCR$^+$), including Bs2, 4, 5, 6, 7, 9 and 10, were linked, 50–95 %, with *malB*. The gene of Bs12 (HCR) was also linked to *malB* as is *uvrA*. The gene of Bs8 (HCR) was linked to *gal*, that of Bs3 (HCR) to *his*. Transduction of *mal*$^+$ from a strain with *uvrA*, or a *mal*$^+$ derivative of Bs12, to Bs2, 4, 5, 6, 7, 9 or 10, yielded about 30 % u.v.-resistant transductants. A *mal*$^+$ transduction with Bs2 *mal*$^+$ as donor and other Bs strains as recipients yielded $< 0{\cdot}1$ % u.v.-resistant transductants. The *malB*-linked *uvr* genes of all mutants (except Bs3 and Bs8) were transducible with *metA*. The quasi-reciprocal crosses and three-point tests suggested the order of markers as *metA malB uvr* (HCR$^+$) *uvr* (HCR). The sensitivity gene of Bs9 was exceptional in that it appeared to lie between *metA* and *malB*. The sensitivity gene of Bs11 could not be mapped because transductants to that strain were not possible, nor did it act as a recipient in sexual recombinations.

The authors are grateful for the excellent technical assistance of Mrs Janet Dougdale-Bradley and to Mrs Jan Neal for her volunteered assistance.

REFERENCES

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

ARBER, W. & LATASTE-DOROLLE, C. (1961). Erweiterung des Wirtsbereiches des Bakteriophagen λ auf *Escherichia coli* B. *Pathol. Microbiol.* **24**, 1012–1018.

BOYCE, R. P. & HOWARD-FLANDERS, P. (1964). Release of ultraviolet light-induced thymine dimers from DNA in *E. coli* K-12. *Proc. natn. Acad. Sci. U.S.A.* **51**, 293–300.

Boyer, H. (1966). Conjugation in *Escherichia coli*. *J. Bact.* **91**, 1767–1772.
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.
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 B_{s-1}. *Genetics* **55**, 193–201.
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.
Hill, R. F. & Feiner, R. R. (1964). Further studies of ultraviolet-sensitive mutants of *Escherichia coli* strain B. *J. gen. Microbiol.* **35**, 105–114.
Howard-Flanders, P. & Boyce, R. R. (1966). DNA repair and genetic recombination: studies on mutants of *Escherichia coli* defective in these processes. *Radiat. Res.* (Suppl.) **6**, 156–184.
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 radition in *Escherichia coli* K-12. *Genetics* **49**, 237–246.
Lederberg, J. & Lederberg, E. M. (1952). Replica plating and indirect selection of bacterial mutants. *J. Bact.* **63**, 399–406.
Lennox, E. S. (1955). Transduction of linked genetic characters of the host by bacteriophage P1. *Virology* **1**, 190–206.
Mattern, I. E., Zwenk, H. & Rörsch, A. (1966). The genetic constitution of the radiation-sensitive mutant *Escherichia coli* B_{s-1}. *Mutation Res.* **3**, 374–380.
Pettijohn, D. & Hanawalt, P. (1964). Evidence for repair-replication of ultraviolet damaged DNA in bacteria. *J. molec. Biol.* **9**, 395–410.
Schwartz, M. (1966). Location of the maltose A and B loci on the genetic map of *Escherichia coli*. *J. Bact.* **92**, 1083–1089.
Setlow, R. B. & Carrier, W. L. (1964). The disappearance of thymine dimers from DNA: an error-correcting mechanism. *Proc. natn. Acad. Sci. U.S.A.* **51**, 226–231.
Van de Putte, P., Westenbroek, C. & Rörsch, A. (1963). The relationship between gene-controlled radiation resistance and filament formation in *Escherichia coli*. *Biochim. biophys. Acta* **76**, 247–256.
Witkin, E. M. (1946). Inherited differences in sensitivity to radiation in *Escherichia coli*. *Proc. natn. Acad. Sci. U.S.A.* **32**, 59–68.
Witkin, E. M. (1947). Genetics of resistance to radiation in *Escherichia coli*. *Genetics* **32**, 221–248.