Genet. Res., Camb. (1969), **14**, *pp.* 111–119
With 6 *text-figures*
Printed in Great Britain

Effect of inhibition of DNA synthesis on u.v. sensitive Bs strains of *Escherichia coli*

BY MICHAEL H. L. GREEN, JOHN DONCH, YOUNG S. CHUNG AND JOSEPH GREENBERG

Palo Alto Medical Research Foundation, Palo Alto, California 94301, *U.S.A.*

(*Received* 26 *March* 1969)

1. INTRODUCTION

In earlier papers (Greenberg, 1967; Donch & Greenberg, 1968*b*, *c*) we examined the genetics of a series of u.v. sensitive (Bs) mutants of *Escherichia coli* B (Hill & Feiner, 1964). Four of the mutants (Bs1, 3, 8 and 12) were known to be unable to reactivate irradiated phage (HCR). The gene responsible for the HCR property* of Bs1 and Bs8 was cotransducible with *gal*$^+$; that for Bs12 with *malB*$^+$ and that for Bs3 with *his*$^+$. The other Bs mutants were HCR$^+$ and the u.v. sensitivity gene of each of them except that of Bs11 was located and found to be cotransducible with *malB*$^+$. These HCR$^+$ mutants were all found to be *exrA* (we shall call them *exrA* to distinguish them genetically from genes (*exr*) responsible for sensitivity to X-rays, but located elsewhere on the chromosome) (Van de Putte, Van Sluis, Van Dillewijn & Rörsch, 1965). Strain Bs11 could not be transduced to *mal*$^+$, nor could sexual recombinants be obtained. Bs11 may be a recombinationless *rec* strain (Donch & Greenberg, 1968*b*).

It was shown that the gene responsible for the u.v. sensitivity and the filamenting properties of strain B was the *lon* gene, which is transducible with *proC*$^+$ (Donch & Greenberg, 1968*a*). We have also shown that the *exrA* gene suppressed the filamenting properties of *lon* mutants whether in a strain B or K 12 background (Donch, Green & Greenberg, 1968). The *exrA* mutants (Bs2, 4, 5, 6, 7, 9, 10) are, therefore, non-filamenting though still *lon*. The genes responsible for the HCR property of strains Bs1, 3, 8 and 12 do not suppress filamentation of *lon* strains. Nevertheless, strains Bs1 and Bs8 are non-filamenting. Both of these have been shown to be doubly mutant from strain B, Bs1 carrying the *exrA* gene, Bs8 carrying a gene which suppresses filamentation and decreases u.v. sensitivity, probably the *sul* gene (suppressor of *lon*) of strain B/r (Donch, Chung & Greenberg, 1969).

Cummings & Mondale (1967) examined the effects of thymine deprivation on the mutants of strain B, including B/r and several of the Bs strains. They found that, whereas strains B and Bs12 were equally sensitive to thymineless death, the other Bs strains were more resistant than B. There was no clearcut relationship between

* In this paper the *uvr* genotype refers to strains of HCR phenotype, apparently, but not proven to be mutated at the *uvrA*, *uvrB* or *uvrC* locus (Howard-Flanders, Boyce & Theriot, 1966).

what was known of the phenotypes of the strains and sensitivity or resistance to death from thymine starvation. Not all HCR strains were sensitive (e.g. Bs1, Bs3 and Bs8); not all strains which could be induced to filament by u.v. were sensitive (e.g. Bs3); and strain Bs4 was exceptionally resistant to thymineless death.

We have re-examined the effect of inhibition of DNA synthesis on the Bs mutants in view of what is now known of their genetics. Since the genetics of strain Bs11 have not been worked out, it will not be discussed in this report. To simplify the procedure we have used nalidixic acid as an inhibitor of DNA synthesis. The effects of nalidixic acid are known to resemble thymine starvation closely (Goss, Deitz & Cook, 1965). It will be shown that the relative sensitivity* of the mutants of strain B to nalidixic acid is similar to their sensitivity to thymine starvation.

In this report we shall furthermore show that all *exrA* mutants of B are less sensitive to the lethal effects of nalidixic acid than strain B. HCR mutations which are known not to suppress filamentation of strain B do not affect the sensitivity of strain B to nalidixic acid. The mutations of strain B which reduce or suppress the tendency to filamentation reduce the sensitivity to nalidixic acid. The anomalously high resistance of the *exrA* strain Bs4 can be explained on the basis of its being an auxotroph that grows poorly under the usual test conditions. The prototrophic derivative of strain Bs4 behaves like other *exrA* strains. Finally, the HCR strain Bs3 is shown to be a double mutant, one gene linked to *his*$^+$, conferring extreme sensitivity to nalidixic acid and a high rate of spontaneous filamentation, while the other suppresses, but not completely, both these effects.

2. MATERIALS AND METHODS

Bacterial strains are shown in Table 1.

Media and treatment with nalidixic acid were described previously (Donch, Green & Greenberg, 1968). Exponentially growing broth cultures were treated with 50 μg/ml nalidixic acid. Strains Bs4*try* and Bs4*try*$^+$ were treated in minimal medium supplemented with 1% vitamin free casamino acids and 80 μg/ml thymine to duplicate the conditions used by Cummings & Mondale (1967) to test for thymineless death. Ten μg/ml tryptophane was added where appropriate.

Transductions were performed with phage P1 as described by Donch & Greenberg (1968*a*). The *exrA*$^+$ gene was cotransduced with *malB*$^+$, the *uvr* genes of strains Bs1 and Bs8 were cotransduced with *gal*$^+$, that of Bs3 with *his*$^+$. Strain Bs4 was transduced directly to *try*$^+$.

3. RESULTS

In Fig. 1 the sensitivity to 50 μg/ml of nalidixic acid of strains B, B/r, Bs1, Bs2, Bs3, Bs4, Bs8 and Bs12 is shown. These are the strains tested by Cummings & Taylor (1966) and Cummings & Mondale (1967) for their sensitivity to thymineless

* In this paper resistant strains are those dying slowly in broth containing 50 μg/ml nalidixic acid, and sensitive strains are those dying rapidly under these conditions.

death. The results with nalidixic acid resemble those for thymineless death. Strains B and Bs12 are sensitive to nalidixic acid; the other strains are resistant though to varying degrees. The survival curves of Bs8 and B/r resemble each other as do those of Bs1 and Bs2. As was true for the results with thymineless death, there is no clear relationship between sensitivity to nalidixic acid and the phenotypes of the strains tested. For instance three of the HCR strains, Bs1, Bs3 and Bs8 are resistant to nalidixic acid and one, Bs12, is sensitive. Two of the strains in which filamentation is induced by u.v., B and Bs12, are sensitive; one, Bs3, is relatively resistant.

Table 1. *Properties of bacterial strains used in this investigation**

Strain	*lon*	*uvr*	*exr*	*sul*	*gal*	*his*	*malB*	Phenotype U.v.	Phenotype Fil	Obtained from
B	−	+	+	+	+	+	−	−	+	R. Hill
B/r	−	+	+	−	+	+	−	+	−	R. Hill
Bs2	−	+	−	+	+	+	−	−	−	R. Hill
HB45	−	+	+	−	−	−	−	+	−	H. Boyer
Bs5	−	+	−	+	+	+	−	−	−	R. Hill
Bs6	−	+	−	+	+	+	−	−	−	R. Hill
Bs7	−	+	−	+	+	+	−	−	−	R. Hill
Bs9	−	+	−	+	+	+	−	−	−	R. Hill
Bs10	−	+	−	+	+	+	−	−	−	R. Hill
Bs4*try thy*	−	+	−	+	+	+	−	−	−	D. J. Cummings
Bs4*try*$^+$*thy*$^+$	−	+	−	+	+	+	−	−	−	Transduction
Bsl	−	−	−	+	+	+	−	−	−	R. Hill
PAM 16	−	−	+	−	+	+	−	−	−	Transduction
PAM 17	−	−	+	+	+	+	+	−	+	Transduction
Bs8	−	−	+	−	+	+	−	−	−	R. Hill
PAM 824	−	−	+	+	+	+	−	−	+	Transduction
Bs3	−	−	+	?	+	+	−	−	+	R. Hill
PAM 3124	−	−	+	+	+	+	−	−	+	Transduction
PAM 3719	−	−	+	?	−	+	−	−	−	Transduction

* Abbreviations are as recommended by Demerec *et al.* (1966). U.v.+ : u.v.-resistant; Fil$^+$: forming long filaments after u.v. irradiation; *sul*: suppresses Lon phenotype.

ExrA *strains*. Strains Bs2, 4, 5, 6, 7, 9 and 10 have been shown to be *exrA* (Donch & Greenberg, 1968*b*). In all of them the u.v.-induced filamentation associated with the parental strain B is suppressed. Cummings & Mondale (1967) showed that strains Bs2 and Bs4 were more resistant to thymine starvation than strain B; and we have shown that strain Bs2 (Donch *et al.* 1968) is more resistant than strain B to nalidixic acid. The sensitivity to nalidixic acid of the other *exrA* strains is shown in Fig. 2. All of these strains are resistant to nalidixic acid. An *exrA* mutation, then, not only suppresses filamentation of strain B but increases its resistance to nalidixic acid.

Strain Bs4. Strain Bs4 will be treated separately. According to Cummings & Mondale (1967) strain Bs4 was more resistant to thymine starvation than all other

Bs strains. In fact, strain Bs4 appeared almost unaffected by thymine deprivation. The medium used for demonstrating thymineless death was minimal medium supplemented with casamino acids. As with other Bs mutants, they used a *thy* mutant of Bs4. However, strain Bs4 itself is an auxotroph, requiring tryptophane (not proline, as stated by Donch & Greenberg, 1968*b*). A casamino acid medium is deficient in tryptophane and a *try* mutant grown in it would, therefore, not be able

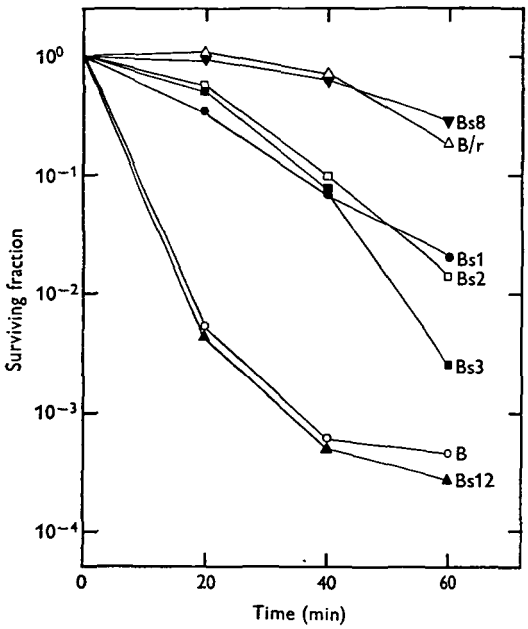

Fig. 1

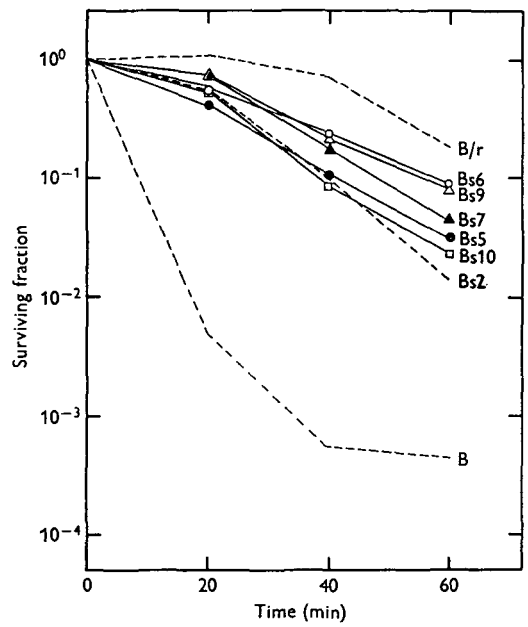

Fig. 2

Fig. 1. Survival of Bs strains during growth in broth containing 50 μg/ml nalidixic acid: ○, B; △, B/r; ●, Bs1; □, Bs2; ■, Bs3; ▼, Bs8; ▲, Bs12.

Fig. 2. Survival of Exr Bs strains during growth in broth containing 50 μg/ml nalidixic acid: ●, Bs5; ○, Bs6; ▲, Bs7; △, Bs9; □, Bs10; controls: – – –, B, B/r, and Bs2.

to synthesize protein. Since thymineless death results from imbalanced growth, the synthesis of RNA and protein in the absence of DNA synthesis, Bs4 would not under the conditions in which it was tested be expected to be sensitive to deprivation of thymine.

The effect of nalidixic acid was tested on the (Cummings & Mondale [*try*, *thy*]) strain Bs4 grown in the casamino acids medium of Cummings & Mondale (1967) to which thymine was added. As seen in Fig. 3, strain Bs4 (*try thy*) under these conditions was almost completely resistant to nalidixic acid. When tryptophane (10 μg/ml) as well as thymine was added to the casamino acids medium, strain Bs4 (*try thy*) was no more resistant than other *exrA* strains. Furthermore, a prototrophic derivative of strain Bs4 tested in casamino acids medium was as sensitive to nalidixic acid as other *exrA* strains. The *exrA* mutation of Bs4 produced a response to nalidixic acid no different from other *exrA* mutations. The high

https://doi.org/10.1017/S0016672300001944 Published online by Cambridge University Press

resistance to nalidixic acid and thymineless death of the Bs4 *thytry* derivative can be attributed to its *try* mutation under the conditions in which it was tested.

Strain Bs1. The HCR strain Bs1 has been shown to contain an *exrA* gene in addition to its *uvr* gene (Mattern, Zwenk & Rörsch, 1966; Greenberg, 1967). It seemed likely that the *exrA* gene, which was responsible for the non-filamenting property of strain Bs1, might also account for its resistance to nalidixic acid (Fig. 1). To test this we made the *exrA*$^+$ derivative of Bs1, PAM 17, by P1 mediated transduction with *malB*$^+$ from strain B251. PAM 17 is HCR and forms filaments

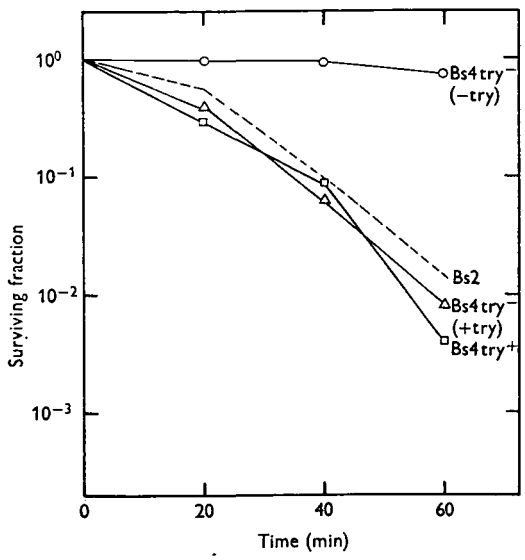

Fig. 3

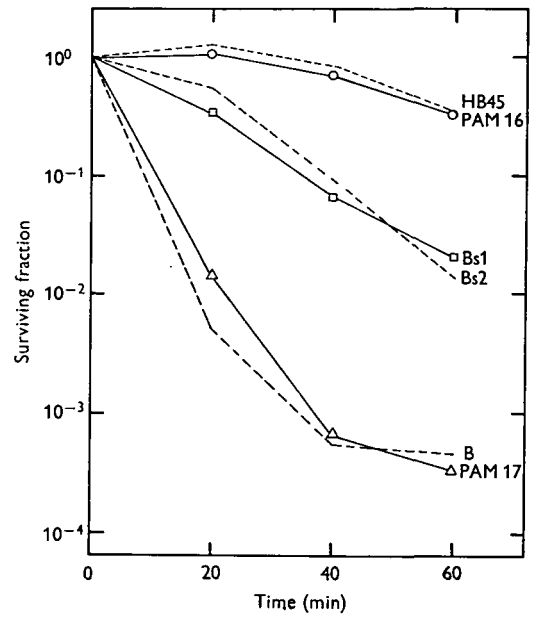

Fig. 4

Fig. 3. Survival of strain Bs4 and its derivatives during growth in casamino acids minimal medium containing 80 μg/ml thymine and 50 μg/ml nalidixic acid: ○, Bs4 *trythy* grown without added tryptophane; △, Bs4 *trythy* grown with 10 μg/ml tryptophane; □, Bs4 *try*$^+$ *thy*$^+$; control: – – – Bs2.

Fig. 4. Survival of strain Bs1 and its derivatives during growth in broth containing 50 μg/ml nalidixic acid: □, Bs1; ○, PAM 16; △, PAM 17; controls: – – – B, HB45 and Bs2.

after irradiation. In contrast to Bs1 PAM 17 (Fig. 4) is almost identically as sensitive to nalidixic acid as strain B and strain Bs12. The *exrA* and not the *uvr* gene is then responsible for the resistance of strain Bs1 to nalidixic acid. Furthermore, the results with PAM 17 indicate that its *uvr* gene, like that of strain Bs12, does not itself increase or decrease sensitivity to nalidixic acid. The irrelevance of the *uvr* gene of strain Bs1 to sensitivity to nalidixic acid is further demonstrated by results with PAM 16 (Fig. 3). In PAM 16 the *uvr* gene of Bs1 was transduced into the auxotrophic B/r derivative HB45. PAM 16 is almost identically as resistant to nalidixic acid as strain HB45 itself.

Strain Bs8. Donch & Greenberg (1968*c*) have shown that strain Bs8, while still *lon* like the parental strain B, contains a *uvr* gene linked to *gal* and a second mutation which acts as a suppressor of the phenotypic expression of *lon*. Bs8 behaves like a *uvr* derivative of strain B/r, and, it can be seen from Fig. 5, is as resistant as strain B/r to nalidixic acid. To determine whether the *uvr* gene was responsible for the nalidixic acid resistance of strain Bs8 we transduced it to strain B, creating strain PAM 824, which is HCR and can be induced by u.v. to

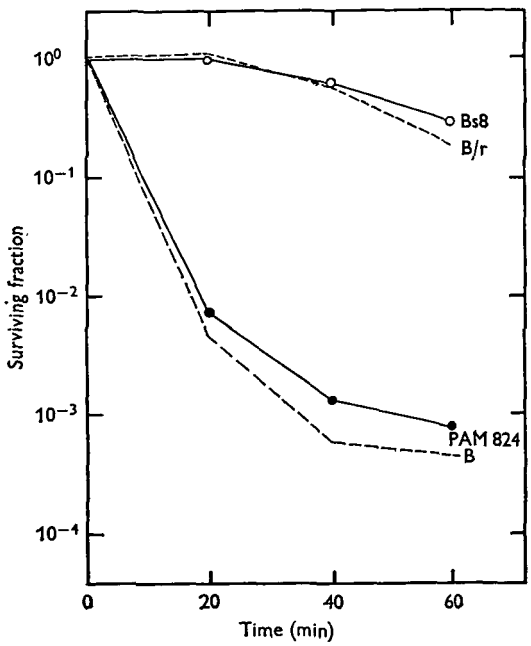

Fig. 5

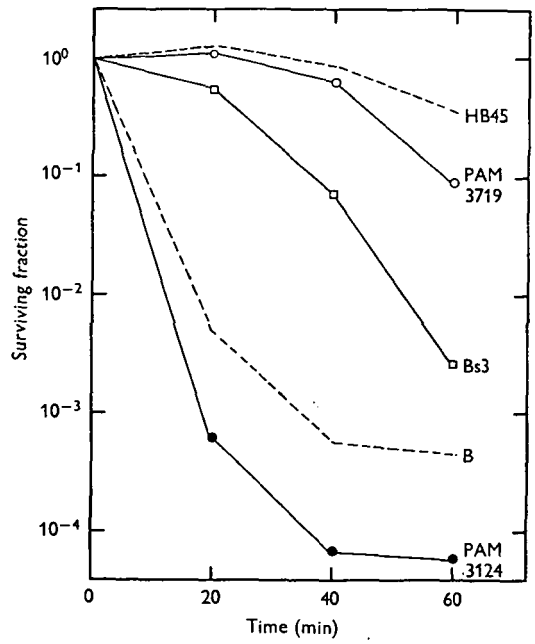

Fig. 6

Fig. 5. Survival of strain Bs8 and its derivative during growth in broth containing 50 μg/ml nalidixic acid: ○, Bs8; ●, PAM 824; controls: – – – B and B/r.

Fig. 6. Survival of strain Bs3 and its derivatives during growth in broth containing 50 μg/ml nalidixic acid: □, Bs3; ●, PAM 3124; ○, PAM 3719; controls: – – – B and HB45.

form filaments. As can be seen in Fig. 5 PAM 824 is as sensitive to nalidixic acid as strain B and, incidentally, as PAM 17 and Bs12 (Figs. 1, 3). The *uvr* gene itself does not affect the sensitivity of strain B to nalidixic acid. It is evident that a second mutation suppresses filamentation and increases resistance to u.v. and to nalidixic acid.

Strain Bs3. Strain Bs3 is HCR and can be induced by u.v. to form filaments. Nevertheless, unlike strain Bs12, it was more resistant to thymine starvation than strain B (Cummings & Mondale, 1967). We found Bs3 to be more resistant than strain B to nalidixic acid (Fig. 1). However, when the *uvr* gene of strain Bs3 was transduced into strain B, creating PAM 3124, the resulting transductant was found to be even more sensitive to nalidixic acid than strains B, Bs12 and PAM 17

(Fig. 6). Furthermore, PAM 3124 was found to form filaments spontaneously, so that in exponentially growing cultures almost all the cells were filamentous. Moreover, the viability of these cells, as measured by their colony-forming capacity, was low, about 1 % of the cells forming viable colonies. We have observed that PAM 3124 is frequently overgrown by mutants less apt to form filaments spontaneously, i.e. resembling strain Bs3.

It is clear that strain Bs3 is modified from strain B in two loci. One gene transducible with *his*$^+$ accounts for its HCR properties. This gene in the *lon* background of strain B increases the tendency to filament and the sensitivity to nalidixic acid. There is another as yet unmapped mutant gene in strain Bs3 which, in effect, reduces but does not eliminate the tendency to filament and reduces the sensitivity to nalidixic acid. Strain PAM 3719 contains the *uvr* gene of strain Bs3 transduced to strain B/r. It can be seen in Fig. 6 that this derivative is more resistant than strain Bs3 to nalidixic acid (also u.v., Donch & Greenberg, 1968*b*). Thus the second mutation in Bs3 is not identical to the *sul* gene in strain B/r (Donch *et al.* 1969).

We have tested the effect of nalidixic acid on a number of other HCR derivatives of strain K12 including AB1884, the *uvrC* derivative (Howard-Flanders *et al.* 1966). All of these strains were resistant to nalidixic acid. It is clear then that the *uvr* gene of Bs3 produces a different phenotype from that in Bs1, Bs12, Bs8 or AB1884.

4. DISCUSSION

When the effect of inhibition of DNA synthesis is reconciled with what is known about the genetics of u.v. sensitive mutants of strain B, some generalizations can be made.

exrA mutations, all of which suppress filamentation in *lon* strains, also cause an increase in resistance of the *lon* strain B to nalidixic acid. It is clear, therefore, that the *exrA* mutation in strain Bs1 (*exrA uvr*) accounts for its non-filamenting properties as well as its resistance to nalidixic acid. Furthermore, the *sul* mutation in strain B/r, which suppresses filamentation in *lon* strain B, also increases resistance to nalidixic acid. It is therefore not surprising that Bs8, which has a *sul* type gene is non-filamenting and resistant to nalidixic acid.

The *uvr* genes of strains Bs1, Bs8 and Bs12 do not influence sensitivity to nalidixic acid in any significant way. When they occur in the unsuppressed *lon* strain B, as in PAM 17, PAM 824 and Bs12, these strains are filamentous and as, but no more, sensitive to nalidixic acid than strain B itself. When they occur in a strain with a suppressed *lon*, as in Bs8, they again do not increase or decrease sensitivity to nalidixic acid. In short, except for the *uvr* gene of strain Bs3, which will be discussed later, *uvr* genes are irrelevant to sensitivity to nalidixic acid.

This leads to the conclusion that the *lon* mutation, whether that of strain B or that in AB1899 *lon* (for we have been able to corroborate our results in derivatives of the K12 strain AB1157), produces a phenotype which is sensitive to inhibition of DNA synthesis. What appears to be sensitive in *lon* strains is the cell division

mechanism. Strains which are prone to filament are sensitive to nalidixic acid. If a second mutation occurs in *lon* strains, which suppresses the tendency to filament (*exrA* or *sul*), the sensitivity to nalidixic acid is also suppressed. It seems reasonable to conclude that the cell division mechanism of an unsuppressed *lon* strain is sensitive to inhibition of DNA synthesis.

This leaves only Bs3, an HCR strain which filaments and is, nevertheless, more resistant to nalidixic acid (and to thymine starvation) (Cummings & Mondale, 1967) than is strain B. It would appear to violate two of the generalizations derived from other data. First, as a filamenting strain it ought to be as sensitive as strain B to nalidixic acid. Secondly, its *uvr* gene ought to be irrelevant to nalidixic acid sensitivity. Strain Bs3 is clearly doubly mutated from strain B. Its *uvr* gene when transduced into strain B produces a phenotype which filaments spontaneously and is even more sensitive to nalidixic acid than strain B itself. This is an exceptional property of the *uvr* gene of strain Bs3 for which we have no explanation. But it is clear that strain Bs3 must contain another mutation which modifies the phenotypic expression of the *lon* gene so that it is less prone to filament and is less sensitive to nalidixic acid. The second gene is not *sul* since the *uvr* gene of Bs3 transduced into strain B/r produces a phenotype significantly more resistant to u.v. (Donch & Greenberg, 1968*b*) and to nalidixic acid than Bs3 itself. Nor is the second gene *exrA*, since Bs3 could not be shown to contain an *exrA* mutation.

It is not surprising that the effect of nalidixic acid, as an inhibitor of DNA synthesis, mimics the effects of thymine starvation on these u.v. sensitive mutants of strain B. It is therefore reasonable to take the results we have obtained with nalidixic acid as probably applying to Cummings & Mondale's (1967) results with thymineless death.

SUMMARY

The effect of nalidixic acid, a specific inhibitor of DNA synthesis, on *Escherichia coli* strain B (*lon*) and its u.v.-sensitive derivatives is examined. Strain B itself is sensitive to nalidixic acid, whereas its u.v.-resistant derivative B/r is resistant.

It is shown that in all *exrA* strains, in which u.v.-induced filamentation is suppressed, resistance to nalidixic acid is increased. Among *exrA* strains, Bs4 is exceptionally resistant to nalidixic acid. This is because nalidixic acid kills only growing cells and strain Bs4, a *try* auxotroph, may grow poorly under the conditions used to test nalidixic acid.

The *uvr* genes of the HCR strains Bs1, Bs8 and Bs12 do not suppress u.v.-induced filamentation nor do they affect the response to nalidixic acid. The *uvr* gene of strain Bs3 is unusual in increasing the tendency to filament and also sensitivity to nalidixic acid.

Strains Bs1, Bs3 and Bs8 are all doubly mutated from strain B, the second mutation (not *uvr*) being responsible for their increased resistance to nalidixic acid as well as partially or completely suppressing filamentation.

It is concluded that the cell division mechanism of (*lon*) strain B is sensitive to inhibition of DNA synthesis. Mutations which suppress the tendency of strain B to filament reduce its sensitivity to inhibition of DNA synthesis.

We are grateful for the excellent technical assistance of Joan Woody, Dorothy Williams and Janet Dougdale-Bradley and for the volunteered assistance of Jan Neal.

This investigation was supported by Public Health Service grant CA 05687-08 from the National Cancer Institute.

REFERENCES

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

CUMMINGS, D. J. & TAYLOR, A. L. (1966). Thymineless death and its relation to UV sensitivity in *Escherichia coli*. *Proc. natn. Acad. Sci. U.S.A.* **56**, 171–176.

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. (1969). Locus for radiation resistance in *Escherichia coli* strain B/r. *Genetics* (in the Press).

DONCH, J., GREEN, M. H. L. & GREENBERG, J. (1968). Interaction of the *exr* and *lon* genes in *Escherichia coli*. *J. Bact.* **96**, 1704–1710.

DONCH, J. & GREENBERG, J. (1968*a*). Ultraviolet sensitivity gene of *Escherichia coli* B. *J. Bact.* **95**, 1555–1559.

DONCH, J. & GREENBERG, J. (1968*b*). Loci of radiation sensitivity in Bs strains of *Escherichia coli*. *Genet. Res., Camb.* **11**, 183–191.

DONCH, J. & GREENBERG, J. (1968*c*). Genetic studies of strain Bs8 of *Escherichia coli*. *Genet. Res., Camb.* **12**, 55–63.

GOSS, W. A., DEITZ, W. H. & COOK, T. M. (1965). Mechanism of action of nalidixic acid on *Escherichia coli*. II. Inhibition of deoxyribonucleic acid synthesis. *J. Bact.* **89**, 1068–1074.

GREENBERG, J. (1967). Loci for radiation sensitivity in *Escherichia coli* strain B_{s-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.

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.

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.

VAN DE PUTTE, P., VAN SLUIS, C. A., VAN DILLEWIJN, J. & RÖRSCH, A. (1965). The location of genes controlling radiation sensitivity in *Escherichia coli*. *Mutation Res.* **2**, 97–110.