**Mutants of *Escherichia coli*
with Altered Surface Properties which are Refractory to
Colicin E2, Sensitive to Ultraviolet Light and which can
also Show Recombination Deficiency, Abortive Growth
of Bacteriophage λ and Filament Formation**

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SUMMARY

Mutants refractory to colicin E2, previous phenotypic symbol RefII, have now been divided into two major phenotypic groups. CetC mutants in distinction to CetB mutants show increased sensitivity to u.v. and to a detergent (DOC). CetC mutants may also show other properties, including abortive growth of bacteriophage λ and defective division. CetB mutants are refractory to E2 at 30° but largely sensitive at 37°. CetC mutants are also refractory at 30° but may be either sensitive or completely refractory at 37°. In contrast to Rec⁻ mutants, CetC strains are not sensitive to gamma rays but two mutants show enhanced DNA breakdown after u.v. irradiation. CetC mutants seem defective in a specific membrane component which renders them insensitive to E2 and alteration of which can affect several aspects of DNA metabolism.

INTRODUCTION

One approach to the study of the functional organization of the bacterial membrane appears to be through the use of certain protein antibiotics, colicins. The general properties of colicins (see Review, Nomura, 1967) suggest that their primary site of action is the cytoplasmic membrane although they do not appear to induce detectable permeability changes in sensitive cells (Nomura, 1964). It is assumed, therefore, that colicins induce subtle changes in the physical properties of the cell membrane, which in turn induce the intracellular changes specific to each colicin. To establish the nature and normal functional role of colicin-specific sites in the cell surface, efforts have therefore been made to isolate mutants, resistant to colicin, with altered membranes. The isolation of mutants possibly of this type, which still adsorb colicin normally but remain refractory to its effect, has been described previously (Hill & Holland, 1967). The mutants included a class which were specifically refractory to colicin E2 at 30° but largely sensitive at higher temperatures. Further studies confirmed that E2 refractivity in one mutant, ASH 101, was temperature-dependent and that the mutant growing at 40° immediately became resistant to E2 after a shift of temperature to 30° (Holland, 1968). At 30° inhibition of cell division and induction of rapid degradation of DNA, characteristic effects of E2 on sensitive cells, were not observed in this mutant. Studies of other mutants refractory to E2 have shown that a small proportion were also

u.v.-sensitive, although this property was not apparently temperature-dependent (Holland, 1967).

In all previous communications E2-refractory strains were designated as Ref11 mutants. In accordance with the recommendations of Clark, Demerec, Adelberg & Hartman (1966), E2-refractory mutants are now identified by the symbol Cet (Colicin E-two). For convenience, mutants of normal u.v. sensitivity are designated CetB, whilst u.v.-sensitive, E2-refractory mutants are designated CetC. In this paper additional CetC mutants are described and the basis of their u.v. sensitivity further examined. The temperature dependence of E2-refractivity in these mutants has been found to be more complex than that of CetB mutants and they have acquired, in addition to u.v. sensitivity, several other mutant characteristics including altered cell surface properties. The properties of these mutants suggest that colicin E2 may normally interact with a part of the cell membrane which is involved in the regulation of cell division and in some aspects of DNA metabolism.

METHODS

The general properties of strains used in this study are shown in Table 1. Mutant strains ASH 101, 110, 111, 112 (Holland, 1967, 1968) and ASH 102, 113 (Holland & Threlfall, 1969) have been studied previously. Strains ASH 114, 115 and 116 are described for the first time. E2-refractory strains ASH 101, 111 and ASH 114 were derived from parental strain ASH 1 (HfrB11, *thi⁻ met⁻ thy⁻ leu⁻ str^r λ⁻*) whilst ASH 102, 112, 113, 115 and 116 were derived from the parental strain ASH 10 (*F⁻ thi⁻ met⁻ thy⁻ leu⁻ lac⁻ str^r λ⁺*). The E2-refractory strain ASH 110 was derived from HfrH (*thi⁻ thy⁻ lac⁻ str^r λ⁻*). The isolation and initial characterization of the mutants has also been described previously (Holland & Threlfall, 1969).

Table 1. *Summary of phenotypic properties of CetB and CetC mutants*

Strain	Sex	Response to E2		Response to				Recom- bination pro- ficiency	Filament forma- tion (+/-)
		25°	37°	u.v.	X-ray	DOC	λ		
Parental ASH 1	Hfr	S	S	R	R	R	S	/*	—
ASH 10	F ⁻	S	S	R	R	R	S	+	—
CetC ASH 110	Hfr	R	R	S	/*	S	S	/	Fil ⁺
ASH 111	Hfr	R	S	S	R	S	S	Rec ⁻	Fil ⁺
ASH 114	Hfr	R	R	S	R	S	R	Rec ⁻	—
ASH 112	F ⁻	R	R	S	R	S	R	Rec ⁻	—
ASH 113	F ⁻	R	S	S	R	S	S	Rec ⁻	—
ASH 115	F ⁻	R	S	S	/	S	S	+	—
ASH 116	F ⁻	R	S	S	/	S	R	+	—
CetB ASH 101	Hfr	R	S	R	/	R	S	/	—
ASH 102	F ⁻	R	S	R	/	R	S	+	—

R denotes resistance, or refractivity; S denotes sensitivity. The response to colicin E2 was determined in liquid-grown cultures as in Fig. 1. With phage λ, three strains, ASH 114, 112 and 116, showed increased but not complete resistance to the phage (see text); * denotes strains not tested for this character.

The RecA strain KMBL 239 (*rec 34*) was obtained from Professor A. Rörsch, and the RecB mutant, JC 4457, was kindly provided by Dr A. J. Clark. Culture methods and production and assay of colicin E2 (P9) obtained from *Salmonella typhimurium* LT 2 906 were described previously (Hill & Holland, 1967).

Irradiation procedures. Ultraviolet irradiation. The source of u.v. was a low-pressure mercury lamp (Hanovia Ltd) used without filter and with an incident dose rate of 6.5 ergs/mm². at 46 cm. For quantitative determination of the u.v. sensitivity of mutant strains, the procedure described previously was used (Holland, 1967). Exponential cultures of various strains grown in tryptone broth (TB) were harvested, washed and resuspended in 0.07 M-phosphate buffer at 10⁸ bacteria/ml. Appropriate dilutions of bacteria were then plated on tryptone (1 % Oxoid tryptone, 0.5 % w/v NaCl) agar plates, irradiated and incubated in the dark for 14 h. at 37° before counting.

Gamma irradiation. Exponential cultures (5 × 10⁸ bacteria/ml.) of mutants in TB media were harvested, washed and resuspended in 0.07 M-phosphate buffer at 10⁸ bacteria/ml.; 2 ml. aliquots in 1¼ in. × ¾ in. glass vials were then irradiated in a Gammacell 200 (Atomic Energy of Canada, Ltd) output 0.13 megarads/h. Irradiated samples were diluted, plated on TB plates, and survival determined after 14 h. at 37°.

Determination of u.v.-induced DNA breakdown. Exponentially growing cultures of strains in Difco nutrient broth (NB) were labelled with H³ thymine (16 µCi/µg. thymine/ml.) in the presence of 100 µg./ml. deoxyuridine, for five generations and harvested when the density reached 3 × 10⁸ bacteria/ml. Labelled cells were washed twice and finally resuspended (2 × 10⁷ bacteria/ml.) in 0.07 M-phosphate buffer pH 7.2 plus 25 µg./ml. cold thymine; 8 ml. samples were irradiated in a thin layer (2 mm.) and then diluted with an equal volume of NB and reincubated in dim light at 37°. Samples (0.5 ml.) were removed at intervals, mixed with 0.5 ml. 10 % cold trichloroacetic acid and cold and hot acid soluble counts analysed as described by Howard-Flanders & Theriot (1966). Full details of labelling procedures and methods of the determination of E2-induced DNA breakdown have been described previously (Holland, 1968).

Growth of bacteriophage λ. Production and assay of λ and λ_{gv} (Jacob & Wollman, 1953) have been described previously (Holland, 1967). One-step growth experiments were carried out by the standard technique based upon that described by Ellis & Delbrück (1939) using *Escherichia coli* C600 as indicator. The medium used in one-step growth experiments consisted of an M9 basal medium (Anderson, 1946) with 1.4 % (w/v) maltose as carbon source and supplemented with 1 % (w/v) Casamino acids (Difco), 40 µg./ml. thymine and 10 µg./ml. vitamin B1. For the assay of intracellular phage, 0.5 ml. aliquots of infected cultures were removed, mixed with an equal volume of 10 % (v/v) chloroform in phage buffer (0.02 M-tris pH 7.2, 0.01 M-MgSO₄, 0.005 % gelatin) and blended vigorously on a Vortex mixer. Finally, the suspension was incubated 10 min. at 37° to complete lysis, and, after cooling, 0.1 ml. samples were assayed for free phage.

RESULTS

Response of Cet mutants to colicin E2

In the initial characterization of E2-refractory mutants (Hill & Holland, 1967), refractivity to colicin was determined in cross-streak tests on nutrient broth agar. The majority of the mutants, although pregrown at 37°, were refractory to E2 at 25° but completely sensitive at 37°. In one mutant, ASH 112, some growth in the presence of E2 was observed at 37° and so sensitivity in this strain was not complete at high temperature. Similar results were again obtained in cross-streak tests with all the mutants examined in this study. When sensitivity to colicin was determined in liquid culture, although all strains grown and tested at 30° were still refractory to E2, the response at 37° varied

widely in different mutants. As shown in Fig. 1, some strains, including ASH 112, were completely refractory to E2 whilst others, e.g. ASH 111 were quite sensitive. Strains ASH 101 and ASH 102, which appeared to be typical of the majority of Cet mutants, showed, as described previously (Holland, 1968), an intermediate sensitivity at 37°. The induction of DNA breakdown by colicin E2 at 37° was also examined in all the mutants. As found previously and as shown in Fig. 2, the extent of E2-induced DNA breakdown at 37° closely parallels the E2-sensitivity of each strain at this temperature.

Fig. 1

Fig. 1. Sensitivity of CetC mutants to colicin E2 at 37°. Exponentially growing cultures in NB medium of various CetC mutants, two CetB mutants (ASH 101 and 102) and a wild-type strain, ASH 10, were adjusted to 10^8 bacteria/ml. After treatment with different concentrations of colicin E2 for 40 min. at 37°, the surviving fractions were determined by plating on NB agar plates.

Fig. 2

Fig. 2. Colicin-induced DNA breakdown in CetC mutants at 37°. Bacteria were labelled by growth in NB media plus H^3 thymine as described in Methods, harvested, washed and re-suspended in NB at 2×10^7 bacteria/ml. E2 was added and the cells reincubated with shaking at 37°. Samples (1.0 ml.) were removed at intervals, mixed with 4 ml. 10% ice-cold TCA and the content of acid-soluble and acid-precipitable radioactivity determined as described previously (Holland, 1968). The colicin concentrations used were 1000 units/ml. for ASH 110, 112; 400 units/ml. for ASH 101, 113 and 111; 100 units/ml. for ASH 10.

Ultraviolet-sensitive Cet mutants. When further properties of Cet mutants were examined they were found to be divisible into two major groups. CetB mutants, e.g. ASH 101 and ASH 102, apparently differ from the wild-type only in their refractivity to colicin E2. CetC mutants, e.g. ASH 112, in contrast have acquired a varied range of additional properties (Table 1). However, CetC mutants, which contribute about 15%

of all *Cet* mutants, i.e. seven out of about 50 tested, have one property in common: they are all u.v.-sensitive. The u.v. sensitivity of several *CetC* mutants at 37° is shown in Fig. 3. The mutants again show a wide range of response with those mutants showing maximum refractivity to E2 at 37° (ASH 112 and ASH 114) being the least sensitive to u.v. The u.v.-sensitivity of ASH 111 varied somewhat in different experiments (see also Holland, 1967) but was usually similar to that shown in Fig. 3.

It was previously reported (Holland, 1967) that *CetC* mutants ASH 110 and ASH 113, like *Rec*⁻ mutants, are still capable of host-cell reactivation (i.e. they are *Hcr*⁺). However, unlike *Rec*⁻ mutants, *CetC* mutants are not particularly sensitive to gamma-irradiation (Fig. 4).

Fig. 3

Fig. 3. Ultraviolet sensitivity of *CetC* mutants at 37°. The *cet*⁺ strain ASH 10 and the *recA* mutant *rec* 34 were included as controls.

Fig. 4

Fig. 4. Gamma-ray sensitivity of *Cet* mutants. The *recB* mutant, JC 4457, an X-ray sensitive strain, was included as a control.

Ultraviolet-induced DNA breakdown in CetC mutants. Howard-Flanders & Theriot (1966) and Willetts & Clark (1969) have shown that whilst at least some *RecB* and *RecC* mutants show a 'cautious' (less than wild-type) DNA breakdown, *RecA* mutants show 'reckless' DNA breakdown after u.v. irradiation. The *CetC* mutant ASH 111 was previously found to show enhanced DNA breakdown after u.v. irradiation (Holland, 1967) and several other *CetC* mutants have now been tested for this property. The u.v.-induced DNA breakdown patterns obtained (Fig. 5) demonstrate that none of the mutants fall clearly into the two categories originally defined by Howard-Flanders & Theriot (1966). Although ASH 114 and ASH 111 and possibly ASH 113 showed increased

breakdown over the parental type after large doses of u.v., they could still be distinguished from typical RecA mutants which characteristically show extensive spontaneous DNA breakdown, have large intracellular nucleotide pools and rapidly degrade their DNA after small doses (50 ergs) of u.v. (Howard-Flanders & Theriot, 1966). With other CetC mutants DNA breakdown after irradiation was similar to the parental strain ASH 10 or the CetB mutant ASH 101.

Detergent sensitivity of CetC mutants. De Zwaig and Luria (1967) reported that some mutants refractory to both colicins E2 and E3 showed increased sensitivity, under certain cultural conditions, to sodium deoxycholate (DOC) and to EDTA. They

Fig. 5. DNA breakdown in CetC mutants after 400 ergs/mm.² u.v. Content of acid-soluble H³ thymine as a percentage of total radioactivity of the culture is plotted against time. The control culture, ASH 10 (*cet*⁺), is isogenic with the CetC mutants ASH 112, 113 and 115 whilst the CetB mutant ASH 101 is shown as an isogenic control for CetC mutants ASH 111 and ASH 114.

suggested that this and other properties of the mutants indicated that refractivity to colicin was associated with a change in properties of some component of the cytoplasmic membrane. When grown in nutrient broth, all CetC mutants showed greatly increased sensitivity to DOC whilst CetB mutants were largely unaffected (Fig. 6). For these tests the bacteria were first grown at 37° and then tested for DOC-sensitivity at either 25° or 37°. The CetC strains were almost equally sensitive at both temperatures as with their response to u.v. As in other tests the mutants showed a wide range of response to the treatment with, to some extent, the mutants most sensitive to u.v. being least sensitive to the detergent. Some CetB mutants also showed slight sensitivity to DOC (e.g. ASH 102) but the effect was much less than that observed with CetC strains.

Other u.v.-sensitive mutants of *Escherichia coli* K 12 including RecA, RecB and Uvr⁻ strains and an Hsp⁻ (host specificity) mutant were also tested for DOC sensi-

tivity and found to be resistant. Increased DOC-sensitivity therefore appears to be a specific change associated with the *CetC* phenotype.

Effect of temperature shift on E2-induced DNA breakdown in CetC mutant ASH 113

Mutants refractory to colicin E2 should conceivably arise through a change in the activity of the nuclease normally activated by the presence of the colicin. Furthermore, since particular strains, e.g. ASH 111 and ASH 113, are very sensitive to E2 at high temperature but refractory at low temperature, it was of interest to determine whether such mutants produced a cold-sensitive nuclease. A culture of ASH 113 was first pre-

Fig. 6. Sensitivity of *CetC* mutants to sodium deoxycholate (DOC). Strains were grown to 5×10^8 bacteria/ml. in NB medium at 37° . The bacteria were then plated, after appropriate dilution, onto NB agar plates containing various concentrations of the detergent. Plates were incubated at 25° for 24 h. and the fraction surviving at each DOC concentration calculated. Similar results were obtained when plates were incubated at 37° .

labelled with H^3 thymine at 37° and colicin E2 was added to several different samples. At various times treated cultures were shifted to 30° and the effect on DNA breakdown determined at intervals. As shown in Fig. 7*b*, temperature shift, even at time zero, did not prevent E2-induced DNA breakdown. The observed reduction in the rate of breakdown is compatible with the reduced activity of a normal enzyme operating at 30° rather than at 37° and in no way indicates instability of a nuclease at low temperature. Moreover, cultures shifted to 30° for at least 20 min. before addition of E2 still showed considerable DNA breakdown upon subsequent addition of E2 (Fig. 7*a*). Similar results were obtained with the *CetC* mutant ASH 111. Neither of these strains therefore produce an E2-specific nuclease which is unstable at low temperature. The results do not, however, exclude the possibility that the mutants produce an altered

nuclease in the form of an aggregate, functioning at both 37° and 30°, which can be assembled at 37° but not at 30°.

The results shown in Fig. 7*a* also indicate a further possible difference between CetB and CetC mutants. Previous studies with CetB mutant ASH 101 (Holland, 1968) showed that this strain produced an altered cellular constituent which, after temperature shift from high to low temperature, immediately underwent a change which rendered the cells insensitive to the bacteriocidal effects of E2. In the present study the

Fig. 7

Fig. 7. Effect of temperature shift on E2-induced DNA breakdown in CetC mutant ASH 113. Strain ASH 113 was labelled with H³ thymine during growth at 37° as described in Fig. 2 and Methods. The labelled bacteria were washed, resuspended at 2×10^7 bacteria/ml. in NB medium and kept at 37° for 10 min. before use.

(a) At time zero E2 (50 units/ml.) was added to each of two cultures, one was incubated at 37° and the other immediately shifted to 30°. E2 was also added to other cultures after 5, 10 and 20 min. incubation at 30° respectively. Release of H³ thymine as cold acid-soluble material was determined, as for Fig. 2, following temperature shift and the addition of colicin. In a control culture (not shown) growing at 30° throughout, addition of colicin E2 produced no increase in the release of soluble H³ thymine over a 2 h. period.

(b) Colicin E2 (50 units/ml.) was added to several cultures of ASH 113 and one culture shifted to 30° at each of the times indicated by the curves. One culture was maintained at 37° throughout.

Fig. 8. Abortive growth of bacteriophage λ in some CetC mutants. The number of infectious centres obtained in one-step growth experiments is plotted against time for each phage-bacterial system. (a) Growth of λ_{gv} on the lysogenic strains ASH 102 (CetB) and ASH 112 (CetC). (b) Growth of λ on non-lysogenic strains ASH 101 (CetB) and ASH 114 (CetC).

Fig. 8

bacteriocidal effects of E2 under conditions of temperature shift were not measured. Nevertheless, the results shown in Fig. 7*a* indicate that in this CetC mutant extensive growth at low temperature is required before E2-refractivity is expressed. The cold-sensitive lesion leading to E2 refractivity in CetB mutants and in at least some CetC mutants may therefore be different.

*Increased resistance to bacteriophage λ amongst *CetC* mutants.* Since the replication of λ and ϕ X174 DNA has been reported to take place at specific membrane sites (Knippers & Sinsheimer, 1968; Salivar & Sinsheimer, 1969), the sensitivity of various *Cet* mutants to phage λ was also determined. So far, out of 30 strains tested, three *CetC* mutants (Table 1) displayed increased resistance of some kind of λ or its virulent derivative λ gv. This was reflected in the formation of minute plaques and reduced plating efficiencies. All three strains (ASH 112, 114 and 116) nevertheless appeared to adsorb λ with an efficiency equal to that of wild-type *cet*⁺ strains. When one-step growth experiments were carried out with ASH 112 and ASH 114, infectious centres were formed at normal frequencies indicating that both adsorption and injection of λ DNA were normal under these conditions. As shown in Fig. 8*b*, however, the average burst-size for λ or λ gv in ASH 114 was only 5. This compares with burst sizes of 30 to 35 in the *CetB* mutant ASH 101 or in *Cet*⁺ parental strains. Growth of λ gv in ASH 112 was found to be even more restricted with a maximum burst size of 1 to 2 phage particles per bacterium compared to 25 to 30 in the *CetB* control ASH 102. Liberation of mature phage was also greatly delayed in the ASH 112 host, but the low burst was not apparently due to lysis inhibition since premature disruption of infected cells with chloroform did not increase the yield. The reason for the restriction of λ multiplication in these *CetC* mutants is as yet unknown, but further experiments will be carried out to determine which particular step in the growth cycle is blocked.

*Other properties of *CetC* mutants.* At least two out of the seven *CetC* mutants so far studied show a further mutant phenotype, filament formation. These strains, ASH 110 and ASH 111, which both plate λ normally, produce, especially on solid nutrient agar media, large numbers of filaments up to 30 μ m. in length, unlike all the other *CetC* mutants so far studied. With one exception (ASH 116) *CetC* mutants do not form mucoid colonies on minimal or nutrient agar. In these respects they are clearly distinct from u.v.-sensitive mutants of the Lon-type (Howard-Flanders, Simson & Theriot, 1964) in which u.v. sensitivity has been attributed to defective control of cellular polysaccharide synthesis (Walker & Pardee, 1967). *CetC* mutants may be further distinguished from Lon-type mutants by their recombination deficiency which is described in the accompanying paper (Threlfall & Holland, 1970).

Table 1 summarizes the main properties of *CetC* mutants described so far, but a further important characteristic of the mutants should be noted. *CetC* strains, in contrast to *CetB* mutants, grow more slowly than wild-type strains in both nutrient and minimal media, and in some cases give reduced (40 to 50 %) colony counts when plated on agar. These characteristics are probably a reflexion of surface defects which may be clearly seen by phase contrast microscopy, particularly with stationary phase cultures. Many free spheroplasts, organisms showing extrusion of almost free spheroplasts and even branched organisms are present. These characteristics have not been observed in *CetB*, *Uvr*⁻, *RecA*, *RecB* and *RecC* mutants examined by us.

DISCUSSION

Adsorption of colicin E2 is normally followed by rapid degradation of DNA and the inhibition of division. *Cet* mutants, refractory to E2, have now been isolated from several *Escherichia coli* K12 strains. These strains still adsorb E2 but remain completely refractory to its effect at 30° although the majority are largely sensitive at 37°. All *Cet*

mutants so far studied show normal sensitivity to colicin E1, bacteriophage BF 23 and to colicin E3, a colicin which appears to utilize the same initial receptor as E2 (Maeda & Nomura, 1966; Hill & Holland, 1967). The mutants also appear unchanged in their sensitivity to colicin K and to bacteriophage T1 (Naysmith, 1967). Colicin E2 refractivity appears therefore to be associated with a highly specific surface or intracellular change rendering interaction with this colicin ineffective.

Mutants refractory to E2 can be divided into two phenotypic groups: those, CetB, which have acquired E2-refractivity only, and those, CetC, which are also u.v.- and DOC-sensitive. Most CetC mutants also appear to be recombination-deficient in some way (Holland & Threlfall, 1969; Threlfall & Holland, 1970). In addition, two CetC mutants show filament formation under normal growth conditions whilst three other mutants support only poor growth of bacteriophage λ . Despite these wide variations in phenotype, genetic studies have shown that *cetB* and *cetC* loci are very closely linked. Furthermore, all the additional characteristics associated with CetC refractivity appear to be the pleiotropic effects of a single gene closely linked to, if not identical with, the *cetC* locus, and not the result of several independent mutations (Threlfall & Holland, 1970). Despite the close linkage of *cetB* and *cetC* loci, the radical differences in the properties of these mutants indicate that at least two distinct cistrons may be involved, and complementation studies are being carried out to test this.

Since colicins appear to exert their effects at the level of the cytoplasmic membrane, some mutants refractory to their action might be expected to have altered membranes. Both the increased detergent sensitivity of the CetC mutants and the microscopic evidence of surface abnormality strongly suggest that the mutants do have altered surfaces. Moreover, preliminary experiments have shown that membrane preparations from strains ASH 111 and ASH 114 also have altered protein patterns on acrylamide gels when compared to wild-types (Samson & Holland, unpublished results).

The complex pleiotropy of CetC mutants may be best explained on the basis of either of the following two models. The first model assumes that the *cet⁺* gene constitutes part of an operon concerned with DNA metabolism and cell surface formation. Mutations in this gene might then arise having strong polar or pseudopolar effects upon adjacent genes (cf. Fink, 1966; Giles, Case, Partridge & Ahmed, 1967; Zipser, 1969). The second model supposes that the *cet⁺* gene determines the synthesis of a component of the cell membrane, malformation of which can directly or indirectly affect u.v. sensitivity, recombinant formation, cell division and, in some cases, growth of λ . Different mutations in the *cetC* gene would then give rise to the pleiotropic effects observed. Further genetic analysis of CetC mutants is now being carried out to test the validity of the first model. To test the second model, a qualitative study of the proteins present in membrane preparations from CetB and CetC mutants and their revertants is in progress. Further investigation of the molecular basis of the u.v. sensitivity of CetC mutants may also aid the understanding of CetC pleiotropy. Results obtained so far indicate that the u.v. sensitivity of these mutants differs from that of all Uvr, Rec and Lon mutants so far described. It is still not clear, however, whether CetC mutants are defective in repair of DNA or in some aspect of cell division which prevents normal recovery from u.v. irradiation.

The mode of action of colicin E2 and the properties of Cet mutants indicate that E2 normally interacts with a unique membrane site. This could be the part of the membrane which binds the DNA and which has been implicated in the cell division

process (Jacob, Brenner & Cuzin, 1963; Ryter, 1968). Comparison of the properties of this membrane fraction which can perhaps be isolated with its attendant DNA (see Tremblay, Daniels & Schaechter, 1969), with those of the remainder of the cell membrane in wild-type and in Cet mutants, should provide a crucial test of our hypothesis.

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