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Genetics of Resistance to Colicins in *Escherichia coli* K-12: Cross-Resistance Among Colicins of Group A

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By using each of the available colicins, we have isolated a large number of colicin-resistant mutants. They included both receptor and tolerant mutants and each was screened for cross-resistance to all other colicins. On the basis of the cross-resistance of these mutants it was possible to place known colicins into two mutually exclusive groups, group A and group B. Mutants selected as resistant to colicins of group A may or may not be cross-resistant to other colicins of group A, but are never resistant to colicins of group B. The reverse also applies. The mutants isolated as resistant to colicins of group A (A, E1, E2, E3, K, L, N, S4, and X) have been divided into 21 phenotypic classes on the basis of their colicin resistance patterns. These include most of the tolerant and receptor mutants previously isolated, some of which were previously shown to also have an increased sensitivity to certain antibiotics and detergents. Type strains from each of the phenotypic classes were therefore tested for sensitivity to a range of antibiotics, detergents, and surfactants that included all those previously used. With these new data, it has been possible to speculate informatively on the mode of action of the different colicins. We have confirmed the position of previously isolated mutations on the *Escherichia coli* K-12 genetic map, and located approximately the loci conferring colicin resistance in some of the newly isolated mutants.

Colicins are bactericidal macromolecules that are produced by some members of the *Enterobacteriaceae*. About 20 different types of colicins have been recognized, all active against *Escherichia coli* or closely related species. For recent reviews, see references 6 and 52.

Different colicins vary in their modes of action, and so far three types of killing action have been observed. Colicins A, B, E1, K, Ia, and Ib appear to affect energy metabolism (2, 19, 29, 36, 39, 43, 51), colicin E2 inhibits deoxyribonucleic acid metabolism (51, 53) and colicins D and E3 inhibit protein synthesis (4, 5, 51, 58, 63).

Despite these differences in biochemical targets, for several colicins it has been shown that the first step in their action is attachment to a receptor on the cell surface (41). It had been assumed that colicins stay on this receptor site, exerting their effect from there (46), perhaps via the cell membrane (10). Recently, however, evidence has appeared suggesting that the colicin E3 molecule, or perhaps part of it, is transmitted to the interior of the cell before exerting its effect (4, 5, 58).

One approach to the elucidation of colicin action has been the study of colicin-resistant mutants. Two different types of colicin-resistant mutants have been described: (i) receptor mutants, which, as the name implies, have lost the colicin receptor function (8, 37), and (ii) tolerant mutants, which are blocked in some step subsequent to binding to the receptor (34, 44, 47). It has become the custom to confine the word resistant to receptor mutants. Here we have used it to describe all forms of resistance, as in some cases no clear distinction can easily be made between tolerant and receptor mutants. Many of the loci conferring colicin resistance have now been mapped (62).

Many of these mutants showed cross-resistance to colicins other than the one with which they were selected. For example, mutants selected as tolerant to colicin E2 might or might not be tolerant to colicin E1. It was hoped that these cross-resistance patterns would elucidate the pathways by which the various colicin effects (or colicin molecules) were mediated. All these studies, however, used a restricted range of colicins (mainly E1, E2, E3, A, and K) and no clear idea emerged of how the colicin effect or colicin molecule is transmitted from the colicin receptor to the individual colicin target.

The present study has used a far wider range

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of colicins than has been used before. By selecting for resistant mutants with each of the available known colicins, and observing their cross-resistance to all the other colicins, a very clear pattern of cross-resistance has emerged, which has important implications for our understanding of the mode of action of colicins. Examples of the various mutant phenotypes have been partially characterized and mapped approximately.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. The noncolicinogenic strains, other than the mutants isolated, and bacteriophages used in these experiments are shown in Table 1. The standard indicator strain used throughout was *E. coli* K-12, strain AB1133, and all colicin-resistant mutants were derived from this strain. The colicinogenic strains used and the colicins they produce are shown in Table 2. Several of the colicins are not defined by many characters at present. An effort has been made to obtain, from different sources, several stocks of the same or different colicinogenic strains producing the one type of colicin, as some have been described merely as the colicin produced by a particular type strain.

Media. Nutrient broth (Difco, 0003) was prepared double strength plus 5 mg of sodium chloride per ml; nutrient agar was blood agar base (Difco, 0045), prepared as directed, without the addition of blood. Soft agar was prepared by mixing equal volumes of nutrient broth and nutrient agar. Minimal liquid medium is that described by Davis and Mingioli (16). Minimal agar was prepared by the addition of 20 mg of agar per ml (Difco, 0140), to minimal liquid medium. Glucose was added as a carbon source at a final concentration of 5 mg/ml to minimal agar. Growth supplements were added at a concentration of 20 μg/ml. EMB agar was eosin methylene blue agar (Difco, 0511) prepared as directed, with the addition of the relevant sugar at a final concentration of 10 mg/ml. Galactose terrazolium agar was as described previously (59).

Chemicals. Sodium deoxycholate and ethylenediaminetetraacetic acid were obtained from British Drug Houses Ltd., sodium dodecyl sulfate came from the Sigma Chemical Co., Triton X-100 was obtained from British Drug Houses Ltd., and

TABLE 1. *Noncolicinogenic bacteria and bacteriophage strains*

Strain	Relevant characteristics[a]	Source or reference[b]
E. coli K-12		
AB1133	*thi argE his proA thr leu ara mtl xyl galK lacY supE* Str^R λ$^-$ F$^-$	1
P118	Tol II mutant of AB1133	1 (50)
P117	Tol III mutant of AB1133	1 (50)
A837	Tol II mutant of C600 *thr leu thi supE lacY* TonA, λ$^-$, F$^-$	2 (44)
A586	Tol VIII mutant of C600	2 (44)
A597	Colicin A-resistant mutant of C600	2 (44)
A845	Tol III mutant of C600	2 (44)
A9	Tol II mutant of AB1133	3 (3)
B1	Tol III mutant of AB1133	3 (3)
ASH 120	Ref IV mutant of HfrH *thyA thi lac* λ$^-$ Also TonB from selection for colicin I resistance	4 (34)
AB259	*thi* λ$^-$ *rel-1* HfrH	1
RC740	*met* HfrC	1
P601	*met*, λ^R F1 *gal*	1
W3101 F2 gal	*galT*, λ$^-$ F2 *gal*	1
Bacteriophages		
BF23		1
φ80 vir	Virulent mutant of φ80	3
T1		1
T5		1
T6		1
C21	Lipopolysaccharide-specific phages	1 (56, 64)
U3		
λ cl90c17	Clear-plaque mutant of λ	3 (55)

[a] The abbreviations and nomenclature are essentially those of Demerec et al. (17) with the exceptions noted by Curtiss (13).

[b] Immediate source: 1, This laboratory; 2, S. E. Luria; 3, B. Rolfe; 4, I. B. Holland.

TABLE 2. *Colicinogenic strains*

Strain	Colicins produced	Source[a]
Citrobacter freundii CA31	A	1, 2, 7
E. coli 23	A[b]	2
E. coli CA18	B, M	7
E. coli K89	B, M	7
E. coli CA23	D, X[c]	7
E. coli K-12 W3110 Str^R (ColD-CA23)	D	6
E. coli K53	E1	7
Shigella dispar P14	E1	7
E. coli CA42	E2	7
S. sonnei P9	E2, Ib[d]	7
E. coli 12-317	E2	1
E. coli K-12 W3110 (Col E3-CA38 I-CA38)	E3[e]	3
E. coli CA46	G	4, 5, 7
E. coli CA58	H	5
Paracolon CA62	I, E1	4, 5, 7
E. coli CA53	Ia	5, 7
Salmonella typhimurium ST4 (Col Ib-P9)	Ib	4
E. coli K-12 (Col Ib-P9)	Ib	7
E. coli K216	K	7
E. coli K235	K, X[f]	7
E. coli 398	L	2
E. coli 206 Md33 TH106 F'*lac* (Col B, M-K260, V-K260)	M, B, V	4
E. coli K260	M, B, V	7
E. coli 284	N, E3	2, 4
E. coli 285	N, E3	2
E. coli II	Q, E1, D, I	2, 4
S. boydii P1	S1	4, 5
S. dispar P15	S4	4, 5
E. coli CA7	V[g]	7
E. coli Ar19a	V[g]	5, 7
E. coli K-12 185II NxII S7a (Col X-K235)	X	2, 4

[a] Immediate source: 1, N. Atkinson; 2, Y. Hamon; 3, D. Helinski; 4, J. Smarda; 5, P. Fredericq; 6, K. Timmis; 7, stocks of this laboratory.

[b] Some evidence of a second, undefined colicin.

[c] The identification of the second colicin, colicin X, is based solely on the correlation of its cross-resistance pattern with that of the colicin X originally from K235.

[d] Produces little detectable colicin Ib.

[e] Produces no detectable colicin I.

[f] Produces little detectable colicin X.

[g] Both CA7 and Ar19a produce colicin V and one other colicin of group B.

ampicillin (Penbritin) was from Beecham Research Laboratories. Phenethyl alcohol was obtained locally.

Isolation of colicin-resistant mutants. Two main methods were used to isolate mutants resistant to the various colicins. Early experiments were done by streaking an overnight culture of the colicinogenic strain across a nutrient agar plate, and then incubating overnight at 37 C. The bacterial growth was killed by exposure to chloroform vapor, and the plate overlaid with soft agar seeded with an overnight culture of AB1133 at a concentration of approximately 10^7 cells/ml. All the resistant colonies appearing in the zone of inhibition were then picked off and streaked on nutrient agar plates so as to give single colonies.

To reduce the possibility of isolating resistant mutants derived from a single clone of cells, in later experiments an overnight culture of a colicinogenic strain was spread over the surface of a nutrient agar plate. After overnight growth at 37 C and chloroform treatment, the plate was overlaid with nutrient agar. Individual cultures of AB1133 were then streaked over the surface of a series of plates so as to give single colonies. Only a few colonies were picked from each streak and subjected to a further single colony isolation.

Except when otherwise stated, all mutants were either selected against the colicin from a colicinogenic strain that had been shown to be producing only one colicin, or from a colicin zone from a polycolicinogenic strain which had previously been separated by electrophoresis (see below).

All mutants were stored as freeze-dried cultures, and working stocks were maintained on nutrient agar slopes at 4 C.

Triple-layer plate test to distinguish tolerant

and receptor mutants. Because of the large number of mutants being screened, and the fact that many colicins are difficult to obtain in liquid medium, a plate test was used to distinguish sensitive, tolerant, and receptor strains. The method used is an adaption and amalgamation of those of Fredericq (25) and Hill and Holland (34). The colicinogenic strain was streaked across a nutrient agar plate and grown overnight at 37 C. The colicinogenic strain was then killed with chloroform and the plate was overlaid with nutrient agar. The strain to be tested was then streaked across the plate at a right angle to the original colicinogenic streak and grown overnight at 37 C. It, too, was then killed by exposure to chloroform vapor, and the plate was overlaid with soft agar seeded with an overnight culture of the indicator strain AB1133 at a concentration of approximately 10^7 cells/ml, and incubated overnight at 37 C.

As the colicin diffuses out and up from the original colicinogenic streak, it will kill any sensitive bacteria, and they will fail to grow. Any receptor or tolerant mutant will be unaffected by the colicin and the cross-streak will continue to grow. As the colicin diffuses into the top layer it will kill the indicator strain, producing what is termed a zone of inhibition. If the cross-streak is a receptor mutant, it will not adsorb the colicin diffusing through the medium, whereas a tolerant mutant will adsorb colicin from the medium, and stop it from reaching the top layer and killing the indicator strain. Thus, receptor mutants should have no effect on the shape of the zone of inhibition, but a cross-streak of a tolerant mutant will be covered by a "cap" of indicator bacteria (Fig. 1). The cap sometimes fails to cover the central part of a streak, indicating that the receptors can become saturated in this area of high colicin concentration. A similar plate test has been developed by P. Fredericq (personal communication).

When many mutants were being tested, large flat dishes (approximately 30 by 30 cm by 3 cm deep) were used. These dishes were made from plate glass, held together with autoclave tape (Scotch brand pressure sensitive tape, Minnesota Mining and Manufacturing [Australia] Pty. Ltd.).

Quantitation of colicin resistance. Crude colicin preparations were obtained by centrifuging overnight cultures of the colicinogenic strain, and sterilizing the supernatant with chloroform. Partial resistance, undetectable on the triple-layer plate test, can be detected by spotting serial twofold dilutions of the colicin preparation in nutrient broth onto the surface of a soft agar overlay seeded with the strain to be tested. By comparing the dilution needed to inhibit the growth of a particular mutant with that of its parent, slight differences in sensitivity to a particular colicin can be detected.

Electrophoretic techniques. In cases where a colicinogenic strain was producing more than one colicin, electrophoresis was used to achieve separation of the colicins. The colicinogenic strain was streaked across a large nutrient agar dish and grown overnight. The bacterial growth was scraped off, and the dish was sterilized by exposure to chloroform vapor. The plate was then subjected to electrophoresis for 16 h at 4 C,

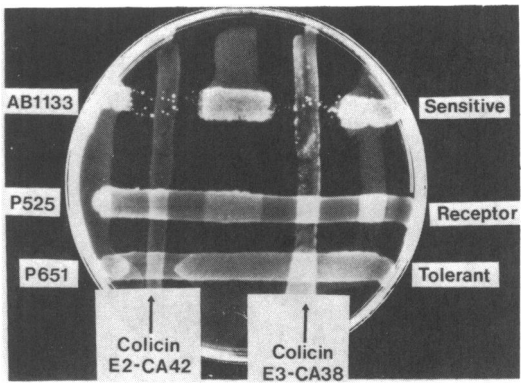

FIG. 1. *Plate receptor test for distinguishing receptor and tolerant mutants. A sensitive strain (AB1133) and a receptor (P525) and a tolerant (P651) mutant were cross-streaked against two colicinogenic strains, and the plate was overlaid with the indicator strain. A tolerant mutant can easily be distinguished from a receptor mutant by its effect on the zone of inhibition. For a fuller explanation, see Materials and Methods.*

with a constant voltage of 200 V. Nutrient broth, diluted twofold in distilled water, was used as an electrolyte. During electrophoresis, the current rose from approximately 30 mA to approximately 150 mA per plate. For the isolation of resistant mutants, the plate was then overlaid with soft agar plus 200 μg of streptomycin per ml, seeded with an overnight culture of strain AB1133 at a concentration of approximately 10^7 cells/ml, and grown overnight at 37 C (Fig. 2).

The colicins of all the multiply colicinogenic strains could be readily separated by this technique with the exception of the colicins of *E. coli* II, where the four zones showed overlap. All the colicins of a given type had a similar electrophoretic mobility although an anomalous situation for colicin V of *E. coli* CA7 and *E. coli* Ar19a is discussed elsewhere (15). Resistant colonies were picked from the colicin zone required and streaked on nutrient agar plates so as to give single colonies. To test a series of mutants for colicin resistance, the plate was exposed to chloroform vapor and overlaid with nutrient agar, and the strains to be tested streaked at right angles to the original colicinogenic streak. After incubation overnight at 37 C, the plate was exposed to chloroform vapor once more, and overlaid with a soft agar layer seeded with the indicator strain for the triple-layer test (Fig. 3).

Bacterial crosses. Bacterial crosses in which F' *gal* strains were used as donors were performed by mixing 1 ml of a log phase F' culture with 5 ml of an overnight culture of the F^- strain. After 1 h of incubation at 37 C, the culture was spun down, resuspended in minimal liquid medium and plated on the appropriate media. Other matings were done by plating 0.1-ml samples of the Hfr and F^- strains directly onto the selective plate.

Recombinants were picked off and streaked for single colonies on nutrient agar plates, before being inoculated onto master plates for replica plating on the appropriate media.

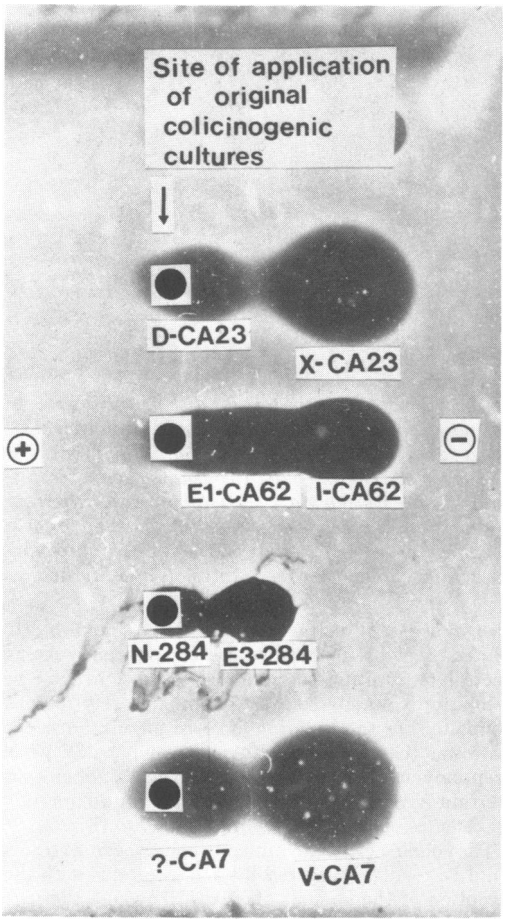

FIG. 2. *Separation of colicin zones by electrophoresis. Colicinogenic strains were spotted onto a large agar plate. After overnight growth, the bacterial growth was removed and a current passed across the plate for 16 h. The plate was then overlaid with the indicator strain, AB1133, to show the zones of inhibition. For a fuller explanation, see Materials and Methods.*

P1 transduction. Phage lysates were prepared by plating various dilutions of phage P1 in a soft agar layer seeded with the donor strain. A plate in which lysis was just complete was selected, the soft agar layer was removed, and an equal volume of nutrient broth was added. This was shaken, and chloroform was added and allowed to stand before centrifuging. The supernatant was collected, and the phage was assayed on AB1133. Transductions were performed by mixing the phage and recipient bacteria in the ratio 1:10 and incubating at 37 C for 30 min before resuspending the bacteria in minimal liquid medium and plating on the appropriate selective medium. Transductants were picked off, streaked for single colonies on nutrient agar plates, and then stabbed into nutrient agar master plates with sterile toothpicks. They were then replica plated onto the appropriate media.

Antibiotic sensitivity tests. The sensitivity of various mutants to a range of antibiotics was tested by overlaying a nutrient agar plate with 5 ml of soft agar seeded with an overnight culture of the strain to be tested, at a concentration of approximately 10^7 cells/ml. A Multodisk (Oxoid Ltd., codes S-1 and 30-9C) was then layered onto the surface of the plate, which was incubated overnight at 37 C. The sensitivity of the various mutants to ampicillin was also determined by plating aliquots of an overnight culture on a series of nutrient agar plates containing varying concentrations of ampicillin. This gave a more accurate indication of the minimal inhibitory concentration of ampicillin for each mutant.

Bacteriophage sensitivity tests. The sensitivity of mutants to various phages was tested by streaking the mutants against each phage on a nutrient agar plate and incubating the plate overnight at 37 C.

Testing sensitivity to detergents and surfactants. The method used is essentially that of Bernstein and Onodera (3). An overnight culture of the strain to be tested was diluted 1:20 in 10 ml of nutrient broth. The reagent was then added at the concentration stated, and the culture was shaken at 37 C for 4 h, at which time a viable count was performed. The log of the percent survival (related to the viable count of the control after 4 h of growth)

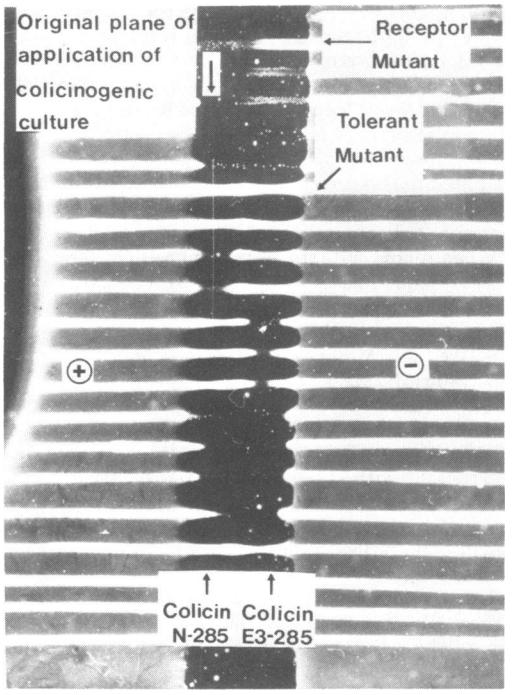

FIG. 3. *Electrophoresis plate test. The plate test for receptor and tolerant mutants and the electrophoresis techniques can be combined to test whether a strain is a receptor or tolerant mutant after separation of the colicin zones of a multicolicinogenic strain by electrophoresis. For a fuller explanation, see Materials and Methods.*

was used as an index of sensitivity. Thus, 2.00 indicates that the agent had no effect on growth over the 4-h period. It will be noted that only Triton X-100 had no effect on the parent strain AB1133.

RESULTS

Use of the triple-layer plate test to distinguish receptor and tolerant mutants. The ability of the triple-layer plate test to distinguish between receptor and tolerant mutants was tested by cross-streaking against two colicinogenic strains, colicin E2 receptor and tolerant mutants. The result (Fig. 1) confirms that these two types of mutants can be distinguished for colicins E2 and E3. Tolerant and receptor mutants could also be distinguished with colicins E1, K, L, and N.

All mutants resistant to colicins A and X, however, appear to give the result expected for a receptor mutant, when cross-streaked against these colicins. To test the ability of the plate test to distinguish receptor and tolerant mutants for colicins A and X, the parent strain, AB1133, was streaked on a sterile membrane filter (Millipore Corp.) lying on the surface of a nutrient agar plate. After overnight growth at 37 C, the bacteria were killed with chloroform vapor, and the membrane was removed and placed on the surface of a nutrient agar plate that had previously been streaked with the two colicinogenic strains, grown overnight at 37 C, killed with chloroform, and overlaid with nutrient agar. The plate was then overlaid with soft agar seeded with AB1133, and incubated overnight at 37 C. As the parent strain has the receptors for the two colicins intact, it should elicit the maximum tolerant result possible for mutants of this strain.

However, when the seeded soft agar layer above the cross-streak was removed and the shape of the inhibition zone was inspected, in both cases it was unaltered indicating that the triple-layer plate test was unable to distinguish receptor and tolerant mutants for colicins A and X. We also have evidence (unpublished data; K. Timmis, personal communication) that the same is true for colicin D. Presumably the cell has too few, or no, receptors for colicins A and X and cannot significantly adsorb the colicins out of the medium. This hypothesis is supported by evidence suggesting that the cell surface has fewer receptors for colicin A than for some other colicins (9).

It was also found that colicin G was chloroform sensitive, and to retain maximal colicin activity, heating at 56 C for 30 min was substituted for chloroform treatment, to kill the bacterial growth at various stages needed, during the plate test.

Colicin S4 exhibited a very weak zone of inhibition and gave rise to large numbers of resistant mutants, making it difficult at times to distinguish the edge of the zone of inhibition.

Isolation of colicin-resistant mutants. Several hundred colicin-resistant mutants were isolated, using each of the available colicins for selection. These initial isolates were screened for their colicin resistance pattern using the triple-layer plate test and a restricted range of colicinogenic strains. In cases where a predominance of one phenotype occurred, only a few were retained for further study.

Approximately 300 mutants were then tested against all the singly colicinogenic strains listed in Table 2 and, after electrophoresis, against the colicins produced by *E. coli* 284, as colicin N is not produced alone by any of the colicinogenic strains.

It immediately became obvious that one could divide the colicins into the two groups (group A and group B) shown in Table 3. Mu-

TABLE 3. *Grouping of colicins by their resistance patterns*

Colicin group	Colicin	Specific colicins used[a, b]
A	A	A-CA31, A-23
	E1	E1-K53, E1-P14, E1-CA62, E1-II
	E2	E2-CA42, E2-P9, E2-12-317, E2-K317
	E3	E3-CA38, E3-284, E3-285
	K	K-K216, K-K235
	L	L-398
	N	N-284, N-285
	S4	S4-P15
	X	X-185II, X-CA23
B	B	B-CA18, B-206, B-K260, B-K89
	D	D-CA23, D-II
	G	G-CA46
	H	H-CA58
	I	Ia-CA53, Ib-ST4, Ib-P9, I-CA62, I-II
	M	M-206, M-CA18, M-K89, M-K260
	S1	S1-P1
	V	V-CA7, V-Ar19a, V-206, V-K260
	Q	Q-II

[a] The nomenclature is that adopted by Nomura (46). In each case, the colicin produced (e.g., E2) is followed by the name of the strain producing it (e.g., CA42), to give, for example, E2-CA42.

[b] In some cases where more than one stock of each colicinogenic strain was obtained, all of the individual stocks were used.

tants selected as resistant to a group A colicin may or may not be resistant to other group A colicins, but are never resistant to any colicin of group B. The reverse also applies. Of approximately 150 mutants that had been selected as resistant to colicins of group B, none showed any resistance to group A colicins (15). This paper deals with 87 mutants that are resistant to colicins of group A.

Resistance to colicins of group A. The mutants resistant to group A colicins can be divided into 21 phenotypic classes on the basis of their colicin resistance patterns. These include both receptor and tolerant mutants. One or more mutants from each class was then tested for resistance to the colicins produced by the remaining multicolicinogenic strains listed in Table 2.

It was found, however, that it was impossible to completely separate, by electrophoresis, the colicins Q and I produced by *E. coli* II. Smarda and Obdrzalek have previously stated that many colicin Q-resistant mutants are also resistant to colicin V (60) so presumably it is a group B colicin. Certainly, no mutant resistant to a group A colicin appeared to be resistant, as far as could be told, to colicin Q.

To check for partial resistance to colicins, twofold serial dilutions of colicins A, E1, E2, E3, and K were spotted onto soft agar overlays seeded with the mutant to be tested. The results are shown in Table 4. Liquid preparations of colicin, with a sufficient colicin concentration, could not be obtained for colicins L, S4, and X. Colicin N could not be obtained free of colicin E3, as both *E. coli* 284 and 285 produce both colicins N and E3.

The results for the complete cross-resistance tests for the type mutants listed are shown in Table 5.

The Bfe and Tsx mutants isolated are the classical receptor mutants for colicins E and K, isolated many times previously (8, 12, 37, 38). The Rcx mutants have not been described before, and are specifically resistant to colicin X. It is impossible to tell from the triple-layer plate test whether they are receptor or tolerant mutants, and colicin X cannot be obtained in liquid media at a concentration sufficient to do adsorption studies.

The Con mutants, so-called because of their conjugation deficiency, have been described elsewhere (14, 59) and are tolerant to colicins K and L. The Tol I mutants (tolerant to colicin K,

TABLE 4. *Quantitation of colicin resistance*

Phenotypic class[a]	Type strain	End-point dilution of colicin preparation[b]				
		A	E1	E2	E3	K
Tol$^+$, Bfe$^+$, Tsx$^+$, Rcx$^+$, Con$^+$	AB1133	9	7	9	7	4
Bfe	P525	0	0	0	0	4
Tsx	P209	8	7	9	7	0
Rcx	P224	6	7	7	7	3
Con	P212	9	7	9	7	0
Tol Ia	P218	0	5	9	7	0
Tol Ib	P210	0	6	8	6	0
Tol IIb	P651	0	0	0	0	0
Tol IIc	P555	0	0	0	0	0
Tol III	P660	0	5	0	0	0
Tol IV	P692	0	5	0	0	4
Tol VII	P689	0	5	2	6	2
Tol VIII	P602	0	0	9	7	2
Tol IX	P596	0	7	9	7	2
Tol X	P661	0	5	7	6	1
Tol XI	P220	1	7	9	7	2
Tol XII	P653	0	7	9	2	4
Tol XIII	P520	0	7	0	0	0
Tol XIV	P530	0	7	0	0	0
Tol XV	P686	0	6	0	0	0
Tol XVI	P516	0	6	2	4	4
Tol XVII	P652	0	0	4	1	0

[a] The complete colicin resistance patterns and descriptions of the various phenotypic classes is given in Table 5.

[b] The figures given in the table are n, where $\frac{1}{2}^n$ is the last dilution of the colicin preparation to give a complete inhibition zone on a lawn of particular mutant. Where $n = 0$, no clearing of the bacterial growth could be seen with an undiluted colicin preparation.

TABLE 5. *Phenotypic classification of mutants isolated*

Phenotypic class[a]	Type strain	No. isolated	Colicin resistance[b]									Type of mutant
Bfe[c]	P525	21	E1	E2	E3			A				Receptor
Tsx[d]	P209	3				K						
Rcx[e]	P224	3									X	
Con	P212	2				K	L					
Tol Ia	P218	3				K	L	A	S4			
Tol Ib	P210	3				K	L	A	S4	N		
Tol IIb[f,g]	P651	10	E1	E2	E3	K	L	A	S4	N		
Tol IIc	P555	9	E1	E2	E3	K	L	A	S4	N		
Tol III	P660	3		E2	E3	K	L	A	S4	N		
Tol IV	P692	1		E2	E3		L	A		N		Tolerant
Tol VII	P689	1		pE2		pK	L	A	S4			
Tol VIII	P602	2	E1			pK		A				
Tol IX	P596	4				pK	L	A		N		
Tol X	P661	3				pK	L	A	S4	N		
Tol XI	P220	1				pK	L	pA	pS4			
Tol XII	P653	1			pE3		L	A	pS4			
Tol XIII	P520	12		E2	E3	K	L	A	S4			
Tol XIV	P530	1		E2	E3	K	L	A		N	X	
Tol XV	P686	2		E2	E3	K	L	A	S4	N	X	
Tol XVI	P516	1		pE2	pE3		L	A				
Tol XVII	P652	1	E1	pE2	pE3	K	L	A	S4	N		

[a] The phenotypic classification was adopted so as to conform with that used previously (44, 47).

[b] The colicins listed are those to which the particular class of mutants is resistant. They have been tested and found to be sensitive to all the other colicins in Table 1. p denotes partial resistance, as described in Table 4, except for colicin S4, where pS4 denotes partial resistance to colicin S4 on the triple-layer plate test.

[c] Also resistant to phage BF23.

[d] Also resistant to phage T6.

[e] This class of mutants has been called Rcs (resistance to colicin X), rather than giving it a more specific Tol classification. As explained in the text, it is impossible to distinguish receptor and tolerant mutants for colicin X.

[f] The classification Tol IIa has been used previously (3, 44) to denote mutants with partial resistance to colicin, and of the Tol II phenotype.

[g] Groups IIb and IIc were differentiated on their sensitivity to detergents and antibiotics (see Tables 6 and 7).

but sensitive to E1, E2, and E3) described previously may be the same as one of the different types described here (45).

The Tol IIb and Tol III mutants are of the types isolated previously (see Table 7), and shown to map *tolA* and *tolB*, respectively (3). The Tol IIc mutants, however, despite the fact that they are resistant to exactly the same colicins as the Tol IIB mutants, were differentiated on their antibiotic, detergent and surfactant sensitivity patterns (see below).

Mutants tolerant to colicin E2 and sensitive to colicins A, E1, and E3 (Tol VII or Ref II) have been described previously (34, 47) and some have been shown to map at the *cet* locus, near *thr* on the *E. coli* genetic map (62). P689, however, was only partially tolerant to colicin E2, and did not show the ultraviolet sensitivity (35) associated with CetA mutants (Davies and Reeves, unpublished data). The Tol VIII class appears to be similar to those mutants shown to map at *tolC*, near *metC* (62, 65).

In all cases, the phenotype of these mutant classes has been extended considerably.

The Tol IX, XI, and XII classes have not been described before, but Tol X has, and been described as a colicin A-resistant mutant (see Table 7). The Tol XIII, XIV, and XV mutants may have been isolated before, but would have been described as Tol III mutants, from which they differ only in their resistance to colicins N, S4, and X. The single Tol XVI mutant is of a previously undescribed phenotype. The single Tol XVII mutant, P652, differs from the Tol I mutants only in that it is partially sensitive to colicins E2 and E3. It does not show the partial sensitivity to colicin E1 exhibited by Tol IIa mutants (3).

Bacteriophage resistance tests. It has been noted several times before that certain colicin-

resistant mutants show cross-resistance to bacteriophages BF23, ϕ80, T1, T5, and T6 (23, 27, 28). All the mutants were therefore tested for their sensitivity to these bacteriophages. None of the mutants were resistant to bacteriophages ϕ80vir, T1, or T5. Only the Bfe mutants were resistant to phage BF23, and only the Tsx mutants were resistant to phage T6.

Sensitivity to antibiotics, detergents, and surfactants. Some colicin-tolerant mutants have previously been shown to have altered susceptibilities to various antibiotics (3, 34, 44, 47). The 21 type mutants listed in Table 4 were tested for their sensitivity to a variety of antibiotics by using Multodisks. The only mutant to show substantial alterations to its pattern of sensitivity was the Tol VIII strain, P602, which had become sensitive to erythromycin, methicillin, fusidic acid, and novobiocin. Others, however, showed increased resistance or increased sensitivity to ampicillin, as judged by the size of the zone of inhibition of growth.

The type mutants were therefore tested for their ability to grow on nutrient agar plates containing varying concentrations of ampicillin. The results are shown in Table 6.

As well as causing changes in antibiotic sensitivity, mutation to colicin resistance can also be accompanied by increased sensitivity to detergents and surfactants (3, 34, 44, 47). The mutants isolated were therefore screened for sensitivity to a range of detergents and surfactants, and the results are shown in Table 7.

Other properties of the mutants isolated. Recently, other changes have been demonstrated in colicin-tolerant mutants. Bernstein et al. (3, 55) have showed that their Tol II and III mutants were mucoid at 30 C on nutrient agar, and have demonstrated a change in the efficiency of plaquing with λcI90c17 on a Tol IVt mutant.

Accordingly, all mutants were screened for changes in sensitivity to λcI90c17. The only mutants to show any resistance were the Tol VII mutant, P689, and the Tol XI mutant, P220. The only mutant to show mucoid growth at 30 C was P220, and it was partially mucoid at 37 C.

The changes in ampicillin sensitivity of some strains suggested that some of the mutants may be altered in their lipopolysaccharide, as some types of ampicillin resistance are accompanied by lipopolysaccharide changes (42). Therefore, all the mutants were tested for their sensitivity to two lipopolysaccharide-specific bacterio-

TABLE 6. *The sensitivity to ampicillin of the mutants isolated*

Phenotypic class	Type strain	Multodisk zone diameter (cm)[a]	Minimal inhibitory concentration of ampicillin (μg/ml)[b]	Sensitivity[c]
Tol$^+$, Bfe$^+$, Tsx$^+$ Rcx$^+$, Con$^+$	AB1133	1.2	2	—
Bfe	P525	1.2	2	—
Tsx	P209	1.1	2	—
Rcx	P224	1.2	2	—
Con	P212	1.3	2	—
Tol Ia	P218	1.0	2	—
Tol Ib	P210	1.0	2	—
Tol IIb	P651	2.0	0.1-1.0	S
Tol IIc	P555	1.3	2	—
Tol III	P660	1.7	0.1-1.0	S
Tol VI	P692	1.0	2	—
Tol VII	P689	1.1	2	—
Tol VIII	P602	1.7	0.1-1.0	S
Tol IX	P596	1.1	2	—
Tol X	P661	1.0	2	—
Tol XI	P220	1.2	2	—
Tol XII	P653	1.1	2	—
Tol XIII	P520	1.9	0.1-1.0	S
Tol XIV	P530	1.0	2	—
Tol XV	P686	1.2	2	—
Tol XVI	P516	1.0	2	—
Tol XVII	P652	2.0	0.1-1.0	S

[a] The diameter of the disk itself is 0.9 cm.

[b] The lowest concentration in micrograms of ampicillin per milliliter at which a noticeable inhibition of growth occurred on nutrient agar plates.

[c] S, Supersensitive; —, no change in sensitivity.

TABLE 7. *Detergent and surfactant sensitivity of mutants isolated*

Phenotypic class	Type of Strain	Sensitivity index in[a, b]					Sensitivities[c, d]
		DOC	SDS	EDTA	PEA	Triton	
Tol$^+$, Bfe$^+$, Tsc$^+$, Rcx$^+$ Con	AB1133	0.5	0.7	1.3	0.1	2.0	
Bfe	P525	0.5	0.6	1.1	0.6	1.9	
Tsx	P209	0.5	0.5	1.3	0.4	2.0	
Rcx	P224	0.6	0.4	1.1	0.2	2.2	
Con	P212	0.6	0.7	0.2	−0.3	1.8	EDTA, PEA
Tol Ia	P218	0.3	0.3	1.3	0.5	2.0	
Tol Ib	P210	0.0	0.3	1.1	0.1	1.9	
Tol IIb	P651	−1.5	0.4	1.0	−0.4	2.0	DOC, pEDTA, PEA
Tol IIc	P555	0.1	0.2	0.9	−0.5	1.7	pEDTA, PEA
Tol III	P660	−1.5	0.6	0.5	−0.6	1.6	DOC, EDTA, PEA, pTriton
Tol IV	P692	−0.1	0.6	1.0	0.3	0.95	pEDTA, pTriton
Tol VII	P689	0.6	0.6	1.3	−0.6	1.8	PEA
Tol VIII	P602	−2.0	−3.3	0.7	−2.1	−1.3	DOC, SDS, EDTA, PEA, Triton
Tol IX	P596	0.6	0.6	1.2	0.1	2.0	
Tol X	P661	0.5	0.8	1.1	0.2	2.0	
Tol XI	P220	0.7	0.5	1.3	0.0	1.9	
Tol XII	P653	0.7	0.5	1.3	0.1	2.0	
Tol XIII	P520	−1.7	0.2	0.3	−0.6	1.7	DOC, EDTA, PEA
Tol XIV	P530	0.0	0.2	0.9	0.2	2.0	pEDTA
Tol XV	P686	0.4	0.8	0.9	0.4	2.0	pEDTA
Tol XVI	P516	−1.4	0.3	1.0	0.1	1.8	pEDTA, DOC
Tol XVII	P652	−1.1	0.8	0.9	0.1	1.3	DOC, pEDTA, pTriton

[a] DOC, Sodium deoxycholate, 5 mg/ml; SDS, sodium dodecyl sulfate, 5 mg/ml; EDTA, ethylenediaminetetraacetic acid, 5 mg/ml; PEA, phenethyl alcohol, 5 μl/ml; Triton, Triton X-100, 10 μl/ml.

[b] The procedure for testing the mutants for their sensitivity to the various detergents is described in Materials and Methods.

[c] pEDTA, Partially sensitive to EDTA; pTriton, partially sensitive to Triton.

[d] Sensitivity to the various agents was defined as when the sensitivity index of a particular mutant was less than −1.0 for DOC and SDS, 0.8 for EDTA, 0 for PEA or 0.9 for Triton X-100. Partial sensitivty for EDTA was when the sensitivity index was between 0.9 and 1.0, and partial sensitivity to Triton X-100 was a sensitivity index between 0.9 and 1.6.

phages, C21 and U3. The indicator strain, AB1133, is resistant to phage C21 and sensitive to phage U3. Any alteration to this pattern has been shown to be accompanied by a change in the lipopolysaccharide (56, 64).

The only mutants to show a change in the sensitivity pattern were the Tol VII mutant, P689, which had become resistant to phage U3; the Tol VIII mutant, P602, which had become partially sensitive to phage C21; and the Tol XI mutant, P220, which had become resistant to U3 and fully sensitive to phage C21.

Comparisons with previously isolated mutants. As mutants of similar, but less completely characterized, colicin resistance patterns to some of those shown in Table 5 had been isolated previously (3, 34, 44, 47, 48) it was necessary to compare, as far as was possible, those mutants with the newly isolated strains. Examples of the previously isolated mutant classes were therefore checked for their resistance to the colicins produced by all the colicinogenic strains listed in Table 2. They were also tested for their sensitivity to the antibiotics, detergents, and surfactants used before. The results are summarized in Table 8.

Mapping of mutants isolated. We have confirmed by P1 transduction that the *bfe* locus maps in the region reported previously (8, 37); the colicin resistance locus in P525 is 56% co-transducible with *argE*.

Mutants resistant to colicin K and bacteriophage T6 (*tsx*) have been shown to map at 9.8 min on the genetic map (12, 64), which should make *tsx* approximately 20% co-transducible with *lac*. We have been unable to confirm this, due to the inability to use *lac* (or *gal*) as a selective marker in the mutants. The parent strain, AB1133, grows sufficiently well on either lactose or galactose as sole carbon source, to make it impossible to select *lac*$^+$ or *gal*$^+$ recombinants or transductants.

TABLE 8. *Comparison of previously isolated mutants with new phenotypic classes characterized*

Strain	Phenotypic class		Colicin resistance pattern		Reference
	Present	Previous	Present	Previous	
A837					44
A9	Tol IIb[a]	Tol II	E1 E2 E3 K L A S4 N	E1 E2 E3 A K	3
P118					50
A845					44
B1	Tol III	Tol III	E2 E3 K L A S4 N	E2 E3 A K	3
P117					50
ASH120	Tol IV	Ref IV	E2 E3 L A N	E2 E3	34
A586	Tol VIII	Tol VIII	E1 pKA[b]	E1 pK pA[b]	44
A597	Tol X	A^R	pK L A S4 N[b]	A	44

[a] These mutants were classified as Tol IIb, rather than Tol IIc, on the basis of their antibiotic and detergent sensitivity patterns.

[b] pK is partial resistance to colicin K.

The *rcx* locus is transferred by HrfH and HfrC, and appears from an examination of the recombinant classes to be between *proA* and the HfrC origin (i.e., between 6.5 and 13 to 14 min). It is not, however, co-transducible with *proA* (6.5 min) or *lip* (14.6 min) at a frequency greater than 4%, and so presumably maps in the region 7.8 to 13.3 min.

The *con* locus, as reported elsewhere (14), maps at approximately 14.5 min.

The colicin resistance loci in the Tol Ia (P218), Tol Ib (P210), Tol IIB (P651), Tol IIC (P555), Tol III (P660), Tol IX (P596), Tol X (P661), Tol XII (P653), Tol XIII (P520), Tol XVI (P516), and Tol XVII (P652) mutants are all transferred by HfrH, and appear to be linked to *gal*. None are transferred by HfrC. Because of the inability to use *gal* as a selective marker, a detailed genetic analysis of these mutants was not performed, but we were able to confirm the position of the relevant loci in the Tol IIb, Tol IIc, Tol III, Tol IX, Tol XII, Tol XIII, and Tol XVII mutants by using them as recipients in crosses using donors carrying the F1 Gal and F2 Gal plasmids. Because of the relatively high frequency of transfer in crosses employing F′ strains as donors, it was possible to isolate Gal^+, bacteriophage MS2 sensitive derivatives of the recipients. In each case, the mutant became sensitive to the relevant colicins, indicating that the relevant loci are located between *lip* (14.6 min) and *chlA* (17.6 min), the region of the chromosome carried by these two plasmids (40), and the wild type is dominant in each case. It seems likely, therefore, that all these mutations map at or near the *tolP*, *-A*, and *-B* loci (3). A more detailed genetic analysis will be necessary to determine if any new loci are located in this area.

The resistance locus in P689, the Tol VII mutant, is transferred early by HfrH, and although it is relatively P1 resistant, P1 transduction has shown it to be 85% co-transducible with *thr*.

It was also possible to isolate several leu^+ transductants that co-transduced this locus, without *thr*. Although the number of transductants was too low to obtain a meaningful co-transduction frequency, the data demonstrate the existence of a new locus, which we have called *tolJ*, between *thr* and *leu*, and probably near 0.1 min on the genetic map.

Although we have not mapped the colicin resistance locus, the Tol VIII mutant, P602, has a phenotype similar (tolerant to colicin E1, but sensitive to E2 and E3) to those mutants shown to map at *tolC*, at 59 min on the genetic map (62, 65).

The resistance loci in the Tol IV, Tol XI, Tol XIV, and Tol XV mutants (P692, P220, P530, and P586) have not been mapped. They are not, however, transferred by HfrH when selecting for either thr^+ or his^+ recombinants, or by HfrC, when selecting for $proA^+$ or $argE^+$ recombinants. They must all map in the region between *his* (38.5 min) and *argE* (78.5 min).

DISCUSSION

An attempt has been made to make a comprehensive study of cross-resistance to colicins in *E. coli* K-12. The only colicins not included in this study are colicins O (31) and S8 (45), the colicin X of Papavassiliou (48), which is apparently different from colicin X-K235 (52), colicin P (24, 30), and the colicin L, described by Fredericq (24), which has been lost. Another colicin L (33) was used here. The more recently

described bacteriocin JF 246 (20) was also not used.

Many of the colicins originally described (21) have now been reclassified. *E. coli* CA42, for example, the type strain for colicin F, has been shown to produce colicin E2 (27), *E. coli* CA62, the type strain for colicin J, has been shown to produce both colicin E1 and I, and the colicin S5 produced by *Shigella dispar* P14 is colicin E1 (26). *E. coli* CA57, the type strain for colicin C has been lost (Fredericq, personal communication), although there is a strain in circulation called CA57, which appears to produce colicin E1. A series of biochemical tests (Davies and Reeves, unpublished data) shows this strain to be *S. dispar*, and not *E. coli*, as CA57 was originally described (21). There may, therefore, have been an error some time since the original classification, and perhaps *S. dispar* P14 (which produces colicin E1) has somehow come to be called *E. coli* CA57.

We have not been able to find any reference to colicins S6 and S7, but they presumably exist, as a colicin S8 has been described (45). The strain producing colicin S2 has been lost, and *S. sonnei* P9, the type strain for colicin S3, has been shown to produce colicins E2 and I (24).

The major finding in this study is the observation that colicins fall into two well defined groups which we have termed A and B (see Table 3). The absence of mutants resistant to colicins from both groups indicates that there must be at least two distinct pathways of colicin action in *E. coli* K-12, with little, if any, interaction between them.

Since cross-resistance within groups A and B is common, and between groups so rare that we have found none by selecting against one colicin at a time, it is surprising that this division into two groups has not been noted before. Hardy et al. (32), however, have divided a group of Col factors into two groups on several grounds, including the relative binding of colicin to *recA*$^-$ and *recA*$^+$ colicinogenic cells. Although they did not use as many colicins as has been used here, the two groups correspond exactly to groups A and B, supporting the theory that there are two very different groups of colicins active on *E. coli* K-12. The lack of cross-resistance between the two groups is even more surprising when one considers that both groups contain colicins that have similar modes of action: colicins D and E3 act on protein synthesis (5, 51, 58, 63), and colicins A and Ia affect energy metabolism (39, 43), although we have not isolated mutants resistant to both colicins.

Fredericq's original study (21) showed several examples of apparent cross-resistance between colicins of the two groups and we have no explanation for this. It is possible that in some instances selection of double mutants occurred due to the use of colicinogenic strains producing more than one colicin.

The invariable resistance of *bfe* mutants to colicin A, also commented on previously by Nagel de Zwaig and Luria (44), was not observed by Fredericq (21). Indeed, mutants specifically resistant to colicins E1, E2, and E3 (presumably *bfe* mutants) provided the basis for combining colicins E1, E2, E3, F, and S5 as colicin E. The discrepancy is probably due to the parent strain used to select the mutants, as Fredericq initially used 6 *S. sonnei* and *E. coli* strains but did not include *E. coli* K-12 (21, 22). A later study (Fredericq, personal communication) included K-12, and substantial cross-resistance between colicins E and A was observed. Had Fredericq originally used only strain K-12, colicin A would be considered a subtype of colicin E, but since its cross-resistance is strain dependent, it is probably better to leave the classification as it is.

Although a large number of mutants was selected, and the groups isolated include most of the previously isolated phenotypes, a few previously described mutant classes were not isolated. These are summarized in Table 9. They include classes Tol IIa and Tol IIIa, mapping at *tolP* in the *tolP,A,B* cluster near *gal*. Because of their partial resistance to colicins (3, 44), it is possible that any such mutants isolated may have been discarded during the initial screening procedures as being sensitive. More recently, mutants mapping at *tolD* (7),

TABLE 9. *Previously described tolerant mutant classes not isolated in this study*[a]

Phenotypic class	Colicin resistance[b]	Reference
Tol IIa	pE1 E2 E3 A K	3, 44
Tol IIIa	pE2 E3 A K	3, 44
Ref III	E3 K	34
Tol V	E1 E2 E3 K^S [c, d]	47
Tol VI	E1 E2 K^S	47
P116	E2 A[e]	50
P137	E2[e]	50

[a] In addition, various different receptor mutants, similar to the Bfe group have been described (34, 50).

[b] p, Partial resistance.

[c] K^S denotes sensitivity to colicin K.

[d] Also probably included in this group is the single Ref VI mutant (34) that is sensitive to colicin K.

[e] These mutants were sensitive to some colicin E2 species (E2-CA42, E2-P9) but tolerant to others (e.g., E2-K317).

tolE (18), *tolF*, and *tolG* (20) have been described.

In addition to revealing the new colicin resistance loci *tolJ* (0.1 min), *rex* (7.8 to 13.3 min) and *con* (14.5 min), this study has revealed a further diversity of phenotypic classes of tolerant mutants mapping at, or near, the *tolP,A,B* cluster at 16.5 min. In addition, four different phenotypic classes of mutants remain unmapped, although they clearly do not map at any of the previously described loci (62). A more detailed genetic analysis, particularly with those mutants mapping near the *tolP,A,B* cluster, will be needed to reveal the full complexity of the various loci.

In the initial model for the general mode of action of colicins put forward by Nomura (46), it was postulated that the colicin molecule adsorbed to a receptor on the surface of a sensitive cell and stayed there, exerting its effect on a specific target by transmitting a message, perhaps via the cytoplasmic membrane (10). Recent work, however, has suggested that colicin E3 enters the cell and acts directly upon its target, the 16*S* ribosomal ribonucleic acid (4, 5, 58). Therefore any model for colicin action must now accommodate the transmission of the colicin E3 molecule, or at least part of it, to the interior of the cell, rather than, or as well as, a colicin-activated specific message.

Indeed, the extensive cross-resistance between colicin E3 and other colicins of group A, especially colicin E2, suggest that other colicins have several steps in common with that of E3, and may also act directly on their targets. Furthermore, although there is as yet no comparable evidence for other colicins acting directly on their targets, most of the recent data on the mode of action of various colicins are at least compatible with the idea that they are themselves transported to their targets (1, 11, 49, 61).

Tolerant mutants, whether they were involved in transport of a colicin molecule, or of a message to the interior of the cell, could show a variety of other defects, depending on where in the transmission process the mutation was located. Colicin-tolerant mutants that also show an increased sensitivity to ampicillin, for example, presumably have a cell envelope altered to such an extent that the cell wall synthesizing machinery, located near the cytoplasmic membrane, is more readily accessible to the ampicillin. As Triton X-100 acts selectively on the cytoplasmic membrane (57), any mutation causing greatly increased sensitivity to this agent is likely to involve changes in the cell envelope structure which are drastic enough to allow greatly increased access to the cytoplasmic membrane itself. It may, in fact, be that this increased access to the cytoplasmic membrane occurs only at specific sites of entry for colicin molecules as postulated recently (15) as P602, although supersensitive to both ampicillin and Triton X-100, is quite viable.

In Table 10, the 21 mutant classes are grouped according to the sensitivity of the type strains to the various antibiotics, detergents and surfactants used. Groups 1 to 7 show a series of changes reflecting what appears to be a gradually increasing alteration of the cell envelope structure. They range from group 1, which includes the receptor mutants, and show no alteration in sensitivity patterns, through to group 7, containing the Tol VIII mutant, P602, which shows greatly increased sensitivity to every detergent and surfactant tested, as well as a range of antibiotics. It appears to be a *tolC* mutant, examples of which have been shown to have a missing membrane protein (55, 65).

The last group (group 8) consisting of five classes (representing 6 of the 87 mutants studied in detail) contains the mutants which do not fit exactly into any of the other seven groups. The Tol XVII mutant is similar to the mutants of group 6, except that it does not show increased sensitivity to phenethyl alcohol. Similarly, the Tol IV mutant differs from those in group 2 only in its partial sensitivity to Triton X-100, and the Tol XVI mutant differs only in its sensitivity to sodium deoxycholate. The Con mutant may belong to group 3. Tol VII differs from group 1 only in that it is sensitive to phenethyl alcohol.

If one now looks at the colicin resistance patterns of the mutants in the groups, it becomes apparent that tolerance to individual colicins arises in different ways. It seems that tolerance to colicins K, L, A, N, and S4 can be accompanied by almost no detectable change in the cell envelope. Full tolerance to colicins E2 and E3, however, only occurs after more substantial changes to the cell envelope. However, once this level of alterations has occurred, the mutants are resistant to all these colicins, even if the changes are much more substantial. It seems, therefore, that tolerance to colicins A, E2, K, L, N, and S4 can be fairly nonspecific, in that once a certain degree of damage has occurred to the cell envelope, the cell in general becomes resistant to the effect of these colicins. The major exceptions to this general rule appear to be the six mutants in group 8.

Resistance to colicins E1 and X, however,

TABLE 10. *Classification of mutant phenotypes on their antibiotic, detergent and surfactant sensitivity, with genetic location of the mutalations*

Group	Phenotypic class	Colicin resistance[a]									Sensitivity	Map position
1	Bfe	E1	E2	E3			A	(BF23)				78.9 min[b]
	Tsx				K			(T6)				9.8 min[b]
	Rcx									X	None	7.8–13.3 min
	Tol XI				pK	L	pA	S4				
	Tol XII			pE3		L	pA	S4				near *gal*[c]
	Tol Ia				K	L	A	S4				near *gal*[d]
	Tol Ib				K	L	A	S4	N			near *gal*[d]
	Tol IX				pK	L	A		N			near *gal*[c]
	Tol X				pK	L	A	S4	N			near *gal*[d]
2	Tol XIV		E2	E3	K	L	A		N	X	pEDTA	
	Tol XV		E2	E3	K	L	A	S4	N	X		
3	Tol IIc	E1	E2	E3	K	L	A	S4	N		pEDTA, PEA	near *gal*[c]
4	Tol IIb	E1	E2	E3	K	L	A	S4	N		AMP, DOC, pEDTA, PEA	near *gal*[c]
5	Tol XIII		E2	E3	K	L	A	S4			AMP, DOC, EDTA, PEA	near *gal*[c]
6	Tol III		E2	E3	K	L	A	S4	N		AMP, DOC, EDTA, PEA, pTriton	near *gal*[c]
7	Tol VIII	E1			pK		A				AMP, DOC, EDTA, PEA, Triton, SDS	59 min[b]
8	Tol IV		E2	E3		L	A		N		pEDTA, pTriton	
	Tol VII		pE2		K	L	A	S4			PEA	0.1 min
	Tol XVI		pE2	pE3		L	A				pEDTA, DOC	near *gal*[d]
	Tol XVII	E1	E2	pE3	K	L	A	S4	N		AMP, DOC,	near *gal*[c]
	Con				K	L					pEDTA, pTriton-EDTA, PEA	14.5 min

[a] p, Partial; other abbreviations defined in Table 7.
[b] From Taylor and Trotter (62).
[c] Locus on F1 Gal and F2 Gal plasmids.
[d] Linked to Gal in Hfr crosses.

seems to be much more specific. The existence of a certain degree of damage is not sufficient to cause tolerance in itself. In both cases, tolerance seems to occur because of mutations at specific sites (for example, besides Rcx, only the Tol XIV and Tol XV mutants are resistant to colicin X).

It should be remembered that the mutants studied were selected as colicin resistant, and that nonspecific structural damage to the cell envelope may well occur without any accompanying colicin resistance pattern. Indeed, Tol VIII shows substantial changes to the cell envelope (Tol VIII mutants mapping at *tolC* have been shown to be missing a membrane protein [54]) but shows tolerance only to colicins E1, K, and A, and not, as one might expect with such widespread structural alterations, to colicins E2, E3, L, N, and S4. Presumably a certain class of envelope mutants damages the membrane in a specific way to varying extents, the damage being at the sites involving colicin action. Damage elsewhere may affect detergent sensitivity, but not colicin tolerance.

Continuing investigations of the membrane protein composition of these mutants should assist in our understanding of colicin resistance and, therefore, the general mode of action of colicins.

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