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Penetration of Colicin M into Cells of *Escherichia coli*

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A new class of colicin M-tolerant mutants of *Escherichia coli* K-12 was isolated. The mutants exhibited an unusually high tolerance in that they were unaffected by colicin titers of 10^6. The tolerance was confined to colicin M. It was mapped at a locus called *tolM*, which is close to *rpsL*. The following gene order was determined: *aroE*, *tolM*, *rpsL*, *cysG*. The tolerance could be caused by a defect in the uptake of colicin M or by a mutation at the site of action. Insensitive *tonA* and *tonB* mutants became sensitive to colicin M upon treatment by osmotic shock, whereas the *tolM* mutants remained insensitive. Trypsin rescue experiments showed that the *tonB*-dependent uptake of colicin M required energy like the other *tonB*-related transport processes. When bound to energy-depleted cells, colicin M prevented adsorption of phage T5. The receptor became accessible to the phage when the cells were energized, except in *tonB* mutants. These data suggest that the function controlled by the *tonB* gene is required for the translocation of colicin M from its initial binding site at the *tonA*-coded receptor protein to the target.

Sensitive cells of *Escherichia coli* require functions specified by the *tonA* and *tonB* genes in order to be killed by colicin M. The *tonA* gene product is a protein located in the outer membrane (8, 9). It serves as a receptor for colicin M and the phages T5, T1, and φ80. Mutants in the *tonA* gene are resistant to these agents. They are also unable to take up iron as ferrichrome complex, and their growth is not inhibited by the structurally similar antibiotic albomycin. *tonB* mutants are also deficient in ferrichrome-mediated iron uptake, and they are unaffected by colicin M, albomycin, T1, and φ80 (4, 6, 17, 18, 22). We describe here a third class of mutants which are completely insensitive to colicin M but fully active with regard to the other *tonA* *tonB*-dependent agents. The interaction of the *tonA*- and *tonB*-controlled functions for the action of colicin M was determined in order to define the functional defect of the tolerant mutants. In our studies we tried to obtain further insight into the functional properties of the outer membrane and its interaction with the cytoplasmic membrane.

MATERIALS AND METHODS

Bacteria and standard procedures. The strains used are listed in Table 1. Growth of cells and phages in tryptone yeast medium or in M9 glucose minimal medium was as described previously (8, 13). The genetic techniques were performed as described by Miller (20). Colicin M-tolerant mutants were isolated by dropping a solution with a titer of 100 onto agar plates containing M9 salts glucose minimal medium supplemented with the required nutrients and seeded with 10^8 cells of *E. coli* 3282 (for complete description of strains, see Table 1). Colicin-insensitive colonies were picked up and cross-streaked over plates onto which filter paper disks impregnated with a solution of albomycin (10 mg/ml) were applied. Of 41 colicin-insensitive colonies, 3 were albomycin sensitive. They were designated by the allele numbers *tolM40*, *tolM41*, and *tolM42* (B. Bachmann, Genetic Stock Center record). They proved to be sensitive to phages T5 and T1 and to colicins B, Ib, and E1. They were as sensitive to ampicillin (0.125 mg/ml), actinomycin D (1 mg/ml), deoxycholate (1%), erythromycin (3 mg/ml), kanamycin (10 mg/ml), spectinomycin (0.4 mg/ml), and fusidic acid (1.9 mg/ml) as the parent strain. The inhibitors were applied on filters, and the diameter of each zone of inhibition was determined.

Mapping of *tolM*. The marker conferring colicin M insensitivity was mapped as follows. A *leu*$^+$ derivative of *E. coli* 3282 *tolM dap lysA thi rpsL*, which was obtained by mating with *E. coli* HfrH, was crossed with *E. coli* KL 228 *leu*, and various marker combinations were selected. To map the *tolM* marker more precisely, *E. coli* AT 2455 *cysG* was transduced with P1 lysates of the three *tolM* derivatives of *E. coli* 3282 *rpsL* to *cysG*$^+$. In addition, *E. coli* HA 121 *aroE rpsL* was infected with a P1 lysate of *E. coli* HA 122 *tolM42* and selected for *aroE*$^+$.

Bypass experiments with colicin M. (i) Procedure 1. The experimental details followed basically those described by Tilby et al. (28). In short, wild-type cells and *tonA*, *tonB*, and *tolM* derivatives of *E. coli* AB 2847 were grown at 37°C with shaking in 100 ml of tryptone yeast medium to a density of about 5×10^8 cells per ml. The cells were centrifuged, and the pellet was suspended in 8 ml of a buffer containing 0.01 M Tris-hydrochloride and 0.03 M NaCl (pH 7.0). The cells were sedimented again, and the washing proce-

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TABLE 1. *Strains of E. coli employed*

Strain	Genotype, phenotype, and/or derivation	Source or reference
E. coli K-12		
W 945 T 3282	*thr leu trp his lysA thy thi dap nagA rpsL*	U. Henning
3282 *tolM40*	Spontaneous colicin M-tolerant derivative of W 945 T 3282	This study
3282 *tolM41*	Same as 3282 *tolM40*	This study
3282 *tolM42*	Same as 3282 *tolM40*	This study
Mo3	P1 transduction of 3282 *tolM40* into AB 2847	S. Mollner
Mo4	P1 transduction of 3282 *tolM41* into AB 2847	S. Mollner
Mo6	P1 transduction of 3282 *tolM42* into AB 2847	S. Mollner
AB 2847	*aroB thi tsx malT*	B. Bachmann
P8	*tonA* spontaneous T5-resistant nonsense derivative of AB 2847	5
41/2	*tonA* spontaneous missense mutant of AB 2847	15
BR 158	*tonB* spontaneous mutant of AB 2847	14
IR 112	*tonB* spontaneous mutant of AB 2847	14
Wo 3	*tonA tonB* spontaneous double mutant of AB 2847	This study
AT 700	*lac gal his aroE argG rpsL mal xyl mtl tsx tonA thi*	B. Bachmann
HA 121	Same as AT 700, but $tonA^+$ by P1 transduction of Tn*10* near $tonA^+$ from 1020	This study
HA 122	*mal thi tolM42*; P1 transduction from donor Mo6 into AT 2455	This study
1020	*lac trp his metB* Tn*10* near $tonA^+$	K. Hantke
AT 2455	*mal cysG*	B. Bachmann
E. coli KL 228	*leu lac thi*; Hfr strain	U. Henning
E. coli Cl 139	(Col B, Col M)	S. Guterman
E. coli Ymel		U. Henning

dure was repeated. The cells were then suspended in 4.5 ml of 33 mM Tris-hydrochloride (pH 7.0), and a 1-ml sample was added to each of four glass tubes. A solution (1 ml) of 40% sucrose in 33 mM Tris-hydrochloride (pH 7.0) was added to two tubes, and the mixture was shaken vigorously with a Vortex mixer; to the other two tubes only buffer without sucrose was added. These procedures were all done at room temperature. The cells were pelleted at 4°C. A colicin M solution (10 μl containing 10 μg of protein) with a titer of 10^5 was added to the cells of two tubes, one of which contained cells treated with sucrose. Colicin M was rinsed into the tubes with 2 ml of ice-cold 0.5 mM $MgCl_2$, which was also added to the other two tubes without colicin M. The cells were immediately suspended with the Vortex mixer and kept in ice for 15 min. Appropriate dilutions were plated on tryptone yeast extract agar plates.

(ii) Procedure 2. Plasmolyzed cells were prepared in a manner similar to the procedure described by Shimizu and Sekiguchi (27). Cells which had been grown overnight in 2 ml of tryptone yeast extract medium were washed with 5 ml of ice-cold M9 salts. The pellet was suspended in 1 ml of a freshly prepared ice-cold solution prepared by mixing 0.8 ml of 0.1 M ethylene glycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetate (pH 7.0) with 0.8 ml of 0.4 M Tris-hydrochloride (pH 7.0) and 6.4 ml of 60% sucrose. After the cell suspension stood for 30 min at 0°C, 0.1 ml was diluted with 0.9 ml of ice-cold M9 salt solution. After a 10-μl sample of colicin M (titer, 10^5) was added (control without colicin M), the suspension was kept on ice for 30 min and then diluted for plating the surviving cells onto tryptone yeast extract agar.

Rescue experiment by treatment with trypsin. About 3.5×10^5 cells of *E. coli* AB 2847 in 1 ml were preincubated for 5 min with the supplements or under nitrogen, as described in the legend to Fig. 2. At zero time 10 μl of colicin M with a titer of 50 was added. After various time intervals 50-μl samples were transferred into 5 ml of M9 salt solution containing 1.25 mg of trypsin. After incubation for 1 h, appropriate dilutions were plated onto tryptone yeast agar, and the colonies were counted after growth overnight.

Source of colicin M. Colicin M was isolated from *E. coli* C1139 essentially as described previously (7). Upon mitomycin induction, this strain produced large amounts of colicins B and M (K. Schaller and R. Dreher, manuscript in preparation). Even at 10^6-fold dilutions (titer, 10^6), the fractions containing colicin M after column chromatography on carboxymethyl cellulose yielded a clear zone of growth inhibition when 10 μl (1 mg of protein per ml) was spotted onto test plates of the indicator strain *E. coli* AB 2847.

Outer membranes. Outer membranes were prepared by the method of Osborn et al. (24), and the proteins were separated by sodium dodecyl sulfate-gel electrophoresis as described by Lugtenberg et al. (19).

Chemicals. The chemicals used were of the highest purity available. Albomycin was a gift from H.-P. Fiedler and H. Zähner of this institute.

RESULTS

Isolation of colicin M-tolerant mutants and mapping of colicin M-tolerant mutations. Colicin M-insensitive mutants with a functional *tonA*-coded receptor protein are by definition tolerant. If they remain fully sensitive to phages T1 and φ80 and to albomycin, they are most likely also not of the *tonB* type. Three such

mutants were isolated from a culture of *E. coli* 3282 (see Table 1 for a full description of the strains used). To exclude *tonA* and *tonB* mutations, which confer insensitivity to colicin M but do not alter other *tonA*- and *tonB*-dependent functions, we performed P1 transductions of the *dapD*$^+$ marker close to *tonA* and of the *trp*$^+$ marker close to *tonB* into strains requiring diaminopimelate and tryptophan, respectively. No change in colicin M sensitivity was observed in 100 *dapD*$^+$ and 100 *trp*$^+$ transductants examined.

Colicin M-sensitive recombinants of crosses between *E. coli* KL 228 as donor and *E. coli* 3282 *tolM rpsL* (selection for *his*$^+$ *leu*$^+$, *lys*$^+$ *dap*$^+$ *leu*$^+$, *thy*$^+$ *leu*$^+$) became all streptomycin sensitive, indicating a linkage between the TolM phenotype and the *rpsL* gene. In addition, P1 cotransduced *tolM* of *E. coli* 3282 *tolM43 rpsL* in 89 of 100 cases tested together with *rpsL* into *E. coli* Ymel. Finally, the cotransduction frequency among *cysG*, *rpsL*, and *tolM* was determined (Table 2). The reduced cotransduction frequency with *tolM* compared with *rpsL* indicated that the loci of the *tolM* mutations were counterclockwise to *rpsL* and very close to 72 min (Fig. 1). This finding was corroborated by the results of studies of the cotransduction frequency of *aroE* with *tolM* and *rpsL* (Table 2 and Fig. 1), which showed a gene order of *aroE*, *tolM*, *rpsL*.

Properties of the colicin M-tolerant mutants. The tolerant mutants showed growth properties and sensitivities to ampicillin, actinomycin D, deoxycholate, and colicins E2 and E3 like those of the parent strains, indicating no major membrane alterations, which were found frequently with other colicin-tolerant mutants (18, 21, 23). Zones of growth inhibition were determined on plates with standard filter paper disks impregnated with solutions of the antibiotics. Six different concentrations were tested above the minimal inhibitory concentrations, which are given above. In addition, growth curves of liquid cultures were recorded with 1% deoxycholate and with various concentrations of penicillin G. There were also no alterations recognizable in the protein patterns of isolated outer membranes after separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (data not shown). Since the tolerance locus mapped in a region in which ribosomal genes are clustered (1), we tested sensitivities to antibiotics acting on ribosomes. The mutants remained fully sensitive to kanamycin, spectomycin, erythromycin, and fusidic acid.

Although all three strains were isolated with a low colicin titer of 100, their tolerances were unusually high. A colicin solution with a titer of

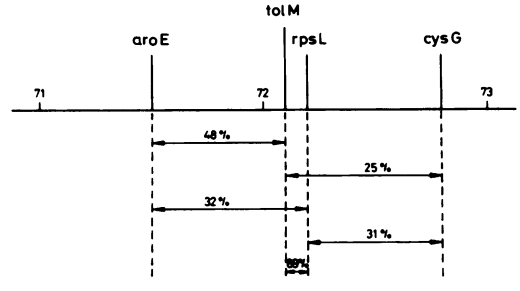

FIG. 1. *Gene order at the tolM locus close to 72 min of the E. coli linkage map, as deduced from the P1 transduction frequencies listed in Table 2.*

TABLE 2. *Genetic analysis of tolM mutants by P1 transduction*

Donor[a]	Recipient[a]	Selected marker	Recombinant class	Cotransduction frequency (%)
3282 *tolM40 rpsL*	AT 2455 *cysG*	*cysG*$^+$	*rpsL tolM*	16
			rpsL tolM$^+$	12
			rpsL$^+$ *tolM*$^+$	70
			rpsL$^+$ *tolM*	2
3282 *tolM41 rpsL*	AT 2455 *cysG*	*cysG*$^+$	*rpsL tolM*	29
			rpsL tolM$^+$	5
			rpsL$^+$ *tolM*$^+$	65
			rpsL$^+$ *tolM*	1
3282 *tolM42 rpsL*	AT 2455 *cysG*	*cysG*$^+$	*rpsL tolM*	26
			rpsL tolM$^+$	5
			rpsL$^+$ *tolM*$^+$	69
			rpsL$^+$ *tolM*	0
HA 122 *tolM42*	HA 121 *aroE rpsL*	*aroE*$^+$	*rpsL tolM*	23
			rpsL tolM$^+$	70
			rpsL$^+$ *tolM*$^+$	1
			rpsL$^+$ *tolM*	42

[a] Both donors and recipients were strains of *E. coli* K-12.

10^6 caused no zone of growth inhibition on plates. When the *tolM* genes were transduced with P1 into *E. coli* AB 2847, the recipients (designated Mo3, Mo4, and Mo6) showed the same high tolerances.

Energization of colicin M uptake. It is assumed that colicins still bound to the receptor at the cell surface can be degraded by trypsin. At a later stage colicins become trypsin resistant and kill cells (16). It has been shown that energy is required for the action of a number of colicins (16). A question arose as to whether the action of colicin M was also energy dependent.

Cells of *E. coli* AB 2847 were grown anaerobically in tryptone yeast medium supplemented with 0.2% glucose and 1% KNO_3 to induce the nitrate reductase. When the cells were also incubated with colicin M under anaerobic conditions, trypsin addition rescued the cells regardless of whether nitrate and formate or glucose were added (Fig. 2). Cells incubated aerobically with colicin M in M9 buffer without an energy source were also fully rescued by trypsin. However, when glucose was added under aerobic conditions, killing occurred rapidly (Fig. 2). Similar results were obtained with aerobically grown cells which had been energy depleted by shaking for 2 h with dinitrophenol in buffer (Fig. 2). The number of viable cells remained nearly constant after an initial decrease of 40%. The moment glucose was added and cells were aerated, colicin M killed the cells. Killing also occurred when cells were diluted in buffer at the same time so that the concentration of colicin M fell below the level of inhibition. This showed that colicin M adsorbed to the cells under energy-depleted conditions and was able to kill cells when they were energized by glucose and respiration. Lactate could replace glucose as a nutrient.

For comparison, experiments with colicins B, E1, and Ib were performed under the same experimental conditions. The behavior of colicin B was very similar to that of colicin M (data not shown). However, colicin E1 killed cells under anaerobic conditions, in contrast to colicin M. Killing by colicin E1 was enhanced under aerobic conditions. Anaerobically grown cells incubated with colicin Ib could be rescued almost completely by treatment with trypsin even when they were shifted to aerobic conditions in the presence of glucose. After aerobic growth, colicin Ib killed cells with kinetics similar to those obtained with colicin M.

Inhibition of the adsorption of phage T5 to cells by colicin M. Colicin M at a concentration of approximately 2,000 molecules per cell was incubated with cells before T5 was added. Binding of colicin M to the tolerant cells could

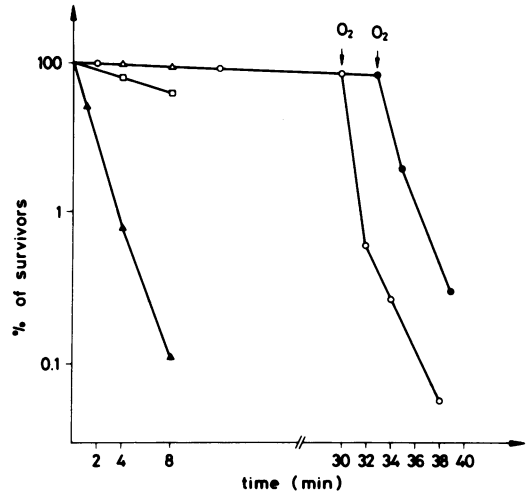

FIG. 2. *Killing of cells of E. coli AB 2847 by colicin M added at zero time. After the time intervals indicated on the abscissa, samples were withdrawn and treated for 1 h with trypsin. Surviving cells were determined by plating onto tryptone yeast agar. Cells were grown anaerobically with 0.2% glucose and 1% KNO_3 and treated with colicin M under anaerobic conditions in M9 salt buffer* (△), *or they were grown with M9 salt buffer supplemented with 0.4% glucose, 1 mM formate, and 1% KNO_3* (□) *or under aerobic conditions with 0.4% glucose* (▲) *before trypsin was added. In another experiment cells were grown aerobically and treated for 2 h with 1 mM dinitrophenol in M9 buffer. They were treated with colicin M under anaerobic conditions for 30 min. The incubation mixture was then diluted 100-fold to prevent further adsorption of colicin M and kept aerobically in the presence of 0.4% glucose for 2, 4, and 8 min until trypsin was added* (○). *In another experiment aerobically grown cells were energy depleted for 2 h by shaking with 1 mM dinitrophenol and treated with colicin M for 33 min anaerobically before they were transferred to aerobic conditions with 0.4% glucose* (●). *They were kept for 2 and 6 min before trypsin was added.*

be demonstrated by the inhibition of T5 adsorption (Fig. 3). The inhibitory effect could be partially reversed by degrading the bound colicin with trypsin (Fig. 3). In these experiments the incubation with trypsin was for only 5 min so that the unblocking of the receptor was less effective than in the trypsin rescue experiments shown in Fig. 2, where trypsin acted for 1 h. The control experiments without added colicin M showed that the adsorption rate of T5 was not affected by trypsin (Fig. 3).

The translocation of colicin M from its initial binding site at the receptor toward its target site was then studied. It was assumed that the inhibition of T5 adsorption took place only as long as the colicin was bound to the receptor. In the

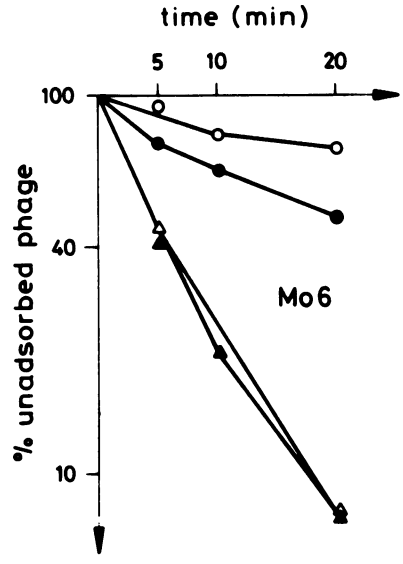

FIG. 3. *Inhibition of adsorption of phage T5 to the colicin-tolerant strain E. coli Mo6 by colicin M. Cells were grown to a density of 3 × 10⁸ cells per ml in tryptone-yeast medium. They were pelleted, washed twice with M9 buffer, and shaken for 2 h at 37°C with 1 mM dinitrophenol in M9 buffer. They were then pelleted, washed three times with M9 buffer, and resuspended in M9 buffer to an optical density of 2.0. Cells (1 ml) were incubated for 5 min with colicin M (10 μl; titer, 10⁴) (○ and ●) or without colicin M (△ and ▲). Then trypsin (50 μg) was added to two samples (● and ▲). After 5 min, phage T5 (0.5 plaque-forming units per cell) was added, and the amount of unbound phage in the supernatant was determined after the times indicated on the abscissa.*

tonA⁺ *tonB*⁺ strain colicin M prevented T5 binding most effectively when the cells were unenergized (Fig. 4). When they were energized by the addition of glucose, inhibition of T5 adsorption decreased from 42 to 16% (Fig. 4). T5 adsorption without colicin M was unaffected by the energy state of the cells (Fig. 4). The behavior of *tonB* cells was of great interest. If the *tonB* function is involved in the translocation of colicin M across the outer membrane, colicin M should prevent T5 adsorption regardless of whether the cells are energized. In fact, colicin M inhibited T5 adsorption to energized and unenergized cells to the same extent (Fig. 5). T5 binding to cells without colicin M was unaffected by the *tonB* mutation (Fig. 5). The degree of inhibition in *tonB* mutants was higher than in unenergized *tonB*⁺ cells. In the latter case (Fig. 4), the cells were not completely energy depleted by the treatment with dinitrophenol, and oxygen had access to the cells during centrifugation with bound colicin. A portion of the colicin could therefore be released from the receptor, which allowed access of T5. In *tonB* mutants colicin M apparently stayed at the receptor protein.

Bypass experiments. To differentiate functionally among *tolM*-, *tonA*-, and *tonB*-related functions, we determined which of the three kinds of defects in mutants could be bypassed by altering the membrane permeability. When

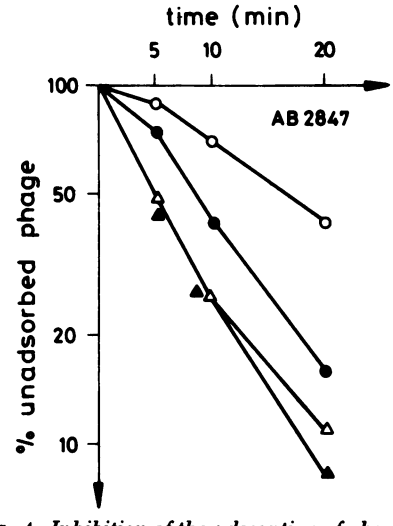

FIG. 4. *Inhibition of the adsorption of phage T5 to wild-type strain E. coli AB 2847 by colicin M. Cells were pretreated with dinitrophenol as described in the legend to Fig. 3. Samples were incubated with colicin M for 5 min (○ and ●) or without colicin M (△ and ▲), centrifuged, and suspended in M9 buffer (○ and △) or in M9 buffer containing 0.4% glucose (● and ▲). Phage T5 was added after 5 min of incubation, and the unadsorbed fraction was determined as described in the legend to Fig. 3.*

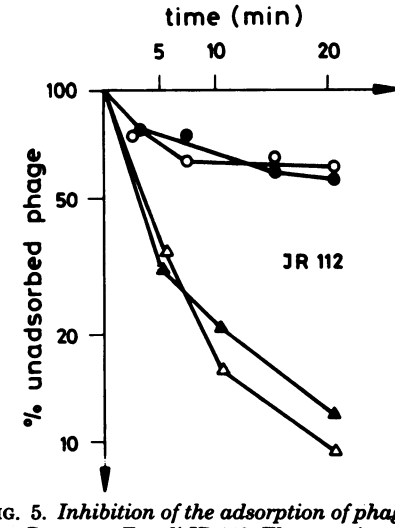

FIG. 5. *Inhibition of the adsorption of phage T5 to the tonB mutant E. coli IR 112. The experiments were performed as described in the legend to Fig. 4.*

colicin M (10 μg/2 ml) was present during osmotic shock, resistant *tonA* and *tonB* mutants became sensitive (Table 3). The bypass of the *tonA* mutation was more effective than that of the *tonB* mutation. *tonA tonB* double mutants became as sensitive as *tonB* mutants (Table 3). In contrast, none of the *tolM* mutants was killed by colicin M upon osmotic shock. In the latter strains the reduction in viable cells by osmotic shock alone was the same as reduction by simultaneous treatment with colicin M.

The *tonA* and *tonB* mutants became sensitive to colicin M to the same extent when plasmolyzed cells were diluted into buffer as described above. The method used to shock the cells osmotically was therefore not a determining factor in the effectiveness of transfer of colicin M from the medium to the target site.

Ferrichrome protects cells against colicin M (4, 6, 22). When colicin M was shocked into cells of the wild-type strain or into *tonA* or *tonB* mutants in the presence of 50 μM ferrichrome, only a slight increase in survivors was observed.

DISCUSSION

Colicin M belongs to the group B colicins (10), which show the common property that *tonB* mutants are tolerant to high doses of all of these colicins. However, the tolerance described in this paper was confined to colicin M. The properties of the tolerant mutants and the map position of the *tolM* gene exclude identity with *tonB* and any other defined type of colicin tolerance (1). It is likely that Davies and Reeves isolated the same type of mutant, which they mapped between 44 and 88 min and called *cmt* (10).

Tolerant mutants may be altered in a step of uptake after binding to the receptor or at the site of action. To our knowledge, the latter type of alteration has not been described, although it might be difficult to differentiate between the two possibilities in cases in which colicins alter the properties of membranes, as has been shown for colicin K (26) and colicin I (29). The action of colicin M leads to cell lysis connected with destruction of the murein (7), yet no murein hydrolase activity could be demonstrated in vitro (K. Schaller and R. Dreher, unpublished data). The tolerance exhibited by the mutants was extremely high since an unidentified colicin solution with a titer of 10^6 had no effect on the growth of cells on plates (drop test). Tolerance is frequently found to be orders of magnitude below the resistance conferred by a receptor mutation (18, 21, 23, 25). The complete insensitivity of the colicin M-tolerant mutants suggests an alteration at the target site rather than one in the uptake system. In this context we performed the bypass experiments. Loss of the *tonA*- and *tonB*-controlled functions involved in colicin uptake could be overcome by osmotic shock. In contrast, *tolM* mutants remained fully insensitive. This result could be taken as evidence in favor of a target site mutation. However, it has been shown that mutants tolerant to the functionally unrelated colicins E3 (18) and L (11) were not killed by osmotic shock treatment in the presence of the colicins. Only insensitivities caused by mutations of outer membrane components could be overcome by osmotic shock (28). This could also hold true for colicin M, in which case the bypass of the lack of the *tonB*-controlled function is very interesting because it would show for the first time that it is required for transfer across the outer membrane. Several lines of evidence relate the *tonB*-controlled function to events in the outer membrane. It is only required for certain uptake systems which involve binding of substrates, phages, and colicins to receptor proteins at the cell surface (2, 3, 5, 12, 17, 18, 22, 30). In the case of infections of cells by phages T1 and φ80, irreversible adsorption, clearly a surface event, required not only the *tonB* function but also energization of the cells (13). In addition, adsorption of T5 was only inhibited by ferrichrome in *tonB* mutants or in energy-depleted $tonB^+$ cells (15). Exactly the same situation applies for the prevention of T5 adsorption by colicin M. Only unenergized $tonB^+$ cells were protected, but *tonB* mutants remained T5 resistant regardless of whether they were energized. Colicin M apparently stays bound to the receptor in *tonB* mutants and in unenergized $tonB^+$ cells. In the colicin-tolerant mutants, cells could be protected against T5 adsorption by colicin M. Energized *tolM* cells with bound colicin adsorbed less T5 than $tolM^+$ cells, but the protection was not as efficient as with *tonB* mutants (data not shown).

TABLE 3. *Action of colicin M on E. coli K-12 AB 2847 and its derivatives*[a]

Strain	Surviving cells (% of input)		
	Shock alone	Colicin M without shock	Colicin M with shock
AB 2847	40	0.7	0.3
P8 *tonA*	35	92	0.32
41/2 *tonA*	34	96	0.37
BR 158 *tonB*	24	100	1.4
IR 112 *tonB*	62	100	1.2
Wo 3 *tonA tonB*	35	100	1.3
Mo3 *tolM40*	27	100	29
Mo4 *tolM41*	22	100	24
Mo6 *tolM42*	10	87	11

[a] The osmotic shock treatment followed procedure 1 (see text).

Jetten and Jetten showed in a comparative study (16) that most likely an energized state of the cytoplasmic membrane is required for the transition from the trypsin-sensitive stage to the trypsin-insensitive stage of colicins E2, E3, and K. We noticed differences in the kinds of energization among various colicins. For example, cells grown anaerobically were effectively killed by colicin E1 under anaerobic conditions in the presence of glucose. Under these conditions they remained largely unaffected by colicin M but were killed when they were aerated. Anaerobically grown cells were insensitive to colicin Ib when treated aerobically or anaerobically. Only aerobically grown cells were killed by colicin Ib after cells had been energy depleted by treatment with dinitrophenol and reenergized by aeration and addition of glucose. These preliminary observations demonstrate that a more detailed investigation will have to be performed to unravel the kind of energy required for the uptake (and the action) of the various colicins. The term membrane potential is not sufficient to describe adequately the mechanism of energization.

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