JOURNAL OF BACTERIOLOGY, Oct. 1969, p. 301-309
Vol. 100, No. 1

Kinetics of Adsorption of Colicin CA42-E2 and Reversal of its Bactericidal Activity

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Received for publication 27 June 1969

The kinetics of killing of *Escherichia coli* K-12 by colicin CA42-E2 have been studied, and the data were used to estimate the adsorption constant of this colicin under various environmental conditions. Evidence was obtained suggesting that the adsorption of colicin occurred in two stages; the earlier stage was reversible and did not lead to the death of the cell, the latter stage was irreversible and bactericidal. Cells which had adsorbed a lethal quantity of colicin could be rescued for a short time by inactivating the adsorbed colicin with trypsin. However, when the metabolic activity of the cells was totally arrested the lethal effect of adsorbed colicin was subject to trypsin reversal over long periods of time.

Previous studies with regard to the kinetics of killing by colicins suggest that the bactericidal effect can result from interaction between a single colicin particle and each cell that is killed and that, as with bacteriophage and phage ghosts, killing is initiated by a specific irreversible adsorption of colicin to the sensitive cell (6, 13, 16, 22, 25). The kinetics of killing have usually been investigated by incubating cells with colicin and sampling and diluting at suitable intervals to determine the number of cells able to produce colonies on nutrient agar. Such techniques do not determine the number of cells actually killed by colicin at the time of sampling but those which have adsorbed a lethal quantity of colicin at that time. With such procedures the kinetics of adsorption of colicin CA42-E2 to *Escherichia coli* K-12 have been examined in various media.

MATERIALS AND METHODS

Bacterial strains. The colicin-sensitive strains used in all experiments were either *Escherichia coli* P501, a streptomycin-resistant derivative of the K-12 Met- strain 58-161 (26), or *Escherichia coli* P503, a thymine-dependent derivative of *E. coli* P501 prepared from that strain by the method of Okada, Homma, and Sonohara (19).

Media. Nutrient broth was prepared from the dehydrated granules of nutrient broth No. 2 (CM67; Oxoid) and nutrient agar from the dehydrated granules of blood agar base (CM55; Oxoid). Minimal salts solution was a solution of salts as formulated by Davis and Mingioli (3). Casamino Acids minimal medium was a 0.2% w/v solution of Casamino Acids (Oxoid) in minimal salts solution at *p*H 7.4; 0.5% glucose w/v was added as a carbon source to produce MCG medium, 50 μg/ml of thymine was added for the growth of *E. coli* P503.

Colicin. Colicin CA42-E2 was prepared by the method of Reeves (22).

Bactericidal assays. Log-phase cultures were grown in nutrient broth or MCG medium at 37 C with shaking to about 3×10^8 cells/ml. Colicin was then added to the culture which was maintained at 37 C. Samples were diluted into sufficient nutrient broth or minimal salts solution to stop further adsorption of colicin. Counts were then made in fourfold replication by the method of Miles and Misra (15). For studying the trypsin inactivation of adsorbed colicin, initial dilutions were made into and incubated with a solution of trypsin (5 mg/ml) in Veronal buffer (*p*H 8.0) at 40 C for 20 min; 2×10^{-3} M 2,4-dinitrophenol (DNP) was present in the system to prevent cell growth during the period of incubation (trypsin-DNP treatment).

Units of colicin activity. The highest dilution of the colicin giving complete inhibition of growth of the sensitive strain P501 on nutrient agar, with the assay method of Goebel, Barry, and Shedlovsky (10), is defined as containing one arbitrary unit (AU) of activity per ml. The term lethal unit (LU) is defined as the minimum quantity of colicin that must be adsorbed to a sensitive cell in order for it to be killed.

Adsorption constant. The number of lethal units adsorbed per bacterium in unit time may be expressed as a fraction of the total number of lethal units of colicin added per ml of bacterial suspension to give an adsorption constant (K) with units $\times$ ml $\times$ min^{-1}.

As the rate of adsorption of colicin was measured in terms of the proportion of bacteria "killed" in a given time, and, as the number of viable organisms fell exponentially only for a limited time (Fig. 1), adsorption rates were determined and compared,

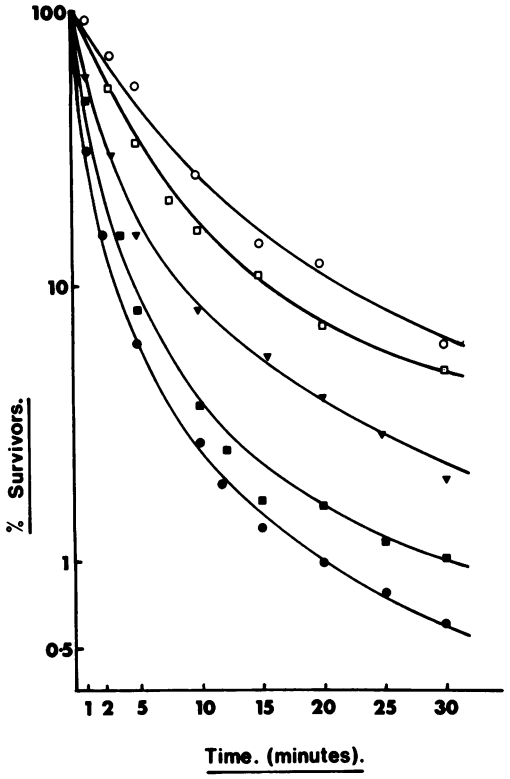

FIG. 1. *Proportion of bacteria surviving in nutrient broth when treated with various concentrations of colicin. Symbols:* ○, *3.5* × *10⁸ cells and 0.2 AU of colicin per ml;* □, *3.2* × *10⁸ cells and 0.5 AU of colicin per ml;* ▼, *3.4* × *10⁸ cells and 1.0 AU of colicin per ml;* ■, *3.4* × *10⁸ cells and 1.5 AU of colicin per ml;* ●, *3.6* × *10⁸ cells and 2.0 AU of colicin per ml.*

under conditions in which the initial bacterial concentration was kept reasonably constant, and in terms of the number of bacteria surviving 1 min subsequent to colicin addition. The proportion of bacteria surviving at that time was determined by extrapolation of the survivor data.

The average number of lethal units (n) adsorbed per bacterium, subsequent to the addition of C lethal units per ml of colicin, was obtained by putting the proportion of cells surviving at 1 min (b) equal to P(o) in the first term of the Poisson distribution $P(o) = e^{-n}$ (23). The adsorption constant (K) was then determined by $K = n/C$ ml × min^{-1}. Colicin was ordinarily titred in terms of arbitrary units per milliliter; however, the number of lethal units contained in an arbitrary unit was initially determined by the method of Reeves (23), and values ranging from 7.6×10^9 to 1.05×10^{10} LU/AU were obtained, with an average value of 9×10^9 LU/AU.

Multiplicity of infection. By analogy to phage systems, the multiplicity of infection (m) has been used to indicate the number of lethal units added per bacterium (16).

RESULTS

Adsorption from nutrient broth and other media. The K for colicin CA42-E2 in nutrient broth was determined from the survivor curve data shown in Fig. 1. The values of K so determined (Table 1) range from 6.1×10^{-11} to 7.3×10^{-11} ml × min^{-1}.

The proportionality between the average number of lethal units (n) adsorbed per bacterium in 1 min and the concentration of added colicin (C) is shown in Fig. 2, from which a value of $K = 6.8 \times 10^{-11}$ ml × min^{-1} was obtained for adsorption in nutrient broth.

When cells were suspended in synthetic media, survivor curve data showed a greater rate of colicin adsorption than in nutrient broth (Table 2). A value of $K = 24.4 \times 10^{-11}$ ml × min^{-1} being found in phosphate buffer and in minimal salts solution at *p*H 7.0.

The rate of adsorption was also found to be *p*H dependent. An optimal rate of adsorption was observed in the region of *p*H 8.0 in tris (hydroxymethyl)aminomethane (Tris) buffer (0.05 M) where $K = 9 \times 10^{-11}$ ml × min^{-1}. The adsorption constant fell to 5×10^{-11} ml × min^{-1} at *p*H 10.0, but was much lower at *p*H 5.0 (2.3×10^{-11} ml × min^{-1}) and at *p*H 4.0 (1.4×10^{-11} ml × min^{-1}). Readjustment of each system to *p*H 7.0 after adsorption had been allowed to occur for 30 min restored the adsorption rates to approximately that at *p*H 7.0 (Fig. 3), indicating that the lowered adsorption rates were not due to inactivation of the colicin.

The addition of Mg^{2+} to Tris buffer (0.05 M) at *p*H 7.0 increased the adsorption constant in this medium (Table 3); the adsorption constants

TABLE 1. *Adsorption constant for colicin in nutrient broth*[a]

Concentration of colicin per ml of culture		Proportion of cells (b) surviving 1 minute after adding colicin	Average number (n) of colicin particles adsorbed per bacterium in 1 min	Adsorption constant ml × min^{-1} from $K = n/C$ (×10^{11})
AU	LU(C)			
0.2	1.8×10^9	0.88	0.12	6.6
0.5	4.5×10^9	0.72	0.33	7.3
1.0	9.0×10^9	0.52	0.65	7.2
1.5	1.35×10^{10}	0.44	0.82	6.1
2.0	1.8×10^{10}	0.30	1.20	6.7

[a] The proportion of *E. coli* P501 cells surviving at 1 min (b) subsequent to adding colicin was read from survivor curves Fig. 1; n was determined by putting b equal to the first term of the Poisson Distribution, $b = e^{-n}$.

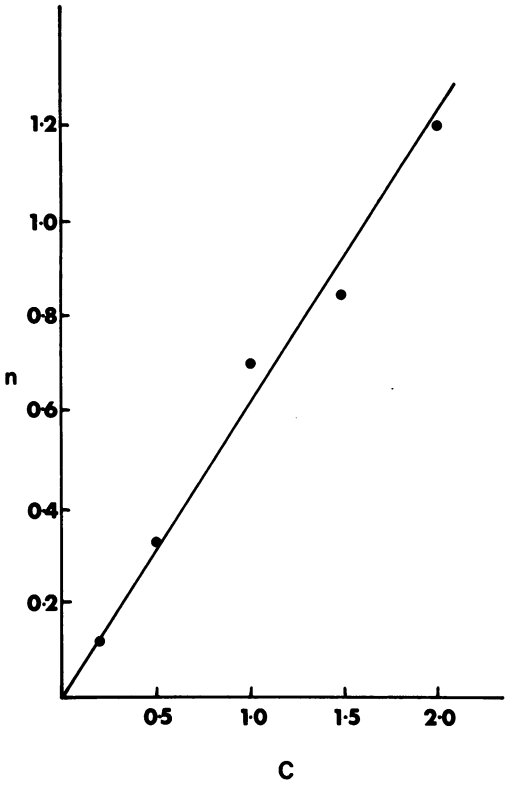

FIG. 2. *Proportionality between the average number of colicin particles adsorbed per cell in 1 min and the concentration of added colicin. With the data from Table 1, n was plotted against C. When C = 1 AU/ml (9 × 10⁹ LU/ml), n = 0.6 and K = 0.6/9 × 10⁹ = 6.8 × 10⁻¹¹ ml × min⁻¹.*

presumably reached a maximum between 10^{-3} and 10^{-4} M Mg^{2+}. However, in the presence of Zn^{2+} the rate of adsorption in phosphate buffer (0.1 M K^+) at *p*H 7.0 was markedly reduced, the adsorption constant falling below 1×10^{-11} ml × min^{-1} at 10^{-3} M Zn^{2+} (Table 4).

When log-phase cells of *E. coli* P503 in MCG medium were treated with colicin prior to rapid filtration, washing with MCG medium at 37 C to remove excess colicin, and resuspended in MCG medium at 37 C, the viable count continued to fall (Fig. 4) until only about 10% survived. The multiplication of the surviving cells was inhibited for at least one generation time. The survival of cells in this system, prior to plateau conditions being reached, appeared greater when they were diluted into nutrient broth prior to plating than when diluted into minimal salts solution.

Log phase cells of *E. coli* P501, added to the above system immediately after filtration, washing, and resuspension of the colicin-treated P503 strain, were found to be considerably affected (Fig. 5); about 40% of the P501 cells appeared to have been killed.

Trypsin rescue of colicin-treated cells. Several colicins are rapidly destroyed by proteolytic enzymes (2, 5). Nomura and Nakamura (18) also showed that the bactericidal action of colicin K was reversed by trypsin. Consequently the possibility of effecting "trypsin rescue" of cells which had adsorbed colicin CA42-E2 was investigated.

Trypsin (0.5 mg/ml) was found to rapidly inactivate colicin CA42-E2 in a Veronal buffer at

TABLE 2. *Binding of colicin in various media*[a]

Medium	Bacteria per ml	Proportion (*b*) of cells surviving in 1 min	Average number (*n*) of colicin particles adsorbed per cell in 1 min	Adsorption constant from $K = n/C$ ml × min^{-1} ($\times 10^{11}$)
Nutrient broth	1.3×10^8	0.92	0.08	5.9
Phosphate buffer (0.1 M K^+) *p*H 7	1.3×10^8	0.72	0.33	24.4
Tris buffer (0.05 M) *p*H 7.0	1.3×10^8	0.89	0.12	8.9
Nutrient broth	0.94×10^8	0.91	0.09	6.8
Minimal salts solution	0.94×10^8	0.72	0.33	24.4
Phosphate buffer (0.1 M K^+) *p*H 7.0	0.94×10^8	0.75	0.29	21.5

[a] Log-phase cells of *E. coli* P501 grown in nutrient broth were harvested by centrifugation, washed in deionized water, resuspended in deionized water, and diluted 1 to 10 into nutrient broth, phosphate buffer at *p*H 7.0 containing 0.1 M K^+, 0.5 M Tris buffer at *p*H 7.0, and diluted salts medium at *p*H 7.0. The proportion of cells surviving at suitable time intervals subsequent to the addition of colicin was determined, and the proportion of cells surviving 1 min subsequent to treatment with colicin [0.15 AU (1.35×10^9 LU) per ml] was obtained by extrapolation.

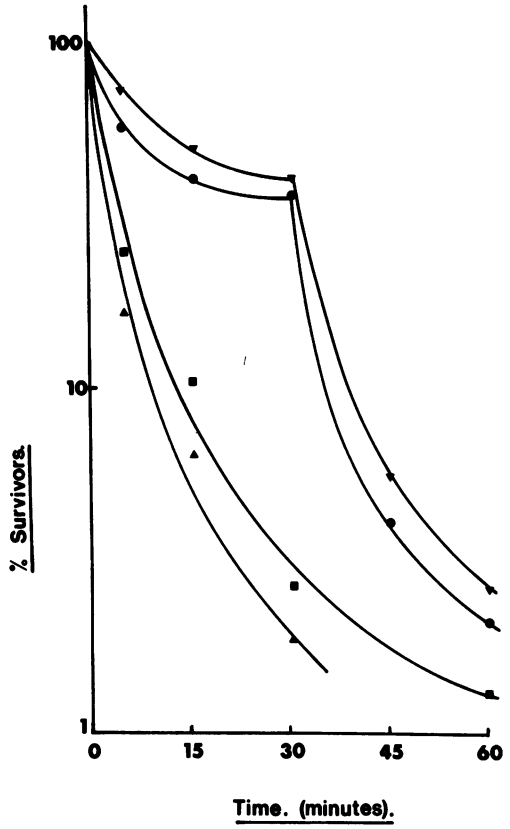

FIG. 3. *Survival of bacteria in Tris buffer (0.05 M), at various levels of hydrogen ion concentration, in the presence of colicin. E. coli P501 cells grown in nutrient broth were washed with Tris buffer (0.05 M) at pH 7.0 and finally resuspended in deionized water. Portions of this resuspension were then added to equal volumes of 0.1 M Tris buffer at pH 10.0, 8.0, 5.0, and 4.0, to give about 1 × 10⁸ bacteria/ml and warmed to 37 C prior to the addition of colicin (0.5 AU/ml). After 30 min, the pH of each cell suspension was adjusted to pH 7. Symbols: ▲, pH 8.0, bacteria surviving at 1 min = 66%, K = 9.2 × 10⁻¹¹ ml × min⁻¹; ■, pH 10.0 bacteria surviving at 1 min = 80%, K = 5.0 × 10⁻¹¹ ml × min⁻¹; ●, pH 5.0, bacteria surviving at 1 min = 90%, K = 2.3 × 10⁻¹¹ ml × min.⁻¹; ▼, pH 4.0, bacteria surviving at 1 min = 94%, K = 1.4 × 10⁻¹¹ ml × min⁻¹.*

*p*H 8.0 at 40 C. However, a significant potentiation of colicin activity was observed on treatment with low concentrations of trypsin for a short time (Table 5). Owing presumably to steric effects, a concentration of 5 mg/ml of trypsin was needed to inactivate colicin adsorbed to cells.

A complete trypsin rescue of log phase cells in nutrient broth treated with colicin (m = 40), could be effected for about 5 min subsequent to colicin addition, the complete trypsin rescue time being extended to 8 min at a lower colicin concentration, (m = 10; Fig. 6).

However, if the colicin were adsorbed in minimal salts solution, in which the rate of adsorption was almost three times faster than in nutrient broth, the interval over which colicin rescue became possible was considerably shortened.

Cells pretreated with trypsin, prior to washing in three changes of cold Veronal buffer (4 C) at *p*H 8.0 were, if anything, more sensitive to colicin action than cells which had not been trypsinized but otherwise identically treated (Fig. 7). Hence the trypsin did not prevent colicin adsorption by modification of the cell receptor.

When colicin treated cells, rescued by trypsin, were washed three times in cold nutrient broth (4 C), resuspended in nutrient broth at 37 C, and then treated with colicin (m = 50), the rate of

TABLE 3. *Effect of Mg^{++} concentration on the binding of colicin*

Medium	Proportion (b)[a] of cells surviving in 1 min	Average number (n) of colicin particles adsorbed per bacterium in 1 min	Adsorption constant $K = n/C$ ml × min^{-1} (×10^{11})
Tris buffer 0.05 M, pH 7.0	0.82	0.20	8.9
+ 10^{-2} M Mg^{++}	0.80	0.22	9.8
+ 10^{-3} M Mg^{++}	0.59	0.53	23.5
+ 10^{-4} M Mg^{++}	0.64	0.45	20.0

[a] Proportion of *E. coli* P501 cells surviving at one minute when treated with 0.25 AU (2.25 × 10^9 LU) colicin per ml.

TABLE 4. *Effect of Zn^{2+} concentration on the binding of colicin*

Medium	Proportion (b of cells surviving in 1 min[a]	Average number (n) of colicin particles adsorbed per cell in 1 min	Adsorption constant $K = n/C$ ml × min^{-1} (×10^{11})
Phosphate buffer 0.1 M K^+, *p*H 7.0	0.72	0.33	24.4
+ 10^{-4} M Zn^{2+}	0.89	0.12	8.9
+ 5 × 10^{-4} M Zn^{2+}	0.98	0.02	1.5
+ 10^{-3} M Zn^{2+}	0.99	0.01	0.75

[a] The proportion of *E. coli* P501 cells surviving at one minute when treated with 0.15 AU (1.35 × 10^9 LU) of colicin per ml.

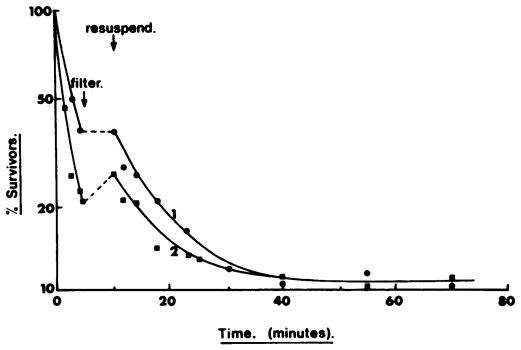

FIG. 4. *Modification of the bactericidal action of adsorbed colicin on dilution of cells. Colicin (10 AU) was added to 30 ml of E. coli P503 culture in MCG medium at zero time. After 5 min of incubation at 37 C, the cells were filtered and washed with warm MCG medium on a membrane (Millipore) and resuspended in 30 ml of fresh MCG medium at 37 C. Curve 1, viable count performed by dilution into nutrient broth; curve 2, viable count performed by dilution into minimal salts solution.*

killing, after the first minute or so, was considerably slower than that occurring in normal cells (Fig. 8), suggesting that fewer colicin receptors were available in the trypsin rescued cells owing, perhaps, to the blocking of these sites by trypsin-inactivated colicin residues.

Metabolic inhibitors and trypsin rescue. The fact that one adsorbed particle of colicin could prove lethal to the cell and that the bactericidal effect of colicin appeared to be initiated, whereas the specifically adsorbed colicin remained external to the cell, as was the case with colicin K (18) and with phage ghosts (12), suggested that the cell contributed in some way to its own destruction by colicin. If this were so, cells in which metabolic activity had been completely halted should be rescuable by trypsin over very much longer periods of time subsequent to colicin adsorption.

Thirty minutes pretreatment with 2×10^{-3} M was found to permit a total trypsin rescue of colicin-treated cells for at least 2 hr subsequent to colicin adsorption (Fig. 9). DNP pretreatment did not prevent adsorption of colicin to cells nor did it reduce their viability. However, pretreatment with 100 μg/ml chloramphenicol for 30 min did not afford appreciable protection against colicin activity (Fig. 10).

DISCUSSION

The data obtained from survivor curves (e.g. Fig. 1) supports the earlier conclusions (13, 16, 22, 25) that one particle of adsorbed colicin can kill a sensitive cell; this is clearly demonstrated by the proportionality existing between the average number of lethal units adsorbed per cell in the first minute, as measured by killing, and the concentration of colicin added (Fig. 2). The ultimate change in slope of such survivor curves

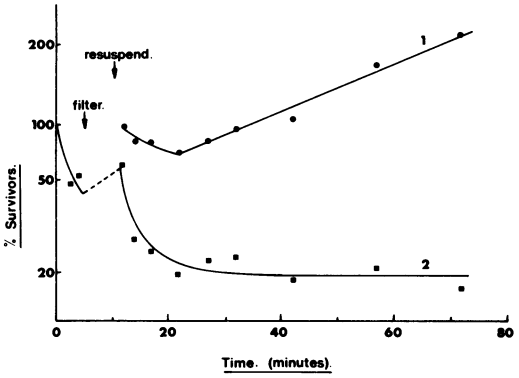

FIG. 5. *Desorption of colicin from cells. Of a log-phase culture of E. coli P503 in MCG medium, 30 ml was treated with colicin (5 AU). After 5 min of incubation, with shaking, at 37 C, unadsorbed colicin was removed by washing the cells with warm medium on a membrane (Millipore). The cells were resuspended in 29.5 ml of MCG medium at 37 C, 0.5 ml of a log-phase culture of E. coli P501 was added, and the mixed cell culture was shaken at 37 C. The number of viable cells of each strain was determined by plating from a suitable dilution in MCG medium onto each of two nutrient agar plates, one of which contained thymine (50 μg/ml), the other not. Curve 1, the survival of E. coli P501, plated on nutrient agar without thymine; curve 2, the survival of E. coli P503 plated on nutrient agar with thymine, allowance being made for the E. coli P501 cells which formed colonies under these conditions.*

TABLE 5. *The inactivation of free colicin by trypsin*[a]

Trypsin concn. (mg/ml)	Titer of colicin (A U/ml) in sample taken at time (min)					
	0	1	2	4	8	15
0.5	540	540	20	Nil	Nil	Nil
0.25	540	540	180	60	Nil	Nil
0.125	540	540	1620	540	60	Nil
0.063	540	540	4860	540	540	60
0.0[b]	540	540	540	540	540	540
0.0[c]	540	540	540	540	540	540

[a] A solution of colicin at 40 C was mixed with an equal volume of a suitable concentration of trypsin in Veronal buffer at *p*H 8.0 and 40 C; samples were removed at suitable intervals of time subsequent to mixing and assayed by threefold serial dilution.

[b] Buffer at 40 C.

[c] Distilled water at 40 C.

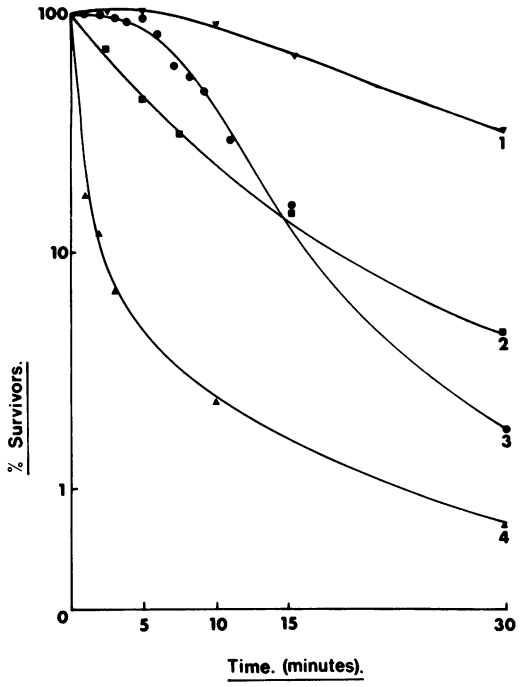

FIG. 6. *Reversal by trypsin of the effect of colicin adsorbed from nutrient broth. Experiment 1: 2.3 × 10⁸ E. coli P501/ml treated with colicin (m = 10), curve 1, samples diluted into trypsin-DNP solution prior to viable count; curve 2, samples diluted directly into nutrient broth for viable count. Experiment 2: 3.6 × 10⁸ E. coli P501/ml treated with colicin (m = 40); curve 3, samples diluted into the trypsin-DNP solution and incubated prior to viable count; curve 4, samples diluted directly into nutrient broth for viable count.*

has been discussed by Hedges (11) and Shannon and Hedges (25) and may well be due to heterogeneity in the number of available receptors on cells within a given population.

The rate of adsorption of colicin, as measured by the rate of killing, was shown to vary considerably with the ionic environment in which adsorption took place. As in the case of T1 phage adsorption by *E. coli* B (8, 9, 20), Mg^{2+}, with an optimum at about 10^{-3} M, appeared to promote the specific irreversible adsorption of colicin but Zn^{2+} was inhibitory. The marked inhibition of colicin adsorption observed at *p*H 5.0 and below was analogous to that found in the phage T2-*E. coli* system by Puck and Tolmach (21). These authors inferred that ionizing carboxyl groups were necessary for phage adsorption, and presumably colicin adsorption shows a similar requirement.

The plateaus observed in survivor curves of colicin-treated cells (Fig. 4 and 5) showed that some cells, although unable to multiply for at least a normal generation time, were eventually able to produce colonies on nutrient agar, suggesting that colicin can interact with cells to prevent their multiplication yet lack the specific interaction necessary for killing. French and Siminovitch (7) reported a similar phenomenon in which cells having adsorbed phage ghosts were able to produce colonies on subsequent plating, although initially their multiplication and protein synthesis had been inhibited.

Two forms of colicin-cell interaction thus appear possible. That these may be sequential steps, as hypothesized for the adsorption of phage T1 to *E. coli* cells by Puck, et al. (20), is suggested by the

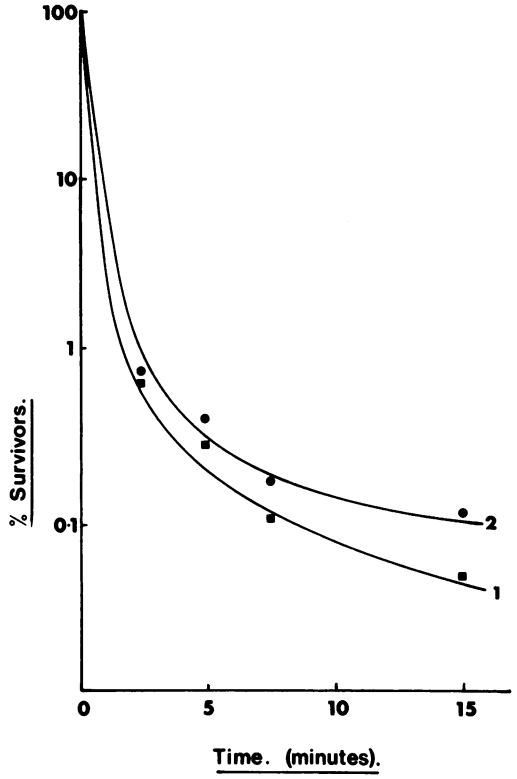

FIG. 7. *Survival of bacteria pretreated with trypsin in the presence of colicin. Nutrient broth-grown E. coli P501, washed in Veronal buffer at pH 8.0 were resuspended in the Veronal buffer and divided into two samples to one of which was added trypsin (5 mg/ml). The samples were then maintained at 40 C for 30 min; each was washed with three changes of cold Veronal buffer and resuspended in nutrient broth at 37 C, and colicin was added. Curve 1, trypsin pretreated bacteria (1.3 × 10⁸ cells/ml) incubated in the presence of colicin (4 AU/ml); curve 2, untreated bacteria (1.5 × 10⁸ cells/ml) incubated in the presence of colicin (4 AU/ml).*

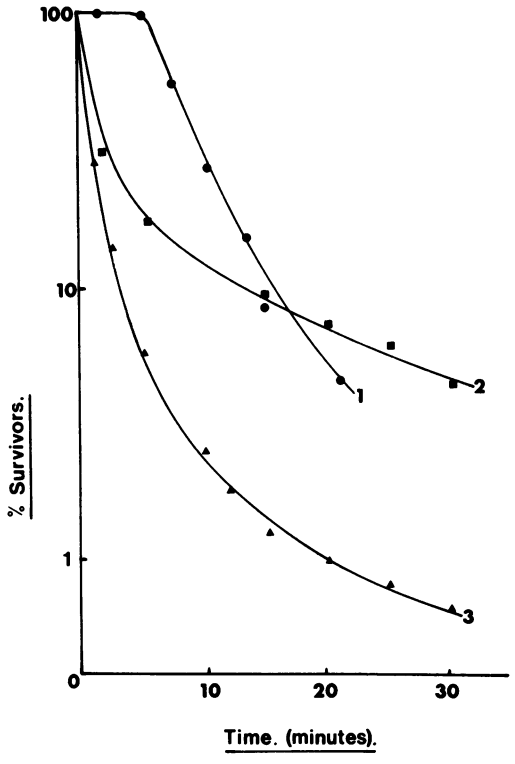

FIG. 8. *Survival of trypsin-rescued bacteria in the presence of colicin. E. coli P501, growing in nutrient broth at 37 C were treated with colicin (m = 50). Curve 1, the viable count in samples after trypsin-DNP treatment; curve 3, the viable count in samples diluted into nutrient broth and plated directly. A sample of the suspension was taken 5 min after colicin addition and incubated (30 min at 40 C) with trypsin-DNP. The cells were then washed three times in cold nutrient broth and resuspended in broth at 37 C. Colicin (m = 50) was added to these rescued cells, and their survival was measured by viable counts, curve 2.*

observations that cells which had adsorbed a lethal quantity of colicin were less likely to die if diluted and plated soon after the initial interaction. Furthermore, death was more likely to occur on dilution into minimal salts solution, in which the ionic environment of the original adsorbing medium was maintained, than when diluted into nutrient broth. Hence, dilution could prevent the lethal effect of adsorbed colicin, the lethality being dependent upon the interval between the initial adsorption and dilution; it was also influenced by ionic factors. This cell rescue observed on dilution could be due to some protective effect of the dilution operation itself. For example, Beppu and Arima (1) have found that when cells which had adsorbed colicin P9-E2 or K235-K were subsequently incubated in the presence of 0.8 M NaCl or sucrose, they were significantly protected from the lethal action of these colicins. However, the observed killing of P501 cells when resuspended with colicin-pretreated P503 cells, in the absence of free colicin (Fig. 5), suggests that colicin, desorbed from P503 cells, was subsequently adsorbed by P501 cells. Hence the phenomenon of dilution rescue seems to involve dissociation of colicin and cells.

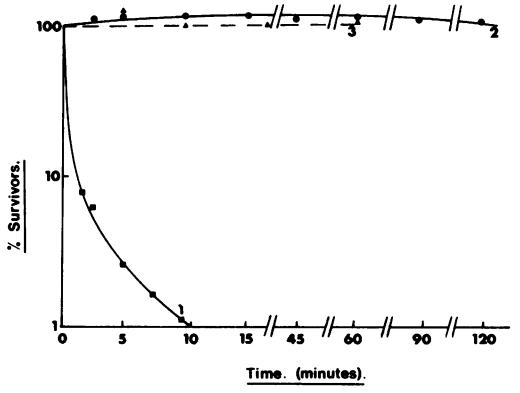

FIG. 9. *Complete trypsin rescue of cells treated with DNP. E. coli P501 was grown in nutrient broth at 37 C with shaking and then pretreated with DNP (2 × 10⁻³ M for 30 min). Three samples were taken, and colicin (m = 100) added to 1 and 2. Curve 1, viable count at intervals after colicin addition; curve 2, viable count of samples taken at intervals after colicin addition and treated with trypsin-DNP before plating; curve 3, viable count of control with no colicin added.*

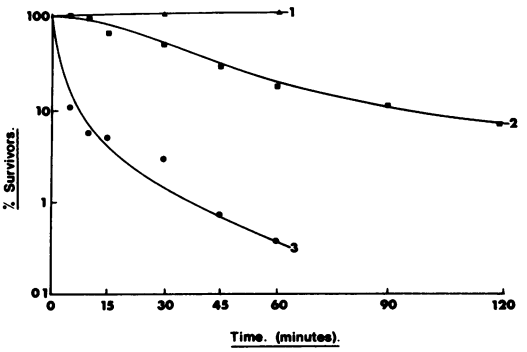

FIG. 10. *Incomplete trypsin rescue of cells pretreated with chloramphenicol. E. coli P501 was grown in nutrient broth at 37 C with shaking and then pretreated with chloramphenicol (100 μg/ml for 30 min). Three samples were taken, and colicin (m = 100) was added to 2 and 3. Curve 1, viable count of cells treated with chloramphenicol alone; curve 3, viable count at intervals after colicin addition; curve 2, viable count of samples taken at intervals after colicin addition and treated with trypsin-DNP prior to plating.*

The foregoing observations are generally compatible with the concept that colicin adsorbs in two stages, a primary adsorption step which is reversible, followed by a secondary step which is irreversible, the transition being influenced by the ionic environment.

The maximum adsorption constant determined for colicin CA42-E2 was about threefold smaller than that reported for colicin ML-E1 (13) and about 10-fold lower than the value reported for the adsorption of phage T4 to *E. coli* (20). The latter authors applied the Von Smoluchowski coagulation equation, as modified by Schlesinger (24) and Delbruck (4) to the phage-host interaction and showed that the phage adsorbed at near the calculated maximum rate (K_{max}), hence that nearly every random collision between phage and bacterium led to adsorption. The maximum theoretical rate of adsorption is given by $K_{max} = 4\ \pi DA$ where

D = the diffusion constant for the adsorbing particle.

A = the radius of a sphere having the same surface area as the host cell.

Applying this model to colicin adsorption, A, = 8×10^{-5} cm (for an *E. coli* cell 1 μm in diameter and 3 μm in length) and $D = 4.2 \times 10^{-5}\ cm^2 \times min^{-1}$, assuming that colicin CA42-E2 is essentially polypeptide (22) and therefore has a partial specific volume of about 0.75 cm^3 per g, that its molecular weight is in the order of 10^5 (23), and that the colicin particle is approximately spherical.

K_{max} is then equal to 4.2×10^{-8} ml $\times$ min^{-1}. The experimental value for K obtained in nutrient broth was about 6.8×10^{-11} ml per min, and thus 1 in 600 random collisions between colicin particles and the bacterial surface results in adsorption with induced killing, although about 1 in 180 random collisions produce this effect in minimal salts solution or in phosphate buffer. The low value for K/K_{max} in the colicin system suggests that either a relatively small area of bacterial surface can function as colicin receptor or that each adsorbed colicin particle has a very low probability of killing the cell. This probability is lower in nutrient broth than in the salt solutions, owing presumably to the enhancement of specific irreversible binding in the latter media.

Although colicin was readily inactivated by trypsin, a significant potentiation of colicin activity was observed when it was treated with low concentrations of trypsin for a short time (Table 5), owing presumably to an increase in the number of lethal units in the system. Thus mild trypsin treatment may activate a relatively large number of effete colicin particles, or it may cause dispersion of multimeric colicin units.

By using suitably low concentrations of colicin, it was clearly possible to rescue with trypsin cells which had already adsorbed a lethal quantity of colicin and were doomed to die. However, such cells could only be rescued for a relatively short time after colicin had been adsorbed from nutrient broth and for an even shorter time in minimal salts solution. This time is comparable to that elapsing before the first biochemical lesion becomes apparent in cells suspended in minimal salts solution with lactate as a carbon source (Reynolds and Reeves, *unpublished data*).

The observation that trypsin can rescue cells to which colicin has adsorbed supports the view that colicin adsorbs to a specific receptor on the cell surface, where it remains exposed to the action of the trypsin. Indeed it would appear likely that the trypsin-inactivated colicin residue remains associated with the receptor blocking further adsorption at this site. For in cells which have been treated with colicin for a second time, subsequent to trypsin rescue from the lethal effects of adsorbed colicin (Fig. 8), a lower level of killing was observed, as well as a marked decrease in the rate of killing compared to that for normal cells. In terms of the "distributed receptor model" of Hedges (11), these observations would imply a marked decrease in the number of receptors available for colicin adsorption in the trypsin-rescued cells and hence that trypsin-inactivated colicin residues remain attached to the receptor sites. The observations of Maeda and Nomura (14) that on trypsin treatment of adsorbed ^{3}H-labeled colicin P9-E2 only about 50% of the label dissociates from the cells also supports this view. It would also seem possible that, as in the case of colicin K (18), the colicin exerts its lethal effect while attached to the external receptor. The observation that pretreatment of cells with a concentration of DNP sufficient to cause a total metabolic paralysis permits complete rescue of colicin-treated cells for at least 120 min after colicin adsorption (Fig. 9) suggests that the cell itself contributes to its own destruction by events which are initiated by colicin receptor interaction as hypothesized by Nomura and Maeda (17). However, because pretreatment with chloramphenicol did not permit appreciable trypsin rescue for longer than 30 min after colicin adsorption (Fig. 10), such events do not appear to involve de novo protein synthesis. This observation and conclusion are similar to that made by Nomura (16) when investigating the mode of action of colicin P9-E2.

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