Resistance of *Escherichia coli* to Osmotically Introduced Complement Component C9

JOHN R. DANKERT

*Department of Comparative and Experimental Pathology, Health Science Center,
University of Florida, Gainesville, Florida 32610*

Received 27 April 1990/Accepted 9 October 1990

Investigation into the action of osmotically introduced C9 in *Escherichia coli* (in the absence of any other complement components) revealed that C9 could inhibit inner membrane respiration and cause a decrease in the viability of cells that were normally complement sensitive. This effect is analogous to the loss of inner membrane function and viability due to the assembly of the C5b-9 complex on these cells. Complement-resistant cells showed no such inhibition of respiration or loss of viability when subjected to the osmotic introduction of C9. The reason for this failure of C9 to affect complement-resistant cells was explored to determine whether this resistance to C9 was due to an inability of proteins in general to be osmotically introduced into the complement-resistant cells. The protein toxins melittin and colicin E1 were shown to be able to kill these complement-resistant cells (as well as complement-sensitive cells) when osmotically introduced into the periplasm. Therefore, cellular resistance to osmotically introduced C9 is not due to an inability of proteins to be introduced into the cells and may be related to a mechanism of cellular resistance to the C5b-9 complex.

Human complement component C9 has been shown to be required for the killing of serum-sensitive strains of *Escherichia coli* bearing assembled C5b-8 sites (BAC1-8 cells) on the outer surface of the cell. The lethal activity of the complete C5b-9 site (or membrane attack complex [MAC]) in the absence of lysozyme involves the inhibition of inner membrane function (2, 7, 13, 20, 32). It is therefore of importance to determine the mechanism by which the MAC can effectively span the outer membrane and periplasmic space to inhibit function in the inner membrane. Some complement-resistant bacterial strains escape killing although MACs are formed on their outer membrane (17–19, 31) and even when a transient inhibition of inner membrane function occurs (6). It has been reported that C9 in the absence of any other complement components has the ability to kill complement-sensitive but not complement-resistant *E. coli* cells (8). This lethal action was expressed only when C9 was introduced into the periplasm, the space between inner and outer membranes of the cells. Contact with the inner membrane is not in and of itself sufficient to explain the inhibition, since it has been shown that the direct addition of C9 alone to inner membrane vesicles does not lead to an inhibition of inner membrane function (7). One explanation is that some factor(s) present in the periplasmic space or outer membrane of these cells allowed C9 to express a membranolytic activity after C9 had gained access to the inner membrane. The ability of osmotically introduced C9 to kill cells would require that C9 have the potential to express a membranolytic function without the C5b-8 complex. A proteolytic fragment of C9 has been shown to form ion-conducting channels in artificial planar bilayers and liposomes (29) as well as inhibiting membrane function in isolated inner membrane vesicles of bacteria (7). Native C9 was unable to perform either of these functions unless the preformed C5b-8 complex was present on the membranes.

The similarity in the spectrum of cell killing of osmotically introduced C9 to that of the assembled MAC suggests that the mechanisms of resistance to these processes may be related. This investigation was undertaken to ascertain sim-

ilarities between osmotically introduced C9 and the action of the assembled MAC on complement-sensitive bacterial cells and to determine whether the resistant cells used in this study were unable to take up proteins (in general) as a result of the osmotic shock procedure.

MATERIALS AND METHODS

Materials. Reagents used for electrophoresis were from Bio-Rad (Richmond, Calif.). All other chemicals were purchased from Fisher Scientific.

Bacterial strains and media. *E. coli* C600 and LP1395 (8) were used. Cells were grown in L broth (Casamino Acids, 3 g/liter; yeast extract, 5 g/liter; NaCl, 5 g/liter) supplemented with 10 g of potassium succinate (pH 7.0) per liter with constant shaking to mid-log phase at 37°C. Cells were washed three times in buffer (10 mM imidazole, 150 mM NaCl, 1 mM MgCl₂, 0.1 mM CaCl₂, pH 7.0) and resuspended in this buffer to an A₆₀₀ of 1.5.

Proteins and sera. Human complement proteins C8 and C9 were purified as described previously (9). Melittin and bovine serum albumin (BSA) were obtained from Sigma. Serum depleted of C9 (R-9) was generated by immunoaffinity adsorption as characterized previously (9). Colicin E1 was purified as described by Cleveland et al. (5). ¹²⁵I-labeled C9 was prepared as described previously (8), using Iodobeads (Pierce Chemical Co.) to a level of 0.3 Ci/g (about 6.5 × 10⁸ cpm/mg), with less than a 10% loss of hemolytic (9) or bactericidal (8) activity.

Preparation of MAC intermediates on cells. Bacterial cells bearing assembled C5b-8 sites (termed BAC1-8 cells) were prepared as described previously (7), using a ratio of 2 ml of R-9 serum to 1 ml of washed cells. The BAC1-8 cells were then washed three times and resuspended to their original density.

Determination of bacterial viability. Aliquots of cells prepared as described above were serially diluted into 0.9% NaCl and plated on 4% tryptic soy agar. Viability was determined 12 to 16 h later by colony counting and were

obtained from quadruplicate platings, expressed as mean $\pm$ standard deviation.

Osmotic shock procedure. Washed cells were resuspended into imidazole buffer (minus CaCl_2) containing 5 mM EDTA and incubated on ice for 10 min. The cells were then injected into buffer containing 1 M sucrose at 37°C (equal volumes). Protein to be shocked into the cells was added to the mixture at least 1 min prior to the dilution into the sucrose solution (protein concentrations include this dilution factor). The cells were incubated for 3 min at 37°C before dilution and plating for the viability assay. Quantitation of radiolabeled C9 into cells by the osmotic shock procedure was performed as described above except that the cells were washed three times in buffer (10 mM imidazole, 150 mM NaCl, 1 mM MgCl_2 , 0.1 mM CaCl_2 , pH 7.0) before assay on a gamma counter (LKB 1272 Clinigamma). For both strains in the absence of osmotic shock, over 98% of the ^{125}I -C9 bound to the cells could be displaced by inclusion of 0.1 mg of unlabeled C9 per ml in the wash buffer. With the shock procedure, the amount of label that was exchangeable in this way was 152 ± 43 molecules per cell for strain C600 and 421 ± 62 molecules per cell for strain LP1395.

Spheroplast preparation. Spheroplasts were prepared by resuspending the washed cells (described above) in 10 mM imidazole–150 mM NaCl (pH 7). The cells were incubated for 30 min at 37°C after the addition of 10 mM EDTA and 50 μg of lysozyme per ml. The suspension was centrifuged ($13,000 \times g$, 10 min), and the pellet containing the spheroplasts was washed three times in 10 mM imidazole–150 mM NaCl (pH 7) and resuspended to the original volume. This preparation resulted in a population of less than one rod per high-powered field of view (magnification of $\times 400$) when viewed directly (not shown).

Oxygen consumption measurements. Cells prepared as described above were placed into the standard bath assembly of a YSI 5300 biological oxygen monitor containing 3 ml of freshly prepared buffer supplemented with 1% potassium succinate (pH 7.0) as previously described (6). For each measurement of respiration, the actual number of bacteria or spheroplasts per milliliter was determined by performing a viability assay by plating for viable colony counts. Inhibition of respiration is expressed as the decrease in the units of slope of an oxygen consumption trace (percent oxygen consumed per 2 min per 10^7 cells) as described previously (6). All oxygen consumption measured in the absence of succinate was $<3\%$ O_2 consumed per 10 min per 10^7 cells, and all succinate-dependent consumption was inhibitable to this level by the addition of 0.1 mM potassium cyanide.

RESULTS

The complement-sensitive *E. coli* strain C600 has been shown to be sensitive to the introduction of C9 into the periplasmic space (8), and this property directly correlated with the complement susceptibility of the strain. It was of interest to determine whether C9 osmotically shocked into the cells caused an inhibition of inner membrane function similar to that seen when C9 was added to intact cells bearing C5b-8 sites. Measurement of the oxygen consumption of cells has been used in the past (28) and more recently (6) as an assay of complement damage to the respiring bacterial inner membrane. Figure 1 presents the tracings of oxygen consumption (as described in Materials and Methods) for C600 cells, for C600 cells subjected to the osmotic shock procedure, and for C600 BAC1-8 cells with or without a preincubation with added C9. Relative to the control, the

FIG. 1. Effects of osmotic shock and formation of the MAC on the oxygen consumption of C600 cells. Oxygen consumption was measured as described in Materials and Methods. A, C600 cells (untreated); B, cells subjected to the osmotic shock procedure in the absence of added protein; C, C600 BAC1-8 cells; D, C600 BAC1-8 cells treated with C9 (10 $\mu\text{g}/\text{ml}$) and allowed to incubate for 15 min at 37°C prior to the assay.

osmotic shock procedure in the absence of added protein caused a small reduction in the capacity of these cells to consume oxygen, whereas the formation of the complete C5b-9 complex (MAC) caused a large inhibition of respiration. The osmotic shock procedure was found to lead to a 5 to 25% decrease in viability in the cells. Sensitivity of the cells to the osmotic shock procedure depended on the concentration of sucrose used and the stage of cell growth at the time of harvest. Cells allowed to reach late log phase were relatively insensitive to the shock procedure (not shown). As this variability was important to the sensitivity of the cells to C9, only the effects of various treatments on a single batch of cells were compared for each set of experiments. Subsequent results of oxygen consumption are expressed relative to the slope of the tracings for a given set of cells.

When C600 cells were osmotically shocked in the presence of C9, a significant inhibition of oxygen consumption was seen (Fig. 2). C600 cells subjected to the osmotic shock procedure in the presence of complement component C8 or BSA displayed no significant inhibition of oxygen consumption or decrease in cell survival. For the complement-resistant strain LP1395, no inhibition of oxygen consumption or decrease in viability was detected by osmotically shocking the cells in the presence of C9 (Fig. 2). Altering the protein concentration during the shock procedure produced a corresponding change in cell survival (Table 1). To quantitate the number of C9 molecules that can be taken up into the cells, the procedure was performed by using ^{125}I -labeled C9. It was found that for strain C600, 170 (standard deviation = 35) molecules of C9 could be bound per cell in the absence of the shock procedure. The osmotic shock resulted in an increase in the number of molecules associated with the cells (315 ± 48), an increase of about 145 additional molecules. For strain LP1395, the background binding of C9 was higher (437 ± 50 per cell), with an increase to 593 ± 45 per cell (156 additional molecules) as a result of the osmotic shock.

To verify that the complement-resistant cells were indeed resistant to osmotically introduced C9, it was of importance to demonstrate that other proteins introduced into the cell

FIG. 2. Respiration (dark bars) and survival (light bars) of C600 (A to C) and LP1395 (D to F) cells subjected to the osmotic shock procedure. Cells were subjected to the osmotic shock procedure in the presence of BSA at 15 μ g/ml (A and D), C8 at 15 μ g/ml (B and E), C9 at 15 μ g/ml (C), or C9 at 50 μ g/ml (F). Respiration is expressed as percent of slope of an oxygen consumption trace relative to the slope for the same preparation of cells not subjected to the osmotic shock procedure. Survival levels were calculated from colony counts (see Materials and Methods) relative to colony counts of untreated cells. Error bars represent $\pm$ standard error of the mean.

this way could affect cell viability. The membrane-disrupting peptide toxin from bee venom, melittin (35), was added directly to cells or osmotically shocked into cells. Both the complement-sensitive and -resistant strains of bacteria were killed by melittin only if it was present during the osmotic shock procedure (Fig. 3). The ability of osmotically introduced melittin to kill the cells when allowed access to the inner membrane is consistent with the proposed membrane-disrupting mechanism of this toxin, which is active against a variety of membrane targets (16, 23). The small size of the melittin molecule (molecular weight [MW], $\approx$ 2,600) relative to that of C9 was of concern since melittin could be envisioned to have easier access to the periplasm. A protein similar in size to C9, the bactericidal protein colicin E1 (MW, $\approx$ 57,000), was tested for the ability to kill both cell types when osmotically introduced into the cells. For cell killing, the colicin E1 molecule requires the vitamin B₁₂ receptor (*btuB*) to gain access to the inner membrane (10, 12). Growth of cells in the presence of excess vitamin B₁₂ represses the synthesis of the receptor (21), and the colicin molecule is unable to bind to the cells. Both strains were

FIG. 3. Sensitivity of bacterial cells to osmotic shock in the presence of melittin or colicin E1. The survival levels of the cells (C600, dark bars; LP1395, light bars), prepared as described in Materials and Methods, were determined under the following conditions: cells incubated with melittin at 15 μ g/ml for 10 min at 37°C (A), cells (grown in 1 μ M vitamin B₁₂) incubated with colicin E1 at 50 μ g/ml for 10 min at 37°C (B), cells subjected to the osmotic shock procedure in the absence of added protein (C), cells subjected to osmotic shock in the presence of melittin at 15 μ g/ml (D), and cells (grown in 1 μ M vitamin B₁₂) subjected to osmotic shock in the presence of colicin E1 at 50 μ g/ml (E). Error bars represent $\pm$ standard error of the mean.

grown, washed, and resuspended in buffer as described in Materials and Methods, with 1 μ M vitamin B₁₂ added to all solutions. For both the C600 and LP1395 cells, addition of colicin E1 under these conditions caused no detectable decrease in viability (Fig. 3). The colicin E1 protein could kill both strains grown in the presence of vitamin B₁₂ through the osmotic shock procedure (Fig. 3), and the survival of the shocked cells was a function of the colicin E1 concentration (Table 1).

The role of the bacterial outer membrane and periplasmic space in this process was investigated by preparing spheroplasts from the cells. The outer membrane and peptidoglycan were removed from the cells by using lysozyme and EDTA as described in Materials and Methods. The spheroplast preparation was such that the spheroplasts could be assayed for viability by colony counting with only a small (5 to 20%) reduction in viability relative to the original population prior to spheroplast formation. Shown in Fig. 4 are the relative rates of oxygen consumption for spheroplasts (from both cell types) and for spheroplasts with added C9 or melittin. Similar to the results described for the ineffectiveness of C9 alone to inhibit bacterial inner membrane vesicles (7), native C9 did not inhibit the respiration or viability of the spheroplasts. In contrast to C9, the respiration of bacterial spheroplasts formed from either strain C600 or strain LP1395 was inhibited by the direct addition of melittin (Fig. 4).

DISCUSSION

It has been reported that C9 has the ability to kill cells when osmotically introduced into the periplasmic space of bacterial cells, and this ability correlates with the complement sensitivity of the bacterial strain (7). Here it has been shown that effective killing of cells by osmotically introduced C9 is associated with an inhibition of respiration of the bacterial inner membrane. This is similar to the effect of C9

TABLE 1. Survival of shocked cells as a function of added protein

Prepn ^a	% Survival (avg $\pm$ SD) ^b at protein concentration (μ g/ml) of:			
	1	5	10	50
C600 + C9	75 $\pm$ 5	32 $\pm$ 4	14 $\pm$ 4	ND
C600 + colicin E1	53 $\pm$ 5	20 $\pm$ 3	4 $\pm$ 2	ND
LP1395 + C9	100 $\pm$ 3	103 $\pm$ 5	106 $\pm$ 7	104 $\pm$ 5
LP1395 + colicin E1	97 $\pm$ 6	71 $\pm$ 8	25 $\pm$ 6	22 $\pm$ 4

^a Cells were prepared and resuspended as described in Materials and Methods.

^b Relative to cells shocked in the absence of added protein. ND, Not done.

FIG. 4. Oxygen consumption of spheroplasts prepared from complement-sensitive (dark bars) or complement-resistant (light bars) cells. Oxygen consumption is expressed relative to that of untreated spheroplasts. A, Spheroplasts incubated with C9 at 15 μ g/ml for 10 min at 37°C prior to dilution into the measuring chamber; B, spheroplasts incubated with melittin at 20 μ g/ml for 10 min at 37°C prior to the measurement; C, oxygen consumption of the spheroplasts in the presence of 0.1 mM potassium cyanide. Error bars represent $\pm$ standard error of the mean.

addition to cells bearing C5b-8 sites in that effective cell killing by the completed MAC is secondary to a number of metabolic responses to complement attack (7, 13, 18, 32), including the inhibition of respiration of the cells (6). C9 osmotically introduced into normally complement-resistant cells does not lead to an inhibition of inner membrane function (respiration) or to a decrease in the viability of the cells. Many complement-resistant bacterial strains can withstand the deposition of the complete MAC without a loss in viability (17, 18, 32). This finding raises the possibility that resistance to C9 introduced into the periplasmic space and resistance to C9 bound to a C5b-8 site may be analogous. The ineffectiveness of osmotically introduced C9 to affect these cells is not due to an inability of the protein to be taken up by the cell. Quantitation of the number of C9 molecules that could be osmotically introduced into a cell suggests that over 100 molecules per cell may be introduced by this method.

The precise location that C9 occupies once taken up into either cell type is not known, and it is possible that C9 is sequestered in different areas, which accounts for the differing susceptibilities. In a study to calculate the size of the periplasmic space in *E. coli* (30), it was determined that 20 to 40% of the cell volume could be taken up by the periplasm under conditions of osmotic stress which could accommodate the soluble protein molecules. The protein toxin melittin was able to kill both cell types only when the cells were subjected to the osmotic shock procedure in the presence of the toxin. The known membranolytic activity of this toxin and the effectiveness of melittin upon bacterial spheroplasts argues that this molecule can be taken up into the periplasmic space of strain LP1395 and contact the inner membrane.

The small size of melittin (MW, $\approx$ 2,600) would allow this protein better access to the periplasm relative to C9 (MW, $\approx$ 70,000); however, it has been reported that the bactericidal proteins colicin E3 (34) and colicin E1 (1) (each of which has a molecular mass of over 55,000 Da) can be osmotically shocked into *E. coli* cells in such a way as to bypass the

required receptor for colicin toxin. The ability to damage both cell types used here by colicin E1 through the osmotic shock procedure suggests that proteins of this size can be introduced in this way. Colicin E1 functions by inhibiting inner membrane function (22), and it has been suggested that one colicin molecule per cell is capable of killing a colicin-sensitive cell (24, 26). The level of cell killing reported here for colicin E1 shocked into the cells is similar to that described previously for this toxin (1). Colicin L-resistant bacterial strains subjected to treatments that increase the permeability of the bacterial outer membrane (14) are also similarly affected. Other proteins, such as the maltose-binding protein, have also been successfully introduced into the periplasm of *E. coli* cells (4).

If the ability of osmotically introduced C9 to kill cells is relevant to the actual killing mechanism of complement, it could be envisioned that the C5b-8 site serves to allow the C9 molecule access to the periplasmic space and inner membrane in a form that allows expression of a membranolytic function. The formation of a channel across the outer membrane is not sufficient to explain the bacterial killing action of the MAC, since many water-filled channels in the form of porins exist across the outer membrane (reviewed in reference 3) and the removal of the outer membrane itself is not sufficient to inhibit colony formation under certain conditions. Although it is accepted that the C5b-9 complex must somehow affect the inner membrane to kill a cell, access to the inner membrane by C9 alone is not in and of itself sufficient to produce an inhibitory activity, as demonstrated by the inability of C9 alone to inhibit the membrane potential of bacterial inner membrane vesicles (7) or spheroplasts. It is possible that for the case of periplasmic C9, some other factor present in the cell provides a step that acts upon C9 in such a way as to lead to the death of the cell. Actively growing, energy-metabolizing cells are relatively more sensitive to complement attack than are the same cells suffering slowed metabolism due to late-log-phase growth (11, 27). In addition, the substrate the cells are grown in (25) or the energy poisoning of actively growing cells (15, 33) alters the effectiveness of complement attack. This finding demonstrates that the killing activity of complement may be dependent on bacterial energy input. The above-mentioned putative factor could require a source of bacterial energy serving to effectively augment the bactericidal activity of both the MAC of complement and osmotically introduced (or periplasmic) C9.

The effects of osmotically introduced C9 and the effects of C9 when added to cells bearing C5b-8 sites are similar with respect to (i) mechanisms of action that involve the inhibition of bacterial respiration and cell death for complement-sensitive cells and (ii) the relationship between resistance to periplasmic C9 and resistance to C5b-9 sites on the bacterial surface. Cells that are resistant to periplasmic C9 are not merely resistant to the osmotic shock procedure used. It is possible that the study of bacterial resistance to complement attack may be aided by determining the mechanism by which bacterial cells are able to either (i) allow periplasmic C9 to express an inhibitory activity or (ii) suppress periplasmic C9.

ACKNOWLEDGMENTS

This work was supported by Public Health Service research grant AI22192 from the NIH.

I thank E. S. Bjers and D. S. Smith for excellent technical assistance.

REFERENCES

1. Bishop, L. J., E. S. Bjers, V. L. Davidson, and W. A. Cramer. 1985. Localization of the immunity protein-reactive domain in unmodified and chemically modified COOH-terminal peptides of colicin E1. *J. Bacteriol.* **164**:237-244.
2. Bloch, E. F., M. A. Schmetz, J. Foulds, C. H. Hammer, M. M. Frank, and K. A. Joiner. 1987. Multimeric C9 within C5b-9 is required for inner membrane damage to *Escherichia coli* J5 during complement killing. *J. Immunol.* **138**:842-848.
3. Brass, J. M. 1986. The cell envelope of Gram-negative bacteria: new aspects of its function in transport and chemotaxis. *Curr. Top. Microbiol. Immunol.* **129**:1-93.
4. Brass, J. M., C. F. Higgins, M. Foley, P. A. Rugman, J. Birmingham, and P. B. Garland. 1986. Lateral diffusion of proteins in the periplasm of *Escherichia coli*. *J. Bacteriol.* **165**:787-794.
5. Cleveland, B. M., S. Slatin, A. Finkelstein, and C. Levinthal. 1983. Structure-function relationships for a voltage-dependent ion channel: properties of C-terminal fragments of colicin E1. *Proc. Natl. Acad. Sci. USA* **80**:3706-3710.
6. Dankert, J. R. 1989. Complement-mediated inhibition of function in complement-resistant *Escherichia coli*. *J. Immunol.* **142**:1591-1595.
7. Dankert, J. R., and A. F. Esser. 1986. Complement-mediated killing of *Escherichia coli*: dissipation of membrane potential by a C9-derived peptide. *Biochemistry* **25**:1094-1100.
8. Dankert, J. R., and A. F. Esser. 1987. Bacterial killing by complement: C9-mediated killing in the absence of C5b-8. *Biochem. J.* **244**:393-399.
9. Dankert, J. R., J. W. Shiver, and A. F. Esser. 1985. Ninth component of complement: self-aggregation and interaction with lipids. *Biochemistry* **24**:2754-2762.
10. Davies, J. K., and P. Reeves. 1975. Genetics of resistance to colicins in *Escherichia coli* K-12: cross-resistance among colicins of group B. *J. Bacteriol.* **123**:96-101.
11. Davis, S. D., and R. J. Wedgewood. 1965. Kinetics of the bactericidal action of normal serum on Gram-negative bacteria. *J. Immunol.* **95**:75-79.
12. DiMasi, D. R., J. C. White, C. A. Schnaitman, and C. Bradbeer. 1973. Transport of vitamin B₁₂ in *Escherichia coli*: common receptor sites for vitamin B₁₂ and the E colicins on the outer membrane of the cell envelope. *J. Bacteriol.* **115**:506-513.
13. Feingold, D. S., J. N. Goldman, and H. M. Kuritz. 1968. Locus of the action of serum and the role of lysozyme in the serum bactericidal reaction. *J. Bacteriol.* **96**:2118-2126.
14. Foulds, J., and T. Chai. 1978. Defeat of colicin tolerance in *Escherichia coli* ompA mutants: evidence for interaction between colicin L-JF246 and the cytoplasmic membrane. *J. Bacteriol.* **133**:158-164.
15. Griffiths, E. 1974. Metabolically controlled killing of *Pasteurella septica* by antibody and complement. *Biochim. Biophys. Acta* **362**:598-602.
16. Habermann, E. 1972. Bee and wasp venoms. *Science* **177**:314-322.
17. Harriman, G. R., E. R. Podack, A. I. Braude, L. C. Corbeil, A. F. Esser, and J. G. Curd. 1982. Activation of complement by serum-resistant *Neisseria gonorrhoea*. Assembly of the membrane attack complex without subsequent cell death. *J. Exp. Med.* **156**:1235-1249.
18. Joiner, K. A. 1988. Complement evasion by bacteria and parasites. *Ann. Rev. Microbiol.* **42**:201-230.
19. Joiner, K. A., C. H. Hammer, E. J. Brown, R. J. Cole, and M. M. Frank. 1982. Studies on the mechanism of bacterial resistance to complement killing. I. Terminal complement components are deposited and released from *Salmonella minnesota* S218 without bacterial death. *J. Exp. Med.* **155**:797-808.
20. Joiner, K. A., M. A. Schmetz, M. E. Sanders, T. G. Murray, C. H. Hammer, R. Dourmashkin, and M. M. Frank. 1985. Multimeric complement component C9 is necessary for killing of *Escherichia coli* J5 by terminal attack complex C5b-9. *Proc. Natl. Acad. Sci. USA* **82**:4808-4812.
21. Kadner, R. J. 1978. Repression of synthesis of the vitamin B12 receptor in *Escherichia coli*. *J. Bacteriol.* **136**:1050-1057.
22. Konisky, J., and H. Tokuda. 1979. Mode of action of colicins Ia, E1, and K. *Zentralbl. Bakteriell. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe* **244**:105-120.
23. Laine, R. O., and A. F. Esser. 1988. Comparison between complement and melittin hemolysis: anti-melittin antibodies inhibit complement lysis. *Biochemistry* **27**:5308-5314.
24. Maeda, A., and M. Nomura. 1966. Interaction of colicins with bacterial cells. *J. Bacteriol.* **91**:685-696.
25. Melching, L., and S. I. Vas. 1971. Effects of serum components on Gram-negative bacteria during bactericidal reactions. *Infect. Immun.* **3**:107-115.
26. Nomura, M. 1964. Mode of action of colicines. *Cold Spring Harbor Symp. Quant. Biol.* **28**:315-324.
27. Rowley, D., and A. C. Wardlaw. 1958. Lysis of Gram-negative bacteria by serum. *J. Gen. Microbiol.* **18**:529-533.
28. Sevag, M. G., and R. E. Miller. 1948. Studies on the effect of immune reactions on the metabolism of bacteria. I. Methods and results with *Eberthella typhosa*. *J. Bacteriol.* **55**:381-392.
29. Shiver, J. W., J. R. Dankert, J. J. Donovan, and A. F. Esser. 1986. The ninth component of human complement (C9), functional activity of the b fragment. *J. Biol. Chem.* **261**:9629-9636.
30. Stock, J. B., B. Rauch, and S. Roseman. 1977. Periplasmic space in *Salmonella typhimurium* and *Escherichia coli*. *J. Biol. Chem.* **252**:7850-7861.
31. Taylor, P. W. 1975. Genetical studies of serum resistance in *Escherichia coli*. *J. Gen. Microbiol.* **89**:57-66.
32. Taylor, P. W. 1983. Bactericidal and bacteriolytic activity of serum against Gram-negative bacteria. *Microbiol. Rev.* **47**:46-83.
33. Taylor, P. W., and H. P. Kroll. 1983. Killing of an encapsulated strain of *Escherichia coli* by human serum. *Infect. Immun.* **39**:122-131.
34. Tilby, M., I. Hindennach, and U. Henning. 1978. Bypass of receptor-mediated resistance to colicin E3 in *Escherichia coli* K-12. *J. Bacteriol.* **136**:1189-1191.
35. Tosteson, M. T., S. J. Holmes, M. Razin, and D. C. Tosteson. 1985. Melittin lysis of red cells. *J. Membr. Biol.* **87**:35-44.