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Effects of Trospectomycin on Serum Sensitivity of *Escherichia coli* UC 9451

J. I. CIALDELLA,[1]* R. G. ULRICH,[2] W. F. LIGGETT,[1] L. D. ADAMS,[3] AND V. P. MARSHALL[1]

Chemical & Biological Screening,[1] Drug Safety Research-Investigative Toxicology,[2] and Biopolymer Research,[3] The Upjohn Company, Kalamazoo, Michigan 49001

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Trospectomycin sulfate, a chemically synthesized analog of spectinomycin, exhibits a broad range of activity against both aerobes and anaerobes, including the etiological agents of sexually transmitted diseases. Its activity in vitro against *Escherichia coli* is considered only moderate. At subinhibitory levels, however, trospectomycin induced changes in a pathogenic strain of *E. coli*, UC 9451, which significantly increased its sensitivity to serum lysis. This strain of *E. coli* shows high-level resistance to serum in vitro, typically growing twofold within a 45-min incubation period. Following exposure to one-fifth the MIC of trospectomycin, >99% of the bacteria were killed in 25% serum within 15 min. Surviving bacteria were static in this level of serum for over 3 h. Killing was due to lysis mediated by both the classical and alternative complement pathways. The bacteria exposed to trospectomycin were enlarged in both diameter and length, but they still grew at rates comparable to those of untreated bacteria. No other visible morphological changes could be directly related to the increase in serum sensitivity. The profile of outer membrane proteins obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis was identical for trospectomycin-treated or untreated bacteria. However, the relative proportion of four major outer membrane proteins varied considerably.

Trospectomycin sulfate (trospectomycin) is a novel aminocyclitol antibiotic with a broad spectrum of activity and has been studied extensively both in vitro and in vivo. In most cases, in vitro antibacterial activity has been predictive of efficacy in animal models. In other cases, however, this correlation has been lacking, with trospectomycin demonstrating greater activity in vivo than that predicted by in vitro results. The activity of trospectomycin in vitro against *Escherichia coli*, for example, is considered only moderate, but animal studies have shown impressive efficacy against experimental *E. coli* infections in vivo. An explanation for these discrepancies may be related to possible synergistic drug-host effects.

Resistance to the bactericidal activity of serum is an important virulence factor of gram-negative bacteria. A primary host defense mechanism, attack and killing by the complement system, is ineffective against serum-resistant organisms. Trospectomycin has previously been shown to induce serum sensitivity in a pathogenic strain of *E. coli*, UC 9451. Following exposure to only one-fifth the MIC of the antibiotic, >99% of the bacteria were killed within 45 min of incubation with serum, while untreated bacteria multiplied twofold. A moderate increase in killing of *E. coli* by polymorphonuclear leukocytes was also observed (4). Other antibiotics, including the lincosaminides (4), paldimycin (3), and cefamandole (14), have been shown to enhance the susceptibility of bacteria to serum. The enhanced serum sensitivity observed appears to be the result of morphological and antigenic alterations in the bacteria induced by exposure to low levels of these antibiotics. In the present study, more detailed analysis was made of the mechanisms involved in the serum sensitivity induced by trospectomycin in *E. coli*.

* Corresponding author.

MATERIALS AND METHODS

Chemicals. Gentamicin sulfate was obtained from Sigma Chemical Co. Trospectomycin sulfate (U-63366F) was synthesized at The Upjohn Co. Antisera to human C1q, C4, C3, C5, C6, C7, C8, C9, and properdin were obtained from Boehringer Mannheim Biochemicals. *E. coli* OK Antisera poly A, B, C, D, and E were purchased from Difco. The molecular weight (MW) standards used in electrophoresis were the Sigma prestained kit for MWs of 27,000 to 180,000 (MW-SDS-Blue kit). Hanks balanced salt solution (HBSS)-HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer was prepared by the addition of 10% HBSS (concentrated 10 times without calcium, magnesium, phenol red, and sodium bicarbonate) to 0.02 M HEPES (pH 7.4). Tris-$MgCl_2$ buffer contained 0.05 M Tris (pH 7.5) and 10 mM $MgCl_2$.

Organism. *E. coli* UC 9451 was a clinical isolate that was obtained from the University of Tennessee. It was highly resistant to both serum lysis and phagocytic killing in our assay. This strain did not express K antigen under the growth conditions that we used, but it did have O antigen. These two antigens are often associated with serum resistance in *E. coli*. The presence of K antigen was determined serologically by slide agglutination by using *E. coli* OK polyvalent antisera. Presence of the O antigen was confirmed by lack of bacterial agglutination in physiological saline or after heating at 100°C for 10 min.

Culture conditions. Stock cultures (10^8 CFU/ml) that were frozen in 0.5-ml portions at −20°C were used as inocula for 100 ml of nutrient broth (pH 7.0). Cultures were incubated at 37°C on a rotary shaker at 200 rpm for 4.5 h. Antibiotic was added after 1 h, which corresponded to the beginning of the log phase of growth. Following incubation, bacteria were washed twice with normal saline and suspended in HBSS-HEPES buffer to 7×10^7 to 10×10^7 CFU/ml.

Serum. Serum was obtained from the clotted blood of healthy volunteers, diluted to the desired concentration in HBSS-HEPES buffer, and used immediately. Serum was

TABLE 1. Serum sensitivity of *E. coli* UC 9451 following exposure to various concentrations of trospectomycin[a]

Trospectomycin concn (μg/ml)	MIC	% Viable bacteria at[b]: 15 min	30 min	45 min
0		120	175	230
3	1/10	30	20	14
6	1/5	10	5	<1
10	1/3	13	5	<1

[a] Bacteria were grown in the presence of trospectomycin for 4.5 h and then washed and incubated with serum (100%) for 45 min, as described in the text.
[b] Results are expressed as percentages of viable bacteria surviving after incubation with serum.

inactivated by heating it at 56°C for 30 min (5). Chelation of serum to inactivate the classical complement pathway was accomplished by the addition of 10 mM EGTA [ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*,*N*′,*N*-tetraacetic acid] and 10 mM $MgCl_2$ (pH 7.2) (Mg-EGTA) for 30 min at 0°C prior to use (8). In certain studies, serum was treated with antisera to specific complement components of the classical or alternative pathway. A portion of 0.1 to 0.5 ml of each antiserum was added per ml of serum. The solution was incubated for 2 h at 0°C and then centrifuged briefly to remove precipitated protein (1). An equivalent volume of heat-inactivated serum containing 0.02% sodium azide was added to controls.

Assay protocol. Serum lysis of *E. coli* was determined as follows. Washed bacteria (0.5 ml) treated with antibiotic or untreated were incubated with 0.5 ml of serum. Serum concentrations ranged from 5 to 100%; this was accomplished by dilution in HBSS-HEPES buffer as necessary. The resulting suspension was mixed slowly by rocking it for 45 min at 37°C in a sterile Nunc cryotube (90 by 125 mm). Duplicate tubes were prepared for each different mixture that was tested. Viable counts were made by using serially diluted samples spread on Trypticase soy agar plates followed by overnight incubation at 32°C. Results are expressed as the percentage of viable bacteria present before and after exposure to serum. In certain studies, serum was heat inactivated or treated with EGTA or specific antihuman complement antibodies prior to use in the assay.

Antimicrobial susceptibility. MICs were determined by broth dilution. The growth conditions in the assay were utilized. The standard inoculum was 5×10^5 CFU/ml. The MIC was defined as the lowest concentration of antibiotic that inhibited visible growth after 18 h of incubation at 32°C. The MIC determined for trospectomycin against *E. coli* UC 9451 was 30 μg/ml, and the MIC of gentamicin was 0.1 μg/ml. Since the growth phase can affect the sensitivity of an organism to serum, the subinhibitory level of trospectomycin or gentamicin to which the bacteria were exposed allowed a rate of growth similar to those of untreated cultures.

Transmission electron microscopy. For electron microscopic examination, samples were pelleted by centrifugation and then fixed with 3% glutaraldehyde in 0.1 M cacodylate (pH 7.2) and allowed to fix for 1 h at room temperature. Following three rinses in buffer (0.1 M cacodylate; pH 7.2), cells were postfixed in 1% osmium tetroxide in buffer for 1 h. Cells were then rinsed three times with distilled water and stained for 30 min in 1% aqueous uranyl acetate. Following two water rinses, cells were dehydrated with graded ethanol concentrations through 100%, cleared in propylene oxide, and embedded in Polybed 812 (Polysciences, Inc.). Thin

TABLE 2. Enhancement of serum killing of *E. coli* UC 9451 treated with subinhibitory concentrations of trospectomycin or gentamicin[a]

% Serum	% Viable bacteria[b]: Untreated	Trospectomycin treated: 1/10 the MIC	Trospectomycin treated: 1/5 the MIC[c]	Gentamicin treated (1/10 the MIC)[c]
2.5	170	110	70	195
5	185	55	36	160
10	167	20	<1	183
25	176	15	<1	120
50	153	14	<1	85

[a] Bacteria were grown in the presence of trospectomycin for 4.5 h and then washed and incubated with various concentrations of serum for 45 min, as described in the text.
[b] Results are expressed as percentages of the viable number of bacteria at time zero that survived after incubation with serum.
[c] The MIC to which the bacteria were exposed reduced growth by <1 log unit.

sections were cut with diamond knives on an MT-5000 ultramicrotome (Dupont-Sorvall), stained with Reynolds lead citrate (15), viewed, and photographed on a Jeol 1200EX scanning-transmission electron microscope.

Membrane preparation. Membrane isolation was based on the procedure of Georgopapadaku and Lui (11). *E. coli* UC 9451 was grown for 4.5 h as described above and then harvested by centrifugation at 10,000 × *g* for 10 min and washed once with 0.05 M potassium phosphate buffer (pH 7.0). The cells were suspended in 2 volumes (wt/vol) of 0.05 M Tris–$MgCl_2$ buffer. DNase was added to the suspension at 5 μg/ml. Lysis of the bacteria occurred following sonication (Fischer Sonic Dismembrator no. 300) for 1.5 min at a setting of 50s (2 times for 45 s each time). Sonic extracts were centrifuged briefly at 10,000 × *g* for 5 min to remove unlysed cells. The supernatant was centrifuged at 45,000 × *g* for 30

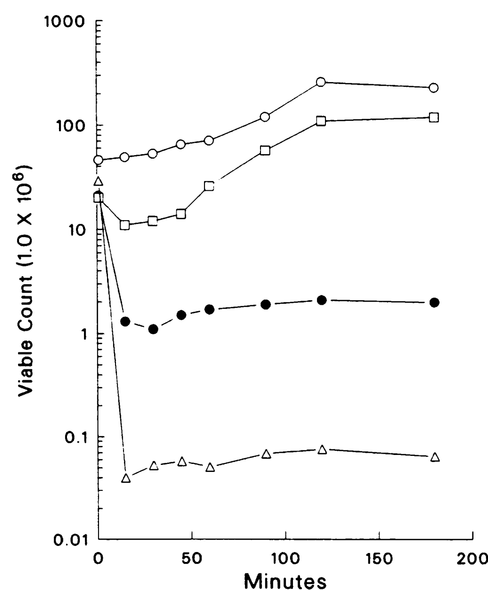

FIG. 1. Comparison of growth inhibition in serum of *E. coli* treated with gentamicin or trospectomycin. Bacteria were grown in the presence of the antibiotics for 4 h, harvested and washed with HBSS-HEPES buffer, and then incubated with serum (100%). ○, Untreated; △, trospectomycin treated at 1/5 the MIC; ●, trospectomycin treated at 1/10 the MIC; □, gentamicin treated.

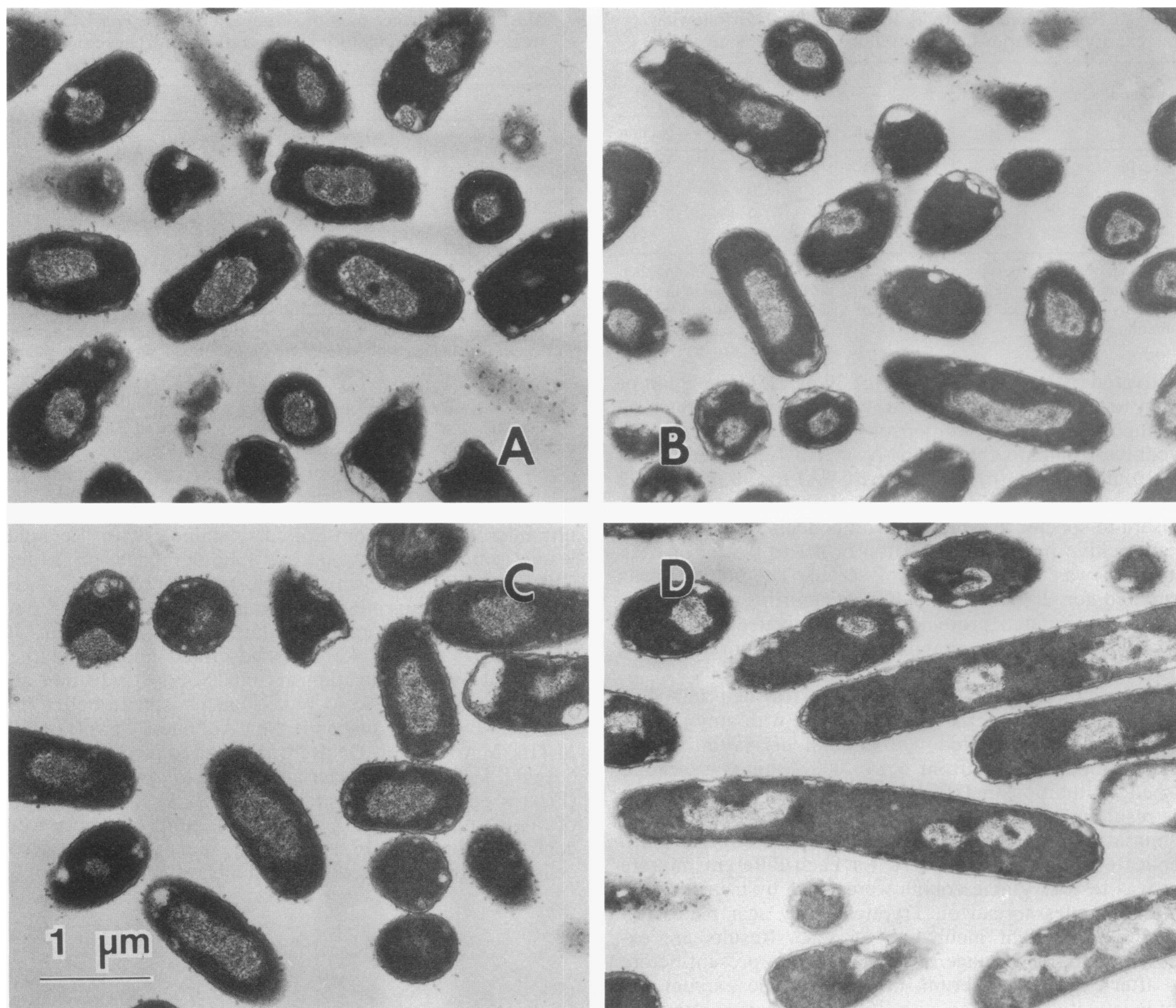

FIG. 2. Transmission electron micrographs of *E. coli* UC 9451 following exposure to trospectomycin. *E. coli* was grown for 4 h in the presence of various concentrations of trospectomycin and was then harvested and washed in HBSS-HEPES buffer. Concentration of trospectomycin: A, 0 μg/ml; B, 3 μg/ml; C, 6 μg/ml; D, 10 μg/ml.

min. Pellets were washed once with the Tris-$MgCl_2$ buffer and recentrifuged. The resulting pellet was then suspended in 3 volumes (wt/vol) of 1% sodium lauroylsarcosinate and incubated for 30 min at room temperature followed by centrifugation at 45,000 × *g* for 30 min. This procedure solubilized the cytoplasmic membrane proteins (7). The pellet containing outer membrane proteins was stored at −20°C.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was performed by using a 10 to 20% gradient gel (16 cm by 16 cm by 1.5 mm). No stacking gel was used. Membrane protein samples were prepared for analysis by the method of Laemmli (12). MW standards (MWs of 27,000 to 180,000) were run simultaneously with membrane samples. Protein bands were stained with an aqueous solution of 0.1% Coomassie brilliant blue R250 in 40% methanol and 10% acetic acid and then scanned on a BioImage Visage 110 image analysis system.

RESULTS

Serum sensitivity. As reported previously (4), a significant increase in serum sensitivity occurred following exposure of *E. coli* UC 9451 to trospectomycin at a subinhibitory level. This strain of *E. coli* is a clinical isolate and exhibited a high level of resistance to serum in our assay. In fact, the bacteria utilize the serum for growth and increase in titer 1.5- to 3-fold with a 45-min incubation period. The serum sensitivity induced by trospectomycin was concentration dependent, with maximal lysis occurring at one-fifth the MIC (Table 1). At this level, >99% of the bacteria were killed at concentrations in serum of ≧10%. Over 50% of the bacteria were killed in 5% serum. High levels of killing were also seen after exposure to only 1/10 the MIC of trospectomycin (Table 2). Surviving bacteria were static in serum for over 3 h (Fig. 1).

As a negative control, gentamicin was also tested in the assay since it has generally been found not to enhance host

TABLE 3. Dimensions of *E. coli* UC 9451 grown in various concentrations of trospectomycin

Trospectomycin concn (μg/ml)	Mean size (μm) of *E. coli*[a]	
	Diam	Length
0	0.69	1.70
3	0.71	1.95
6	0.75	2.10
10	0.80	3.35

[a] Mean size is based on measurements of 75 to 100 cells. Cells which were measured were not in advanced stages of replication.

defense mechanisms (6, 13, 17). *E. coli* was exposed to 1/10 the MIC of gentamicin since this was the highest level of antibiotic which allowed a growth rate comparable to that of untreated bacteria. Growth rate was greatly diminished in the presence of higher subinhibitory levels. Bacteria grown in the presence of gentamicin were only slightly inhibited by a very high concentration in serum (Table 2). However, surviving bacteria began to grow again within 90 min (Fig. 1). The subinhibitory levels of both trospectomycin and gentamicin to which *E. coli* was exposed reduced growth by <1 log unit. So, both control and antibiotic-treated bacteria were in the late exponential growth phase when they were harvested for assay. Therefore, differences in serum sensitivity of the bacteria were not due to differences in their phase of growth.

Morphology. Morphological alterations in *E. coli* following exposure to various concentrations of trospectomycin were observed in transmission electron micrographs. After growth in trospectomycin, bacteria were found to increase in diameter and length in a dose-dependent manner when they were compared with untreated control cultures (Fig. 2). At the highest level tested, the diameter of the bacteria increased approximately 15%, but an increase in length of twofold was found (Table 3). Elongated cells with multiple nuclear regions were also observed. Interestingly, at the concentration used in most experiments (6 μg/ml, one-fifth the MIC), the average diameter of bacteria exposed to trospectomycin increased <10% compared with that of untreated bacteria, and there was only a 20% difference in length. Yet, at this level of exposure, *E. coli* became highly susceptible to killing by serum (>99%). Increased concentrations of trospectomycin did not change the rate or percentage of killing by serum. Serum sensitivity was also observed after exposure to 1/10 the MIC (3 μg/ml) of trospectomycin, but morphological changes were even more minor. *E. coli* was viable and replicated at all levels of trospectomycin tested. At 3 and 6 μg/ml, there was <1 log unit difference in titers compared with those of untreated bacteria.

Mechanism of serum lysis. The bactericidal effects of serum are mediated by the activated components of either the classical or the alternative complement pathway. In order to determine the mechanism of killing by serum of trospectomycin-treated bacteria, various inhibitors of each pathway were added to serum prior to incubation with *E. coli* (Table 4). Killing was not observed when trospectomycin-treated bacteria were incubated with heat-treated serum, in which both complement pathways would have been inactivated. Partial reversal of killing was observed when the classical pathway was inactivated with Mg-EGTA or when it was blocked by the removal of C1q or C4 through the addition of antihuman C1q or C4 antibody to the serum. Partial reversal of killing was also observed when the alternative pathway was blocked by the removal of properdin with the corresponding antiserum. Only removal of proteins common to both pathways, such as C3 or components of the membrane attack complex, totally eliminated the bactericidal activity of the serum against trospectomycin-treated *E. coli*. Killing was still found even after treatment of serum with antihuman C9 antibody. However, attachment of the $\overline{C5b678}$ complex to the bacterial membrane has some effect on disrupting membrane integrity even in the absence of C9. So, some killing could still occur.

TABLE 4. Reversal of killing by serum of trospectomycin-treated *E. coli* UC 9451 by blocking the classical and alternative complement pathways

Serum treatment	Complement pathway blocked[a]	% Viable bacteria[b]	
		Untreated	Trospectomycin treated
Normal	None	170	0.03
Heat treated	C and A	155	127
Mg-EGTA	C	163	47
Antihuman properdin Ab[c]	A	140	60
Antihuman C1q Ab	C	135	41
Antihuman C4 Ab	C	127	30
Antihuman C3 Ab	C and A	143	105
Antihuman C5 Ab	C and A	137	171
Antihuman C6 Ab	C and A	164	115
Antihuman C7 Ab	C and A	183	196
Antihuman C8 Ab	C and A	144	171
Antihuman C9 Ab	C and A	225	36

[a] C, Classical; A, alternative.

[b] Results are expressed as percentages of the viable number of bacteria at time zero that survived after 45 min of incubation with normal serum (100%) or serum to which inhibitors of the classical or alternative pathway were added.

[c] Ab, Antibody.

The bactericidal activity of the serum was accompanied by cell lysis. As seen in the transmission electron micrographs in Fig. 3, only trospectomycin-treated bacteria were lysed in the presence of serum. Lysis was found at all the sub-MICs to which *E. coli* was exposed from 1/10 to 1/3 the MIC. Even though bacteria treated with 1/10 the MIC exhibited only partial serum sensitivity, the susceptible bacteria were lysed.

Analysis of outer membrane proteins. Trospectomycin was shown to inhibit protein synthesis in susceptible strains (A. L. Laborde and R. Mourey, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 166, 1987). Although the levels of antibiotic used in our assay did not appreciably affect the growth rate, these concentrations could still cause interference with or alter the synthesis of enzymes or proteins that are important in the assembly of cell surface characteristics, thus exposing or partially exposing complement and antibody receptors at the outer membrane. The profiles of outer membrane proteins obtained by sodium dodecyl sulfate-gradient gel electrophoresis (Fig. 4) were identical for both control and trospectomycin-treated *E. coli*. However, the relative proportions of four major outer membrane proteins varied considerably, as shown in the densitometric analysis of the gel (Fig. 5 and Table 5). Since the relative proportions of these proteins can vary during the normal course of growth (1), the outer membranes were isolated from both cultures during the late exponential phase of growth. The two major peaks in untreated *E. coli* were the first and third ones. They made up a nearly equivalent amount of the total density. Following treatment of the bacteria with trospectomycin, the fourth peak in this

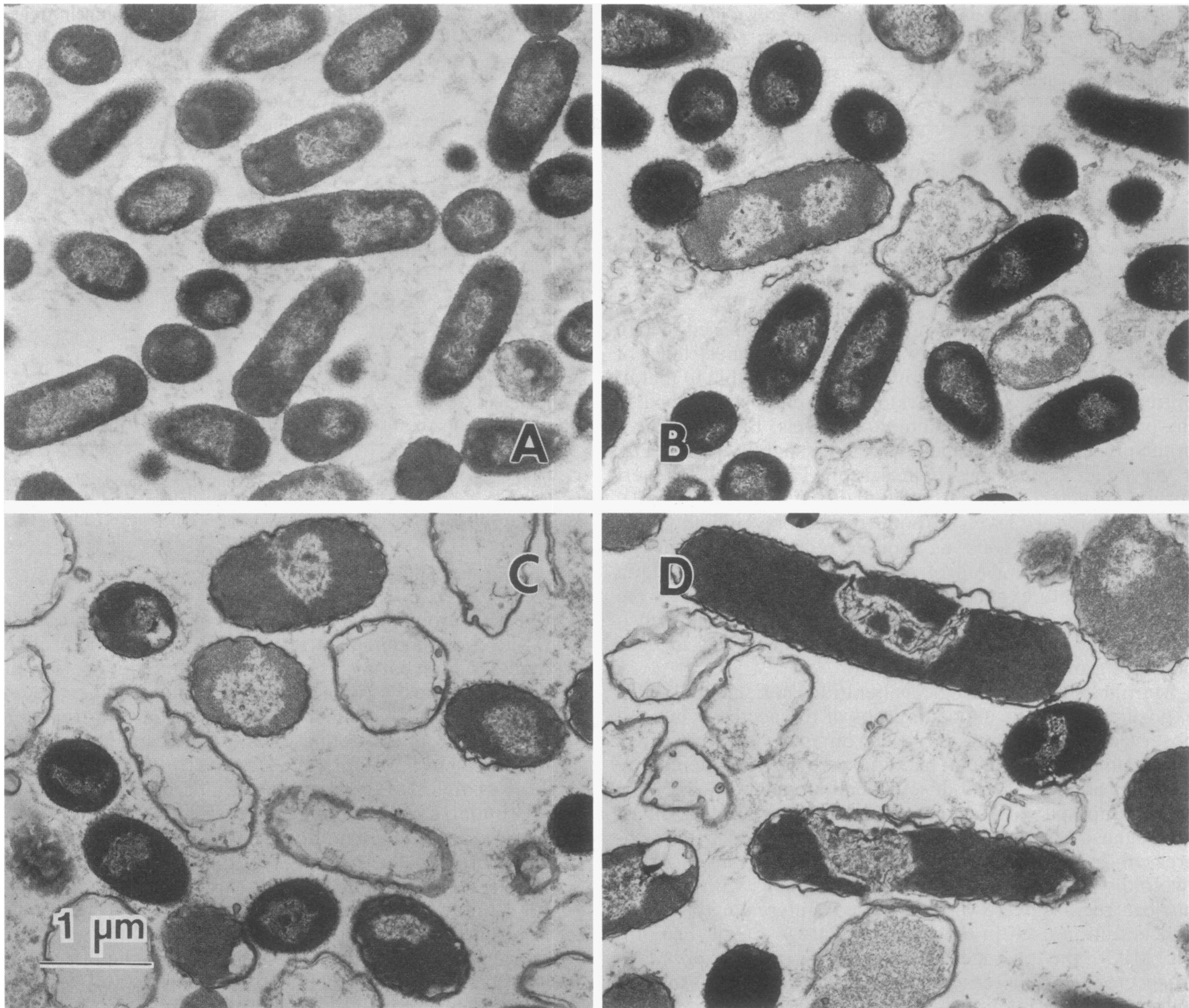

FIG. 3. Transmission electron micrographs of trospectomycin-treated *E. coli* incubated in serum. *E. coli* was grown for 4 h in the presence of various concentrations of trospectomycin and was then harvested and washed in HBSS-HEPES buffer. Washed bacteria were incubated for 10 min in 10% serum. Concentration of trospectomycin: A, 0 µg/ml; B, 3 µg/ml; C, 6 µg/ml; D, 10 µg/ml.

series of membrane proteins was by far the most predominant. The first and second peaks were still proportionally similar to that observed in untreated *E. coli*. The ratios of the third and fourth peaks seemed to vary considerably between the treated and untreated bacteria.

DISCUSSION

Subinhibitory levels of trospectomycin significantly enhanced lysis of a pathogenic strain of *E. coli*, UC 9451, by human serum. The trospectomycin-induced serum sensitivity was dose dependent, with >99% of the bacteria being killed in 10% serum following exposure to only one-fifth the MIC. The surviving bacteria were static in serum for over 3 h. At this level of antibiotic, there was less than a 1 log unit difference in growth between control and treated bacteria. Both antibiotic-treated and untreated bacteria were in the late exponential phase of growth when they were harvested for assay. Therefore, differences in serum sensitivity were not due to differences in the stage of their growth cycle.

Serum lysis of the trospectomycin-treated bacteria was complement mediated and involved both the classical and alternative pathways. Only inhibition of both pathways totally eliminated bacterial killing by serum. The classical pathway is activated by complexing of antigen to specific antibody (immunoglobulin G [IgG], IgM) before C3 fixation occurs. Activated enzymes of the alternative pathway assemble on the target membrane, usually without antibody involvement. The sugar component of the microbial membrane often initiates the reaction sequence (16). Thus, killing of trospectomycin-treated bacteria appeared to proceed with or without antibody involvement.

The activated $\overline{C5b\text{-}9}$ complex on the cell membrane induces alterations and lesions which ultimately lead to cell death because of the loss of vital cellular constituents. However, actual lysis of bacterial cells requires the presence of lysozyme in serum (18). In the transmission electron micrographs of the *E. coli* incubated in serum, general lysis of the bacteria exposed to trospectomycin was observed.

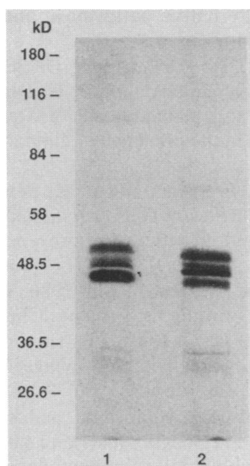

FIG. 4. Gradient electrophoresis of outer membrane proteins from *E. coli* treated with trospectomycin or untreated. *E. coli* was grown for 4 h in one-sixth the MIC of trospectomycin prior to membrane isolation. Lane 1, Membrane proteins from treated *E. coli*; lane 2, membrane proteins from untreated *E. coli*. kD, Kilodaltons.

However, because partial reversal of killing was found after inhibition of specific components of the classical or alternative complement pathway, killing and lysis was not due to random activation of the $\overline{C5b\text{-}9}$ complex by lysosomal enzymes which can also occur (5).

The alterations in trospectomycin-treated *E. coli* that caused serum susceptibility have not been definitely established. Transmission electron micrographs of bacteria following exposure to trospectomycin showed morphological variations. These bacteria were elongated and had increased diameters compared with untreated organisms. However, only minor dimensional changes were present in bacteria that had already become highly susceptible to serum lysis. So, the dramatic morphological changes (twofold increase in length at one-third the MIC) did not correlate entirely with serum sensitivity.

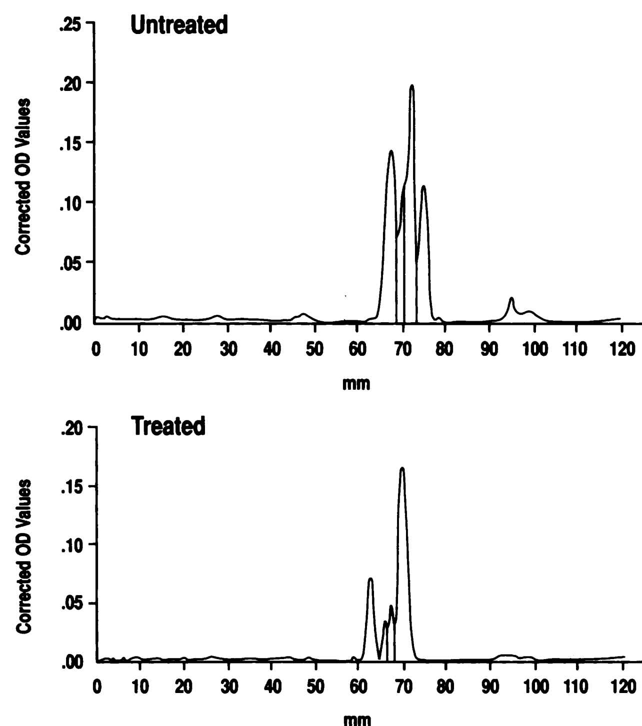

FIG. 5. Densitometric analysis of the protein profiles obtained by gradient polyacrylamide gel electrophoresis of outer membranes from *E. coli* treated with trospectomycin or untreated (see Table 5). OD, Optical density.

TABLE 5. Densitometric analysis of the protein profiles obtained by gradient polyacrylamide gel electrophoresis of outer membranes from trospectomycin-treated or untreated *E. coli*[a]

Peak	Untreated		Treated	
	IOD[b]	% of total protein	IOD	% of total protein
1	0.399	23.1	0.149	15.0
2	0.140	8.1	0.036	3.6
3	0.386	22.4	0.067	6.8
4	0.270	15.6	0.381	38.4

[a] Analysis of protein profiles shown in Fig. 5.
[b] IOD, Integrated optical density.

Strains of *E. coli* capable of evading complement lysis generally express O antigen, K antigen, or both. The O antigen is a polysaccharide side chain constituent in the lipopolysaccharide. Smooth strains which contain O antigen are generally resistant to the lethal action of complement. Rough strains in which the lipopolysaccharide lacks a peripheral carbohydrate residue are highly susceptible to lysis. Serum resistance is related not only to the presence of O antigen but also to the amount. The K antigen is a sialic acid capsule which again generally protects the organism from complement lysis. Resistance to serum is also associated with the presence of certain outer membrane proteins, such as the *traT* protein of *E. coli*, which mediates surface exclusion and the *iss* protein (2). The presence of one or more of these antigens or proteins alone is not always sufficient to confer resistance. The combination of determinants that are present and the amount of each one that is expressed are also important. Even the orientation of proteins on the surface of the cell can alter serum sensitivity. In certain serum-resistant strains of bacteria, the $\overline{C5b\text{-}9}$ complex binds to the outer membrane, but killing does not occur because the complex does not align properly.

Since trospectomycin inhibits protein synthesis, any one or all of these resistance factors could be affected. The production of certain proteins or antigens could be interrupted or altered, and in turn, the makeup of proteins and antigens as well as their orientation on the cell surface would change. Other antibiotics which were shown to enhance phagocytosis of bacteria were also shown to inhibit the production of surface virulence factors by these bacteria. Clindamycin, which enhances the phagocytosis of *Streptococcus pyogenes*, was also shown to reduce, but not totally inhibit, production of the M and T antigens by the organism (9). Clindamycin (10) and ampicillin (K. L. Cates and L. Caparas, 21st ICAAC, abstr no. 658, 1981) were found to inhibit capsule formation in *Haemophilus influenzae* and bacteroides, respectively.

E. coli UC 9451 did not exhibit K antigen in our assay. O antigen is present both before and after exposure to trospectomycin. It is possible, however, that the amount of O antigen is reduced following growth in the presence of the antibiotic, thus revealing receptor sites for complement or antibody to bind. Again, no difference was detectable in the

electrophoretic analysis of outer membrane proteins from trospectomycin-treated and untreated bacteria. However, the ratio of four major proteins did vary. Conceivably, reduction in the levels of key membrane proteins and surface antigens or a change in their ratio could alter the orientations of proteins on the membrane, thus revealing receptor sites for complement or antibody to bind or allowing binding in the proper orientation.

LITERATURE CITED

1. **Allen, R. J., and G. K. Scott.** 1980. The effect of purified lipopolysaccharide on the bactericidal reaction of human serum complement. J. Gen. Microbiol. **117:**65–72.
2. **Binns, M. M., J. Mayden, and R. P. Levine.** 1982. Further characterization of complement resistance conferred on *Escherichia coli* by the plasmid genes *traT* of R100 and *iss* of ColV,I-K94. Infect. Immun. **35:**654–659.
3. **Cialdella, J. I., R. G. Ulrich, and V. P. Marshall.** 1988. Augmentation of serum bactericidal activity by paldimycin. J. Antibiot. **41:**660–666.
4. **Cialdella, J. I., J. J. Vavra, and V. P. Marshall.** 1986. Susceptibility of bacteria to serum lysis or phagocytosis following growth in subinhibitory levels of lincosaminide or spectinomycin related antibiotics. J. Antibiot. **39:**978–984.
5. **Cooper, N. R.** 1984. The complement system, p. 119–130. *In* D. Stites, J. Stobo, H. Fudenberg, and J. Wells (ed.), Basic and clinical immunology. Lange Medical Publications, Los Altos, Calif.
6. **Efrati, C., T. Sacks, N. Ne'emann, M. Lahau, and I. Ginsburg.** 1976. The effect of leukocyte hydrolases on bacteria. Inflammation **1:**371–407.
7. **Filip, C., G. Fletcher, J. Wulff, and C. Earhart.** 1973. Solubilization of the cytoplasmic membrane of *Escherichia coli* by the ionic detergent sodium-lauroylsarcosinate. J. Bacteriol. **115:** 717–722.
8. **Fosgren, A., R. H. McLean, A. F. Michael, and P. G. Quie.** 1975. Studies of the alternative pathway in chelated serum. J. Lab. Clin. Med. **85:**904–912.
9. **Gemmell, C. G., P. K. Peterson, D. Schmeling, Y. Kim, J. Matthews, L. Wannamaker, and P. G. Quie.** 1981. Potentiation of opsonization and phagocytosis of *Streptococcus pyogenes* following growth in the presence of clindamycin. J. Clin. Invest. **67:**1249–1256.
10. **Gemmell, C. G., T. Spear, and P. K. Peterson.** 1983. Morphological changes in *Bacteroides fragilis* and *Klebsiella pneumoniae* attributable to growth in the presence of various antibiotics. Eur. J. Clin. Microbiol. **2:**1–7.
11. **Georgopapadakou, N., and F. Lui.** 1980. Penicillin-binding proteins in bacteria. Antimicrob. Agents Chemother. **18:**148–157.
12. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
13. **Nishida, M., Y. Mine, S. Nonoyama, and Y. Yokota.** 1976. Effect of antibiotics on the phagocytosis and killing of *Pseudomonas aeruginosa* by rabbit polymorphonuclear leukocytes. Chemotherapy (Basel) **22:**203–210.
14. **Perna, P., A. Andreana, M. Dilillo, R. Utili, and G. Ruggiero.** 1983. Sensitivity of germs exposed to subinhibitory concentrations of antibiotics to the action of serum and hepatic phagocytes. Boll. Soc. Ital. Biol. Sper. **59:**1683–1686.
15. **Reynolds, E. S.** 1963. The use of lead citrate at high pH as an electronopaque stain in electron microscopy. J. Cell Biol. **17:**208–212.
16. **Roitt, I. M., J. Brostoff, and D. Male.** 1986. Complement, p. 7.1–7.14. *In* Immunology. C. V. Mosby Co., St. Louis.
17. **Root, R., R. Isturiz, A. Molavi, J. Metcalf, and H. Malech.** 1981. Interactions between antibiotics and human neutrophils in killing of staphylococci. J. Clin. Invest. **67:**247–259.
18. **Rozenberg-Arska, M., J. C. Parsuis, E. Y. Jaarsma, and J. Verhaef.** 1986. Bactericidal, bacteriolytic, and opsonic activity of human serum against *Escherichia coli*. J. Med. Microbiol. **22:**143–149.