Surface Manifestations of Antibiotic-Induced Alterations in Protein Synthesis in Bacterial Cells

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Scanning electron microscopy of changes in bacteria induced by antimicrobial agents which interfere with cell wall synthesis revealed morphological alterations which correlated well with their mechanism of action. The present studies were undertaken to investigate the presence and characteristics of alterations in surface morphology resulting from the action of antibiotics known to interfere with intracellular protein synthesis. Strains of Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa were exposed to kanamycin, chloramphenicol, and tobramycin during various phases of bacterial growth. A spectrum of morphological changes related to concentration of drug and duration of exposure was observed which was similar to those induced by penicillin or cephalothin. Cells were also exposed to sulfamethoxazole with similar results. The morphological abnormalities observed may be surface reflections of specific abnormalities of intracellular protein synthesis or may represent a final common pathway of drug-induced injury at many sites within or on bacterial cells.

Studies of antibiotic-induced alterations in bacterial morphology have contributed to a better understanding of the mechanism of action of these agents. The majority of early studies were limited to those agents which interfere with cell wall synthesis (6, 7, 11) and were pursued with most vigor at the time when antibiotics first became available. These initial studies utilized light and phase-contrast microscopy. Later, the transmission electron microscope provided a means of studying ultrastructure and permitted correlation of morphology with cellular physiology and biochemistry (1, 17).

More recently, the effects of cell wall-active antibiotics on the surface morphology of specific bacteria have been demonstrated with the scanning electron microscope (9, 14, 15). Penicillin G and cephalothin have been observed to

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<table>
<thead>
<tr>
<th>Organism</th>
<th>Source</th>
<th>Penicillin G</th>
<th>Cephalothin</th>
<th>Chloramphenicol</th>
<th>Kanamycin</th>
<th>Tobramycin</th>
<th>Sulfamethoxazole</th>
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<tr>
<td>S. aureus</td>
<td>ATCC 6538 (209)</td>
<td>0.008</td>
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<td>12.500</td>
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<td>—</td>
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<td>S. aureus</td>
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<tr>
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<td>CDC Phoenix 156 (serotype 055B5)</td>
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<td>3.900</td>
<td>15.600</td>
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<tr>
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<td>B53-145c</td>
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<td>—</td>
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<tr>
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<td>Clinical isolate (Bricker strain)</td>
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<td>—</td>
<td>—</td>
<td>—</td>
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cause a progression of characteristic changes in the morphology of staphylococci and other microorganisms (14); these were interpreted as surface reflections of phenomena occurring within the bacterial cell wall, since these drugs are known to act at the peptidoglycan component of the inner layer (18). These studies were expanded in the present investigations to determine whether antimicrobial agents whose site of action is intracellular would likewise cause surface alterations.

**MATERIALS AND METHODS**

The microorganisms studied included standard test strains and clinical isolates obtained from patients at University Hospital, Columbus, Ohio (Table 1). Trypticase Soy Broth (BBL) was inoculated with each organism and incubated at 37°C for 18 hr. A sample was then subcultured into fresh broth to obtain cultures in the logarithmic phase of growth (4 to 6 hr for the organisms studied) and 12-hr-old cultures.

Penicillin G, cephalothin, kanamycin, chloramphenicol, and sulfamethoxazole were diluted in sterile, distilled, demineralized water. Tobramycin was obtained in solution from Eli Lilly & Co., Indianapolis, Ind. The effects of final antibiotic concentrations equivalent to 0.1, 1.0, and 10 times the minimal inhibitory concentration (MIC) for the test organisms were studied (Table 1). Specified concentrations of each antibiotic were added to cultures in the logarithmic phase of growth (4 to 6 hr for the organisms studied) and 12-hr-old cultures.
mic phase of growth (4 hr) and to 12-hr cultures. Each of the test organisms was exposed to the appropriate antimicrobial agent for 15, 30, 60, 120, and 180 min and 18 hr at 37 C. In each study, control cultures were manipulated in an identical manner except that they were exposed to drug-free diluent.

The methods utilized for further preparation of specimens and the details of scanning electron microscopy have been previously reported (13, 14); in the present studies, 0.9% NaCl was used to wash the organisms prior to fixation.

RESULTS

Figures 1a-1d demonstrate the effects of increasing concentrations of penicillin G on a penicillin-susceptible strain of Staphylococcus aureus; as previously described (14), a progression of changes occurred characterized by the appearance of discrete blebs on the cell surface (Fig. 1b) which, with subsequent enlargement of the cells, resulted in forms resembling "cobblestones" or "raspberries" (Fig. 1c) and ultimately in the formation of spheroplasts (Fig. 1d). Similarly, penicillin G resulted in a spectrum of concentration-dependent changes in Escherichia coli (Fig. 1f-1h); low concentrations of the antibiotic resulted in elongation (Fig. 1f), presumably owing to interference with cell division but not cell

![Fig. 2](http://aac.asm.org/)

**FIG. 2.** (a, b) Staphylococcus aureus, exposed to 1.0 MIC of kanamycin for 3 hr. (c) Escherichia coli, untreated control. (d) E. coli, exposed to 0.1 MIC of kanamycin for 3 hr. (e) E. coli, exposed to 1.0 MIC of kanamycin for 3 hr. (f) E. coli, exposed to 1.0 MIC of kanamycin for 3 hr. (g) E. coli, exposed to 1.0 MIC of kanamycin for 3 hr (h) E. coli, exposed to 10 MIC of kanamycin for 3 hr.
Fig. 3. (a) Staphylococcus aureus, exposed to 1.0 MIC of chloramphenicol for 3 hr. (b) Escherichia coli, exposed to 0.1 MIC of chloramphenicol for 3 hr. (c) E. coli, exposed to 1.0 MIC of chloramphenicol for 3 hr. (d) E. coli, exposed to 10 MIC of chloramphenicol for 3 hr. (e) S. aureus, exposed to 1.0 MIC of tobramycin for 3 hr. (f) S. aureus, exposed to 1.0 MIC of tobramycin for 3 hr.
growth, and higher concentrations induced mid-cell defects (Fig. 1g) and spheroplasts (Fig. 1h).

When the same strain of *S. aureus* was exposed to kanamycin, an aminoglycoside antibiotic, similar intact and collapsed spheroplasts were observed (Figs. 2a and 2b). Kanamycin-induced changes in *E. coli* were also similar to those seen after exposure to penicillin G; specifically, elongation (Fig. 2d), discrete defects on the cell surface producing cells with a "prickly" appearance (Fig. 2e), "raspberry" or "cobblestone" forms (Fig. 2f), large smooth cells consistent with intact spheroplasts (Fig. 2g), and forms consistent with collapsed cell membranes (Fig. 2h) were seen.

The similarity between the morphological alterations induced by a cell wall-active antibiotic, i.e., penicillin G, and those caused by an antibiotic known to interfere with intracellular protein synthesis, i.e., kanamycin, suggested that the changes observed might be independent of the specific site of action of these drugs. Other antimicrobial agents which interfere with various levels of protein synthesis were therefore investigated. Chloramphenicol, which acts at the ribosomal level at a stage after the binding of messenger ribonucleic acid and during peptide synthesis to prevent final condensation of amino acids and growth of nascent polypeptide chains

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**Fig. 4.** *Pseudomonas aeruginosa*, untreated control. (b) *P. aeruginosa*, exposed to 0.1 MIC of tobramycin for 3 hr. (c) *P. aeruginosa*, exposed to 1.0 MIC of tobramycin for 3 hr. (d) *Staphylococcus aureus*, untreated control. (e) *S. aureus*, exposed to 1.0 MIC of sulfamethoxazole for 3 hr. (f) *S. aureus*, exposed to 1.0 MIC of sulfamethoxazole for 3 hr. (g) *Escherichia coli*, untreated control. (h) *E. coli*, exposed to 1.0 MIC of sulfamethoxazole for 3 hr.
(2), induced spheroplast formation in S. aureus (Fig. 3a) and in E. coli (Figs. 3b-3d); however, in the phase of elongation of E. coli a subtle difference was noted in that discrete defects along the shaft of the organisms appeared as fusiform swellings (Fig. 3b) rather than sacculous outpouchings (14, 15). Similarly, tobramycin, a new aminoglycoside antibiotic which interferes with intracellular protein synthesis at a stage prior to that of chloramphenicol (16), induced spheroplast formation in both a penicillin-resistant strain of S. aureus (Fig. 3c and 3f) and a strain of Pseudomonas aeruginosa (Fig. 4a-4c).

To evaluate further the possibility that the drug-induced surface alterations observed were not entirely related to the specific site of action of antibiotics utilized in individual studies, strains of S. aureus and E. coli were exposed to increasing concentrations of sulfamethoxazole. A sulfonamide was specifically chosen because it is a chemical, rather than a biological, antimicrobial agent, and because it seemed necessary to study a drug affecting neither cell wall nor intracellular protein synthesis; sulfonamides interfere with the normal utilization of p-aminobenzoic acid (3). Figures 4d-4h illustrate the similarity of changes resulting from sulfamethoxazole action to those described above.

From these studies, it appeared that antimicrobial agents whose site of action is intracellular rather than on the cell surface result in morphological alterations consistent with spheroplast formation and essentially similar to those induced by cell wall-active agents. Although studies are presently in progress to substantiate this observation, a simple morphological comparison with biochemically proven cell wall-defective staphylococci (5) revealed these to be morphologically similar to those illustrated.

In all experiments, a part of each bacterial population remained intact and resembled untreated controls. The number of organisms exhibiting drug-induced morphological alterations increased with the concentration of drug and the duration of exposure; but, at 10 MIC, a spectrum of changes was frequently seen in the same field (Fig. 3d) including morphologically intact organisms, elongated forms, and intact and collapsed spheroplasts. These phenomena were interpreted as reflecting the heterogeneous nature of any bacterial population and substantiate the studies of Greenwood and O'Grady (10), who described a variety of responses of specific microorganisms to the penicillins. Although similar morphological changes were observed in 12-hr cultures, they were less frequent than in cells in the logarithmic phase of growth; this difference was not qualitatively, significant and likewise may reflect the heterogeneity of the bacterial populations studied.

DISCUSSION

Although the penicillins, the cephalosporins, cycloserine, vancomycin, rifampin, and bacitracin are the antimicrobial agents commonly classified as cell wall-active drugs, variants with altered cell walls have been described with streptomycin, erythromycin, chloramphenicol, and tetracycline (8, 19). These latter observations have been substantiated by the morphological changes noted in the present study. Lack of a better appreciation of this phenomenon may be the result of past limitations in examining large numbers of bacterial cells in the same specimen at magnifications sufficiently high to clearly demonstrate alterations in surface morphology; this problem has been overcome in great part by the availability of scanning microscopy techniques.

The present studies demonstrate that antimicrobial agents whose site of action is thought to be intracellular may cause morphological alterations which are similar to those induced by cell wall-active drugs. Whether kanamycin, chloramphenicol, and sulfamethoxazole specifically interfere with cell wall synthesis, directly injure the cell wall, or cause specific abnormalities of intracellular protein synthesis or intermediary metabolism which simply are reflected at the surface of cells is not evident from these studies. The hypothesis that forms consistent with spheroplasts represent a final common pathway of drug-induced injury at many sites within or on bacterial cells is also worthy of consideration. In defense of the latter are studies demonstrating that lysozyme (12, 20) and antibody (4) induce wall-defective forms; preliminary scanning microscopy studies in our laboratory with lysozyme and specific antibody have demonstrated morphological alterations similar to those described above.

At present, the significance of these observations in clinical infection must be considered with caution, but it is hoped that these data will stimulate a reevaluation of present concepts of the nature and role of morphological variants of bacteria exposed to a variety of antibacterial factors.

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LITERATURE CITED