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Morphological Changes of *Escherichia coli* Induced by Bicyclomycin

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Electron microscopic studies with *Escherichia coli* revealed that bicyclomycin inhibits septum formation and converts the cells to filamentous forms. The antibiotic induced high undulation and numerous blebs of the outer membrane. Sometimes cytoplasmic contents leaked into the lumen of the bleb through a disrupted region of the membrane. Breakage of the outer membrane or blebs led to cell lysis. Electron-dense masses of amorphous material and vesicles were found in the cytoplasm.

Bicyclomycin is a cyclic dipeptide antibiotic which has a unique structure and inhibits the growth of some gram-negative bacteria (3, 5-7). The mechanism of action of this antibiotic has been investigated with *Escherichia coli* (10, 11, 13). Bicyclomycin blocks biosynthesis of lipoprotein covalently linked to peptidoglycan (13). The drug binds to the inner membrane proteins, which are distinguished from penicillin-binding proteins (10). The olefinic double bond of bicyclomycin seems to react with the sulfhydryl group of the receptor proteins, although the whole molecule is required for the drug-receptor interaction (11).

We studied morphological alterations in bicyclomycin-treated *E. coli*, and the results are presented in this paper.

MATERIALS AND METHODS

Antibiotic. Bicyclomycin was generously given by Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan.

Growth conditions of the organism. *E. coli* ATCC 27166, a mutant hypersensitive to bicyclomycin, was grown in Lennox broth (10, 13). Exponentially growing cells (absorbance at 660 nm, 0.2 to 0.4) with or without antibiotic treatment were used for electron microscopic studies. The viable cell number was determined by colony formation on Lennox medium solidified with 1.5% agar.

Electron microscopy. The cells were fixed by the standard OsO_4 fixation schedule of Ryter and Kellenberger (9). A culture (10 ml) was poured into 1.1 ml of the fixative, centrifuged at 3,000 × *g* for 10 min, and further fixed in 1% OsO_4 overnight. After being washed with 0.5% uranyl acetate, the cells were enrobed in 2% agar, dehydrated in graded solutions of ethanol and acetone, and embedded in Spurr resin (12).

Ultrathin sections were cut with a DuPont diamond knife on a Porter-Blum MT-2 ultramicrotome, picked up on Formvar-coated copper grids, and stained with uranyl acetate and lead citrate (8). All sections were examined in a JEM 7A electron microscope at 80 kV.

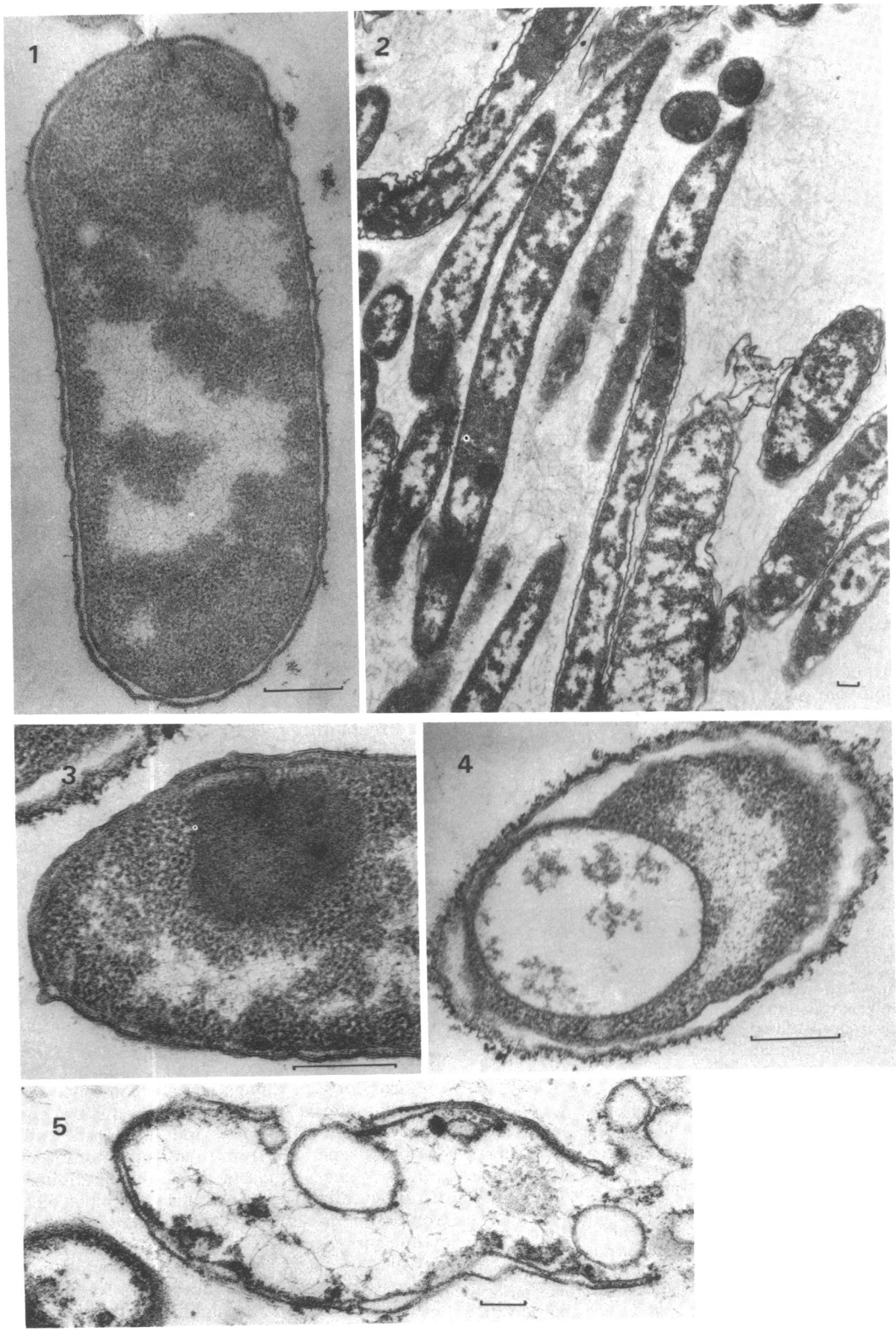
RESULTS

Bactericidal action of bicyclomycin. The viable cell number of *E. coli* ATCC 27166 was not significantly changed within 1 h, but was markedly reduced from ca. 10^6 to ca. 10^3 cells per ml at 3 h after exposure to bicyclomycin at a concentration of 20 μg/ml. The bactericidal effect was observed at drug concentrations higher than the minimal inhibitory concentration (3 μg/ml), as reported previously (7).

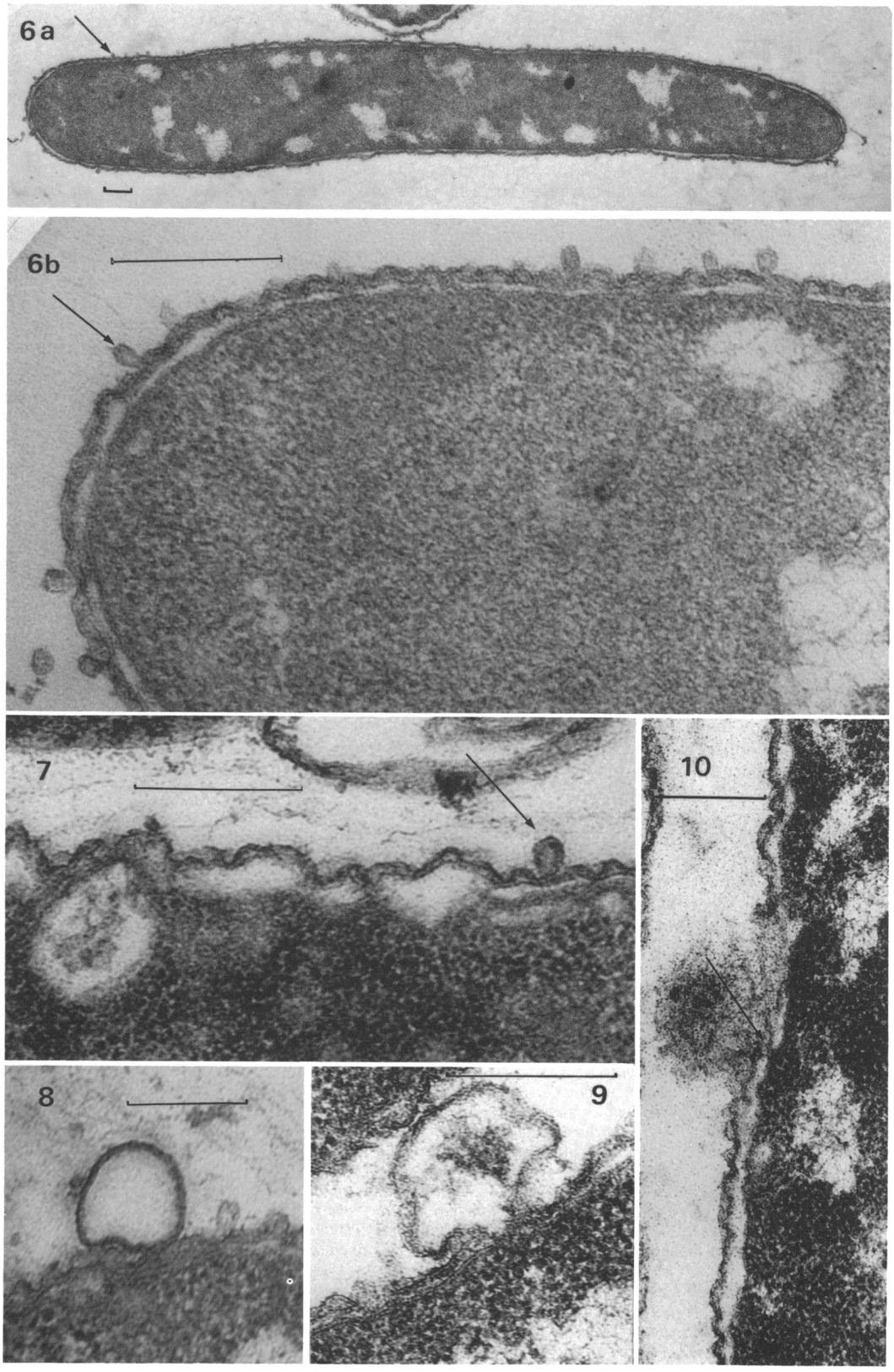
Electron microscopy of the morphological effects of bicyclomycin on *E. coli*. Figure 1 shows a section of a normally growing cell fixed by the technique of Ryter and Kellenberger (9). The untreated cell is surrounded by an undulated cell wall with a smooth surface. The cytoplasm is filled with ribosomes and a less electron-dense area of fibrous nuclear material. This figure shows the typical structure of an exponentially growing cell.

When bicyclomycin was introduced into a logarithmic-phase culture, *E. coli* cells did not show any morphological alterations after the initial 30 min. Prolonged exposure to the antibiotic brought about the failure of septum formation and eventually resulted in filamentous cells. Figure 2 was obtained from a culture treated with the drug for 2 h. Elongated cells contained the nuclear material in a more dispersed state, and some showed irregularly undulated cell walls distinctly separated from the cell membrane by a large space and parts of the cell periphery disrupted. Filamentous cells also contained electron-dense masses of amorphous material in the cytoplasm (Fig. 2 and 3).

After the 3-h treatment, a large vacuole surrounded by a single membrane was frequently observed in cells with a highly undulated outer layer (Fig. 4). In some cells, disruption of the cell wall as well as the cell membrane caused loss of



FIGS. 1–5



FIGS. 6–10

cytoplasmic material, but preserved a number of vesicles (Fig. 5).

Another morphological alteration of *E. coli* due to the action of bicyclomycin was the appearance of numerous blebs on the cell wall, which were detected in the cells treated for 2 h (Fig. 6). Nuclear materials were fragmented and dispersed in cells with many blebs. Blebs seemed to be derived from the outer membrane and not to include the mucopeptide layer (Fig. 6 and 7). They became large (Fig. 8), and sometimes cytoplasmic contents leaked into the lumen of enlarged blebs through a disrupted region of the cell membrane (Fig. 9). Further disruption of the bleb membrane led to the extrusion of cellular contents and eventually to cell lysis. Figure 10 appears to show an early stage of such a lytic process.

DISCUSSION

Bicyclomycin blocks septum formation in *E. coli*, resulting in filamentous cells. Disturbance of the coordination between the biosynthetic processes of cellular structures, especially of the cell wall and cell membrane, due to the presence of the drug induces the formation of numerous blebs on the cell surface as well as a highly undulated outer membrane. Disruption of the bleb membrane leads to cell lysis. In these respects, the morphological effect of bicyclomycin is similar to that of penicillin (1).

Polymyxin and aminoglycoside antibiotics also cause blebs to form on the cell membrane of *E. coli* within 10 min (2, 4). Bleb formation by bicyclomycin takes longer, indicating that bicyclomycin reacts slowly with the receptor. The result is in accord with the previous observation (10) that the binding of the antibiotic to *E. coli* inner membrane proteins requires ca. 30 min.

At a later stage of drug treatment, involution of the cell membrane or degradation of the dense mass of amorphous material or both may yield vesicles bounded by a single membrane.

The biochemical mechanism of action of bicyclomycin seems to be different from that of penicillin. Bicyclomycin inhibits biosynthesis of lipoprotein covalently linked to peptidoglycan (13). Cell wall peptidoglycan synthesis, observed by the in vivo uptake of diaminopimelic acid and the in vitro transpeptidase reaction, is not significantly affected by bicyclomycin (unpublished data). The antibiotic binds to inner membrane proteins of *E. coli*; and bicyclomycin-binding proteins are different from penicillin-binding proteins (10). The olefinic double bond seems to react with the sulfhydryl group of inner membrane proteins (11).

The inhibition by bicyclomycin of lipoprotein synthesis occurs before the loss of viability and morphological changes (13). However, the relationship of the lipoprotein synthesis inhibition to the lethal or morphological effect remains to be determined.

From the results of the current morphological study and previous biochemical studies on the mechanism of action of bicyclomycin, the antibiotic seems to affect the cell membrane of bacteria.

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LITERATURE CITED

1. **Burdett, I. D. J., and R. G. E. Murray.** 1974. Septum formation in *Escherichia coli*: characterization of septal structure and the effects of antibiotics on cell division. J. Bacteriol. **119**:303–324.
2. **Iida, K., and M. Koike.** 1974. Cell wall alterations of gram-negative bacteria by aminoglycoside antibiotics. Antimicrob. Agents Chemother. **5**:95–97.

FIG. 1. *Microphotograph of an ultrathin section of E. coli ATCC 27166 without treatment of bicyclomycin. Bars represent 0.2 μm in all figures.*

FIG. 2. *Cells treated with bicyclomycin (20 μg/ml) for 2 h. Multinucleate and aseptate filaments formed. Undulated cell walls are separated from the cell membrane in most cells, and dense masses can be seen in many cells. All of the remaining figures show cells exposed to 20 μg of antibiotic per ml.*

FIG. 3. *Electron-dense mass of amorphous material in the cytoplasm of a cell treated with the drug for 2 h.*

FIG. 4. *A cell, exposed to the antibiotic for 3 h, shows a large vacuole surrounded by a single membrane.*

FIG. 5. *Lysed cell after treatment with bicyclomycin for 3 h. Several vesicles can be seen.*

FIG. 6. *(a) Numerous blebs can be seen on the surface of a cell which was exposed to the drug for 3 h. (b) Part of the cell from (a), magnified. The arrow indicates one of the blebs, which is clearly connected to the outer membrane.*

FIG. 7. *Part of a cell treated with the antibiotic for 3 h. A loose structure can be seen in the outer layer of cell membrane. Membrane involution is seen underneath the cell wall.*

FIG. 8. *Large bleb on the surface of a cell exposed to bicyclomycin for 3 h.*

FIG. 9. *Projection of cytoplasmic contents into the lumen of a bleb limited by an outer membrane.*

FIG. 10. *Burst of cytoplasmic contents through a crack in the cell envelope.*

3. **Kamiya, T., S. Maeno, M. Hashimoto, and Y. Mine.** 1972. Bicyclomycin, a new antibiotic. II. Structural elucidation and acyl derivatives. J. Antibiot. **25**:576–581.
4. **Koike, M., K. Iida, and T. Matsuo.** 1969. Electron microscopic studies on mode of action of polymyxin. J. Bacteriol. **97**:448–452.
5. **Maag, H., J. F. Blout, D. I. Coffen, T. V. Steppe, and F. Wong.** 1978. Structure, absolute configuration, and total synthesis of an acid-catalyzed rearrangement product of bicyclomycin. J. Am. Chem. Soc. **100**:6786–6788.
6. **Miyoshi, T., N. Miyairi, H. Aoki, M. Kohsaka, H. Sakai, and H. Imanaka.** 1972. Bicyclomycin, a new antibiotic. I. Taxonomy, isolation and characterization. J. Antibiot. **25**:569–575.
7. **Nishida, M., Y. Mine, T. Matsubara, S. Goto, and S. Kuwabara.** 1972. Bicyclomycin, a new antibiotic. III. *In vitro* and *in vivo* antimicrobial activity. J. Antibiot. **25**:582–593.
8. **Reynolds, E. S.** 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**:208–212.
9. **Ryter, A., and E. Kellenberger.** 1958. Etude au microscope electronique de plasmas contenant de l'acide deoxyribonucleique. I. Les nucleoides des bacteries en croissance active. Z. Naturforsch. Teil B **13**:597–605.
10. **Someya, A., M. Iseki, and N. Tanaka.** 1978. Binding of bicyclomycin to inner membrane proteins of *E. coli*. J. Antibiot. **31**:712–718.
11. **Someya, A., M. Iseki, and N. Tanaka.** 1979. Active groups of bicyclomycin and the reaction with thiols. J. Antibiot. **32**:402–407.
12. **Spurr, A. R.** 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26**:31–43.
13. **Tanaka, N., M. Iseki, T. Miyoshi, H. Aoki, and H. Imanaka.** 1976. Mechanism of action of bicyclomycin. J. Antibiot. **29**:155–168.