ACTION MECHANISM OF 3′, 4′-DIDEOXYKANAMYCIN B AND GENTAMICIN AS OBSERVED BY ELECTRON MICROSCOPY

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The antibacterial activity of 3′, 4′-dideoxykanamycin B and gentamicin against *Pseudomonas aeruginosa* was examined by electron microscopic ovservation of ultrathin sections. It was found that these antibiotics effected breakage of the cell wall in various places and caused a defect of one end of the bacterial cells. The presence of electron-dense granules was observed in the cytoplasm which had become diluted by the escape of its contents and the entrancing water.

The mechanism of the resistance of kanamycin-resistant bacteria was shown by UMEZAWA and others[1~3], and a new derivative of kanamycin B, 3′, 4′-dideoxykanamycin B, was prepared on the basis of their studies. This new compound has approximately the same antibacterial spectrum as gentamicin, an amino-glycoside antibiotic, and is especially effective against *Pseudomonas aeruginosa*, such infections are cured with difficulty. In order to examine the action mechanism of these antibiotics, observations were made of morphological changes in these bacteria, using an electron microscope[4].

Materials and Methods

The bacterial strain used was *Pseudomonas aeruginosa* No. 12, kindly supplied by Prof. HOMMA of the Institute of Medical Science, University of Tokyo. This bacterium was precultured in a Tryptosoya broth (Nissan) for 18 hours and then used to inoculate heart infusion broth (Nissan) to make a 5% concentration. This broth was shake-cultured at 37° and, in its early logarithmic growth phase, 3′, 4-dideoxykanamycin B (Meiji Seika Kaisha, Ltd.) or gentamicin (Shionogi and Company. Ltd.) was added to make a final concentration of 4 or 0.4 μg/ml, respectively. Bacterial cells were then collected periodically as samples for electron microscopy. The collected cells were fixed by the KELLENBERGER-RYTER method[5], dehydrated in ethanol series, and embedded in an epoxy resin[6]. Ultra-thin sections were cut on an LKB ultramicrotome, and double-stained with uranyl acetate and lead citrate[7]. A high-resolution electron microscope, Akashi Model S-500, was used.

Results

Fig. 1 shows an ultrathin section of normal *Pseudomonas aeruginosa* No. 12. The cell wall, cytoplasmic membrane, ribosomal granules, and nucleus are observed. There are protrusions on the cell wall.

One hour after application of 3′, 4′-dideoxykanamycin B, the cell wall is severed at several places, though there is almost no change in the cytoplasm (Fig. 2). Three hours later, breakage of the cell wall, a partial defect of the cells, and dilution of the cytoplasmic contents are observed (Figs. 3, 4, and 5). In some cases, although the outer layer of the cell wall had been severed, the granular layer between the cell wall and cytoplasmic membrane remained intact (Fig. 4). The phenomenon of the appearance of a grain-like defect on one end of the cell (Fig. 5) was often observed by phase-contrast microscopy.

Six hours after application of the chemical, the presence of electron-dense granules was observed in the cytoplasm which had become diluted due to the escape of its contents through the severed

Fig. 1 An ultrathin section of normal *Pseudomonas aeruginosa* No. 12. Some protrusions on the cell wall are observed.
CW : (cell wall), CM : (cytoplasmic membrane), N : (nucleus), R : (ribosome)

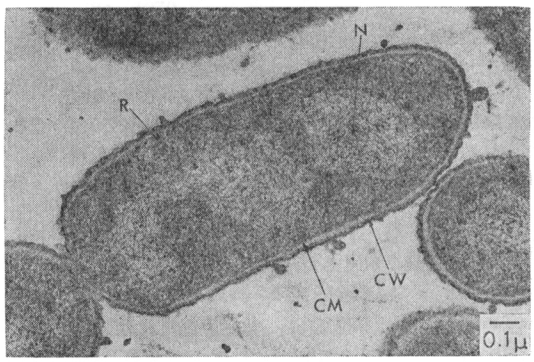


Fig. 2 One hour after application of 3′,4′-dideoxykanamycin B (4 μg/ml). There is almost no change in cytoplasm but the cell wall is severed in several places.

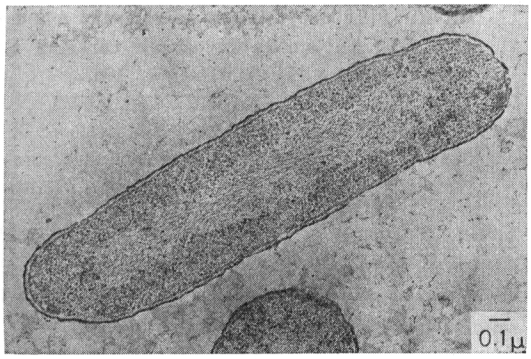


Fig. 3 Three hours after application of 3′,4′-dideoxykanamycin B. The cell wall is severed in several places but there is almost no change in the cytoplasm.

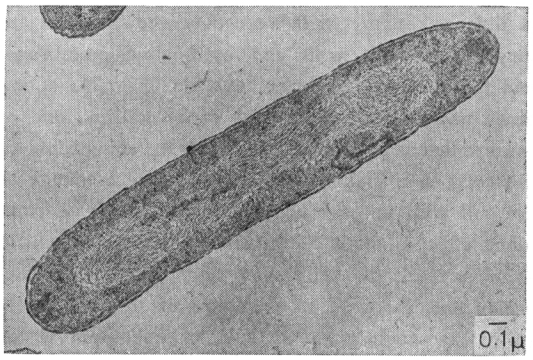


Fig. 4 Higher magnification of a part of Fig. 3. The outer layer of the cell wall is broken but the granular layer present between the cell wall and cytoplasmic membrane is still intact.

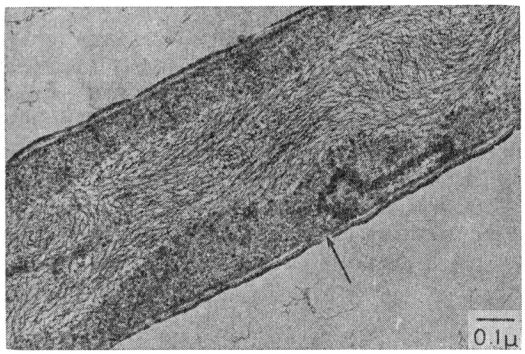


Fig. 5 Three hours after application of 3′,4′-dideoxykanamycin B. There is a defect in part of the bacterial cell, the cell wall is broken in several places, and the cytoplasm has become diluted. A grain-like defect in one end of the bacterial cell was often observed by phase-contrast microscope.

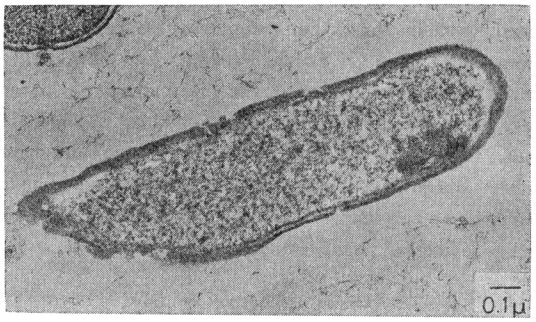


Fig. 6 Six hours after application of 3′,4′-dideoxykanamycin B. Both cell wall and cytoplasmic membrane are broken and the cell contents have flowed out. The cytoplasm has become diluted, and substances considered to correspond to granules observed by phase-contrast microscopy are present.

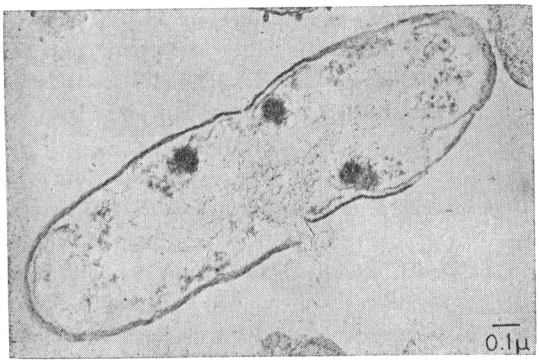
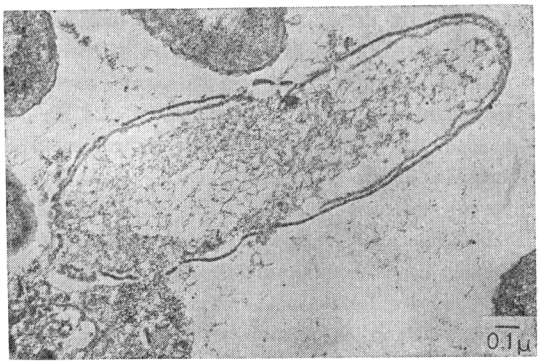


Fig. 7 Six hours after application of gentamicin (0.4 μg/ml). Phenomena approximately similar to those seen after the application of dideoxykanamycin B are observed.



cytoplasmic membrane (Fig. 6). In phase-contrast microscope observations, electron-dense granules appeared when the density of the cells became low.

In the case of gentamicin, effects similar to those of dideoxykanamycin B were observed, such as injury to the cell wall and cytoplamic membrane, and the escape of cellular contents (Fig. 7). Combined use of gentamicin and carbenicillin, one of the synthetic penicillins, resulted in elongation of the cells to a filamentous shape, with breakage of the cell wall in several places, indicating a combined effect of both chemicals. When carbenicillin alone is used, the cells show only filamentous elongation and there is no breakage of the cell wall. as in the application of dideoxykanamycin B or gentamicin.

Discussion

It has been believed that the action mechanism of aminoglycoside antibiotics was the inhibition of protein synthesis by binding with the 30 S subunit of ribosomes but the present series of electron microscopic observations seems to indicate that dideoxykanamycin B and gentamicin damage the surface layer of the bacterial cell, such as the cell wall, rather than the cytoplasm of *Pseudomonas aeruginosa* No. 12. Further studies are now being made on the action of these antibiotics on other strains of *Pseudomnas aeruginosa* and on *Escherichia coli*. The action of streptomycin, kanamycin, and other amino-glycoside antibiotics on *Pseudomonas aeruginosa* and *Escherichia coli*, and the problem of drug-resistant *Pseudomonas aeruginosa* are being investigated.

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