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Cell Wall Alterations of Gram-Negative Bacteria by Aminoglycoside Antibiotics

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After treatment with aminoglycoside antibiotics, *Escherichia coli* B and *Pseudomonas aeruginosa* P28 showed numerous blebs on the cell wall surface.

Aminoglycoside antibiotics are known to inhibit protein synthesis at the level of the 30*S* ribosomal subunit (6). In addition to the effect on protein synthesis, streptomycin damages the membrane of *Escherichia coli*, causing a rapid efflux of potassium (3) and the excretion of nucleotides (1). Morphological studies should contribute to an understanding of the relationship between these two observations. For these studies, *E. coli* B and *Pseudomonas aeruginosa* P28 (furnished by Y. Homma, Institute of Medical Science, Tokyo University, Japan) were used. Aminoglycoside antibiotics, dideoxykanamycin B (10 μg/ml) and streptomycin (10 μg/ml) (Meiji Seika Kaisha Ltd., Tokyo), gentamicin (10 μg/ml) (Shinonogi Pharmaceutical Co., Ltd., Osaka), spectinomycin (10 μg/ml) (Japan Upjohn Ltd., Tokyo), or kasugamycin (10 μg/ml) (Banyu Pharmaceutical Co., Ltd., Tokyo) were added to logarithmic-phase cultures in nutrient broth at 37 C, and at various intervals after the addition, each culture was examined for viable cell count and prepared for electron microscopy. For electron microscopy, the cells were fixed with Kellenberger and Ryter's OsO_4 solution (4), dehydrated through the graded alcohol solutions, and embedded in Epon 812 (5). The specimens were cut with a Reichert OmU2 ultramicrotome and examined with a JEM 100B electron microscope.

The culture viability was reduced by about 10^{-3} within 10 min by contact with dideoxykanamycin B, gentamicin, or streptomycin, but was not affected by spectinomycin and kasugamycin (Fig. 1).

In the electron microscopic studies, the untreated cell was surrounded by the undulated cell wall with a smooth surface (Fig. 2), whereas on cells treated with dideoxykanamycin B for 30 min, numerous blebs derived from the outer layer of the cell walls were observed (Fig. 3, 4, and 5). These blebs appeared within 10 min after addition of the drugs, which coincides with the period for reduction of cell viability. Similar alterations were observed on cells treated with other aminoglycoside antibiotics (Table 1), but were not observed with chloramphenicol, tetracycline, and erythromycin. These observations indicate that the wall alterations are specific effects of aminoglycoside antibiotics, but are unrelated to the presence of streptamine in the chemical structure.

Furthermore, it has been reported that both the bactericidal action of streptomycin and kanamycin and the effect on nucleotide excretion were antagonized by chloramphenicol, tetracycline, and erythromycin (1, 2, 7). An *E. coli* B culture, to which chloramphenicol, tetracycline, or erythromycin (at concentration of 20 μg/ml) had been added 2 min earlier, was treated with dideoxykanamycin B, streptomycin, spectinomycin, or kasugamycin at

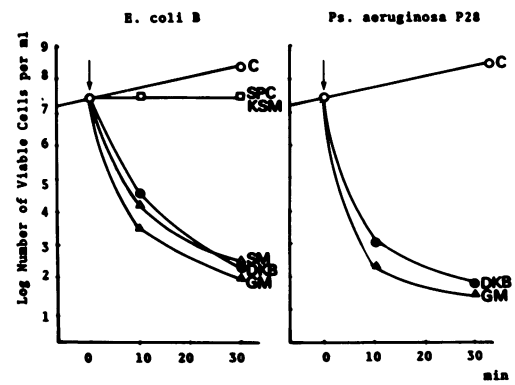
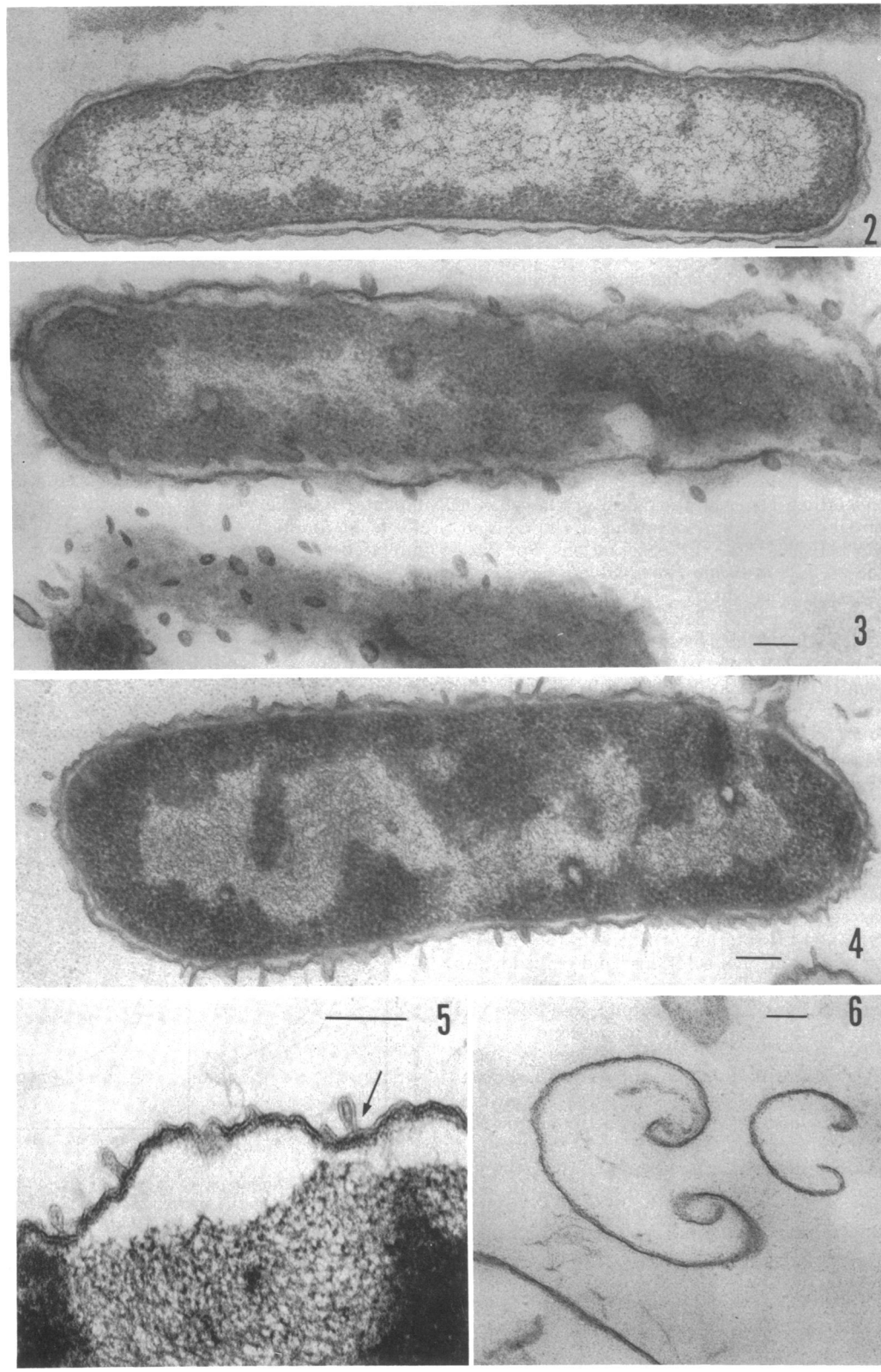


FIG. 1. *Effect of aminoglycoside antibiotics on the growth of E. coli B and P. aeruginosa P28. Symbols:* ○, *control (without drugs);* ●, *10 μg of dideoxykanamycin B (DKB) per ml;* ▲, *10 μg of gentamicin (GM) per ml;* △, *10 μg* of streptomycin (SM) per ml; □, *10 μg of spectinomycin (SPC) or kasugamycin (KSM) per ml.*



FIGS. 2–6

TABLE 1. *Effect of chloramphenicol, tetracycline, and erythromycin pretreatment on killing and cell wall alteration of E. coli B by dideoxykanamycin B, streptomycin, spectinomycin, and kasugamycin*[a]

Determination	Survivors (%)													
	No treatment	DKB	SM	SPC	KSM	CP	TC	EM	CP + DKB	TC + DKB	EM + DKB	CP + SM	CP + SPC	CP + KSM
Exposure time (min)														
0	100	100	100	100	100	100	100	100						
30	200	0.02	0.06	100	100	100	100	100	100	100	60	100	100	100
Cell wall alteration	−	+	+	+	+	−	−	−	−	−	−	−	−	−

[a] DKB, dideoxykanamycin B; SM, streptomycin; SPC, spectinomycin; KSM, kasugamycin; CP, chloramphenicol; TC, tetracycline; EM, erythromycin. −; No bleb-formation on the cell wall; +; bleb-formation. Exponentially growing cultures of *E. coli* B were divided into four portions. The first portion was kept in normal exponential growth. The second was treated with an aminoglycoside antibiotic (10 μg of DKB, SM, SPC, or KSM per ml) for 30 min. The third was treated with a non-aminoglycoside antibiotic (20 μg of CP, TC, or EM per ml) for 30 min. The fourth, to which CP, TC, or EM had been added 2 min earlier, was treated with DKB, SM, SPC, or KSM for 30 min. Samples were taken from each reaction mixture for viable counts and electron microscopy study.

37 C for 30 min. Chloramphenicol, tetracycline, and erythromycin blocked completely not only the bactericidal action, but also the cell wall alteration caused by dideoxykanamycin B and streptomycin (Table 1). With bacteriostatic agents such as spectinomycin and kasugamycin, the cell wall alteration was also blocked by chloramphenicol.

However, no effect was observed on treatment of an isolated cell wall fraction from *E. coli* B and *P. aeruginosa* P28 (Ribi cell fractionator) with dideoxykanamycin B (Fig. 6).

From these results, it is suggested that the action of the aminoglycoside antibiotics on the cell wall is an indirect effect due to the dependence on ribosomal function for cell envelope biosynthesis.

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FIG. 2. *Thin section of a normally growing cell of E. coli B. ×60,000. Markers represent 100 nm.*

FIG. 3. *Thin section of E. coli B cell treated with 10 μg of dideoxykanamycin B per ml for 30 min. Numerous blebs on the cell wall are seen. A tangential section of the cell wall with many blebs is seen at the bottom. ×63,000.*

FIG. 4. *Thin section of P. aeruginosa P28 cell treated with 10 μg of dideoxykanamycin B per ml for 30 min. Many blebs from the cell wall can been observed. ×63,000.*

FIG. 5. *A higher magnification of P. aeruginosa P28 cell treated with 10 μg of dideoxykanamycin B per ml for 2 h. A longitudinal section of a bleb (arrow) shows that the blebs derive from the outer layer of the cell wall. ×120,000.*

FIG. 6. *Thin section of an isolated cell wall fraction treated with dideoxykanamycin B. ×45,000.*