CELL WALL SYNTHESIS BY *STAPHYLOCOCCUS AUREUS* IN THE PRESENCE OF PROTEIN SYNTHESIS INHIBITORY AGENTS

II. ELECTRONMICROSCOPIC STUDY

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Our previous reports dealt with electron-microscopic studies of ultra-thin sections of *Staphylococcus aureus* FDA 209P JC which had been exposed to spiramycin and clindamycin (7-chloro-lincomycin). These revealed formation of cross walls inside the bacterial cells, and noticeable thickening and multilayering of cell walls. Subsequently, we have extended our study to other protein-synthesis-inhibiting antibiotics including macrolides, lincomycins, tetracyclines, aminoglycosides and chloramphenicol to obtain the following findings (Plates 1~7).

1. With its MIC, each of these antibiotics caused thickening of cell walls. The thicking was especially remarkable with macrolides, lincomycins and chloramphenicol.

2. Tetracyclines caused conspicuously abnormal cell division in addition to the thickening of the cell walls.

3. Clinically isolated staphylococcal strains highly resistant to these antibiotics remained unchanged after exposure showing no thickening of their cell walls as was observed in sensitive strains following similar exposure.

4. A study in which clinically isolated staphylococcal strains resistant to these antibiotics were used, revealed that in each group of antibiotics there are close correlations between cross resistance and cell wall thickening, thereby indicating the availability of a visual means for confirming cross resistance.

Organisms used in this study were preincubated in Trypto-soya Broth (Nissan) for 18 hours, then Heart Infusion Agar (Nissan) was added and mixed to make plates containing 10^6 cells/ml. Cylinders were placed on the plates, then erythromycin (EM),

Plate 1. *S. aureus* FDA 209P JC following exposure to erythromycin. Extremely thickened cell walls were observed.

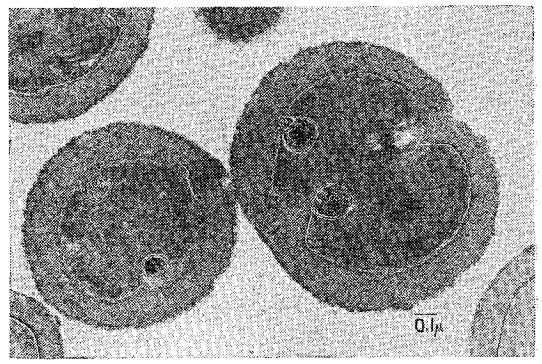


Plate 2. A clinically isolated *S. aureus* strain resistant to erythromycin, following exposure to erythromycin. Cell walls were not so thickened as seen in sensitive strains.

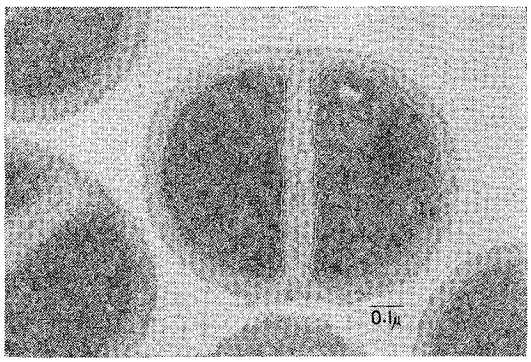


Plate 3. Clinically isolated *S. aureus* strain resistant to erythromycin (the same organism shown in Plate 2), following exposure to spiramycin. Cell walls were as thickened as seen in Plate 1.

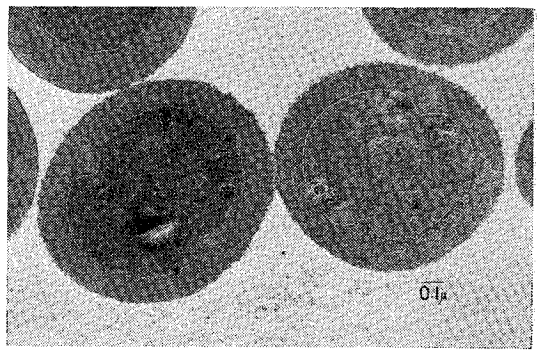


Plate 4. *S. aureus* FDA 209P JC following exposure to tetracycline. Cell walls were thickened and abnormal cell division was observed.

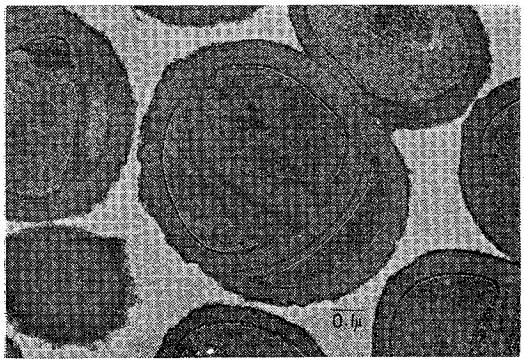


Plate 5. *S. aureus* FDA 209P JC following exposure to minocycline. Same phenomena as with Plate 4 were observed.

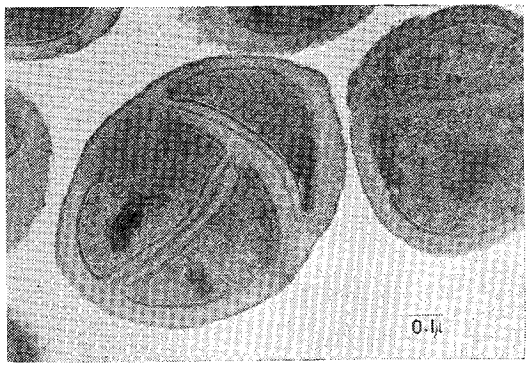


Plate 6. A clinically isolated *S. aureus* strain, resistant to tetracycline, following exposure to tetracycline. All walls were not so thickened and abnormal cell division was not so prevalent as seen in the sensitive strains.

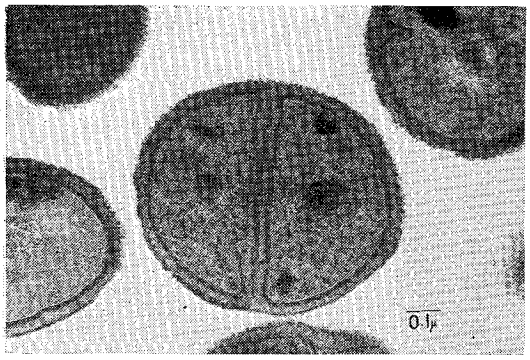
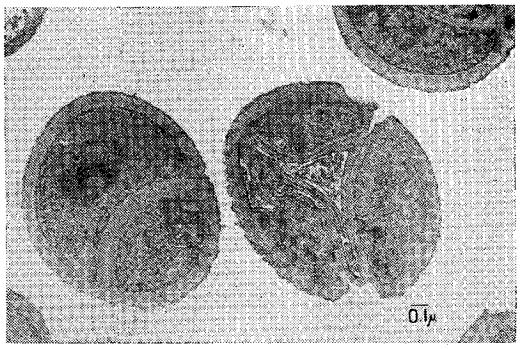


Plate 7. A clinically isolated *S. aureus* resistant to tetracycline (the same organism shown in Plate 6), following exposure to minocycline. Thickened peripheral cell walls and abnormal cell divisions were observed.



spiramycin (SPM), tetracycline (TC), and minocycline (MINO) (each in a concentration of 10 mcg/ml) were filled into each cylinder. The plates were cultivated for 24 hours at 37°C to make the inhibitory circle, and the boundary of the circle was cut out to obtain agar blocks. With the resistant strains the inhibitory circle was not produced. The agar just outside the cylinder was cut out. The blocks were fixed by KELLENBERGER's method and embedded by LUFT's method. Ultra-thin sections were prepared with LKB ULTROTOME and double-strained with uranyl acetate and lead citrate. Specimens were then examined with the electron-microscope Akashi S-500.