NOTES

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

ELECTRONMICROSCOPIC STUDY

MASAFUMI NAKAO, EIRYO KITANAKA, KAZUYORI OCHIAI and SHOZO NAKAZAWA

Department of Microbiology, Kyoto College of Pharmacy, Kyoto, Japan

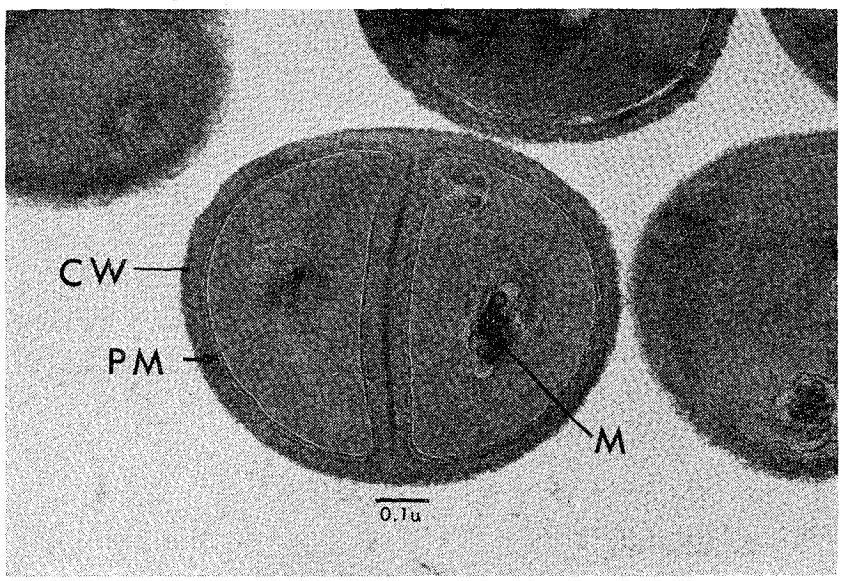
(Received for publication October 2, 1971)

In our laboratory, for several years, the relation between the mechanism of action of antibiotics and the morphological changes of bacterial cells exposed to antibiotics have been studied with electronmicroscopy and phase-contrast microscopy. This paper concerns our electronmicroscopic study on how the biochemical phenomenon previously reported by R. HANCOCK *et al.*[1] and J. J. JOSTEN *et al.*[2] relates to the morphological changes in organisms after their exposure to protein synthesis-inhibitory agents such as spiramycin (SPM) and clindamycin (CLDM). The electronmicroscopic samples were prepared by modified KELLENBERGER's method[3] and by LUFT's method.[4]

Staphylococcus aureus FDA 209 P JC was preincubated in Tryptosoya Broth (TSB) (Nissan) for 18 hours, and then admixed with TSB to make plates containing 10^7 cells/ml. Cylinders were placed on the plates, then SPM or CLDM (each in a concentration of 10 μg/ml) was poured into the 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 blocks. The blocks were fixed with 1% osmium tetraoxide dissolved in KELLENBERGER buffer solution, dehydrated with alcohol series, and embedded in epoxy resin.

Ultrathin sections were prepared with Lkb Ultraotome 4801 A and double-stained with uranyl acetate and lead citrate. Specimens were then examined with Akashi S 500. The electronmicrographs revealed that, as compared with the cell walls of intact organisms, those of the organisms exposed to antibiotics markedly thickened, often with the formation of multilayers (Figs. 1~6). Moreover, the electrodensity of the cytoplasm was higher in drug-treated cells than in intact cells. These phenomena, most marked in cells exposed to CLDM, were observed also in cells

Fig. 1. The electron-micrograph of intact *S. aureus*.



exposed to spiramycin and two other protein synthesis-inhibitory agents (lincomycin and chloramphenicol).

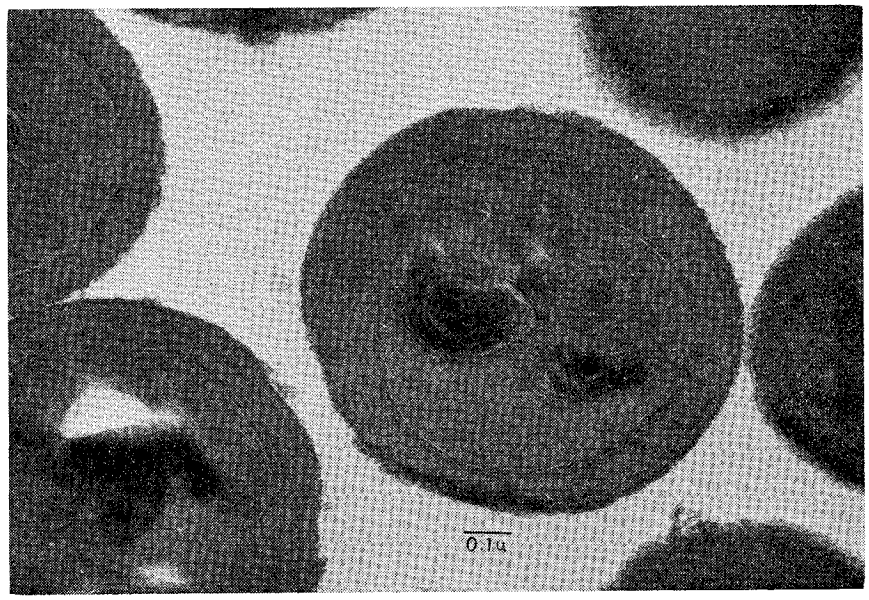
In their study using *Streptococcus faecalis*, SHOCKMAN *et al.*[5,6] have observed the thickening of bacterial cell walls in the presence of chloramphenicol and amino acid starvation. But they did not notice such a degree of thickness and formation of multilayers of cell walls as we indicated in this paper. The significance and the mechanism of such thickening and formation of multilayers of bacterial cell walls are now under study, morphologically and biochemically, at our laboratory.

Acknowledgements

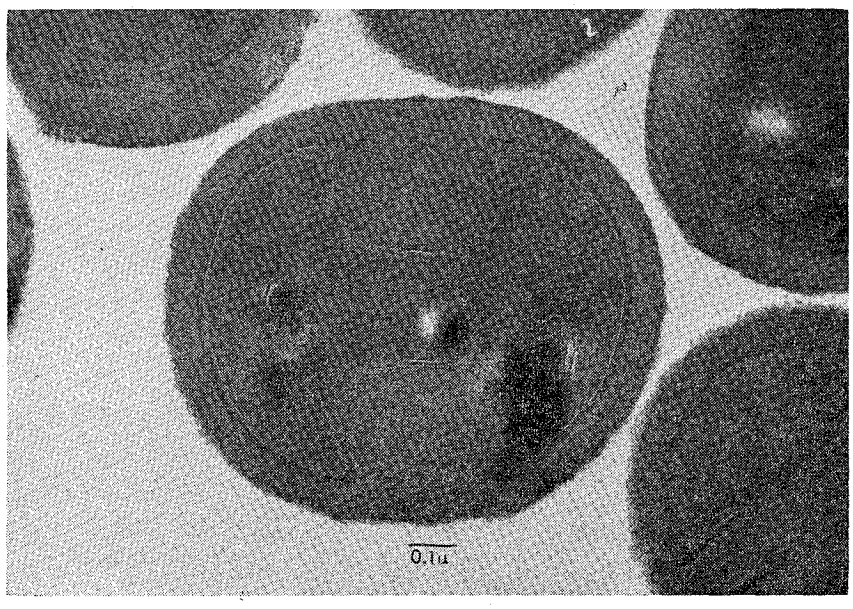
The authors appreciate the advice of Dr. ATSUSHI

Fig. 2. The electron-micrographs of *S. aureus* exposed to SPM.
Thickened cell walls were observed.

(a)



(b)



(c)

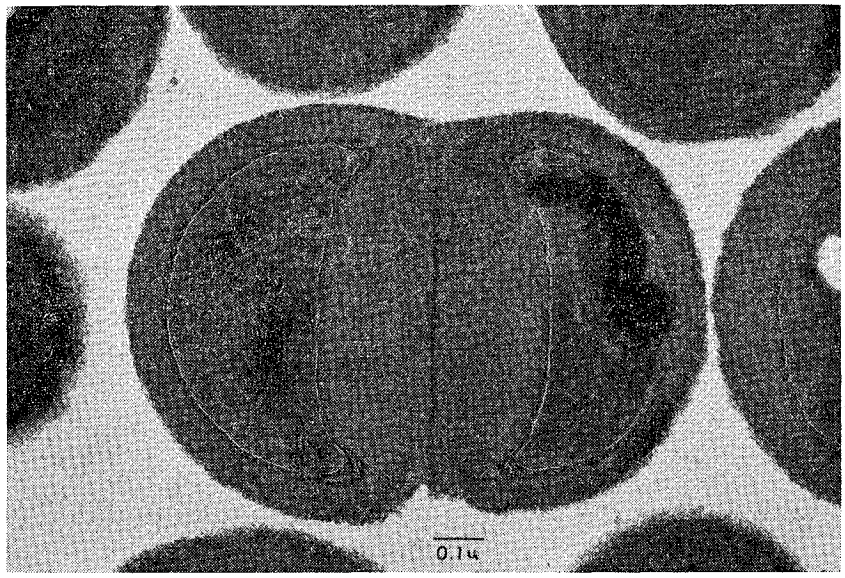
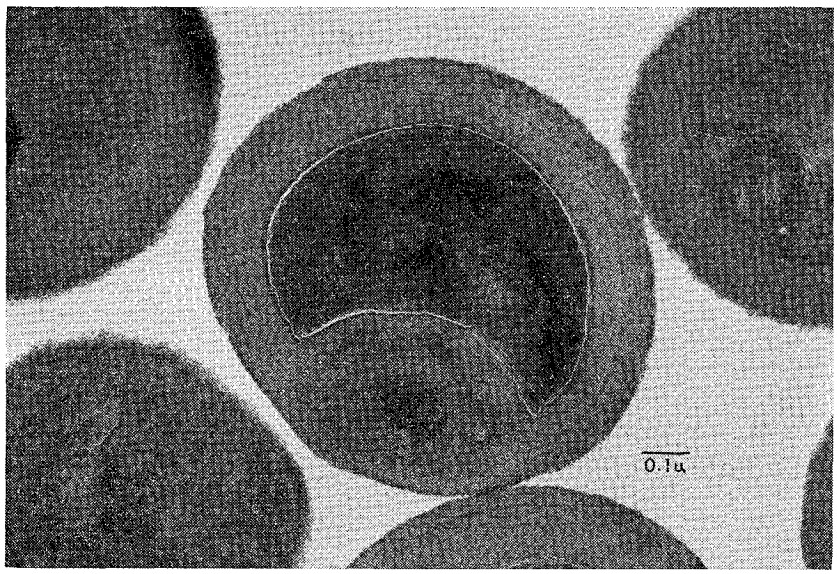


Fig. 3. The electron-micrographs of *S. aureus* exposed to CLDM. Thickened cell walls in multiple layers were observed.

(a)

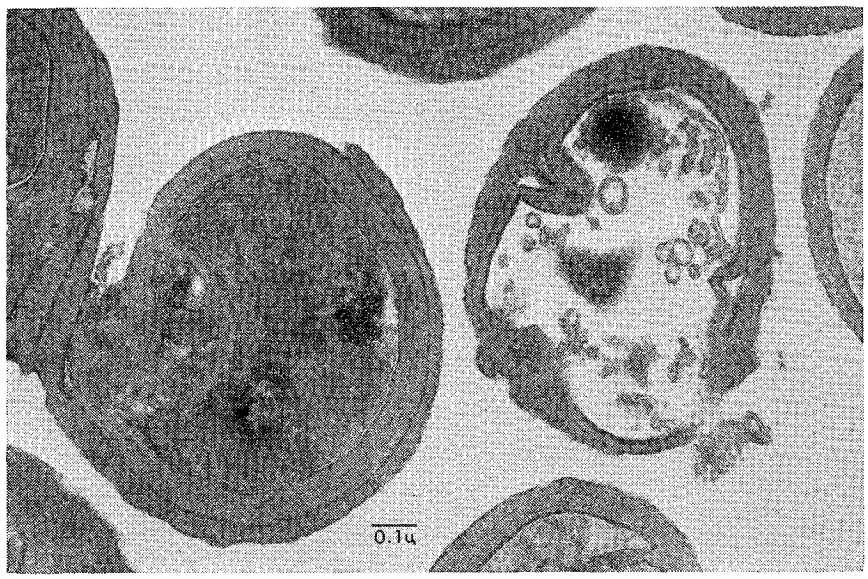


SUGANUMA and Dr. TERUO TANINO of this institute for the electronmicroscopic studies.

References

1) HANCOCK, R. & J. T. PARK: Cell-wall synthesis by *Staphylococcus aureus* in the presence of chloramphenicol. Nature 181 : 1050~1052, 1958
2) JOSTEN, J. J. & P. M. ALLEN: The mode of action of lincomycin. Biochem. Biophys. Res. Comm. 14 : 241~244, 1964
3) KELLENBERGER, E.; A. RYTER & J. SECHAUD: Electron microscope study of DNA-containing plasms. II. Vegetative and mature phage DNA as compared with normal bacterial nucleotide in different physiological states. J. Biophys. Biochem. Cytol. 4 : 671~678. 1958
4) LUFT, J. H.: Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9 : 409~414, 1961
5) HIGGINS, M. L. & G. D. SHOCKMAN: Early

(b)



changes in the ultrastructure of *Streptococcus faecalis* after amino acid starvation. J. Bact. 103 : 244~254, 1970

6) HIGGINS, M. L.; H. M. POOLEY & G. D. SHOCKMAN: Reinitiation of cell wall growth after threonine starvation of *Streptococcus faecalis*. J. Bact. 105 : 1175~1183, 1971