

## FURTHER STUDIES ON THE PLASMA MEMBRANE OF *STAPHYLOCOCCUS AUREUS*

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Robertson has proposed the concept that all biological membranes have the same "unit structure" (Robertson, 1957 and 1959; Moody and Robertson, 1960). In an early study (Suganuma, 1961), the plasma membrane of *Staphylococcus aureus* was seen as only a single line with an over-all thickness of about 50 Å. However, in a later paper (Suganuma, 1964), it was reported that the plasma membrane of the *S. aureus* cell treated with 1 N HCl solution appeared as a unit membrane. It was thought that treatment with HCl might leach out cytoplasmic material which can mask the inner dense stratum of the unit membrane (Suganuma, 1964). In a subsequent effort (Suganuma, 1965), a section of a plasmolyzed *Staphylococcus* cell subjected to mechanical disruption demonstrated the triple-layered structure of both the plasma membrane and the cell wall. These observations support the concept that the plasma membrane of *Staphylococcus* possesses the unit membrane structure. However, the evidence published so far is based on cells subjected to drastic treatment, and demonstration of unit membrane structure in the *Staphylococcus* after the usual methods of handling has been lacking.

This paper reports the successful demonstration of unit membrane structure in *Staphylococcus aureus* subjected to the usual methods of fixation, embedding, and sectioning, except for a prolongation of the fixation procedure. The results permit additional confidence to be placed in the general validity of the unit membrane hypothesis, and suggest that, after brief fixation, followed by the usual methods of embedding, sectioning, and staining, the components of the unit membrane may be confused and obscured; the outer component by constituents of the cell wall; and the inner dense lamina by cytoplasmic material adjacent to it (Suganuma, 1965).

### MATERIALS AND METHODS

A strain (FDA 209-P) of *Staphylococcus aureus* was cultivated on agar for 5 hr. Colonies to be sectioned were fixed for 18 hr with cold *s*-collidine-buffered osmium tetroxide solution (Bennett and Luft, 1959). After dehydration, the specimens were embedded

in Epon epoxy resin (Luft, 1961). All sections were stained with uranyl acetate according to the method of Watson (1958) and were studied with an Hitachi 11-A electron microscope.

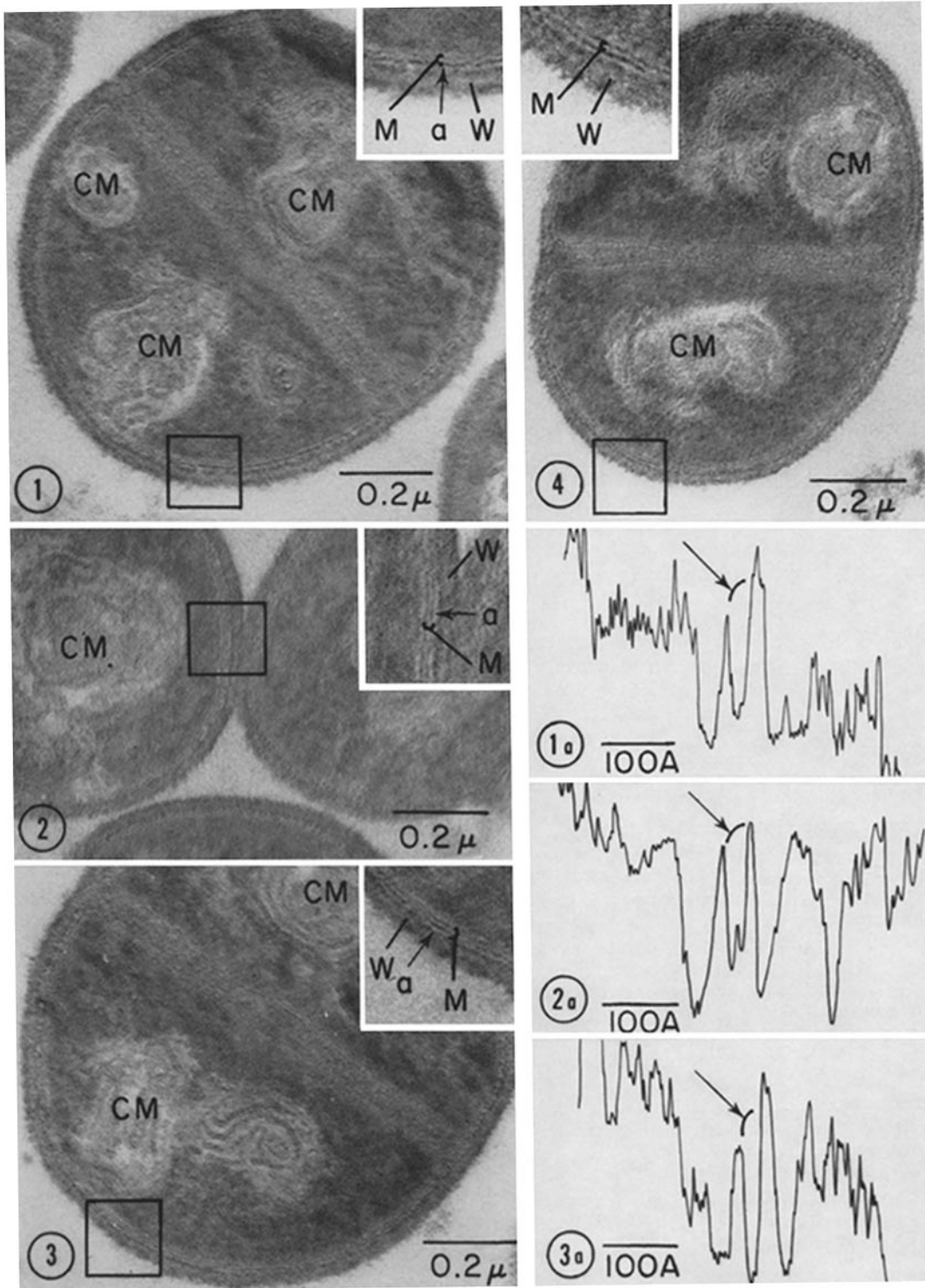
### RESULTS AND DISCUSSION

Figs. 1 through 4 show sections of cells treated as mentioned above. Surrounding each cell is a concentric series of alternating light and dark layers. The fine structure of the membranes is relatively well preserved, although other structures of the cytoplasm and the nuclear sites may be slightly damaged. One can see membranous structures (*CM*) in the cytoplasm (Suganuma, 1963). The cell wall (*W*) consists of two dark layers and an intervening light layer. Inside the cell wall the cytoplasm can be seen, part of which is slightly detached from the cell wall and is surrounded by plasma membrane. This plasma membrane (*M*) clearly exhibits unit membrane structure. These interpretations about the cell wall (*W*) and the plasma membrane (*M*) seem reasonable because the figures are similar to those of the plasmolyzed cell demonstrated in a previous paper (Suganuma, 1965).

Figs. 1 *a* through 3 *a* show densitometric tracings of the areas indicated by arrows (*a*) in Figs. 1 through 3 (insets). The tracing across the plasma membrane shows two density peaks separated by approximately 50 Å.

Sager and Palade (1957) have studied the structure and development of the chloroplast in *Chlamydomonas*. They have shown that after a long fixation in osmium tetroxide at room temperature the membranous structures of the cell appeared generally in a state of satisfactory preservation, but other structural elements (nucleus, pyrenoid) showed evidence of extensive extraction.

Specimens such as those shown in this paper, fixed in osmium tetroxide for a longer time than usual, also show evidences of considerable extraction of constituents of cytoplasm. However, the fine structure of the membranes is well preserved. Moreover, a separation of the cell membrane from the cell wall has been brought about. As a result



FIGURES 1 through 4 Sections of *Staphylococcus aureus* cells cultivated on agar for 5 hr and fixed in osmium tetroxide for 18 hr. Figs. 1, 2, and 3,  $\times 80,000$ ; Fig. 4,  $\times 70,000$ . The regions within the squares are enlarged (insets). The outermost two dark layers and the intervening light layer are attributed to the cell wall (*W*). The plasma membrane (*M*) and the cytoplasmic membranous structure (*CM*) show the unit membrane structure. Insets 1, 2, and 3,  $\times 130,000$ ; Inset 4,  $\times 180,000$ .

FIGURES 1a through 3a Densitometric tracings of the plasma membrane. The lines traversed in taking the tracings are indicated by arrows (*a*) in the insets of Figs. 1 through 3. The peak-to-peak distance between the two dense lines of a unit membrane ( $\downarrow$ ) is approximately 50 Å.

of this separation, the plasma membrane is seen clearly to have a unit structure.

Recently, there have been reports by many authors that the plasma membrane of bacteria has a unit structure. Robinow (1962) has stated that the best views of the membranes were obtained when the protoplast was pulled away from the wall by some means or other. For example, in plasmolyzed cells of *B. subtilis* (Fitz-James, 1960; van Iterson, 1961) or in *E. coli* of slight degeneration (Ogura, 1963), a unit structure of the plasma membrane has been observed. A long fixation in osmium tetroxide mentioned above may produce an effect resembling slight plasmolysis.

The plasma membrane of *S. aureus* appears to share the unit structure with *Bacillus* and *E. coli*, though under some preparative conditions the unit membrane characteristics may be very difficult to see. Thus, it seems that Robertson's unit structure of the plasma membrane is characteristic of many bacteria and may have the universality

claimed for it, not only in animal cells, but also in bacterial cells.

Recently, Saito (1965) demonstrated that a membrane which appears single in an exactly focused image may show a triple-layered structure, closely resembling the unit membrane, in a out-of-focus image, due to the presence of interference fringes. However, it is clear that the unit structure presented here is not a "false" unit membrane by the same tests as described in Saito's paper.

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#### BIBLIOGRAPHY

1. BENNETT, H. S., and LUFT, J. H., 1959, *s*-Collidine as a basis for buffering fixatives, *J. Biophysic. and Biochem. Cytol.*, **6**, 113.
2. FITZ-JAMES, P. C., 1960, Participation of the cytoplasmic membrane in the growth and spore formation of *Bacilli*, *J. Biophysic. and Biochem. Cytol.*, **8**, 507.
3. VAN ITERSON, W., 1961, Some features of a remarkable organelle in *Bacillus subtilis*, *J. Biophysic. and Biochem. Cytol.*, **9**, 183.
4. LUFT, J. H., 1961, Improvements in epoxy resin embedding techniques, *J. Biophysic. and Biochem. Cytol.*, **9**, 409.
5. MOODY, M. F., and ROBERTSON, J. D., 1960, The fine structure of some retinal photoreceptors, *J. Biophysic. and Biochem. Cytol.*, **7**, 87.
6. OGURA, M., 1963, High resolution electron microscopy on the surface structure of *Escherichia coli*, *J. Ultrastruct. Research*, **8**, 251.
7. ROBERTSON, J. D., 1957, New observations on the ultrastructure of the membranes of frog peripheral nerve fibers, *J. Biophysic. and Biochem. Cytol.*, **3**, 1043.
8. ROBERTSON, J. D., 1959, The ultrastructure of cell membranes and their derivatives, *Biochem. Soc. Symp. (Cambridge, England)*, No. 16, 3.
9. ROBINOW, C. F., 1962, On the plasma membrane of some bacteria and fungi, *Circulation*, **26**, 1092.
10. SAGER, R., and PALADE, G. E., 1957, Structure and development of the chloroplast in *Chlamydomonas*. I. The normal green cell, *J. Biophysic. and Biochem. Cytol.*, **3**, 463.
11. SAITO, A., 1965, The recognition of interference fringes appearing in electron micrographs of ultrathin sections, *J. Electron Microsc.*, **14**, 101.
12. SUGANUMA, A., 1961, The plasma membrane of *Staphylococcus aureus*, *J. Biophysic. and Biochem. Cytol.*, **10**, 292.
13. SUGANUMA, A., 1963, Some features of membranous structures in *Staphylococcus aureus*, *J. Infect. Dis.*, **113**, 179.
14. SUGANUMA, A., 1964, The plasma membrane of *Staphylococcus aureus* treated with HCl solution, *J. Cell Biol.*, **21**, 290.
15. SUGANUMA, A., 1965, Fine structure of *Staphylococcus aureus*, *Ann. New York Acad. Sc.*, **128**, 26.
16. WATSON, M. L., 1958, Staining of tissue section for electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, **4**, 475.