THE PLASMA MEMBRANE OF *STAPHYLOCOCCUS AUREUS* TREATED WITH HCL SOLUTIONS

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Robertson has advanced the concept that all lipoprotein membranes in and around cells have the characteristic triple-layered 'unit membrane structure" (8–10). Other authors have reported occasional exceptions. Thus Lillibridge (6) described a single-layered membrane in pepsiniferous cells in human stomach, and Karrer (4) has mentioned certain vesicles in lung phagocytes as being surrounded by thin, single-layered membranes differing from those conforming to Robertson's unit membrane structure. In two previous studies (11, 12), the plasma membrane of *Staphylococcus aureus* was seen as a single-layered structure about 50 A thick, resembling the exceptions to the unit membrane structure mentioned above. In the course of subsequent efforts, it has been possible to demonstrate a typical triple-layered unit membrane in the case of *S. aureus*, using a special preparative technique. This demonstration suggests that the plasma membrane of *S. aureus* may possess a true unit membrane structure, part of which may be masked when observed after the usual preparative techniques for electron microscopy.

MATERIALS AND METHODS

A strain (FDA 209-P) of *Staphylococcus aureus* was cultivated on agar for 4 hours. Colonies to be sectioned were fixed with cold *s*-collidine-buffered (1) osmium tetroxide solution. After dehydration the specimens were embedded in Luft's Epon (7) epoxy resin. A part of the colonies was treated with 1 N HCl solution at 60°C for 10 minutes before fixation. All sections were stained with uranyl acetate according to the method of Watson (13) and were studied with RCA-2c and HU-11A electron microscopes.

RESULTS AND DISCUSSION

Figs. 1 and 2 show sections of cells cultivated on agar for 4 hours, without HCl treatment. Surrounding each cell is a concentric series of alternating light and dark layers. The outermost dense layer (*1*) and the next light layer (*2*) are identified as belonging to a cell wall (*W*). The second dense layer (*3*) appears to be a component of the protoplast and is identified as the plasma membrane (*M*) of the cell on the basis of evidence brought forth in previous papers (11, 12). The plasma membrane (*M*) appears here again as a single layer with an over-all width of approximately 50 A.

Fig. 3 shows sections of cells treated with 1 N HCl solution at 60°C for 10 minutes before fixation. The fine structure of the membranes is relatively well preserved, although other structures of the cytoplasm and the nuclear sites are dam-

FIGURES 1 and 2 Sections of cells cultivated on agar for 4 hours and fixed in osmium tetroxide. Fig. 1: × 130,000; Fig. 2: × 110,000.

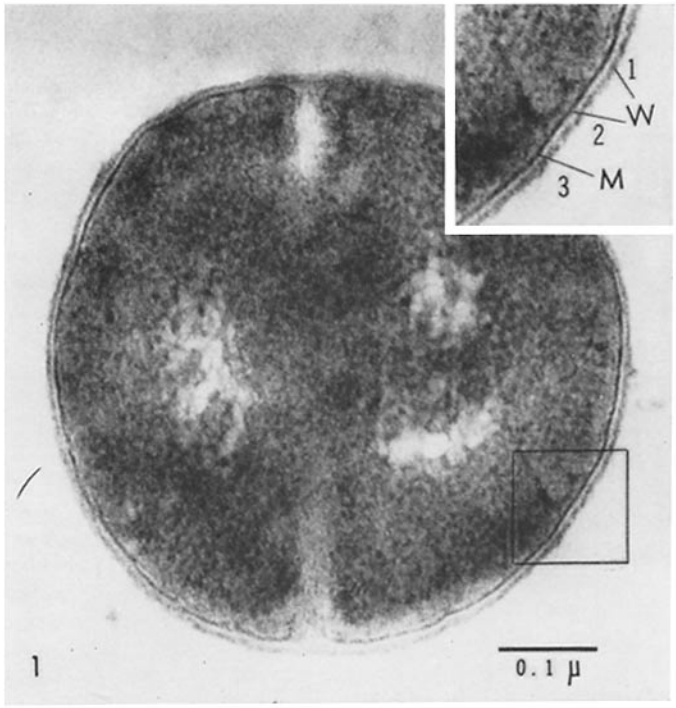
The regions within the squares are enlarged (insets). Layers *1* and *2* are cell wall (*W*), and layer *3* is the plasma membrane (*M*). Inset 1: × 180,000; Inset 2: × 180,000.

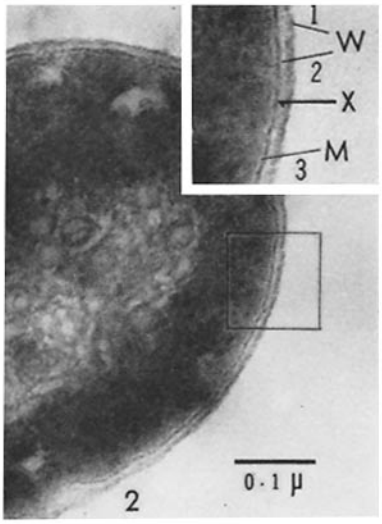
FIGURE 2 *x* Densitometric tracing of the area indicated by the arrow (X) in inset of Fig. 2. Peak-to-peak distance between two dense lines is approximately 50 A.

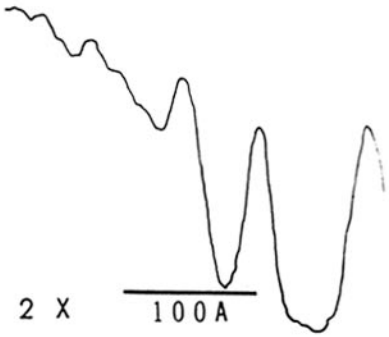
FIGURE 3 Sections of cells (from the same culture that was used for Figs. 1 and 2) treated with 1 N HCl solution at 60°C for 10 minutes before fixation. The plasma membrane (*M*) and the cytoplasmic membranous structures (*CM*) show a unit membrane structure. × 130,000.

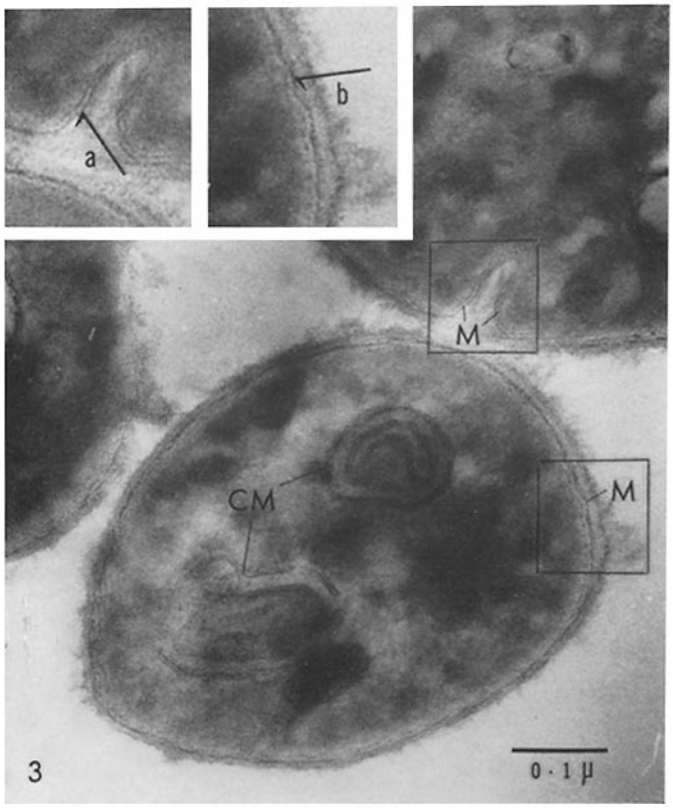
The regions within the squares are enlarged (insets). × 200,000.

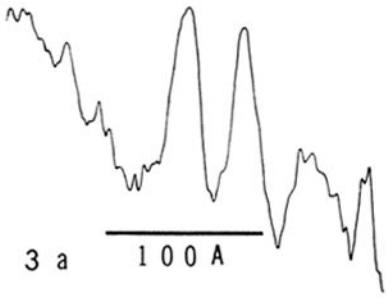
FIGURES 3 *a* and *b* Densitometric tracing of the plasma membrane. The tracing points are indicated by arrows (*a*) and (*b*) in insets of Fig. 3. Peak-to-peak distance between two dense lines of a unit membrane is approximately 50 A.

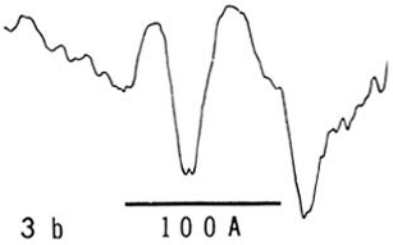












aged. The plasma membrane (*M*) displays two dark layers separated by an intervening light layer and has an over-all width about 70 A. One can see membranous structures (*CM*) in the cytoplasm. These structures are thought to be composed of infolding of the plasma membrane, invaginated from the cell surface.

Figs. 3 *a* and 3 *b* show densitometer tracings of the areas indicated by arrows (*a*) and (*b*) in Fig. 3 (insets). The tracing across the plasma membrane shows two density peaks separated by approximately 50 A. This can be compared with the tracing across the plasma membrane of the microvilli of frog intestine, showing two density peaks separated by 50 A (11).

Kellenberger and Ryter (5) have reported that the plasma membrane of *Escherichia coli* is always single-layered and approximately 60 to 80 A wide. This is another example, among bacteria, of a plasma membrane which was thought not to be a unit membrane. According to Conti and Getter (2), however, there are some indications that the plasma membrane of *E. coli* is a unit membrane.

Although at the present time one can not be sure why the plasma membrane of the cell treated with 1 N HCl solution appears as a unit membrane, two reasons can be postulated: (*a*) a part of the unit membrane may be masked by some other cell material which is removed by 1 N HCl treatment, or (*b*) because the plasma membrane has much more lipid content, the density is so much increased that the real structure is undetectable with the ordinary methods of preparation.

It is suggested that the first reason might be the more reasonable. Actually, it should be pointed out that in Fig. 2 (inset) there is a light zone inside of layer *3* (*M*) which corresponds to the light zone seen in Fig. 3. The inner boundary of the unit membrane in Fig. 2 would seem to be masked by the dense cytoplasm.

Fig. 2 *x* shows a densitometer tracing of the area indicated by the arrow (*X*) in Fig. 2 (inset). The tracing shows two density peaks, although one of them is not distinct because of the adjacent dense material of the cytoplasm. The distance between the two density peaks is approximately 50 A.

Therefore, the plasma membrane of *Staphylococcus aureus* may possess a true unit membrane structure, a part of which may be masked. Treatment with HCl may leach out the cytoplasmic material which masked the inner dense stratum of the unit membrane.

Recently Edwards and Stevens (3) demonstrated the fine structure of *Listeria monocytogenes*. They stated that the dense line at the edge of the cytoplasm was not always discernible because its density is similar to that of the ground cytoplasm.

It may be possible to clarify the structure of the plasma membrane either by ultrathin sectioning of free protoplasts or by the study of isolated plasma membranes. The latter are, however, very difficult to isolate and to purify.

Additional studies should be made in order to clarify further the fine structure of the plasma membrane of *Staphylococcus aureus*.

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