THE PLASMA MEMBRANE OF *STAPHYLOCOCCUS AUREUS*

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As electron microscopic techniques have improved, the fine structure of bacterial cells has come to be understood more clearly. There are, however, many details yet to be elucidated. I propose to report here some observations on the plasma membrane of *Staphylococcus aureus*, using sectioning techniques. Bradfield (1956) has already described some structural features of *Staphylococcus aureus* using the thin sectioning method.

MATERIALS AND METHODS

A strain of *Staphylococcus aureus* was cultivated on agar or in broth for 5 hours or more. Colonies to be sectioned were fixed either with cold buffered (Bennett

and Luft, 1959) osmium tetroxide or with potassium permanganate (Luft, 1956) solution. After dehydration the specimens were embedded in Luft's Epon (Luft, 1961) epoxy resin and sectioned in a direction perpendicular to the surface of the agar. Some sections were stained with uranyl acetate according to the method of Watson (1958). Sections were studied with an RCA-2C type electron microscope.

RESULTS AND DISCUSSION

Surrounding each cell is a concentric series of alternating light and dark layers. For purposes of convenience, they are numbered from *1* to *5* as shown in Fig. 1. Layer *1* is the outermost dense layer, layer *5* is the cytoplasmic matrix of the cell. Layers *1* and *2* are identified as belonging to a relatively rigid cell wall (*W*). Layer *3* appears to be a component of the protoplast, and is identified as representing the plasma membrane of the cell (*M*). This identification is based on the following three points:

Under certain circumstances, one can see indentations of layers *3*, *4* and *5*, whereas layers *1* and *2* show undisturbed circumferential contours in the regions characterized by indentations (*C*) as shown in Fig. 2. These indentations are identified as caveolae intracellulares (Yamada, 1955). It is possible that these represent manifestations of pinocytosis or of some other transport mechanism involving vesiculation of the membrane. The fact that layer *3* participates in forming these caveolar indentations is the first point leading to the identification of layer *3* as the plasma membrane of the cell.

Fig. 3 shows a section of a dividing cell fixed in potassium permanganate. The regions indicated by *X* and *Y* show an early stage of cross-wall formation. One can see that layer *3* infolds, forming two dense layers between which is another dense layer coming from layer *1* and carrying with it a lighter layer corresponding to layer *2*. Consequently, the cross-wall shows three dense layers and four light layers. Fig. 4 shows a section of a dividing cell fixed in osmium tetroxide. The regions *X* and *Y* show an early stage of cross-wall formation. The upper and lower parts of layer *3* join at the inner edge of the cross-wall. This is a second point supporting the interpretation that layer *3* is the plasma membrane of the cell (Chapman, 1959).

Inside the cytoplasm is an area of reduced density containing very delicate filaments (*N*). This is tentatively identified as the nucleus. Of particular interest is the occurrence of a constriction of the nuclear material in the plane of the incipient transverse cell wall of the dividing cell.

Fig. 5 shows the completed cross-wall. It is made up of three dense layers and four light layers, as in the previous example. A continuity of layer *3* with membranous structures (*L*) (Glauert and Hopwood, 1959; Glauert and Hopwood, 1960) in the cytoplasm is observed. This is the third point supporting the view that layer *3* represents the plasma membrane of the cell.

The dense layer here numbered (*3*) and identified as the plasma membrane appears in the electron micrographs of Murray, Francombe, and Mayall (1959) who suspected that it might be a component of the cell wall since it seemed to be adherent to the major portion of the cell wall in their material. However, in the present study layer *3* may diverge from the cell wall showing that it is not in fact a part of it, as suspected by Murray *et al.*

Layer *3*, which has been thus identified as the plasma membrane of the *Staphylococcus* cell, always appears as a single dense layer about 50 A thick. It corresponds to the layer of *Escherichia coli* identified as the cytoplasmic membrane by Kellenberger and Ryter (1958), but is somewhat thinner. If these interpretations are correct, they represent an exception to Robertson's concept that all biological membranes have the same "unit" structure (Robertson, 1957; Robertson, 1959; Moody and Robertson, 1960).

To demonstrate the difference between a typical unit membrane as seen in the plasma membrane of animal cells and the staphylococcal plasma membranes under closely similar conditions, the following experiment was made. The small intestine of a frog and colonies of *Staphylococcus aureus* were fixed separately with osmium tetroxide. Before embedding, the inner surface of intestine was brought into contact with the surface of colonies, so that the microvilli of the intestine and the *Staphylococci* could be included in the same section and studied in the same electron micrograph.

Fig. 6 shows one of the examples. The upper portion of the picture shows the microvilli and the lower portion shows a section of *Staphylococcus*. The areas within the squares are enlarged, as shown in Figs. 7 and 8. The plasma membrane

of the microvilli (*P*) has the appearance typical of the unit membrane. It displays two dark layers separated by an intervening light layer, with an over-all width of about 70 A (Sjöstrand and Zetterqvist, 1956; Palay and Karlin, 1959). The plasma membrane of the *Staphylococcus* (*3*) appears as only a single line with an over-all width of about 50 A.

Figs. 9 and 10 show densitometer tracing of the area between the ink marks (*i*) on Figs. 7 and 8. The tracing across the plasma membrane of the microvilli shows two dense peaks separated by 50 A, but that across the plasma membrane of *Staphylococcus* shows only a single peak with an over-all width of about 50 A.

It is thus clear that the plasma membrane of *Staphylococcus aureus* differs from that of frog intestinal microvilli. The difference in appearance between the two plasma membranes must reflect differences in their molecular structure.

Recently P. Mitchell (1957) obtained isolated staphylococcal membranes in a reasonably clean state. He reported that the lipoprotein particles are fragments of the plasma membrane, and that the weight of the membrane or lipoprotein fraction (*ca.* 10 per cent of the cell dry weight) is sufficient to make a layer only 5 mμ thick on the surface of the protoplast. Since the membrane is composed of about 40 per cent lipid and 20 per cent protein, it could form about one monolayer of lipid and one of protein. On the other hand, the plasma membranes which have the typical unit membrane appearance contain an amount of lipid which can be accounted for only when arranged in a bimolecular leaflet (Gorter and Grendel, 1925). These chemical data correlate well with the morphological data presented.

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FIGURE 1

Section of a cell cultivated in broth for 5 hours, fixed in osmium tetroxide. Layers *1* and *2* are cell wall (*W*) and layer *3* is the plasma membrane (*M*). Layers *4* and *5* are attributed to the cytoplasm. × 140,000.

FIGURE 2

Section of a cell cultivated on agar for 4 hours, fixed in osmium tetroxide. Indentations of layers *3*, *4*, and *5* are seen, forming caveolae (*C*). × 120,000.

FIGURE 3

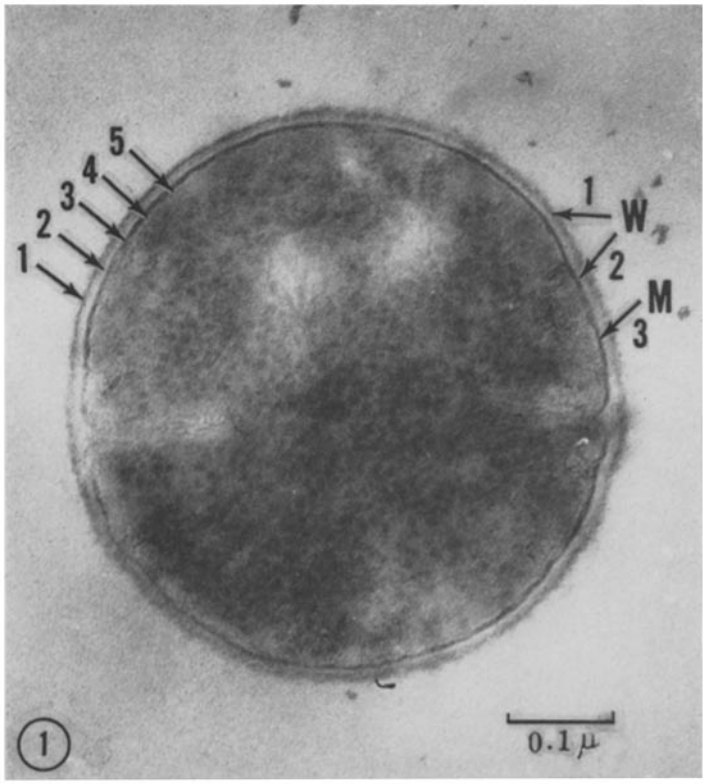
Section of a dividing cell cultivated on agar for 4 hours, fixed in potassium permanganate. The layers of the cross-wall (*X* and *Y*) are continuous with the outside layers. × 100,000.

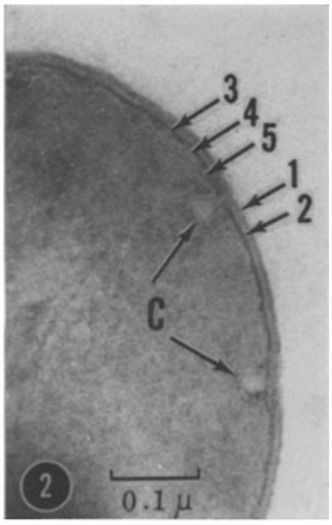
FIGURE 4

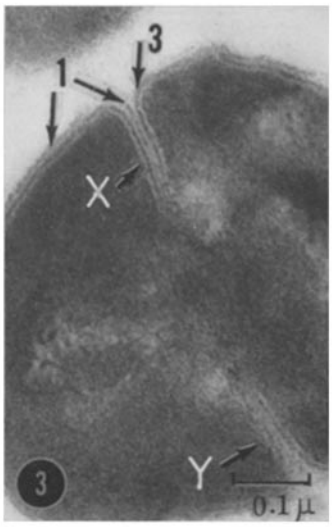
Section of a dividing cell cultivated on agar for 5 hours, fixed in osmium tetroxide. Layer *3* joins at the inner edge of the cross-wall (*X* and *Y*). Nuclear material (*N*) is constricted in the plane of the incipient transverse cell wall. × 150,000.

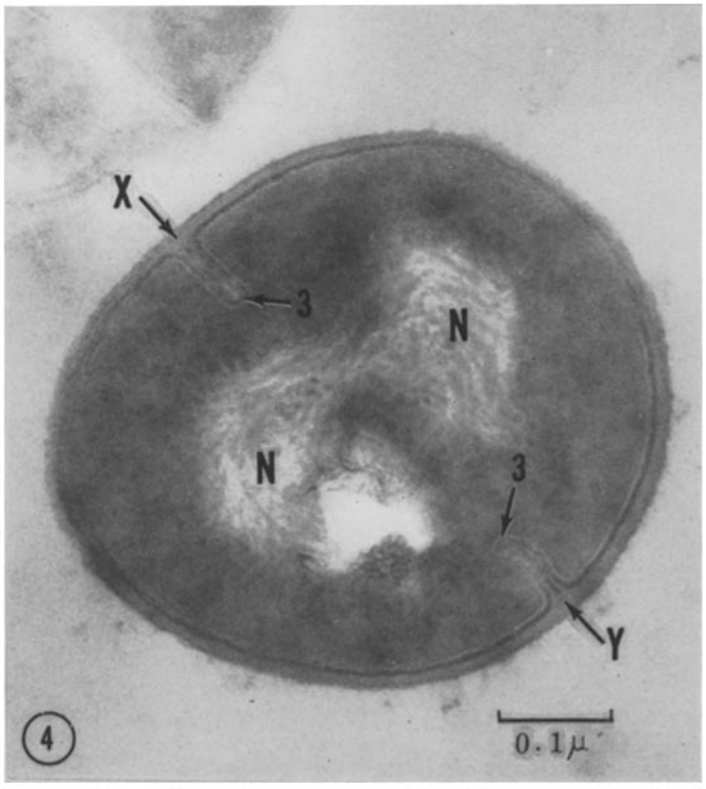
FIGURE 5

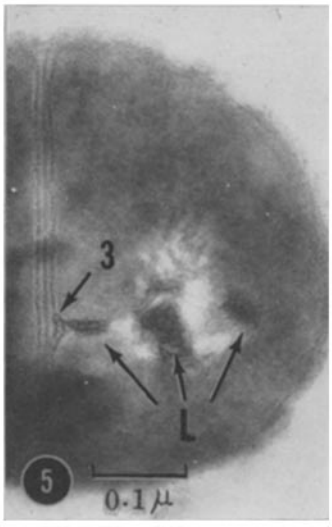
Section of a cell cultivated on agar for 5 hours, fixed in osmium tetroxide. Layer *3* is continuous with the membranous structures (*L*) in the cytoplasm. × 130,000.


1
2
3
4
5
W
M
0.1 μ


3
4
5
1
2
C
2
0.1 μ


1
3
X
Y
3
0.1 μ


X
3
N
N
3
Y
4
0.1 μ


3
L
5
0.1 μ

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BIBLIOGRAPHY

1. BENNETT, H. S., and LUFT, J. H., s-Collidine as a basis for buffering fixatives, *J. Biophysic. and Biochem. Cytol.*, 1959, **6,** 113.
2. BRADFIELD, J. R. G., Organization of bacterial cytoplasm, *in* Bacterial Anatomy, *Symp. Soc. Gen. Microbiol.*, 1956, **No. 6,** 296.
3. CHAPMAN, G. B., Electron microscopic observations on the behavior of the bacterial cytoplasmic membrane during cellular division, *J. Biophysic. and Biochem. Cytol.*, 1959, **6,** 221.
4. GLAUERT, A. M., and HOPWOOD, D. A., A membranous component of the cytoplasm in *Streptomyces coelicolor*, *J. Biophysic. and Biochem. Cytol.*, 1959, **6,** 515.
5. GLAUERT, A. M., and HOPWOOD, D. A., The fine structure of *Streptomyces coelicolor*. I. The cytoplasmic membrane system, *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 479.
6. GORTER, E., and GRENDEL, F., On bimolecular layers of lipoids on the chromocytes of the blood, *J. Exp. Med.*, 1925, **41,** 439.
7. KELLENBERGER, E., and RYTER, A., Cell wall and cytoplasmic membrane of *Escherichia coli*, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 323.
8. LUFT, J. H., Permanganate—A new fixative for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 799.
9. LUFT, J. H., Improvements in epoxy resin embedding techniques, *J. Biophysic. and Biochem. Cytol.*, 1961,**9,** 409.
10. MITCHELL, P. D., Structure and function of microorganism, *in* The Structure and Function of Subcellular Components, *Biochem. Soc. Symp.*, 1959, **No. 16,** 73.
11. MOODY, M. F., and ROBERTSON, J. D., The fine structure of some retinal photoreceptors, *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 87.
12. MURRAY, R. G. E., FRANCOMBE, W. H., and MAYALL, B. H., The effect of penicillin on the structure of staphylococcal cell walls, *Canad. J. Microbiol.*, 1959, **5,** 641.
13. PALAY, S. L., and KARLIN, L. J., An electron microscopic study of the intestinal villus, *J. Biophysic. and Biochem. Cytol.*, 1959, **5,** 363.
14. ROBERTSON, J. D., New observations on the

FIGURE 6

Section of *Staphylococcus* and of microvilli of intestine of a frog, fixed in osmium tetroxide, taken simultaneously on the same film. Regions within the squares are enlarged and shown (Figs. 7, 8). × 60,000.

FIGURE 7

The portion of the plasma membrane of the microvilli (*P*) indicated by the upper rectangle in Fig. 6. The typical appearance of a unit membrane is demonstrated. The densitometric tracing (inset) was made between two ink marks (*i*). × 430,000.

FIGURE 8

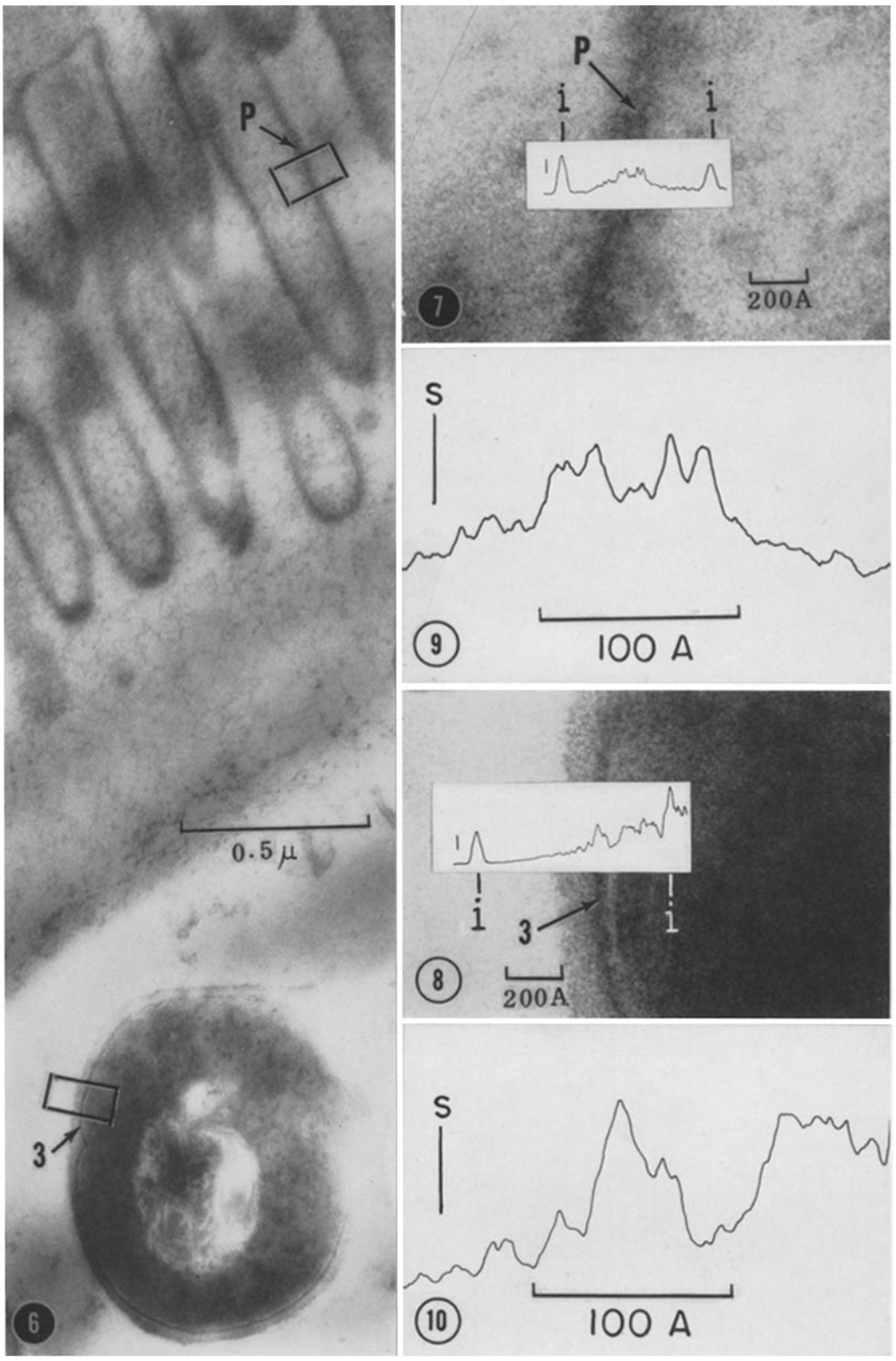
An enlargement of the part of the *Staphylococcus* outlined in Fig. 6. The plasma membrane appears as a single dense line (*3*), about 50 A wide. The densitometric tracing (inset) was made between two ink marks (*i*). × 430,000.

FIGURE 9

Enlargement of center portion of densitometric tracing shown in Fig. 7. Line *S* indicates the slit size in the densitometer, relative to the curve. The actual slit length is $\frac{2}{3}$ mm and slit width is $\frac{1}{60}$ mm. Subsidiary peaks are present within the two main peaks. × 3,100,000.

FIGURE 10

Enlargement of center portion of densitometric tracing shown in Fig. 8. Line *S* indicates slit size relative to the curve. × 3,100,000.


P
0.5 μ
3
6
P
i
i
200A
7
S
100 A
9
i
3
i
200A
8
S
100 A
10

ultrastructure of the membranes of frog peripheral nerve fibers, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 1043.

15. ROBERTSON, J. D., The ultrastructure of cell membranes and their derivatives, *in* The Structure and Functions of Subcellular Components, *Biochem. Soc. Symp.*, 1959, **No. 16**, 3.
16. SJÖSTRAND, F. S., and ZETTERQVIST, H., Functional changes of the free cell surface membrane of the intestinal absorbing cell, *in* Electron Microscopy, Proceedings of the Stockholm Conference, September, 1956, (F. S. Sjöstrand and J. Rhodin, editors), New York, Academic Press, Inc., 150.
17. WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 475.
18. YAMADA, E., The fine structure of the gall bladder epithelium of the mouse, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 445.