FINE STRUCTURE OF *LISTERIA MONOCYTOGENES*

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ABSTRACT

EDWARDS, MERCEDES R. (New York State Department of Health, Albany) AND ROY W. STEVENS. Fine structure of *Listeria monocytogenes*. J. Bacteriol. **86**:414–428. 1963.—Cells of *Listeria monocytogenes*, at different stages of growth, were fixed with osmium tetroxide and treated with uranyl acetate. The material was dehydrated in alcohol, embedded in prepolymerized methacrylate, and studied in thin sections. In most of the micrographs, the plasmalemma (or plasma membrane) showed a pattern of three dense lines, each ca. 25 A thick, alternating with two light zones, each ca. 30 A thick. The outer light zone was regularly bridged by strands of dense material, and the inner one was not. The dense line at the edge of the cytoplasm was not always discernible because of its similarity in density with the ground cytoplasm, although it could be easily demonstrated in lysed cells and in protoplasts. The latter were found to be limited by a pair of dense lines, each ca. 25 A thick, bounding a light core ca. 30 A thick. This structure corresponds to a "unit" membrane, but it represents only a part of the plasmalemma of the intact cell; it was therefore interpreted as being more complex than a single unit membrane. Intracytoplasmic membranes of various configurations were clearly shown to be extensions of the plasmalemma. They may branch repeatedly and anastomose to form a complicated honeycomb-like organelle or organelles of different appearances, sometimes lamellate. The lamellar bodies are envisioned as resulting from spiraled ingrowths. The various kinds of ingrowths of the plasmalemma were designated "plasmalemmosomes" to indicate their origin; however, some of these organelles in *Listeria* were similar to those described in different bacteria by other authors. Plasmalemmosomes have been found in both aerobically and anaerobically grown cells. Another outstanding feature in many micrographs was the nucleoid, which contains dense fibrils measuring 25 to 50 A in diameter. These fibrils frequently appeared to be coiled and were of the order of magnitude ascribed to deoxyribonucleic acid molecules.

The investigations of a number of bacteria in electron micrographs of thin sections in recent years have been reviewed by Robinow (1960), Murray (1960, 1962), and Glauert (1962). Most of these studies were of aerobic forms of only a few genera, although there are a few interesting reports on photosynthetic bacteria (Giesbrecht and Drews, 1962; Cohen-Bazire and Kunisawa, 1963).

The need for a thorough study of the fine structural details of both nonphotosynthetic anaerobic bacteria and the microaerophilic forms prompted us to make a survey with the electron microscope of typical representatives of these bacteria. A report on *Actinomyces bovis* (Edwards and Gordon, 1962*a*) revealed uncommon types of intracytoplasmic membranes, and proved that the choice of that organism was rewarding. The present report deals in detail with *Listeria monocytogenes*, a pathogenic microaerophilic organism of wide distribution and unusual adaptive nature. It grows under aerobic conditions, but does best under reduced oxygen tension, with excellent growth in a carbon dioxide atmosphere (Seeliger, 1961). If intracytoplasmic membranes of bacteria are related to the respiratory enzyme chains, as are the membranes of mitochondria in higher cells, morphological changes might be expected to follow shifts in mode of respiration from aerobic to anaerobic and *vice versa*.

MATERIALS AND METHODS

L. monocytogenes Pirie 1940, strain 6057, was employed in this study. The culture was examined for morphological and staining characteristics prior to transfer to Tryptone (Difco; 1% Tryptone, 0.5% sodium chloride, pH 7 to 7.2) and Tryptose (Difco; 2% Tryptose, 0.2% glucose, 0.25% sodium hypophosphate, pH 7.5) broth or agar (2% agar added to broths when solid medium

was used). The cultures were kept at 25 or 37 C, in air or under anaerobic conditions.

Preparation for electron microscopy followed, with slight modification, the procedure of Kellenberger, Ryter, and Séchaud (1958). Cells were harvested at intervals of 1 to 8 days. They were transferred directly from the surface of the agar into the fixative. When broth culture was used, a prefixation was accomplished by addition of 0.7 ml of 1% OsO_4 in acetate-Veronal buffer (pH 6.1) to 7.0 ml of culture and immediate centrifugation. The pellet was resuspended in 2 ml of the same 1% OsO_4 solution with 0.2 ml of Tryptone medium or 0.1% Casamino Acids (van Iterson, 1961). In most cases, traces of calcium or magnesium, or both, were added to the fixative solution. The material was left at room temperature for 3 to 5 hr, and, after a change to fresh fixative solution, was kept in a refrigerator overnight. The samples were pre-embedded in agar and treated with 0.5% uranyl acetate in acetate-Veronal buffer (pH 6.1) for 1 to 2 hr. (The pH of the final solution was 5.2.) In a few cases, 10% sucrose was added to the OsO_4 solution. When plasmolysis was wanted, the cells were treated with 30 to 35% sucrose for 2 to 10 min prior to their fixation.

After dehydration in ethanol, the material was infiltrated at room temperature with a mixture of *n*-butyl-methyl methacrylate (9:1) monomer, which was renewed several times. The material was then transferred into a fresh methacrylate mixture containing benzoyl peroxide (0.5 to 1%) and placed in a refrigerator for 24 hr, or in some cases for 2 to 8 days. Finally, it was embedded in the same mixture which was prepolymerized prior to its use by slow shaking in a water bath at 70 C for about 30 min. The blocks were hardened at 46 C overnight. In a few cases, Vestopal W (Merlin Jaeger, Geneva, Switzerland) was substituted for methacrylate; in such cases, dehydration was in acetone.

Thin sections, exhibiting gray interference color, were cut with a Caracas diamond knife in a Porter-Blum microtome, and picked up on carbon Formvar coated copper grids. Some sections were stained with lead acetate, lead hydroxide (Watson, 1958), or chromyl chloride (Bullivant and Hotchin, 1960). The sections were examined in a Siemens Elmiskope I (Siemens & Halske, Berlin, West Germany) at original magnifications of 20,000 to 40,000 ×; further enlargements were obtained photographically.

RESULTS

Cells harvested from different media, grown under aerobic or anaerobic conditions, did not show significant differences in fine structure and will be considered together with reference to growth conditions made only when necessary for clarification of a particular point.

Cell wall. The cells are encased in apparently rigid walls (Fig. 1 to 3) of an average width of 30 mμ. In cells fixed at a plasmolyzed stage, the wall is partially separated from the cytoplasm and may show outer and inner layers slightly denser than its middle layers (Fig. 18). In general, however, this distinction is not found. The border of the cell wall is sometimes very irregular in shape (Fig. 9, 11, and 13). On the other hand, the demarcation of the innermost layer of the wall is frequently impossible because of its adhesion to the plasma membrane. Adhesion between cell wall and plasma membrane was suggested by Mitchell and Moyle (1956) as a characteristic of gram-positive bacteria. In most of our micrographs, the density of the cell wall is similar to that of the cytoplasm (Fig. 11 and 13), although in some examples it is denser (Fig. 2, 3, and 10). This may depend on the capability of the fixative to penetrate into the cytoplasm through the cell wall, where sometimes it accumulates, or on the quantity of ribonucleoprotein particles present in the cytoplasm. Chapman (1959*a*) has also shown that the density of the cell wall (or of its layers) may vary within the same bacterium. Material of foreign origin, as pointed out by Glauert (1962), frequently is adsorbed at the periphery of the wall (Fig. 2, 5, and 15). The possibility that this material may be excreted by the bacteria and freed into the surrounding medium should not be excluded, but it does not seem to be typical capsular material, since the latter may be readily recognized in electron micrographs of encapsulated bacteria, e.g., in pneumococci studied in this laboratory (*unpublished observations*). The adsorption of various substances to the outermost layer of the cell wall may be promoted by treating the cells prior to fixation with salt solutions. In certain circumstances, a relatively thick coat may be added to the wall, and this may account for reported observations of a capsule in *Listeria* (Smith and Metzger, 1962). Capsules were not present in our preparations.

Cell wall in division. Listeria, as a gram-positive

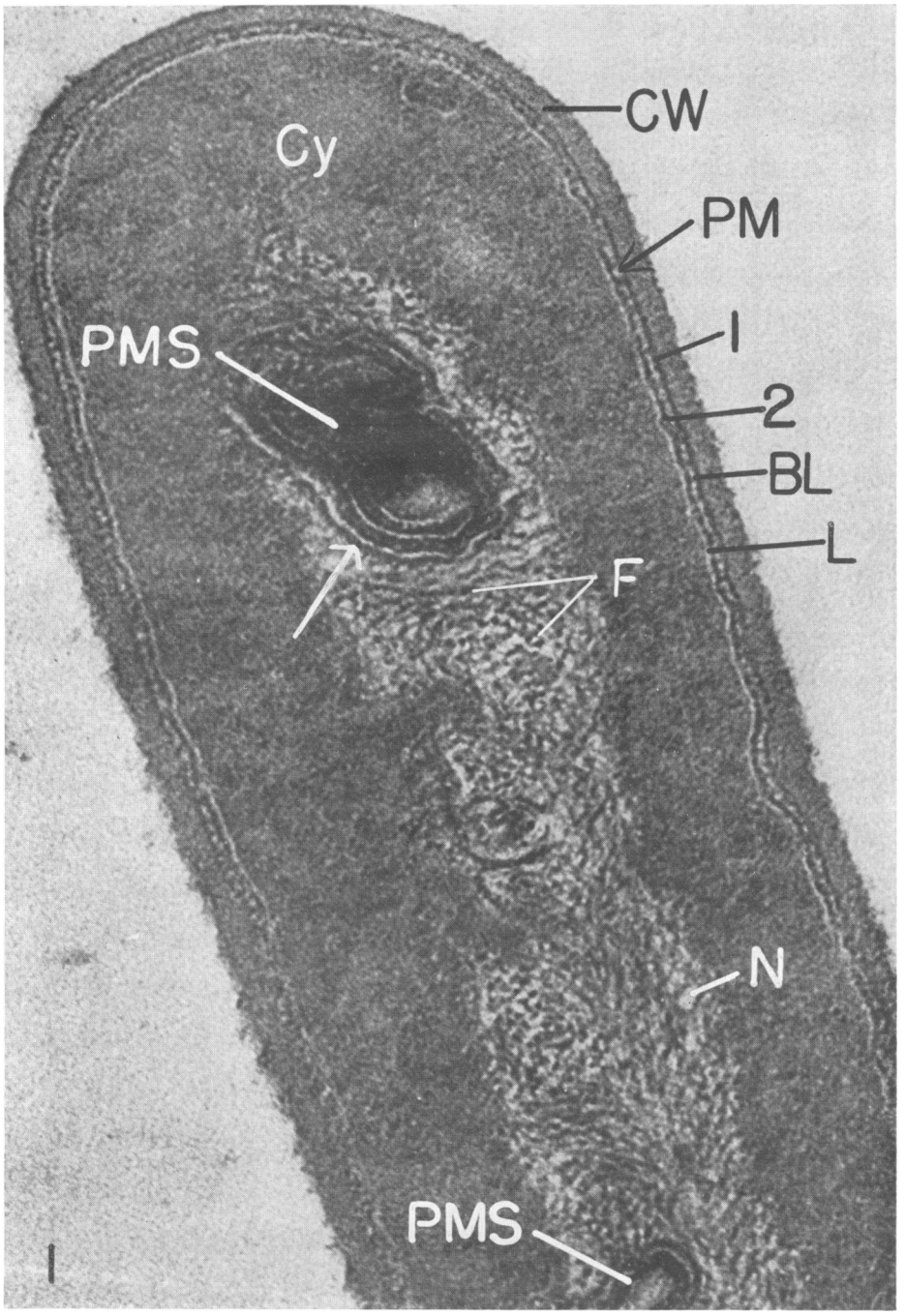

FIG. 1. *Cell in preparation for division. The cell wall (CW) is lined by the plasma membrane (PM). The latter shows two dark lines (1 and 2) and two light layers, the bridged (BL) and the nonbridged (L) layers. The cytoplasm (Cy) lies between the plasma membrane and the nucleoplasm (N). In the nucleoplasm are seen dense fibrils (F), which may appear coiled or as dots in cross section. Parts of two plasmalemmosomes (PMS) are shown apparently associated with the nuclear apparatus. Note, in the upper one, the alternation of bridged and nonbridged layers (arrow). Sucrose was added to the fixative solution. Section stained with chromyl chloride. Magnification, 200,000×. [All figures are electron micrographs of thin sections of cells of Listeria monocytogenes. Some sections were stained prior to their examination. References to the stain used are found with the descriptions of figures. Unless otherwise stated, the cells were fixed with 1% osmic acid in acetate-Veronal buffer (pH 6.0 to 6.2) with addition of Tryptone or Casamino Acids, calcium chloride, and traces of magnesium, and then treated with 0.5% uranyl acetate in the same buffer.]*

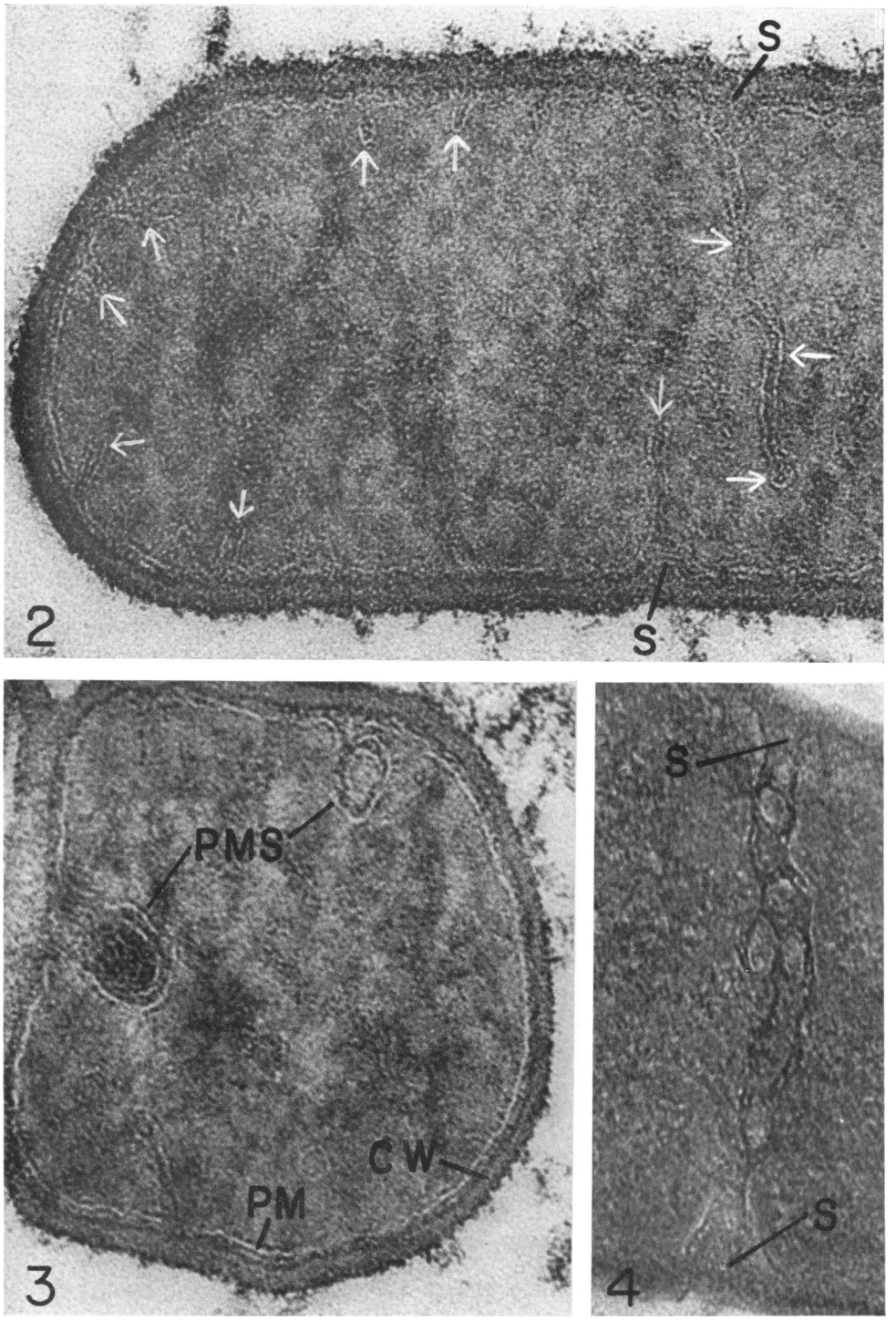

FIG. 2. *View of peripheral cytoplasm. It is packed with tiny dense granules which are found in rows or isolated. Arrows point at intrusions of the plasma membrane which at two sites appear to precede the ingrowth of the cross wall (or septum S). Note that this formation is out of register. Magnification, 200,000×.*

FIG. 3. *Recently divided cell showing two plasmalemmosomes (PMS), one of which is at the septum (left-hand side). Cell wall (CW) and plasma membrane (PM) are also indicated. Magnification, 200,000×.*

FIG. 4. *Early stage of septum formation. The cytoplasm of the dividing cell is crossed by a chainlike structure (an ingrowth of the plasma membrane) connecting both sides of the developing septum (S). Magnification, 200,000×.*

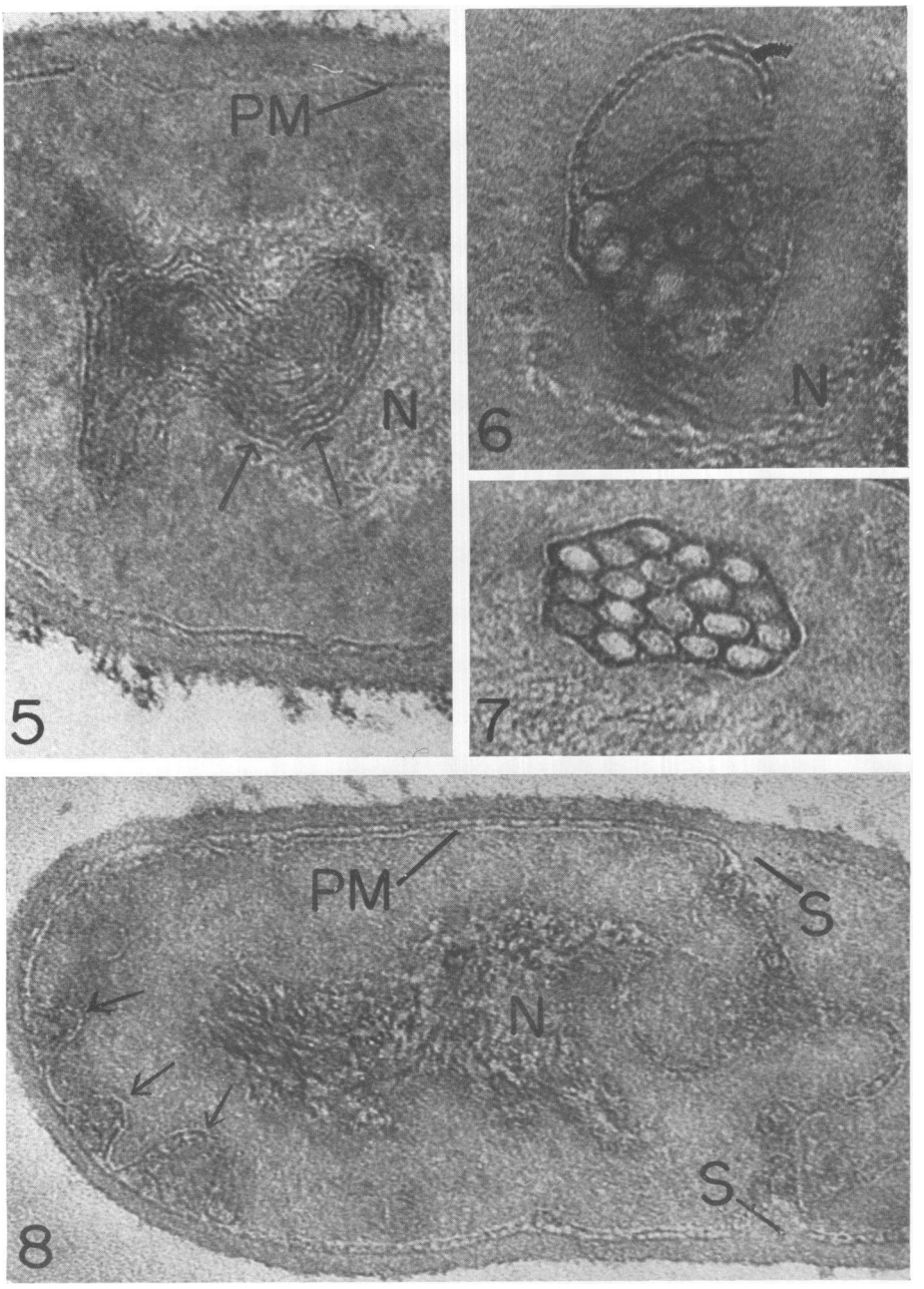

FIG. 5 to 7. *Portions of cells showing plasmalemmosomes. In Fig. 5, the plasmalemmosome appears associated with the nuclear area (N) and has a lamellar feature. Note the identity of its bridged and non-bridged layers (arrows) with those of the plasma membrane (PM). In Fig. 6, it is shown that the layers of the plasmalemmosome may extend themselves by a system of loops or branches which may anastomose, thus giving rise to an alveolar structure. In Fig. 7, the plasmalemmosome has a honeycomb appearance. Magnification, 200,000×.*

FIG. 8. *Portion of a dividing cell showing a row of plasmalemmosomes (arrows) at the periphery of the cell and a ribbon at the center where septation is occurring. Cross-wall material (S) and nuclear material (N) are also indicated. Magnification, 140,000×.*

bacterium, follows in general the scheme of cell division given by Robinow (1945) in that the initial stages of cytokinesis involve an inward growth of the plasma membrane preceding the ingrowth of the cross wall (septum in Fig. 2, 4, 8, and 15). The cross wall, as a closing annular diaphragm, is formed by a gradual synthesis of wall material which is deposited in continuation with the original wall inwards from the periphery of the cell. The deposition of this new material seems not only to be led by the plasma membrane, but also is located inside of its infoldings (Fig. 2, 4, and 8). In *Bacillus subtilis*, Glauert, Brieger, and Allen (1961) also found that pockets of membranes originating at the plasma membrane appeared to initiate the growth of cross walls. Chapman (1959*b*) described plasma membrane septation in bacterial cells. His micrographs show invaginations of the plasma membrane at zones of cell division, a process in general similar to that of *Listeria*. Complete septa (Fig. 3 and 11) are lined at both sides by the plasma membrane. The newly divided cells may stick together for some time after the cross wall is completed, as has been observed by others (Robinow, 1945; Dawson and Stern, 1954; Bisset, 1956). The cross wall is not always perpendicular to the original wall (Fig. 11). As seen in Fig. 2, the infoldings of the plasma membrane at the points of septum formation are out of line; this would explain the finding of oblique cross walls. In some cases (Fig. 9), the tips of the ingrowing septum appear open, that is, not enveloped by the plasma membrane. This could be due to the plane of sectioning.

Plasma membrane. The plasma membrane, or plasmalemma, in most of our micrographs (Fig. 1) shows a pattern of two dense lines (ca. 25 A wide) alternating with two light zones (ca. 30 A each). (In this paper we have used the terms, plasma membrane and plasmalemma, synonymously without preference for one or the other.) The light zone toward the cell wall is regularly crossed by bridges of dense material (ca. 20 A in width); the other is not. For the sake of simplicity, these light zones will be designated as the bridged and nonbridged layers. In micrographs of heavily stained preparations, as in Fig. 11, a third dense line (ca. 25 A in width) is seen at the edge of the cytoplasm. This is the innermost limit of the plasma membrane, but it is not always visible because its density is similar to that of the ground cytoplasm. However, it may be readily found in lysed cells and in protoplasts (Fig. 19). The study of plasmolyzed cells and freed protoplasts greatly helped with the delimitation of the plasma membrane and the interpretation of its structure. An electron-microscopic study of plasmolyzed bacterial cells has also been recently reported by Cota-Robles (1963). In the earliest stages of plasmolysis (Fig. 16 to 18), it may be seen that the bridged layer and the outermost dense layer (BL and 1 in Fig. 1 and 20) are integral parts of the plasma membrane. As the cytoplasm shrinks away from the cell wall, a process similar to vesiculation occurs at the bridged layer (Fig. 16), the bridges being pulled apart but not breaking immediately, or not breaking all at the same time. At this point of distension (Fig. 17 and 18), the continuity through the bridges between the middle and outer dense layers of the plasma membrane is readily seen. If the contraction of the cytoplasm is carried further, a tearing along the bridged layer occurs, the cell wall is disrupted, and the protoplast is freed. The latter, greatly enlarged, is limited by a single "unit" membrane (Fig. 19) of the type described by Robertson (1959, 1960). The plasma membrane of the intact cell (Fig. 20) is then interpreted as a complex structure, possibly corresponding to a double "unit" membrane. A view of the plasma membrane in tangential section is given in Fig. 14. The convoluted design indicates that there are many entrances of the membrane into the cytoplasm in that area. It may also represent a cut tangential to the base of a complex membranous organelle.

Plasmalemmosomes (internal membrane systems). Vast systems of membranes, variable in size and shape, are located in the cytoplasm and nuclear area of *Listeria*, and are found to be developed by intrusions of the plasma membrane, as has already been suggested for other bacteria (Fitz-James, 1960; Giesbrecht, 1960; Glauert and Hopwood, 1960; Glauert et al., 1961; van Iterson, 1961; Imaeda and Ogura, 1963). They may be simple invaginations (Fig. 2 and 8), a spiraled structure (Fig. 13), or complex organelles of various appearances (Fig. 5 to 7, 9, and 10). Their attachment to the plasma membrane is not always seen at the plane of sectioning (Fig. 1, 5 to 7, and 10), but in Fig. 9, 12, and 13 the continuity between the layers of both plasmalemmosome and plasmalemma is readily

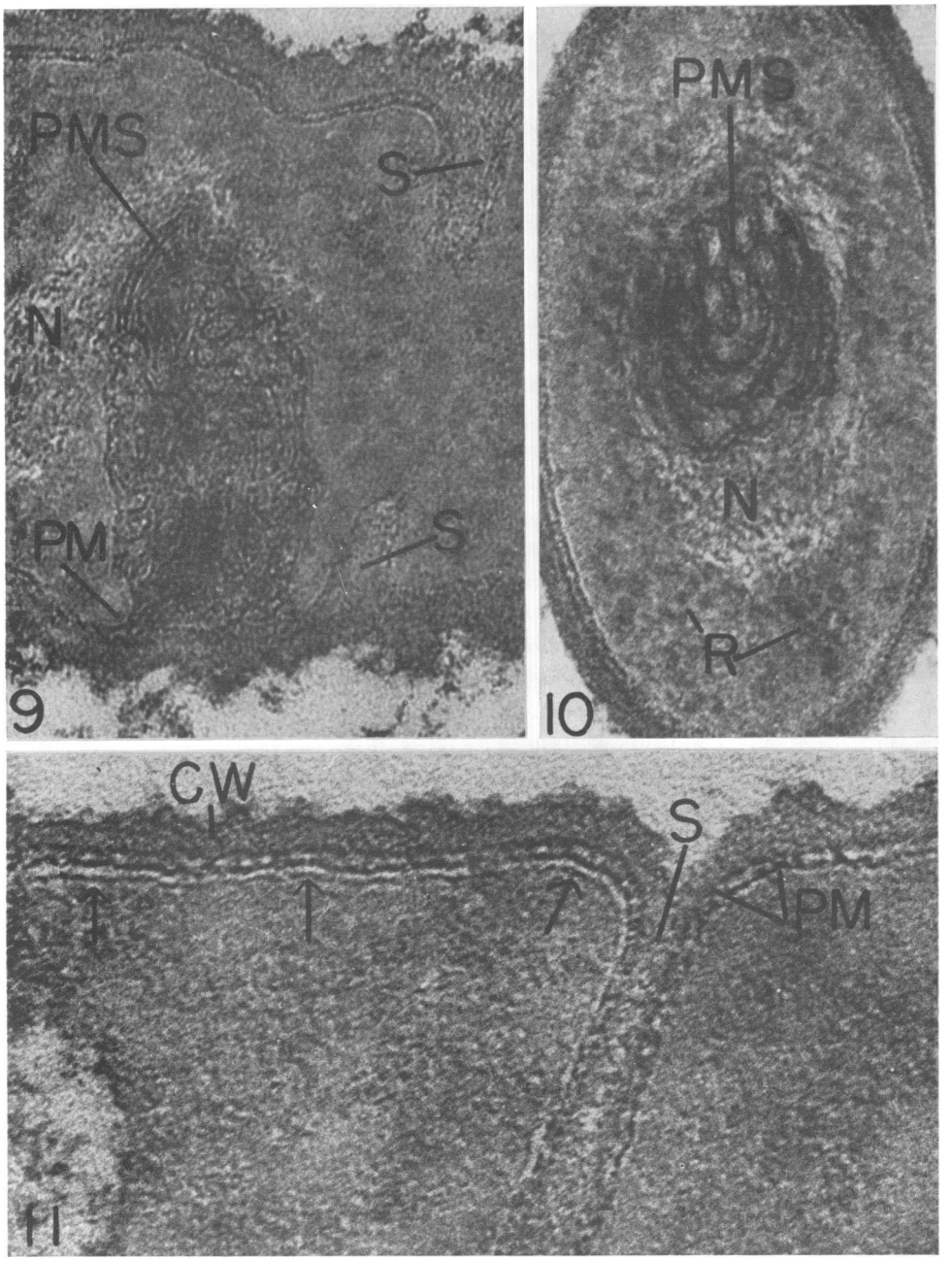

FIG. 9. *Portion of a dividing cell. The plasma membrane (PM) appears interrupted at the tips of the ingrowing septum (S), probably due to the plane of sectioning. A large plasmalemmosome (PMS) reaching the nuclear area (N) is shown attached to the plasma membrane (PM). Magnification, 200,000×.*

FIG. 10. *Anaerobically grown cell. In the nuclear region (N) is seen a plasmalemmosome (PMS), which is part lamellate and part alveolar. Ribosomes (R) appear scattered in the cytoplasm and around the nuclear material. Magnification, 200,000×.*

FIG. 11. *Portion of a divided cell showing the cell wall (CW) and the plasma membrane (PM), which lines both cytoplasmic sides of the septum (S). Note the innermost limit of the plasma membrane, the third dark line at the edge of the cytoplasm (arrows). Section stained with chromyl chloride. Magnification, 300,000×.*

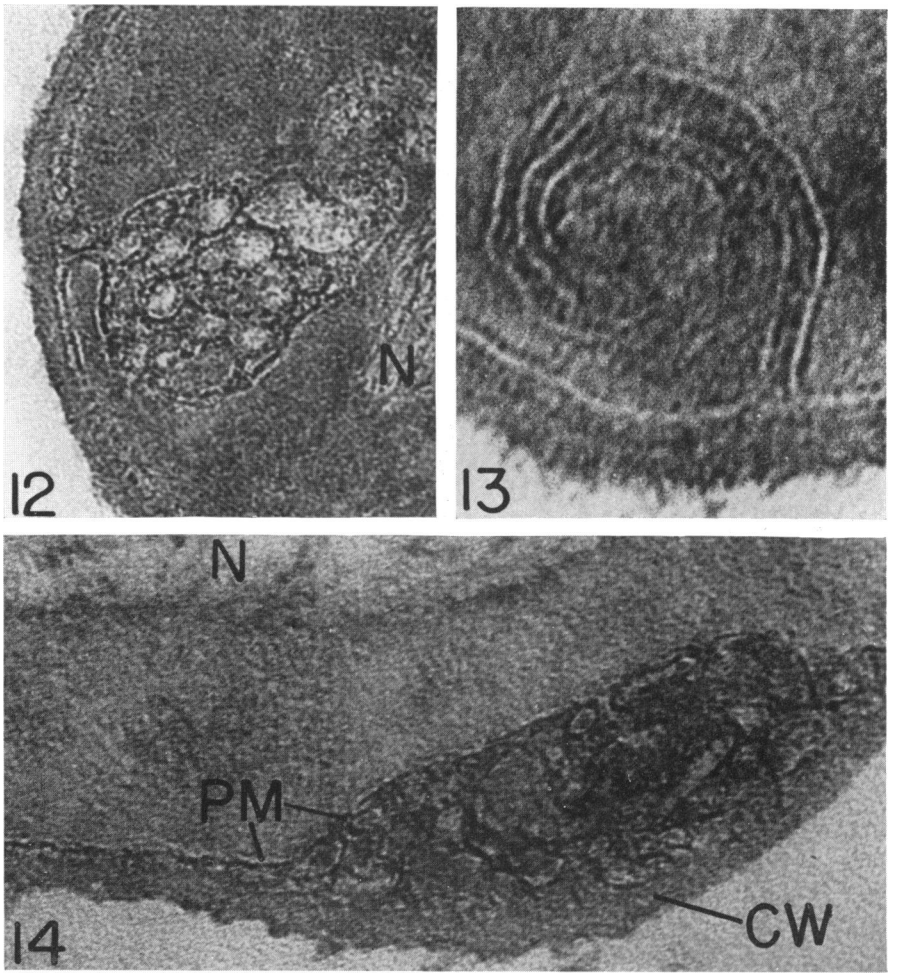

FIG. 12. *Peripheral part of a cell to show another example of the continuity between the layers of the plasma membrane and the plasmalemmosome. Nuclear fibrils are seen in the nucleoplasm (N). Treated with sucrose (30%) for 2 min prior to fixation. Section stained with chromyl chloride. Magnification, 200,000×.*

FIG. 13. *Small portion of the border of a cell greatly enlarged to show a spiral growth of the plasma membrane. Magnification, 400,000×.*

FIG. 14. *Small portion of the border of a cell. The plasma membrane (PM) in tangential section displays a convoluted design. Parts of the nuclear region (N) and of the cell wall (CW) are also indicated. Section stained with chromyl chloride. Magnification, 300,000×.*

found. Plasmalemmosomes, as we have designated such internal membranes (Edwards, 1962), are found in practically all the cells examined, no matter under what conditions the cells were grown, even in cultures kept under anaerobiosis for 2 weeks or longer, through many transfers (Fig. 10 and 15).

The location of the plasmalemmosomes inside the cytoplasm varies. They are found at cross walls (Fig. 3), as well as at any point along the periphery of the cell, at times reaching its center. The frequent finding of these organelles in the nuclear region may be more than mere coincidence (van Iterson, 1961). They are present before and after cell division, but their greater development occurs with the earlier steps of cytokinesis. The fact that they are frequently present at regions of cross-wall formation, as extensions of the plasma membrane at the inner rim of the ingrowing wall, is indicative of some relation with synthesis of wall material, as recently suggested for *Mycobacterium* sp. by Im-

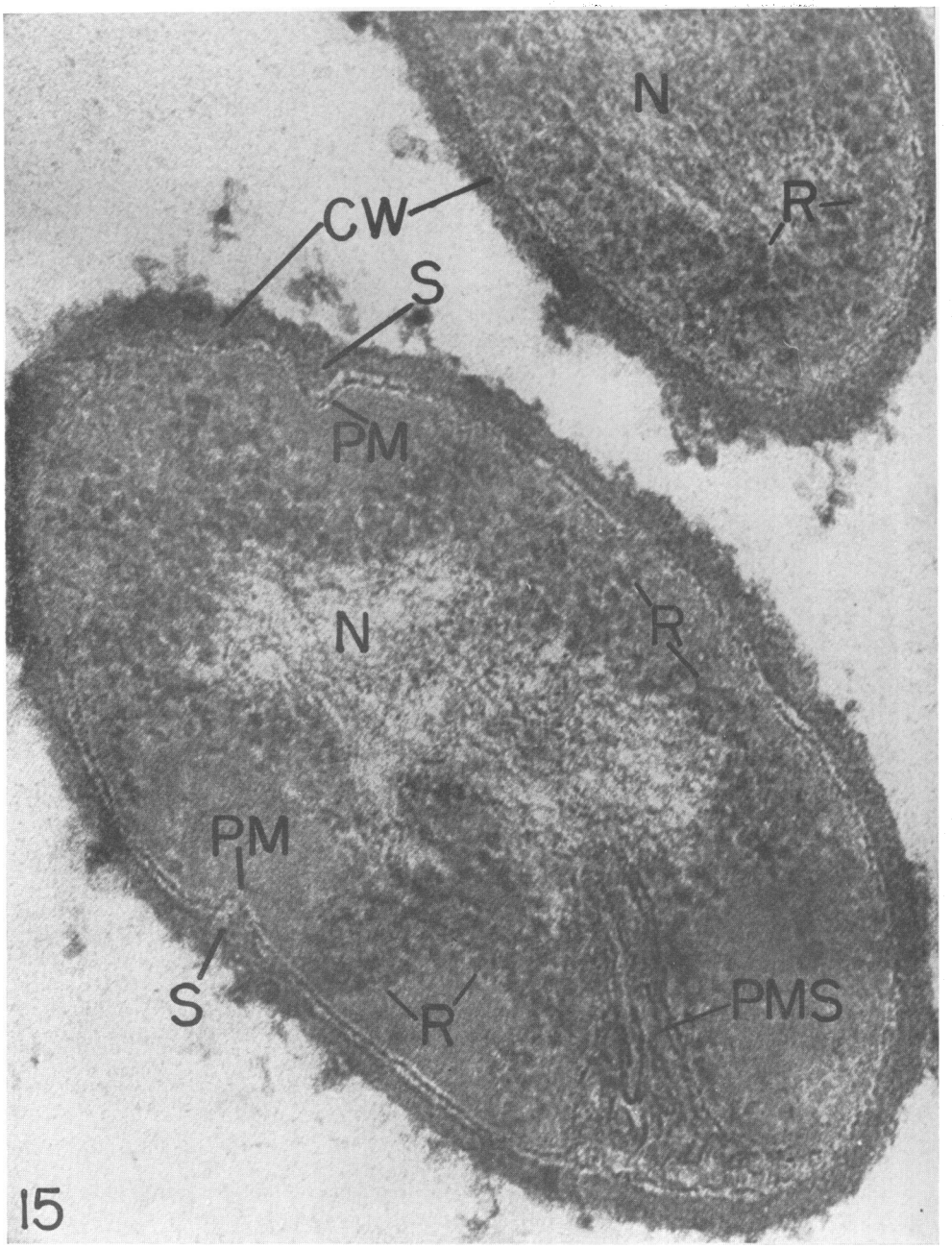

FIG. 15. *Cells grown anaerobically. Numerous ribosomes (R) are found in the cytoplasm around the nucleoplasm (N). The cell wall (CW), the plasma membrane (PM), and a plasmalemmosome (PMS) are also represented. Note that at the starting points of a septum (S), the plasma membrane appears as pockets inside of which the wall material is deposited. At this very early stage of septum development, typical plasmalemmosomes may be missing in that area. Magnification, 200,000×.*

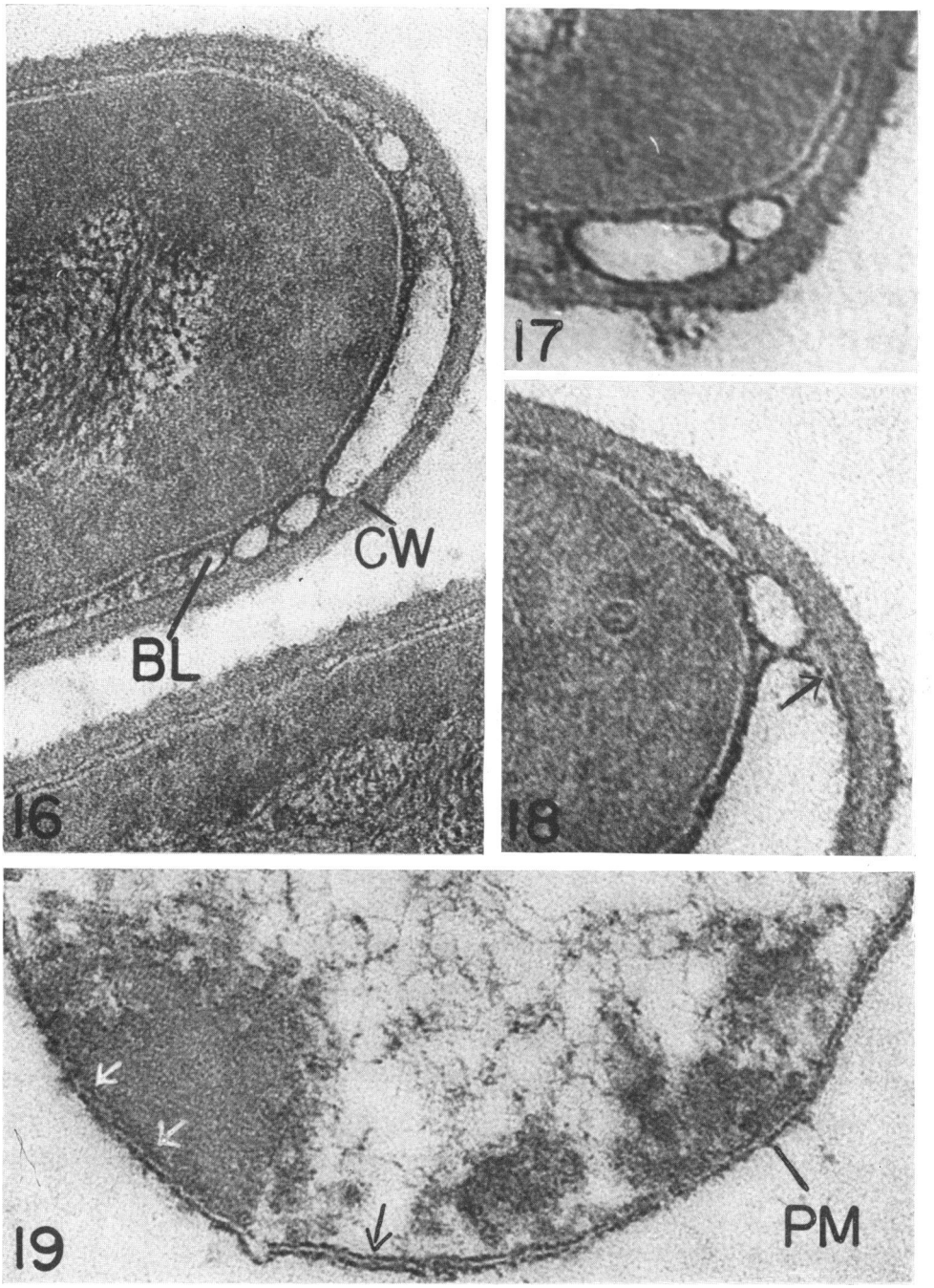

FIG. 16. *Parts of two cells submitted to hypertonic conditions. In the upper cell, the cytoplasm is pulled away from the cell wall (CW), the separation being initiated at the bridged layer (BL). The bridges are distended and seen as continuation between the middle and outer dense layers of the plasma membrane. Section stained with chromyl chloride. Magnification, 200,000×.*

FIG. 17 and 18. *Structure of the plasma membrane, which becomes more distinct at the early stages of plasmolysis, is shown in detail. Arrow in Fig. 18 points to the outer dense layer of the plasma membrane; at this point the inner limit of the cell wall may also be seen, as a layer of electron density similar to that of the adjacent layer of the plasma membrane. Magnification, 280,000×.*

FIG. 19. *Part of a free protoplast, of round shape, greatly enlarged in volume. The plasma membrane appears as a "unit" membrane (PM). Note that its innermost layer is readily seen at places where the ground cytoplasm is loose or absent (black arrow), but not visible or slightly outlined (white arrows) where mass of dense cytoplasm is present. Section stained with chromyl chloride. Magnification, 200,000×.*

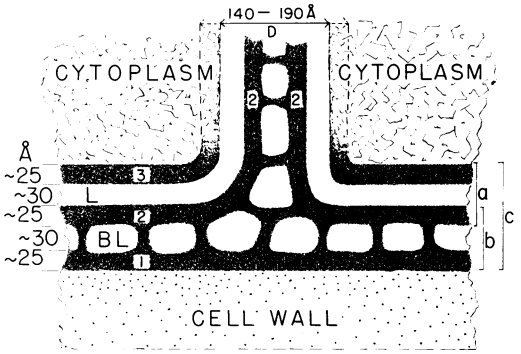

FIG. 20. *Diagrammatic interpretation of the plasmalemma and its ingrowth into the cytoplasm. Three dense lines (1, 2, and 3) alternate with two light zones (the bridged BL and the nonbridged L layers). The innermost dense line (3) is not always discernible but may be seen in heavily stained preparations, in lysed cells, and in protoplasts. According to interpretation of others, part* a, *as well as part* b (*each ca. 80 A*) *could be taken as the "unit" membrane, but in our interpretation the whole complex* c *corresponds to the plasmalemma (or plasma membrane). The outermost dense layer (1) is adherent to the cell wall and not found in the ingrowth. The diameter of the membranes of the plasmalemmosome (D), as measured in various micrographs, varies between 140 and 190 A.*

aeda and Ogura (1963). This correlation between bacterial organelles and cross-wall formation was first shown by Chapman and Hillier (1953), who demonstrated that the "peripheral bodies" moved centripetally after the appearance of septa in dividing cells of *B. cereus*. Sometimes the plasmalemmosome may have a chainlike appearance (Fig. 4), separating the two daughter cytoplasms, or it may appear as a ribbon (Fig. 8).

Cytoplasm. As in other bacteria (Murray, 1960; Glauert, 1962), the cytoplasm of *Listeria* may be packed with tiny dense granules (less than 100 A in diameter), sometimes in rows (Fig. 2) and appearing as short rough filaments. It is interesting that cells grown under anaerobic conditions frequently contain larger granules (100 to 200 A in diameter) located around the nuclear region, and sometimes apparently associated with nuclear fibrils (Fig. 10 and 15). In the latter case we believe it safe to identify the granules as ribosomes (ribonucleoprotein particles), since they resemble the ribosomes of higher cells in size and feature. Other usually conspicuous cytoplasmic structures, such as metaphosphate granules or lipidic bodies, were not found in our preparations of *Listeria*.

Nuclear apparatus. The nuclear apparatus or nucleoid of *Listeria* shows the same general features found in other bacteria (Chapman and Hillier, 1953; Kellenberger et al., 1958; Ryter and Kellenberger, 1958; Chapman, 1959*a*; Hopwood and Glauert, 1960; Robinow, 1962*a*). The nucleoplasm has a low density as compared with the cytoplasm, and it contains thin fibrils (25 to 50 A in diameter) which at times appear as rows of beads or twisted filaments (Fig. 1). A ropelike appearance, such as that found in a small coccus by van Iterson and Robinow (1961), is also seen in some of our micrographs (Fig. 1 and 8). Similarly, bundles of fibrils run parallel to one another or may be twisted in a helicoidal fashion. Evidence has been accumulating that such fibrils are sections of a long deoxyribonucleic acid molecule (Kellenberger, 1960; Kleinschmidt and Lang, 1961), coiled and unbroken inside the nucleoid, as in a ball of yarn. Such interpretation seems to agree with the "one circular chromosome" theory advocated by bacterial geneticists (Jacob and Wollman, 1961; Hayes, 1962). Giesbrecht (1961) described chromosomelike appearances in *B. subtilis*, which are at divergence with the view above. In *Listeria* the appearance and arrangement of the nuclear fibrils do not support any comparison with chromosomelike structures, such as those of higher cells. However, the appearance of the fibrils varies with the cell age, their morphological changes apparently following the steps of cell division. In Fig. 1, for instance, the cell is at an early stage of division, and its nuclear fibrils are very dense and conspicuous throughout the long nucleoid. Soon after the formation of the new nucleoids, the fibrils may still be clearly delineated (Fig. 8 and 9), but later they lose their identity, as was also observed by Glauert (1962).

DISCUSSION

The plasma membrane of *Listeria* is complex, involving more than the three standard (dense, light, and dense) layers usually attributed to a "unit" membrane (Robertson, 1959, 1960). A similarly complex structure was described by Fitz-James (1960) in members of the genus *Bacillus*. One of his interpretations was that the entire space between the cell wall and the cytoplasm is occupied by the plasma membrane.

Agreeing with this interpretation, the overall thickness of the plasma membrane in *Listeria*, as seen in cross sections, is close to twice that of a unit membrane, i.e., 120 to 150 A. In our diagrammatic interpretation, the plasma membrane has three dense layers (1, 2, and 3 of Fig. 20), each varying in width from 15 to 35 A. These alternate with two light zones (BL, the bridged, and L, the nonbridged, layers), each with an average thickness of 30 A. If part *a* of Fig. 20 is interpreted as a unit membrane, it would agree with the description by Glauert et al. (1961) of the plasma membrane in *B. subtilis*. According to these authors, the plasma membrane is separated from the wall by a space which is crossed at places by thin bridges of dense material. However, in the description of the same organism by van Iterson (1961), the outer layer of the plasma membrane is adjacent to the cell wall, thus corresponding to line 1 in our diagram. Van Iterson's (1961) interpretation (as indicated, e.g., in Fig. 14 of her article) would correspond to part *b* in our diagram (Fig. 20). In their study of a *Mycobacterium* sp., Imaeda and Ogura (1963) also arrived at the latter interpretation. Present attempts to reconcile these divergent points of view are impractical, owing to variation in techniques and to the necessarily subjective interpretation of electron micrographs. Our concept of the plasma membrane, as it appears in *Listeria*, corresponds to a doubling of the usual unit membrane found in some other bacteria. However, the limiting membrane of *Listeria* protoplasts (Fig. 19) comprises only a part of this complex, i.e., part *a* in Fig. 20. The latter may be assumed to account for the osmotic properties of the plasma membrane in the intact cell, since the protoplast appears to retain its physiological integrity. On the other hand, one might ask what is the functional role of the other part of the intact plasma membrane, the conspicuous bridged part which is bordered by the two dense layers (1 and 2 of Fig. 1 and 20). In the earliest stages of plasmolysis, we found (Fig. 17 and 18) a perfect continuation between these layers, the outermost of which is then distinct from the cell wall. Since the bridges are found also in the plasmalemmosomes (Fig. 5 and 6), these structures are probably not simply means of attachments to the wall, as stated by Glauert (1962). Finally, of all the layers represented in Fig. 20, the middle dense layer (or line 2), which appears most consistently in cross sections of bacterial plasmalemma, would seem to be the backbone of the entire structure (Fitz-James, 1960) regardless of interpretation.

As has been shown in other bacteria (Stuart, 1959; Glauert and Hopwood, 1959, 1960; Fitz-James, 1960; Giesbrecht, 1960; Glauert et al., 1961; Koike and Takeya, 1961; Edwards and Gordon, 1962*a*; Robinow, 1962*b*; Imaeda and Ogura, 1963), the plasma membrane of *Listeria* is also continuous with a system of internal membranes variable in size and shape. It has been proposed (Edwards, 1962) that these intracytoplasmic membranes be designated "plasmalemmosomes" to indicate their origin, and to avoid terms which have been in use for morphological entities of higher cells. The expression "peripheral bodies" (Chapman and Hillier, 1953) is not sufficiently descriptive for the many forms of cytoplasmic organelles observed in *Listeria*, and it does not indicate a relationship to the plasma membrane. Fitz-James (1960) introduced the term "mesosome" for organelles attached to the spore septum and also to the septum of dividing cells of *Bacillus* spp. The same designation has been used by Ohye and Murrell (1962). However, "meso" (or mesaxon of nerve fibers), as taken from Robertson (1959), was referred to as a "double membrane leading from some included structure to the outside" or "might also be used for cytoplasmic structures connected to the outside by a double membrane." None of these cases seems to apply to bacteria. In *Listeria*, there is no structure included in the cytoplasm and connected to the outside, as is the case in a mesaxon (Peters, 1960, Fig. 1; Peters, 1961, Fig. 1A). Furthermore, "mesosome" is a synonym for "phallosome," a characteristic structure in the male genitalia of *Culicidae* (Matheson, 1944, Fig. 6 and 7; Kenneth, 1960). Such a term does not seem appropriate for bacteria. The morphological identification of organelles of bacteria with mitochondria of higher cells was advanced by Mudd and his collaborators (Mudd et al., 1951; Mudd, 1954), has since been supported by some investigators (Shinohara, Fukushi, and Suzuki, 1957; Niklowitz, 1958; Shinohara et al., 1959; Chapman, Hanks, and Wallace, 1959; Giesbrecht, 1960; Drews, 1960), and questioned by others (Mitchell, 1959; Murray, 1960; Fitz-James, 1960;

Glauert, 1962). Our findings of similar plasmalemmosomes equally abundant in cells of *Listeria* grown either aerobically or anaerobically cast doubt upon the validity of their identification with mitochondria *propria*. In this laboratory, plasmalemmosomes have been found in other anaerobic and microaerophilic organisms such as *A. bovis* (Edwards and Gordon, 1962*a*) and *Lactobacillus arabinosus* (*unpublished observations*); the latter, as are other lactobacteria, is well-known to lack the cytochrome systems (Wood, 1961; Dolin, 1961*a*, *b*) characteristically found in mitochondria (Chance and Williams, 1956). The picture becomes even more confused if we consider those bacteria which contain cytochrome systems but are devoid of plasmalemmosomes; this is the case in *Escherichia coli*, which has not been convincingly demonstrated to have internal membranes. We examined five strains of *E. coli* and failed to find plasmalemmosomes in any of them. However, we do not suggest that the plasmalemma and plasmalemmosomes are devoid of enzymatic activities. On the contrary, it is tempting to speculate that in *Listeria*, as in other bacteria, these membranes serve as sites of multienzyme systems, not only those of the respiratory chain but also others involved in uptake and active transport of nutrients, in output of end products, and in synthetic processes such as the deposition of wall material.

The development of plasmalemmosomes in *Listeria* is undoubtedly associated with the plasmalemma. Although it has been impossible to ascertain whether the latter participates as a whole in the ingrowths, we believe that all layers but the outermost (1 in Fig. 20) are represented in the plasmalemmosome. As with the plasma membrane, the innermost layer of the plasmalemmosome is not always visible. Since the middle layer of the plasmalemma is the one most readily traced into the plasmalemmosome, the development of the latter is envisioned as starting by an infolding of the middle layer enclosing the bridged layer and being enveloped by the nonbridged layer. This is an oversimplified description, but it serves to interrelate these structures.

The highly developed plasmalemmosomes of *Listeria* are laminated (lamellar bodies), as found in mycobacteria (Koike and Takeya, 1961; Imaeda and Ogura, 1963) and in *Dermatophilus congolensis* (Edwards and Gordon, 1962*a*), or alveolar (honeycomblike), as seen in published micrographs (Takeya et al., 1959; van Iterson, 1961; Glauert et al., 1961), and yet of different configurations. The lamellar bodies, as interpreted by Koike and Takeya (1961) may be derived from a spiral ingrowth of the plasma membrane (Fig. 13). Such spirals as found in serial sections are visualized in three dimensions, as slices through a jelly-cake roll. The alveolar type may arise either from spiraled or straight elements; in the former case with secondary interconnections by means of transversal membranes and in the latter by branching or infolding of the original elements and by an anastomosing process (Fig. 6). In any case, plasmalemmosomes of older or resting cells frequently have a vesicular appearance, showing profiles of membrane-enclosed spaces, i.e., parts devoid of any visible structure. If at this point we recall that such spaced lamellar structures may be extended into most of the regions of the *Listeria* cell, we have here, in small dimensions, some of the characteristics of the endoplasmic reticulum of higher cells. This similarity, however, does not support a real identification of the former bacterial system with the latter, because the bacterial cell lacks a nuclear envelope which is conceptually necessary for the outgrowth of the endoplasmic reticulum proper (Porter, 1961).

Finally, despite its tiny size, *Listeria* has a remarkable system of intracytoplasmic membranes (plasmalemmosomes), which are similar to those described but not identical with larger bacteria (bacilli, mycobacteria, and actinomycetes). These membranes are in *Listeria* of sizes and configurations more variable than those mentioned above. They are also relatively more abundant. This may suggest a relation to the uncommon adaptive nature of this bacterium to different environmental conditions.

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