JOURNAL OF BACTERIOLOGY, Oct. 1967, p. 1189-1205
Vol. 94, No. 4

Fine Structure of *Bacillus megaterium* During Synchronous Growth

D. J. ELLAR,[1] D. G. LUNDGREN, AND R. A. SLEPECKY

Department of Bacteriology and Botany, Biological Research Laboratories, Syracuse University, Syracuse, New York 13210

Received for publication 17 July 1967

A fine-structure study of synchronously dividing *Bacillus megaterium* revealed the sequence of events involved in the division of the cell. First, a mesosome develops as a concentric fold of the plasma membrane at the site of septum formation. The mesosome contains membrane-bound vesicular structures, 300 to 500 A in diameter, plus a large membrane-bound structure, 2,000 A in diameter. These larger vesicles are peculiar to mesosomes in this stage of division and are not observed in the mesosomes involved in spore septum formation. The transverse septum originates within the mesosome and remains enclosed during its subsequent growth across the cell. An intimate association is observed between mesosome vesicles, mesosome membrane, and the growing edge of the transverse septum. Prior to completion of the septum, the membranes bounding the mesosome fuse, and further wall thickening occurs within the structure formed by this fusion. At this time, the septum only equals the parent cell wall in thickness. The doubling in thickness of the septum, which is required for the production of two normal daughter cell walls, occurs during a second phase of wall thickening, which is characterized by the appearance of a constriction at the base of the septum. As the constriction widens, the wall in this region thickens, forming the typical rounded poles of the daughter cells. Capsular synthesis at the poles occurs during this second phase of wall thickening. Throughout the division process, the nuclear material appears to be associated at one end with a mesosome at or near the pole of the cell and at the other end to the mesosome involved in septum formation. This association frequently takes the form of a stalk-like extension of the mesosome penetrating into the chromatin fibrils.

Electron microscopy of the division process in gram-positive bacteria has shown that the growth of the cross wall is preceded by the inward proliferation of the plasma membrane (13, 18, 30, 36, 39). Several workers have observed "peripheral bodies" or mesosomes in the vicinity of the developing cross wall (5, 11, 20, 23), and it has been suggested that they function in some manner in the orderly synthesis of cell wall material. Evidence to date, however, is not adequate to determine the relationship between the mesosome, the infolding plasma membrane, and the new cross wall, nor is it possible to determine the precise sequence of changes in fine structure which occur when the cell divides. The use of a synchronously dividing culture of *Bacillus megaterium* offers a more useful method for studying both the nature and time of appearance of these morphological changes during cell division.

[1] Present address: Department of Microbiology, New York University School of Medicine, New York, N.Y. 10016.

MATERIALS AND METHODS

The organism used was *B. megaterium* ATCC 19213 grown on a defined sucrose salts medium (SS) prepared according to Slepecky and Foster (37). For these studies, the medium was supplemented by the addition of 0.02% Trypticase (BBL).

Culture technique. Details of the culture procedures and the modified filtration method of Maruyama and Yanagita (25) which were used to obtain division synchrony have been previously reported (19). In the present experiments, when the culture reached late log phase (166 Klett units, measured with a no. 54 filter), the cells were collected on filters and aseptically pooled into a sterile 3-liter Fernbach flask and placed on a reciprocating shaker at 30 C. The entire filtration process took less than 5 min and was performed at room temperature.

Electron microscopy. Samples (10 ml) of the culture were removed from the Fernbach flask at intervals of 1 hr and were fixed immediately with osmium by the agar block method of Kellenberger, Ryter, and Séchaud (24). The procedures for postfixation and embedding were the same as those previously described for *B. cereus* (32). Sections were cut on an

MT-2 Porter-Blum microtome, transferred to carbon-coated copper grids, and poststained with lead hydroxide (42). Preparations were examined in a Phillips 200 microscope at 80 kv with a 20-μ objective aperture. Micrographs were made on Kodak (3.25 by 4 inches) Projector slide plates (contrast) and enlarged photographically. Final magnification of the micrographs is included in the figure legends.

RESULTS

In the growth system, after inoculation, the cells divided synchronously for 4 hr. At this time, sporulation began and was complete by the 10th hr after inoculation. Details of growth kinetics are published elsewhere (19). Cells sectioned during the period of synchronous division were observed to be in various stages of cross wall formation and to possess a varying number of mesosomes.

Structure of the mesosome. In *B. megaterium*, all mesosomes have the same basic structure and originate as an invagination of the plasma membrane which subsequently expands into the cytoplasm. Although they vary in size, all mesosomes appear to contain vesicular structures 300 to 500 A in diameter (Fig. 1–6) which are themselves bounded by a membrane similar in appearance to the plasma membrane. In this study, including some of the serial sections, concentric membrane lamellae were only rarely seen in the mesosomes; it would appear, therefore, that the structures seen are in fact true vesicles.

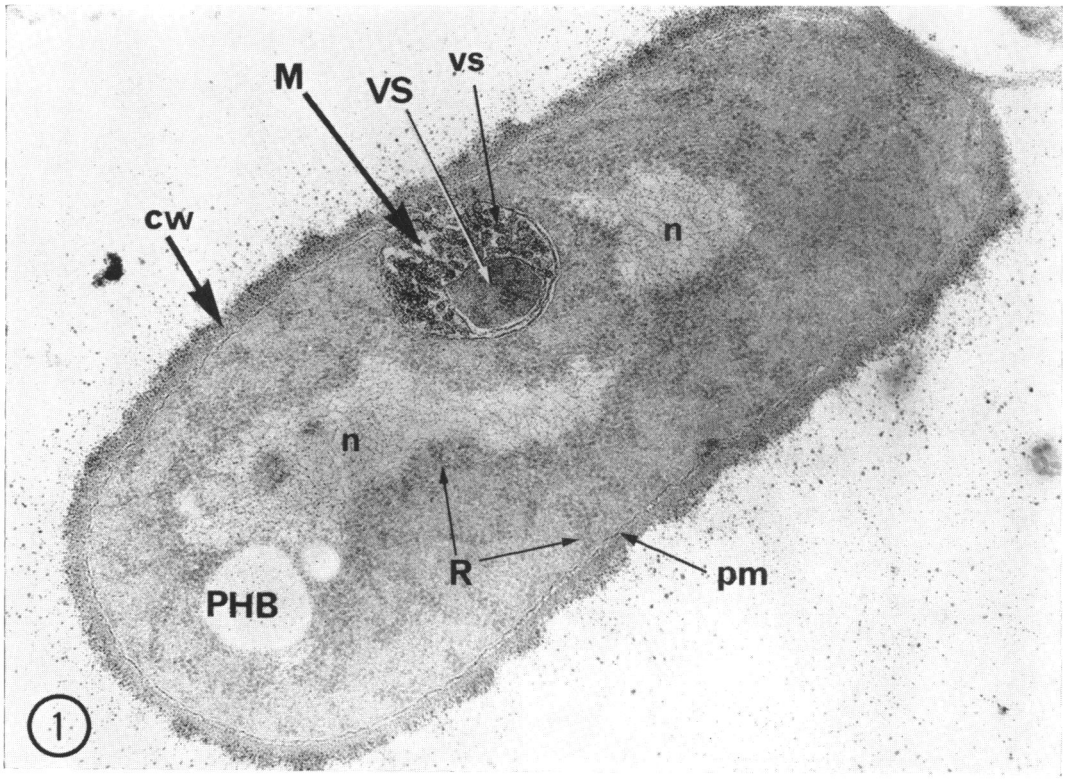
Cell division and the role of the mesosome. At the outset of cell division, a mesosome develops as a concentric fold of the plasma membrane at the site of future septum formation (Fig. 1, 2, 5). In cross section this therefore frequently appears as two separate structures. At this point in development, the membranes at the base of the mesosome are separated by a considerable distance (2,000 A), and the structure consequently appears to be in open connection with the space between the cell wall and the plasma membrane (Fig. 1, 2). As the mesosome enlarges, a second distinct structure is observed within the mesosome in addition to the 300 to 500 A membrane-bound vesicles. This is a much larger membrane-bound vesicle, approximately 2,000 A in diameter, and is filled with granular material (Fig. 1, 2, 5, 6). The chemical composition of this material has not been determined. Except for these two structures, the mesosome contains no additional visible material. The new cross wall begins to appear at the base of the mesosome, projecting into the cytoplasm at right angles to the old cell wall (Fig. 3, 4, 5, 18, 19). At the same time, the space at the base of the mesosome narrows considerably, so that the only connection between the contents of the mesosome and the extracytoplasmic region is through the 30 to 60 A space which separates the plasma membrane from the cell wall (Fig. 4).

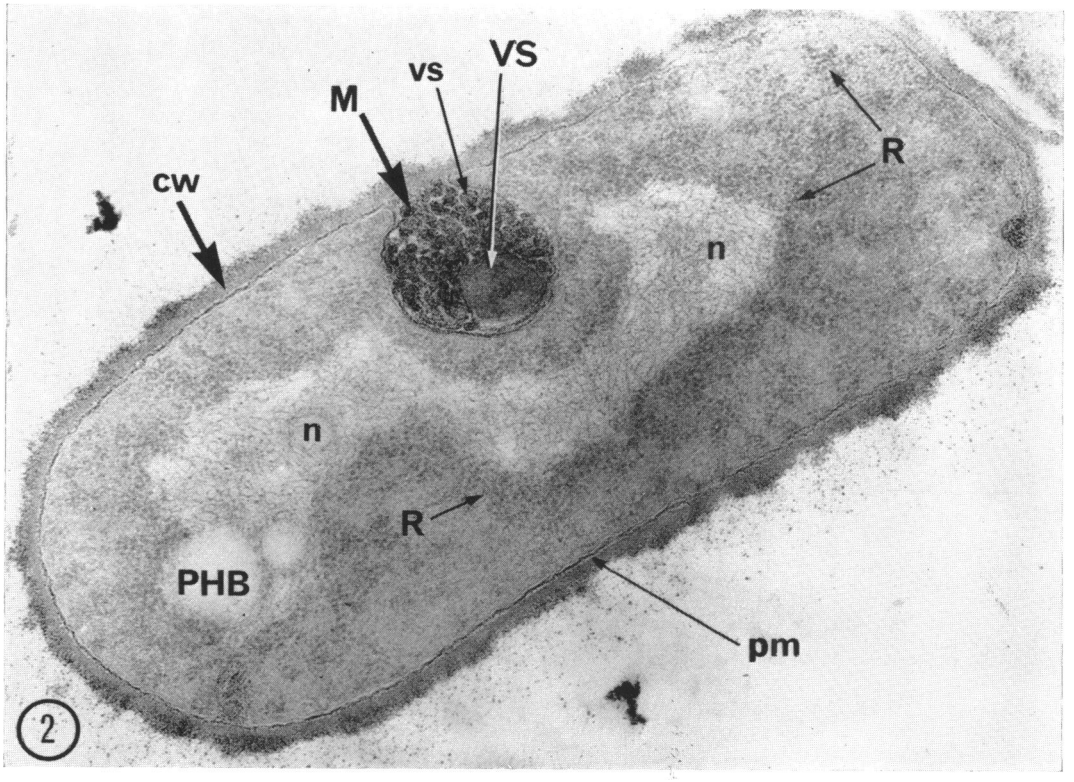
This stage and the subsequent events leading to the completion of the new cross wall are characterized by an intimate association between mesosome vesicles, mesosome membrane, and the growing edge of the transverse septum. Figure 4 shows this association to be such that the mesosome membrane is folded through 90° to cover the growing edge. At its base, the newly developing transverse septum measures approximately 300 to 400 A, which is the same thickness as that of the parent cell wall. At the growing edge, however, the septum is generally only half this thickness (Fig. 3, 4). Before the transverse septum is complete, the extension of the plasma membrane over the surface of the mesosome fuses, and the final stage of this wall synthesis occurs within the structure formed by this fusion (Fig. 6). The exact point at which this fusion occurs has not been determined, but it probably occurs early in development when the new wall has progressed half the distance to the cell center. An exact determination of the time of fusion requires further careful analysis of serial sections, since what appear to be separate mesosomes are frequently revealed in serial sections to be fused. After fusion, it is interesting to observe that the resulting structure is bipartite, with each half distributed to opposite sides of the developing septum (Fig. 6). The growing region of the transverse septum is half the thickness of the completed wall (Fig. 6), and the region of synthesis is in intimate contact with the vescular contents of the mesosomes.

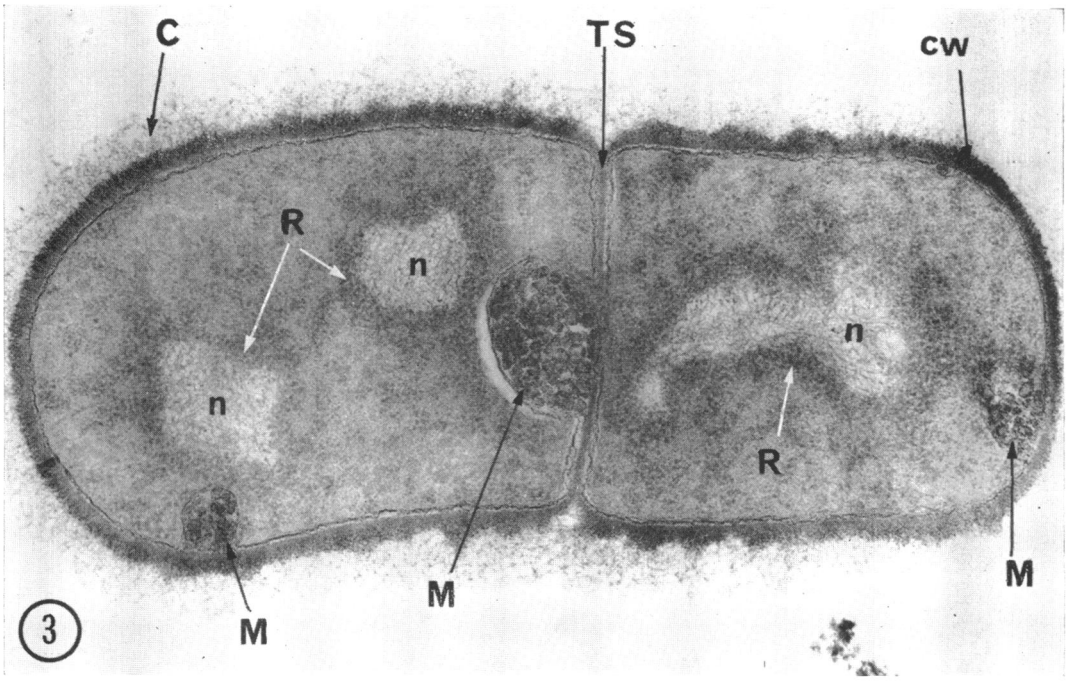
Even after the new transverse septum was continuous across the cell, it remained only 300 to 400 A thick. If two normal daughter cells are to be formed, this new wall must double in thickness at some later stage. Since this organism is surrounded by a thick capsule (Fig. 20, 21), this too must be formed at a later time. An examination

FIG. 1. *Section of a cell at 2 hr demonstrating the cell wall (cw); plasma membrane (pm); poly-β-hydroxybutyrate granule (PHB); nuclear material (n) arranged in the form of axially disposed filaments or fibrils. A layer of densely stained ribosomes surrounds the nucleus and extends to the plasma membrane in the form of strands (R). The mesosome (M) at the site of septum formation contains 300 to 500 A vesicular structures (vs) together with a 2,000 A vesicle (VS).* × *61,000.*

FIG. 2. *Serial section of the cell in Fig. 1 demonstrating the continuity of mesosome (M) structure. The axially disposed nuclear material (n) is now revealed as a continuous filament.* × *60,000.*


M
VS
vs
cw
n
n
R
pm
PHB
1


M
vs
VS
R
cw
n
n
R
PHB
pm
2



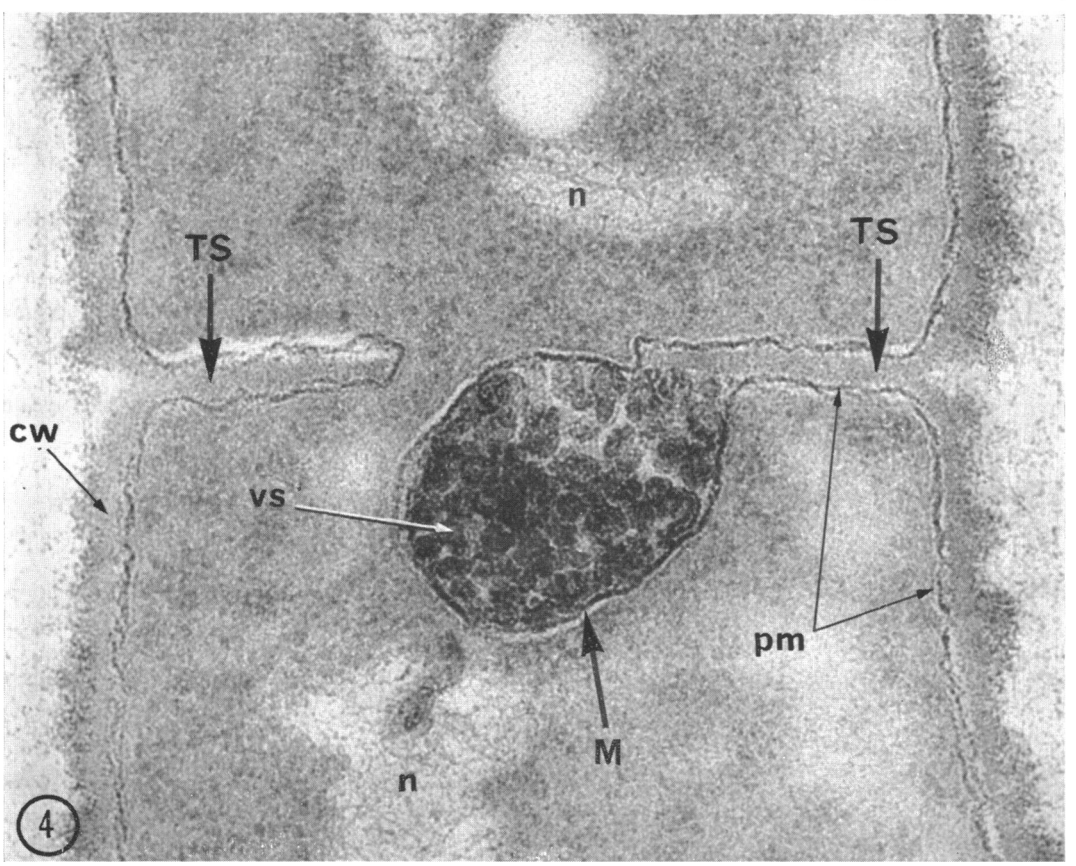
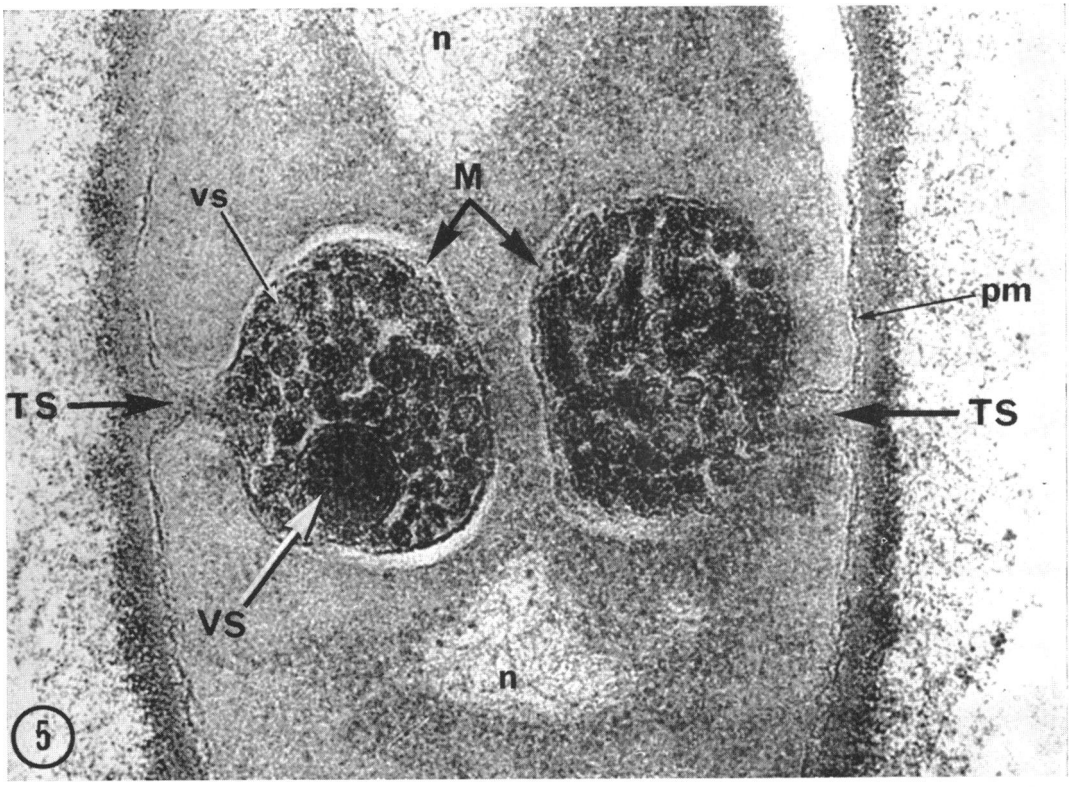


FIG. 3. *Section of a cell at 3 hr demonstrating the developing transverse septum (TS) enclosed within the infolding plasma membrane bounding the mesosome (M). Additional mesosomes (M) are seen to be close to the nuclear material (n) at the poles of the cell. Capsular material (C) surrounds the cell. See legend of Fig. 1 for explanation of other abbreviations.* × *50,600.*

FIG. 4. *Part of a serial section of the cell in Fig. 3 demonstrating the close association between the transverse septum (TS), the plasma membrane (pm), and the mesosome vesicles (vs). An association between nuclear material (n) and mesosome (M) can also be seen.* × *115,000.*



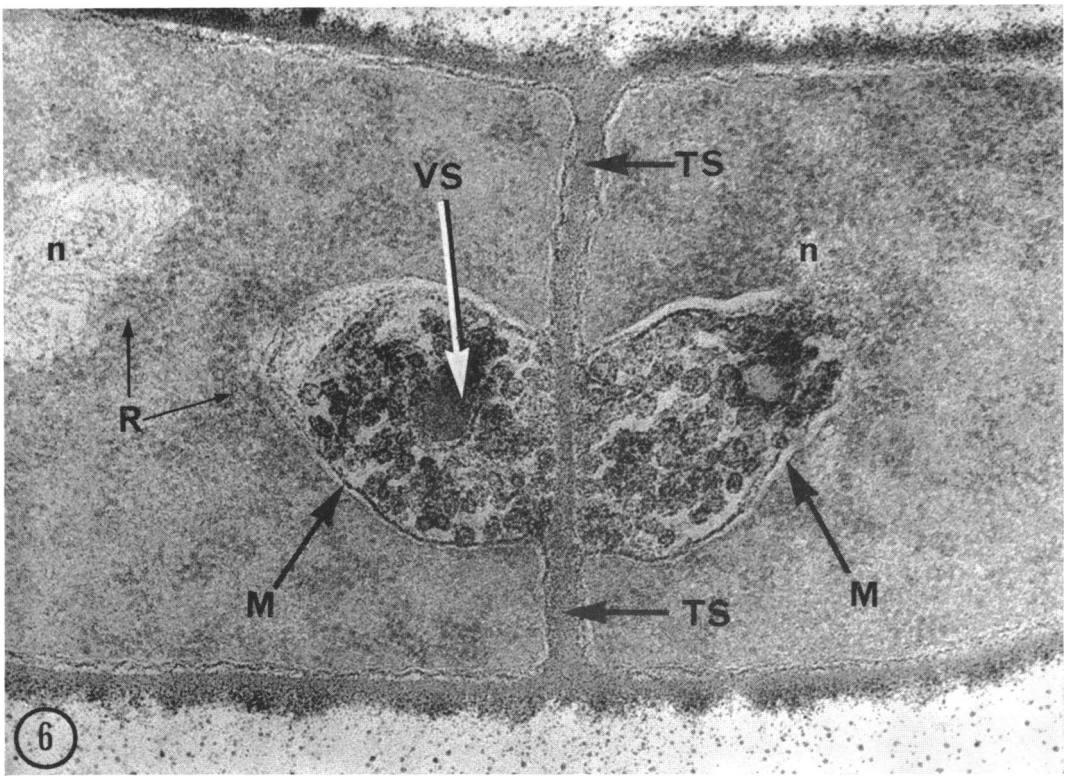


FIG. 5. *Part of a section of a cell at 3 hr demonstrating the structure of the concentric mesosome (M) just prior to fusion of the membranes bounding it. Division of the nuclear material (n) has taken place at this time. The mesosome contains both types of vesicular structure described in Fig. 1.* × *106,000.*

FIG. 6. *Part of a section of a cell at 3 hr where the membranes bounding the mesosome have fused, forming a bipartite structure. Half of the resulting structure is distributed to each side of the transverse septum (TS) which is now continuous across the cell. At its center, the completed septum is only half the thickness of its base.* × *92,000.*

of thin sections revealed that both the wall thickening and capsule formation occur during the process of separation of the two daughter cells, which in this organism takes place some time after the completion of the transverse septum. This separation begins with the appearance of a constriction between the subsequent daughter cells (Fig. 8, 9, 10), which initially contains no capsular material. At the same time, the cell wall begins to thicken in the constriction region (Fig. 11, 12, 13). The continued constriction between the cells is marked by the appearance of capsular material and the further thickening of the wall until the pole of the cell assumes its characteristic rounded shape (Fig. 14). The cell wall does not achieve its normal thickness, however, until constriction is complete and the two daughter cells have completely separated.

Connection between dividing chromatin and mesosomes. The use of a synchronously dividing culture facilitates a more efficient analysis of several features of actively dividing cells. The cell's nuclear material appears in the form of an axially disposed filament of fine fibrils 15 to 20 A in diameter (Fig. 1, 2, 20, 21). On occasion, a cell at the outset of division appears to contain two distinct chromatin regions, but, when serial sections are analyzed, the chromatin is revealed as a continuous filament (Fig. 1, 2). Several reports in the literature (20, 23, 35) indicate the existence of a connection between the bacterial chromatin and the plasma membrane via the mesosome. Although a statistical analysis is lacking, it appears from the micrographs (Fig. 3, 4, 15–19) that, when the cell is actively dividing, the chromatin is attached at one end to a mesosome located at or near the cell pole and at the other end to the mesosome involved in transverse septum formation. The nature of this attachment is not apparent, but, in some instances (Fig. 6, 15–19), the mesosome or some part of it appears to be embedded in the chromatin fibrils. Frequently this connection takes the form of a densely staining stalk-like structure arising from the membrane bounding the mesosome (Fig. 15–19).

Capsular structure. B. megaterium possesses a well-defined capsule continuous over the entire cell surface, which is demonstrable as strands of granular material approximately 25 to 45 A in width (Fig. 20, 21). In some micrographs these strands appear to intertwine to form a reticulum-like structure (Fig. 21). The thickness of this capsule is approximately 1,000 A, compared to 400 A for the cell wall. Accurate measurement is frequently rendered difficult, however, for there is no clear distinction between the origin of the capsule and the outer surface of the cell wall, and, at higher magnifications (Fig. 24, 25), similar granules (30 to 40 A) are seen in the cell wall.

Fine structure of the plasma membrane and cytoplasm. A careful examination of the plasma membrane and its mesosomal extension stained with lead hydroxide reveals an asymmetric structure (Fig. 1, 4, 7, 11, 15, 25). Although the membrane was composed of the typical dark-light-dark pattern, the outer dark band facing the cell wall invariably appeared approximately twice as thick (35 A) and more heavily stained than the inner band (20 A) surrounding the cytoplasm. In several instances, micrographs revealed that both dark bands appeared to be composed, at least in some regions, of spherical subunits whose diameter equalled the thickness of their respective layers (Fig. 24, 25).

Although the structure of the bacterial cytoplasm with its constituent ribosomes appeared typical, the ribosomes did exhibit a differential staining depending on their location within the cell. The chromatin was invariably surrounded by a layer of densely staining ribosomes 800 to 1,000 A thick (Fig. 1, 2, 3, 20). From this layer, strands of these densely staining ribosomes radiated out toward the plasma membrane.

Spore septum formation. Spore formation begins at 4 hr and is complete by the 10th hr after filtration. Initially, the fine structure of this process resembles transverse septum formation in that a concentric invagination of the plasma membrane leads to the formation of a vesicular mesosome with an associated segregation of chromatin material. In sporulation, however, no cell wall is synthesized at the base of this invagination, and the membranes bounding the mesosome progress toward the cell center where they fuse, forming the double membrane of the spore septum (Fig. 21). These mesosomes were devoid of the 2,000 A granular structure seen in the mesosomes concerned with transverse septum development. Upon completion of the spore septum, it rounds out and proliferates toward the cell pole forming the forespore membrane and enclosing the spore chromatin. The subsequent stages of spore maturation follow the pattern already described (12, 13, 30, 35).

DISCUSSION

The results of this investigation demonstrate the precise nature of the association between the developing transverse septum and the mesosome. It is apparent that the growing cross wall originates within the area enclosed by the mesosome, and moreover that the subsequent inward growth of the wall is preceded by a corresponding inward proliferation of the mesosome. This meso-

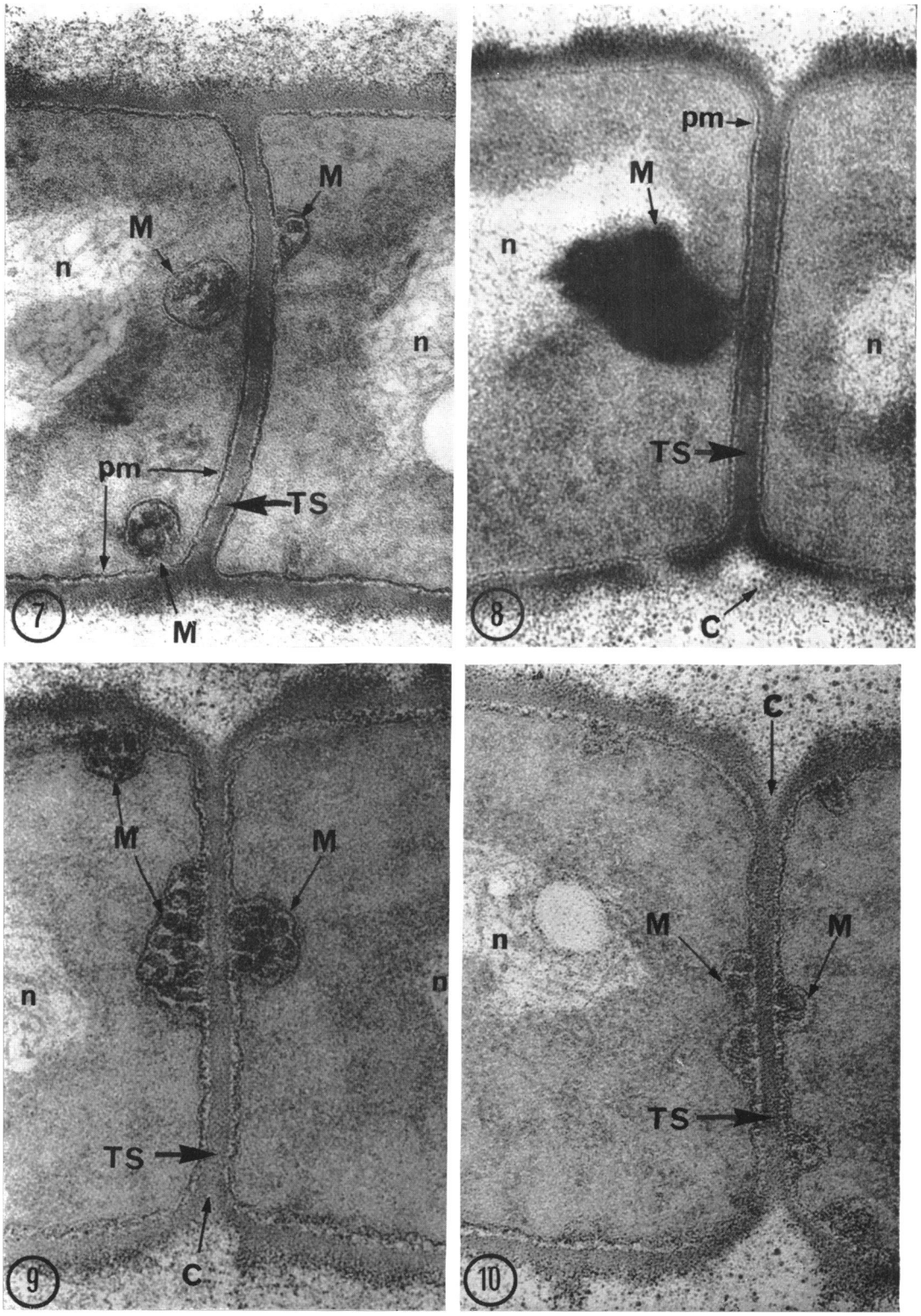


FIG. 7–10. *Series of sections of the newly formed transverse septum, demonstrating the appearance of a constriction between the cells and the initiation of wall thickening. The mesosomes (M) as described in Fig. 6 persist during this process. See legend of Fig. 1 for abbreviations. Fig. 7 and 8, × 107,000; Fig. 9 and 10, × 100,000.*

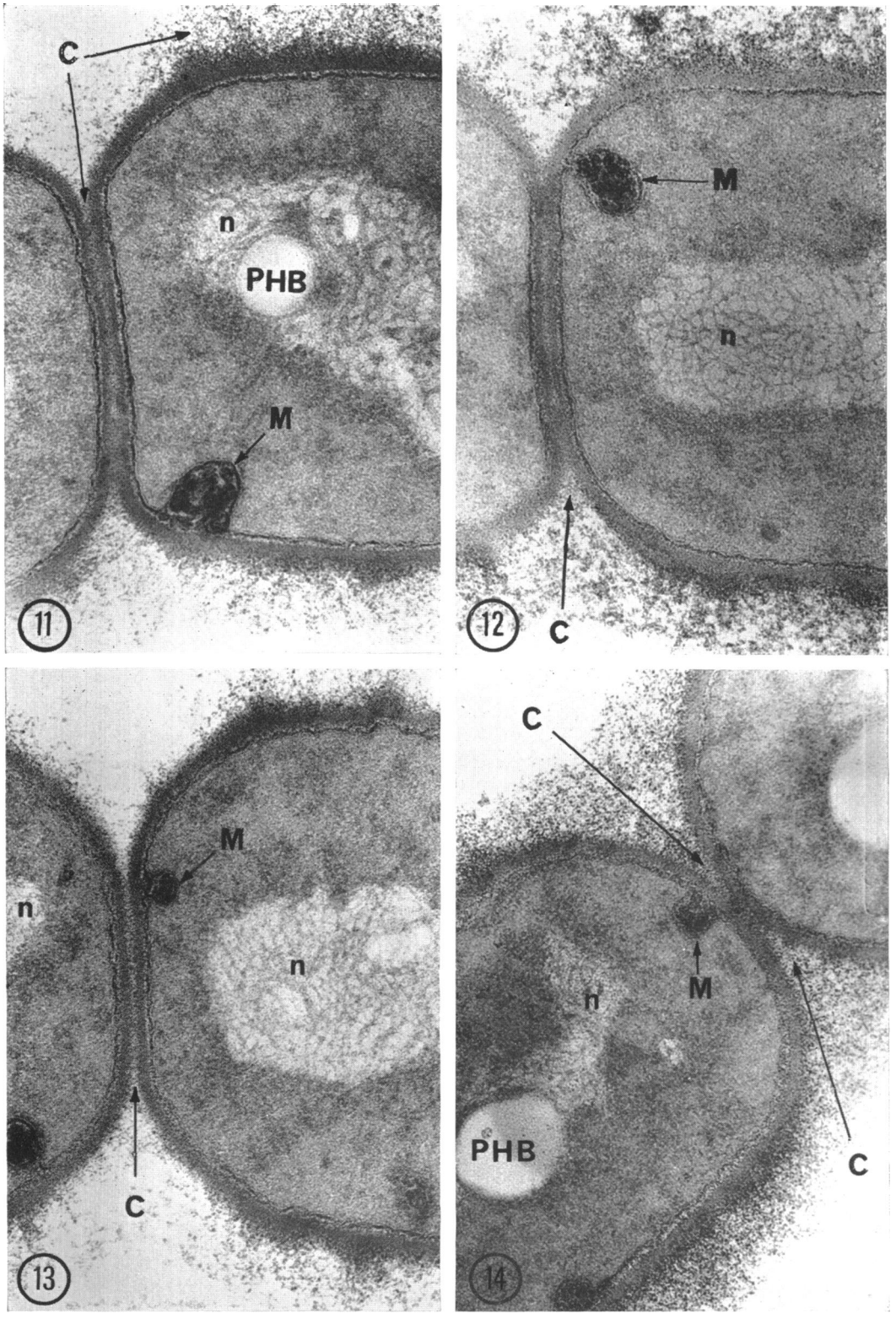
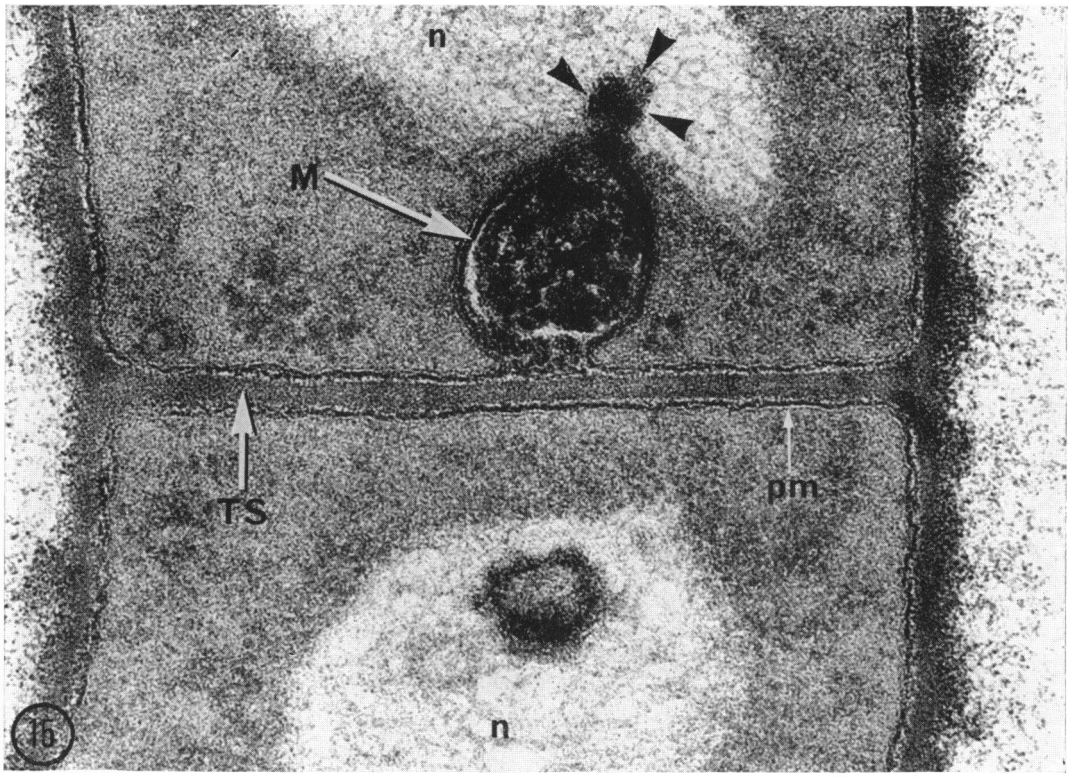
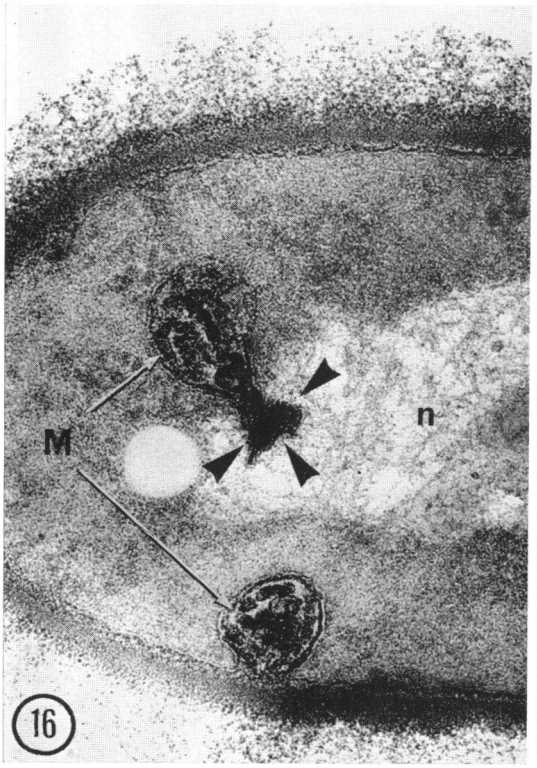


FIG. 11–14. *Series of sections demonstrating the final stages of constriction, a wall thickening, and capsule formation which lead to the production of two mature daughter cells. Wall thickening is seen to progress towards the pole. Fig. 11, × 107,000; Fig. 12 and 13, × 100,000; Fig. 14 × 70,000.*





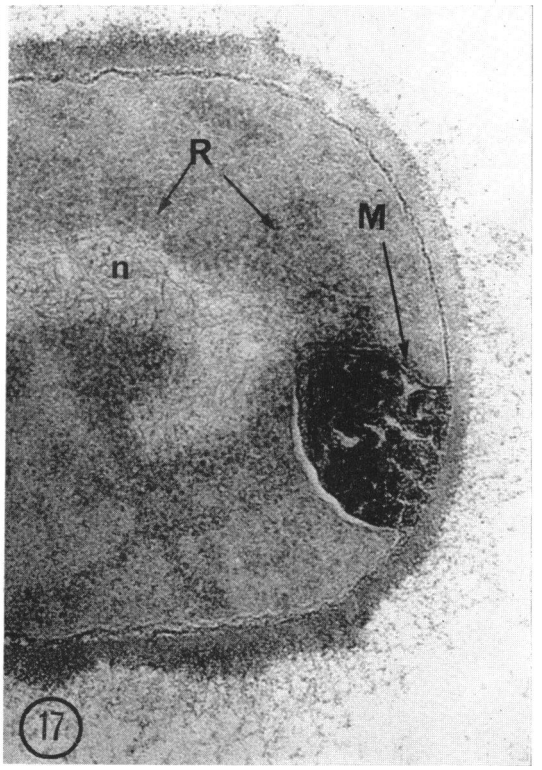


FIG. 15. *Part of a section through the completed transverse septum demonstrating the densely stained stalk-like extension of the membrane bounding the mesosome (triple arrow heads) penetrating into the nuclear fibrils.* × *110,000.*

FIG. 16. *Part of a section through the pole of the cell demonstrating a stalk-like extension of the membrane bounding the mesosome in this region (triple arrow heads) penetrating into the nuclear fibrils.* × *109,000.*

FIG. 17. *Part of a section through the pole of the cell demonstrating the close association between the membrane bounding the mesosome (M) and the nuclear material.* × *77,000.*

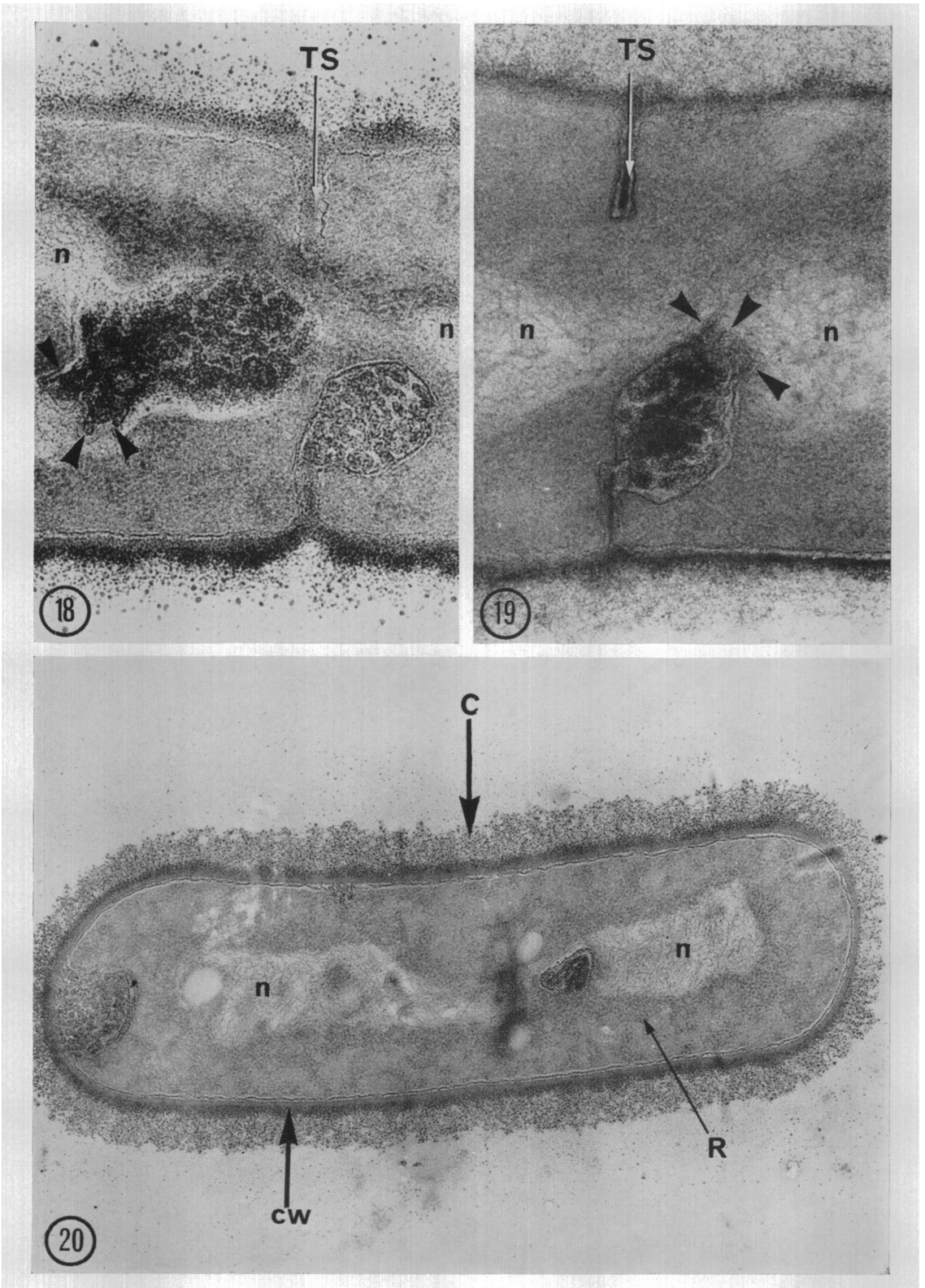
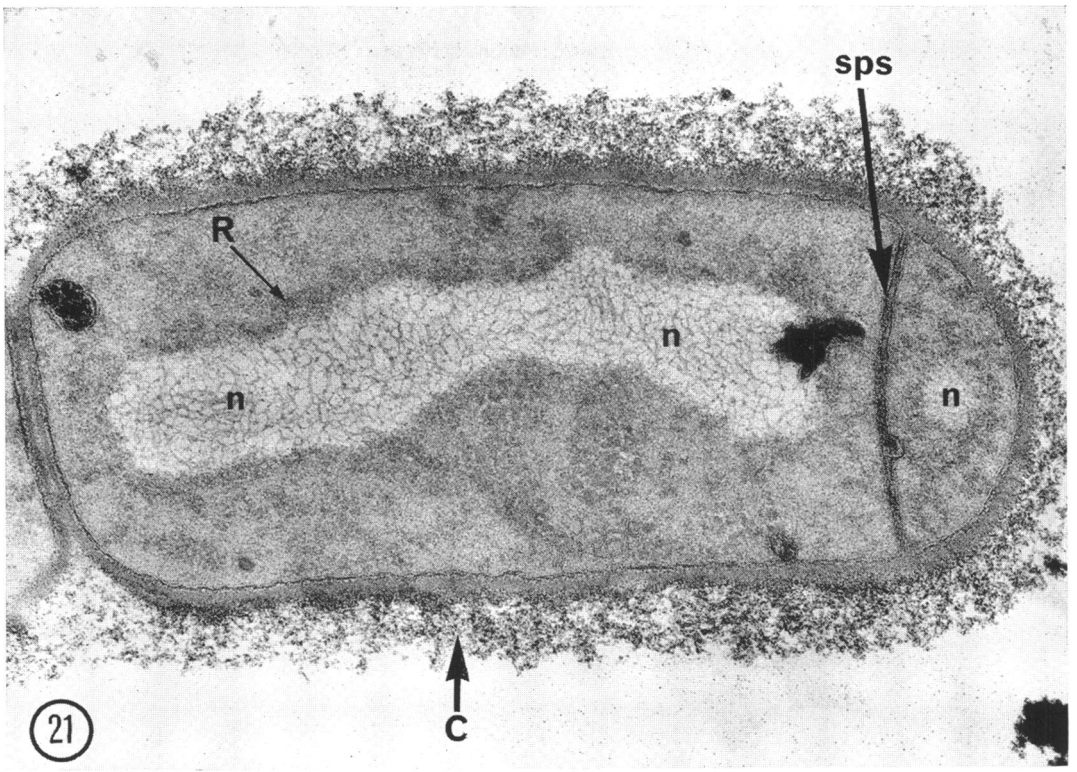


FIG. 18 and 19. *Portions of sections through the developing transverse septum (TS) demonstrating densely stained stalk-like extensions of the membrane bounding the mesosome (triple arrow heads) penetrating into the nuclear material. Fig. 18, × 81,000; Fig. 19, × 72,000.*

FIG. 20. *Section of a cell at 1 hr demonstrating the well-defined capsule (C). Densely stained ribosomes (R) can be seen surrounding the nuclear material (n) and extending outwards to the plasma membrane. × 40,000.*



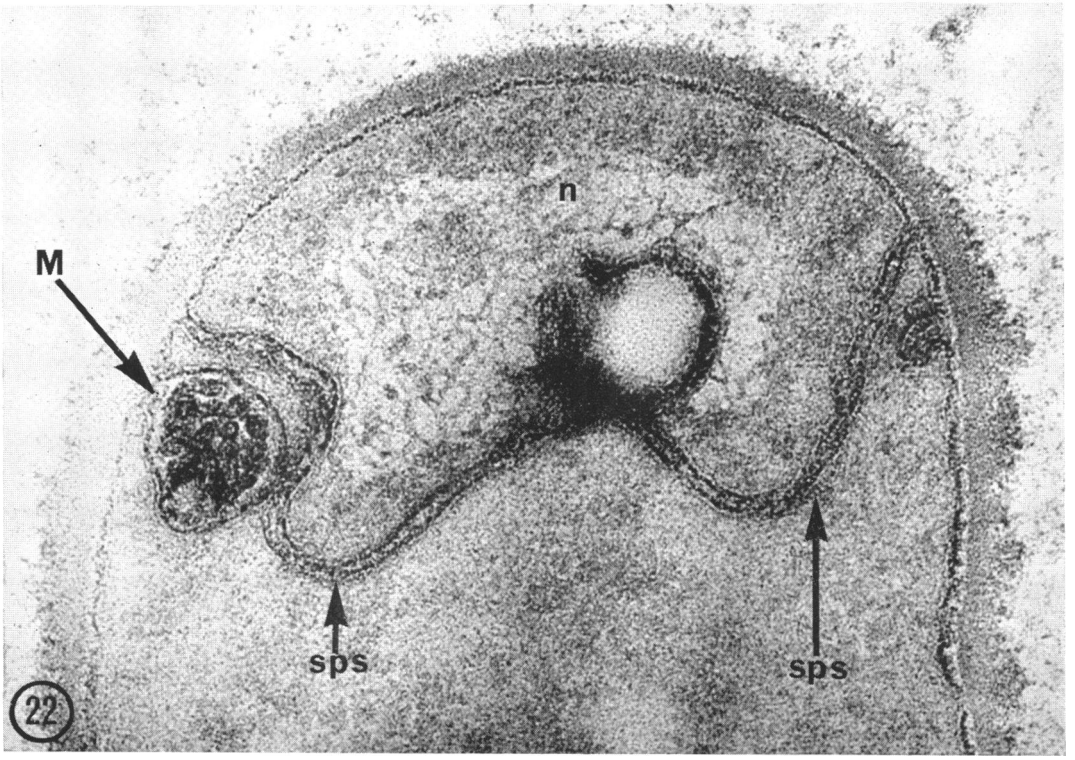
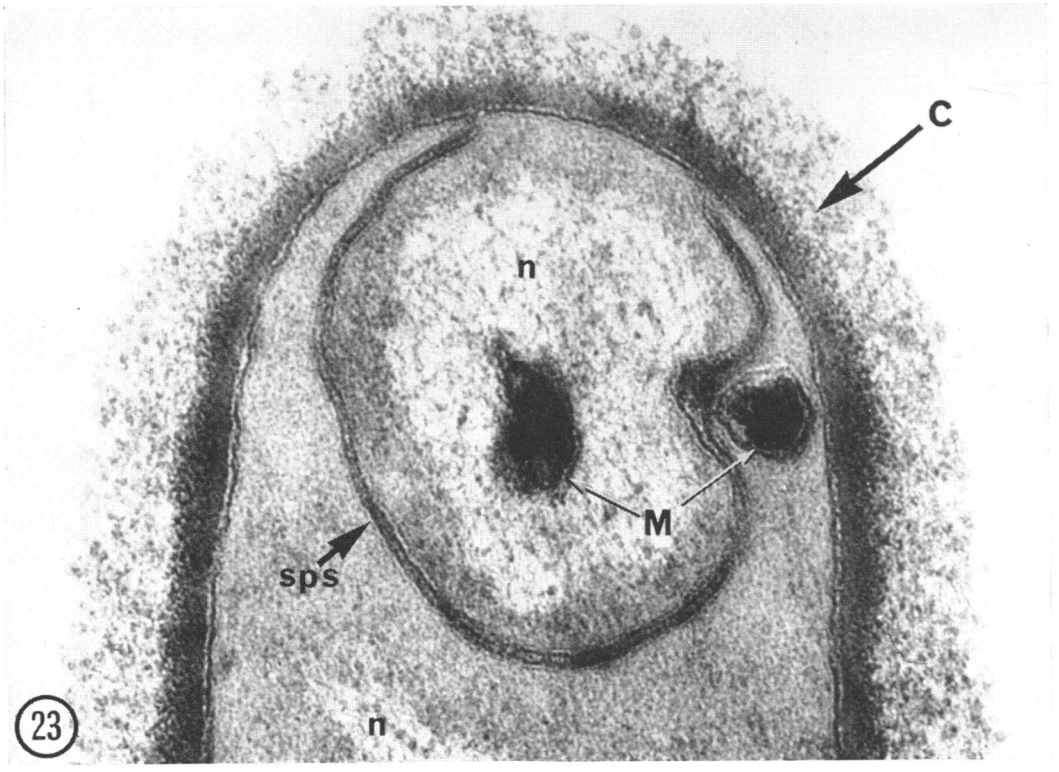


FIG. 21. *Section of a cell at 6 hr demonstrating the completed spore septum (sps). Division of the nuclear material (n) has occurred with subsequent segregation into the developing forespore. The typical capsule (C) is seen to persist through this stage. Densely stained ribosomes are again apparent.* × *51,000.*

FIG. 22. *Enlargement of the terminal portion of the developing forespore illustrating the folding of the spore septum (sps) and its movement towards the cell pole.* × *126,000.*



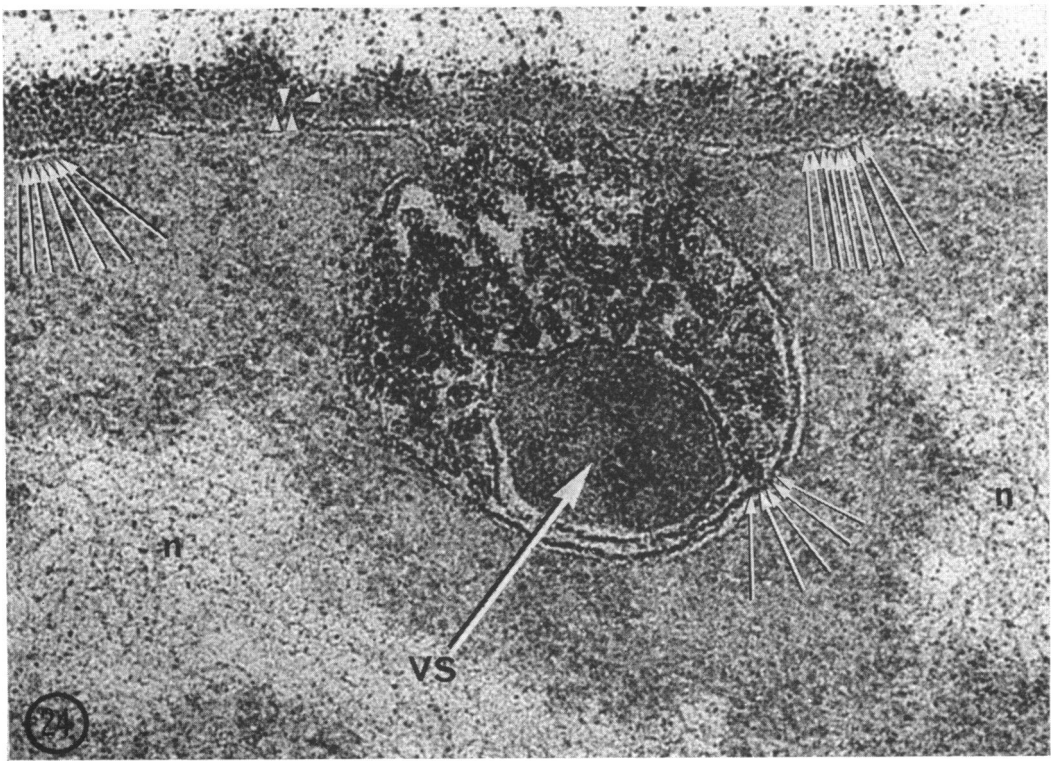


FIG. 23. *Enlargement of the terminal portion of the forespore demonstrating a later stage in the process described in Fig. 22.* × *102,000.*

FIG. 24. *Enlargement of part of the concentric mesosome from Fig. 1. Approximately spherical subunits can be seen in the inner and outer layers of the membrane bounding the mesosome and of the plasma membrane (small arrows). Granules are also evident within the cell wall (triangles).* × *305,000.*

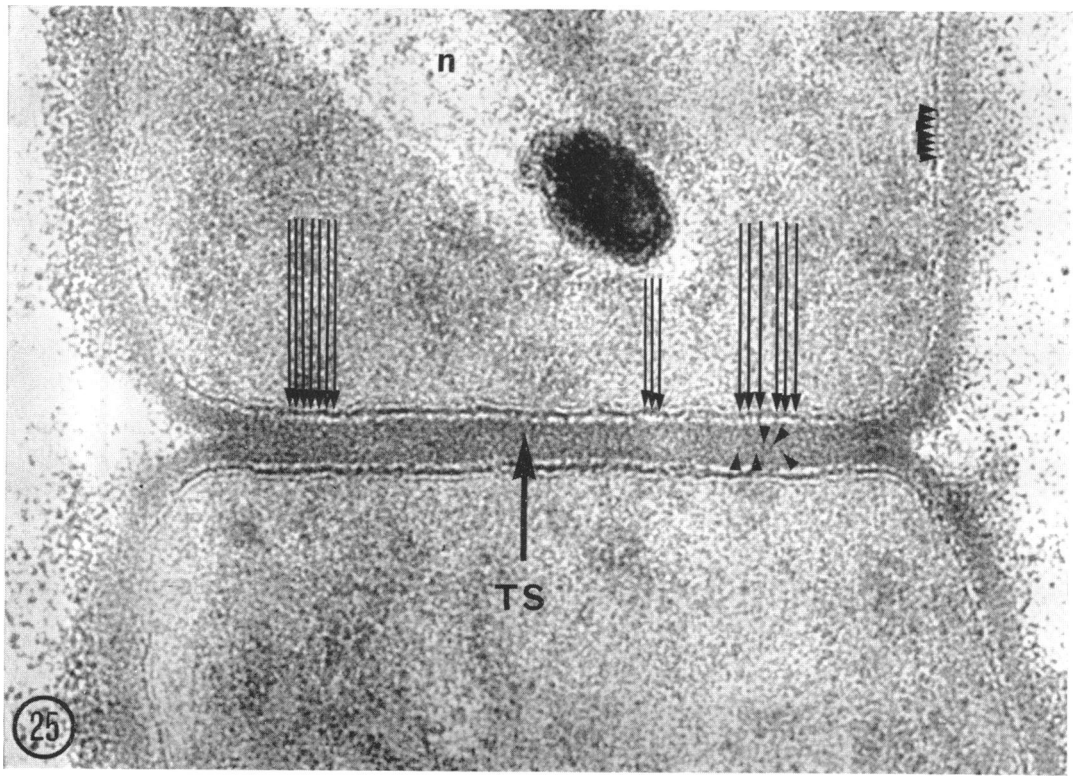


FIG. 25. *Enlargement of the completed transverse septum (TS) demonstrating subunits in the outer layer of plasma membrane (small arrows) and the granular nature of the cell wall (triangles).* × *140,000.*

some is formed by a concentric infolding of the plasma membrane which subsequently expands into the cytoplasm. The transverse septum is thus isolated from the remainder of the cytoplasm during the entire period of its development.

Cole and Hahn (9) have suggested that, in streptococci, moving equatorial membrane sites are responsible for laying down new wall material, whereas Chung et al. (7) found that single or multiple membrane areas in the center of the rod were the active sites of cell wall synthesis in *B. megaterium*. Both studies used immunofluorescent techniques and, as Fitz-James (14) has pointed out, they do not determine whether the septal membranes play an active role in wall synthesis. Discussing the partitioning of the genome, Mazia (26) suggested, "that the process of the partitioning of all kinds of cells in division employs the same general principle: formation of a partition by feeding membrane substance from within." That this is the case in *B. megaterium*, is evident from the micrographs which show that the structure resulting from the fusion of the membranes bounding the mesosome is partitioned and distributed to opposite sides of the newly developed transverse septum. However, the question which could not be resolved until now concerned the process by which the growth of the new cell wall was directed in space. Rogers (34) has proposed that the arrangement of previously deposited wall components controls this directional synthesis, with the membrane merely supplying the necessary biosynthetic machinery. Implicit in this suggestion is the further possibility that the growth of the transverse septum controls the direction of growth of the underlying membrane. Our observation that the development of the mesosome precedes the appearance of any cross wall material would seem to indicate that the *initiation* of the wall, at least, is determined by the membrane. This of course does not rule out the possibility that subsequent transverse septum development is directionally determined by this initial growing wall region. It is clear from the micrographs, however, that the spatial relationship between the developing septum and the membrane is indeed a close one. This pattern of septum development appears to differ from that observed

by Chapman (5) in an unidentified organism. However, in this early study, the resolution of wall and membrane was not sufficient to distinguish satisfactorily between the two structures.

As the mesosome, followed by the growing transverse septum, moves toward the cell center, the membranes at the base of the mesosome continue to form around the newly synthesized cell wall. This apparent spatial isolation of the developing transverse septum by the mesosome stimulates even more interest in the contents of this structure. Added to the morphological localization described here is the requirement for a localization of the biochemical processes leading to the growth of the wall and membranes. Evidence for this has been discussed by Rogers (34) and includes the demonstration by Fitz-James and Hancock (15) that the initial lesion caused by both penicillin and streptomycin in logarithmically growing cells consists of a distortion of the normal transverse septum-wall interrelationship. In the light of this information, the structures which we observe in the mesosome are especially interesting. The requirement for new membrane synthesis could conceivably be supplied by the large numbers of 300 to 500 A membrane vesicles. Mazia (26) noted the alignment and fusion of membrane-bound vesicles which occur in the partitioning of animal cells and suggested that a similar process might occur in the bacteria. Using short exposures to potassium tellurite, Jacob et al. (22) have succeeded in labeling the membrane of growing *B. subtilis* cells. Their results showed that during subsequent growth the tellurite was not diluted randomly, but was located at the cell poles and as a strip in the central region of the cell.

The means by which cell wall precursors are assembled into the form of a new transverse septum is not known. The large 2,000 A membrane-bound vesicles which we observe within the mesosome contain material resembling the cell wall in appearance, and they are similar in structure to those previously reported by Giesbrecht (17). The presence of these structures may indicate that cell wall synthesis involves an initial synthetic step at some distance from the growing edge. This would then be followed by a second step in which this presynthesized material is incorporated into the existing cell wall. A two-step polymerization process of this kind has been suggested by Rogers (33).

Measurements of the developing transverse septum suggest that it grows by addition of material at the free edge. Although this edge is, therefore, much thinner than normal, the region immediately behind this growing edge shows normal wall thickness. This observation was reinforced by an examination of cells in the later stages of septum development after the membranes bounding the mesosome and the growing edges had fused (Fig. 6, 9). It was apparent that the septum was of normal thickness except for a thinner central region in the area of fusion and that final thickening involved only this central region.

After the completion of the transverse septum, a separation must occur to produce the two daughter cells. However, the newly developed cross wall only *equals* the thickness of the parent cell wall. It seems, therefore, that a second period of cell wall synthesis must occur during the separation of the two cells. Since this organism also possesses a capsule, this too must be synthesized at a later time. Cole (8) stressed the importance of determining the time and place of replication of capsular material, not only in view of its possible usefulness as a marker for following wall synthesis, but also to establish whether there is an actively moving site for its synthesis. The present results clearly show that a second period of wall thickening accompanies the separation of the daughter cells. During this period, as the wall doubles in thickness, the capsule is formed at the outer surface. It is important to note that this secondary phase of wall growth does not involve addition and assembly of wall components to a growing edge, but rather an overall thickening of an already defined structure. Two conclusions can reasonably be drawn from these findings. (i) The synthesis of the complete cross wall occurs in two stages which are separated in time, and (ii), in a chain of cells, it is conceivable that both types of synthesis are taking place at the same time in the same cell. However, since capsule synthesis is associated only with the second phase of transverse septum development, the sites of its synthesis would be confined to constricting regions at the cell poles. This finding provides an explanation, in terms of fine structure, for the observation of Meynell and Meynell (28) that the ends of each cell become encapsulated before the equator. Other studies by Meynell and Lawn (27) demonstrated that in *B. anthracis* the capsule is not produced until late in the exponential growth phase. In *B. megaterium*, micrographs reveal a well-defined capsule surrounding the cells throughout the entire growth phase and also during sporulation.

In their study of *B. subtilis*, Ryter and Jacob (36) demonstrated an association between the replicating chromatin and the plasma membrane via the mesosome, and thereby lent support to the idea of a structural coordination between cell and chromatin division, proposed by Jacob, Brenner, and Cuzin, (21). The present micro-

graphs confirm that a close association between mesosome and chromatin does occur, and that in synchronously dividing cells the chromatin appears to be associated at one end to a mesosome at the pole of the cell and at the other to the mesosome involved in transverse septum formation. The fusion of the membranes bounding the mesosome and subsequent partition into the daughter cells seem to be the means whereby this association of chromatin and membrane is perpetuated. The attachment of the chromatin at the cell pole appears to be permanent, whereas the attachment to the mesosomes in the center of the cell only arises during the period of septum formation. These observations are in accord with the findings of Eberle and Lark (10) that the relationship of the site of the attached chromosome to the site of septum formation is decided within one generation and thereafter is permanent. The determining factor would thus be the site of transverse septum formation. Assuming the existence of a permanent attachment of chromatin at the pole of the cell, Eberle and Lark further reasoned that the deoxyribonucleic acid templates, which replicated when one cell divided repeatedly to form a chain, would be found in cells at the end of the chain. Using autoradiography, they were able to confirm this. This demonstration by Eberle and Lark together with the finding that moving equatorial sites are involved in wall (9) and membrane (22) synthesis, strongly suggests that these processes are linked in some way. The physical nature of the link between membrane and nuclear material is still not clear, but membrane profiles have been seen penetrating into the chromatin fibrils in *B. cereus* (12). In this study, the membrane bounding the mesosome was frequently observed to possess a stalk-like appendage or plate which was embedded in the chromatin (Fig. 15–19).

The remarkable similarity in appearance between the cell wall and the capsule is interesting in view of Tomcsik's finding (40) that these two structures in *B. megaterium* are essentially similar in chemical composition. Structurally, the capsule resembles the mesh-like capsule seen by Baker and Loosli in *Diplococcus pneumoniae* (3). An examination of the cell wall at high magnification reveals that it contains granules approximately 35 A in diameter. This observation remains to be correlated with our studies of the surface of *B. cereus* walls by use of negative staining (*unpublished data*). The latter show a repeating subunit of 60 A in the cell wall arranged in a rectangular pattern. A similar pattern has been observed in *Clostridium tetani* by Takagi et al. (38) and in *B. polymyxa* by Baddiley (2) using shadowed preparations.

The asymmetric staining pattern of the plasma membrane seen in these studies may result as Fitz-James (14) and Glauert et al. (18) have suggested from the masking by the cytoplasm of its inner profile. However, this suggestion does not explain the observation by Murray and Watson (29) that in *Nitrobacter agilis* the inner profile is more dense than the outer profile. Murray and Watson suggested that the extreme density of this layer might represent a localization of some metal enzyme complex. The consistency with which we observe the dense outer profile suggests a similar conclusion. With regard to this obvious difference between the two layers of the plasma membrane, it may be relevant to note that during the process of septation it is the outer layer which is directly in contact with the developing septum, whereas the available evidence indicates that it is the inner layer of the mesosome membrane which is in contact with dividing chromatin. It is conceivable, therefore, that the structural differences between the layers may in fact reflect different biochemical functions.

Results of recent studies utilizing X-ray diffraction and other techniques (1, 4, 31, 43) have strengthened the idea that membranes from a variety of sources may be composed of subunits. Fuhs (16) has recently demonstrated the presence of spherical subunits in the chromatophore membranes and plasma membrane of *Rhodospirillum rubrum*. On occasion, spherical subunits were seen in our micrographs in the inner and outer profiles of both the mesosome and plasma membranes.

We have no explanation for the peculiar pattern of densely staining ribosomes observed in this organism, beyond stating that it may reflect differential metabolic activity at the ribosome level. Although the fine structure of spore formation in the aerobic bacilli has been carefully elucidated (12, 13, 30, 35), it is noteworthy that the pattern of mesosome formation, proliferation, and membrane fusion, which characterizes transverse septum development, is also common to the formation of the spore septum. The important difference is that in the latter case no cell wall material is synthesized within the membranes bounding the mesosome. However, after the forespore membrane is complete, material which resembles the cell wall in chemical composition, namely cortex, is synthesized between these membranes (41). The absence from the sporal mesosomes of the 2,000 A vesicles repeatedly seen in the mesosomes involved in

transverse septum formation supports the idea that they play a special role in cell wall synthesis.

ACKNOWLEDGMENTS

This investigation was supported by Atomic Energy Commission contract NYO 2038-3 (D. L.) and by National Science Foundation grant NSF-GB3816 (R. S.).

We wish to thank R. Pfister for the use of the Phillips electron microscope and Z. Celkis for technical assistance.

LITERATURE CITED

1. ABRAM, D. 1965. Electron microscope observations on intact cells, protoplasts, and the cytoplasmic membrane of *Bacillus stearothermophilus*. J. Bacteriol. **89**:855–873.
2. BADDILEY, J. 1964. Teichoic acids and the bacterial cell wall. Endeavour **23**:33–37.
3. BAKER, R. F., AND C. G. LOOSLI. 1966. The ultrastructure of encapsulated *Diplococcus pneumoniae* type I before and after exposure to type specific antibody. Lab. Invest. **15**:716–730.
4. BLAISIE, J. K., M. DEWEY, A. E. BLAUROCK, AND C. R. WORTHINGTON. 1965. Electron microscope and low angle X-ray diffraction studies on outer segment membranes from the retina of the frog. J. Mol. Biol. **14**:143–152.
5. CHAPMAN, G. B. 1959. Electron microscope observations on the behaviour of the bacterial cytoplasmic membrane during cellular division. J. Biophys. Biochem. Cytol. **6**:221–224.
6. CHAPMAN, G. B., AND J. HILLIER. 1953. Electron microscopy of ultra-thin sections of bacteria. I. Cellular division in Bacillus cereus. J. Bacteriol. **66**:362–373.
7. CHUNG, K. L., R. Z. HAWIRKO, AND P. K. ISAAC. 1964. Cell wall replication. 1. Cell wall growth of *Bacillus cereus* and *Bacillus megaterium*. Can. J. Microbiol. **10**:43–48.
8. COLE, R. M. 1965. Symposium on the fine structure and replication of bacteria and their parts. III. Bacterial cell-wall replication followed by immunofluorescence. Bacteriol. Rev. **29**:326–344.
9. COLE, R. M., AND J. J. HAHN. 1962. Cell wall replication in *Streptococcus pyogenes*. Science **135**:722–723.
10. EBERLE, H., AND K. G. LARK. 1967. Chromosome segregation in *Bacillus subtilis*. J. Mol. Biol. **22**:183–186.
11. EISERLING, F. A., AND W. R. ROMIG. 1962. Studies of *Bacillus subtilis* bacteriophages: structural characterization by electron microscopy. J. Ultrastruct. Res. **6**:540–546.
12. ELLAR, D. J., AND D. G. LUNDGREN. 1966. Fine structure of sporulation of *Bacillus cereus* grown in a chemically defined medium. J. Bacteriol. **92**:1748–1764.
13. FITZ-JAMES, P. A. 1960. Participation of the cytoplasmic membrane in the growth and spore formation of bacilli. J. Biophys. Biochem. Cytol. **8**:507–528.
14. FITZ-JAMES, P. C. 1965. A consideration of bacterial membrane as the agent of differentiation. Symp. Soc. Gen. Microbiol. **15**:369–378.
15. FITZ-JAMES, P. C., AND R. HANCOCK. 1965. The initial structural lesion of penicillin action in *Bacillus megaterium*. J. Cell Biol. **26**:657–667.
16. FUHS, G. W. 1966. Spherical subunits in photosynthetic membranes of two *Cyanophyceae* and the bacterium *Rhodospirillum rubrum*. Arch. Mikrobiol. **54**:253–265.
17. GIESBRECHT, P. 1960. Über organisierte Mitochondrien und andere Beinstrukture von *Bacillus megaterium*. Zentr. Bakteriol. Parasitenk. Abt. I Orig. **179**:538–582.
18. GLAUERT, A. M., E. M. BRIEGER, AND J. F. ALLEN. 1961. The fine structure of vegetative cells of *Bacillus subtilis*. Exptl. Cell Res. **22**:73–85.
19. IMANAKA, H., J. R. GILLIS, AND R. A. SLEPECKY. 1967. Synchronous growth and sporulation of *Bacillus megaterium*. J. Bacteriol. **93**:1624–1630.
20. ITERSON, W. VAN. 1965. Symposium on the fine structure and replication of bacteria and their parts. II. Bacterial cytoplasm. Bacteriol. Rev. **29**:299–325.
21. JACOB, F., S. BRENNER, AND F. CUZIN. 1963. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **28**: 329–348.
22. JACOB, F., A. RYTER, AND F. CUZIN. 1966. On the association between DNA and membrane in bacteria. Proc. Roy. Soc. (London) Ser. B. **164**:267–278.
23. KAKEFUDA, T., J. T. HOLDEN, AND N. M. UTECH. 1967. Ultrastructure of the membrane system in *Lactobacillus plantarum*. J. Bacteriol. **93**: 472–482.
24. KELLENBERGER, E., A. RYTER, AND J. SÉCHAUD. 1958. Electron microscope studies of DNA-containing plasms. II. Vegetative and mature DNA as compared with normal bacterial nucleoids in differing physiological states. J. Biophys. Biochem. Cytol. **4**:671–676.
25. MARUYAMA, Y., AND T. YANAGITA. 1956. Physical methods for obtaining synchronous cultures of *Eschericia coli*. J. Bacteriol. **71**:542–546.
26. MAZIA, D. 1965. The partitioning of genomes. Symp. Soc. Gen. Microbiol. **15**:379–394.
27. MEYNELL, G. G., AND A. M. LAWN. 1965. Inheritance of capsule and the manner of cell wall formation in *Bacillus anthracis*. J. Gen. Microbiol. **39**:423–427.
28. MEYNELL, G. G., AND E. MEYNELL. 1966. The biosynthesis of poly-D-glutamic acid, the capsular material of *Bacillus anthracis*. J. Gen. Microbiol. **43**:119–138.
29. MURRAY, R. G. E., AND S. W. WATSON. 1965. Structure of *Nitrosocystis oceanus* and comparison with *Nitrosomonas* and *Nitrobacter*. J. Bacteriol. **89**:1594–1609.
30. OHYE, D. F., AND W. G. MURRELL. 1962. Formation and structure of the spore of *Bacillus coagulans*. J. Cell Biol. **14**:111–123.
31. PARK, R. B. 1965. Substructure of chloroplast lamellae. J. Cell Biol. **27**:151–161.

32. PFISTER, R. M., AND D. G. LUNDGREN. 1964. Electron microscopy of polyribosomes within *Bacillus cereus*. J. Bacteriol. **88**:1119–1129.
33. ROGERS, H. J. 1963. The bacterial cell wall. The result of adsorption, structure or selective permeability? J. Gen. Microbiol. **32**:19–24.
34. ROGERS, H. J. 1965. The outer layers of bacteria: the biosynthesis of structure. Symp. Soc. Gen. Microbiol. **15**:186–219.
35. RYTER, A. 1965. Etude morphologique de la sporulation de *Bacillus subtilis*. Ann. Inst. Pasteur **108**:40–60.
36. RYTER, A., AND F. JACOB. 1964. Etude au microscope électronique de la liason entre noyau et mésosome chez *Bacillus subtilis*. Ann. Inst. Pasteur **107**:384–400.
37. SLEPECKY, R. AND J. W. FOSTER. 1959. Alterations in metal content of spores of *Bacillus megaterium* and the effect of some spore properties. J. Bacteriol. **78**:117–123.
38. TAKAGI, A., K. NAKAMURA, AND M. VEDA. 1965. Electron microscope studies of the intracytoplasmic membrane system in *Clostridium tetani* and *Clostridium botulinum*. Japan. J. Microbiol. **9**:131–143.
39. TOMASZ, A., J. D. JAMIESON, AND E. OTTOLENGHI. 1964. The fine structure of *Diplococcus pneumoniae*. J. Cell Biol. **22**:453–467.
40. TOMCSIK, J. 1956. Bacterial capsules and their relation to the cell wall. Symp. Soc. Gen. Microbiol. **6**:41–67.
41. WARTH, A. D., D. F. OHYE, AND W. G. MURRELL. 1963. Location and composition of spore mucopeptide in *Bacillus* species. J. Cell Biol. **16**:593–609.
42. WATSON, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. J. Biophys. Biochem. Cytol. **4**:475–478.
43. WEIER, T. E., T. BISALPUTRA, AND A. HARRISON. 1966. Subunits in chloroplast membranes of *Scenedesmus quadricauda*. J. Ultrastruct. Res. **15**:38–56.