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Electron Microscope Observations on Intact Cells, Protoplasts, and the Cytoplasmic Membrane of *Bacillus stearothermophilus*

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ABSTRACT

ABRAM, DINAH (Purdue University, Lafayette, Ind.). Electron microscope observations on intact cells, protoplasts, and the cytoplasmic membrane of *Bacillus stearothermophilus*. J. Bacteriol. **89**:855–873. 1965.—Negatively stained preparations of protoplasts and fragments of cytoplasmic membranes from cells of *Bacillus stearothermophilus* ruptured by treatment with sonic oscillation, partial lysis with lysozyme, autolysis, or phage infection were examined electron microscopically. Specimens of intact cells also were examined by the same technique. The following structural details were revealed. Intact or nearly intact, partially swollen, elongated protoplasts and their ghosts have a characteristic differentiated surface texture and can easily be distinguished from the cell wall. Infoldings of the cytoplasmic membrane can be observed in these protoplasts, to which flagella are attached; the latter originate via hooks from "basal structures" that are in close association with the cytoplasmic membrane or part of it. Abundant intracytoplasmic membranous elements, which appear to be tubular or vesicular, can be seen in whole cells of three of the strains studied. The fine structure of the cytoplasmic membrane and probably that of its intracytoplasmic infoldings was observed on flattened and folded membrane fragments, one layer thick. Structural units, roughly spherical, 65 to 85 A in diameter, were present on one side of the cytoplasmic membrane, facing the cytoplasm. They were attached loosely to the membrane by fine stalks, 40 to 60 A long, and were easily detached, probably leaving the stalks behind them on the membrane. While the greater stability of membranes from thermophiles made this study of the fine structure possible, the structural units described were demonstrated also on cytoplasmic membranes from mesophiles.

In most bacteria, the relatively rigid cell wall is responsible for the shape of the cell and protects the protoplast from the high internal osmotic pressure of the cytoplasm. When the cell wall is damaged, or removed partially or entirely, spheroplasts or protoplasts form, assuming a spherical shape. These spherical bodies can be maintained as such if protected from low osmotic pressure. However, usually they are fragile and easily collapse upon mechanical manipulations, even in the presence of osmotic stabilizers. In hypotonic media, lysis occurs, and, under controlled conditions, round ghosts of spheroplasts or protoplasts can be observed. When spheroplasts or protoplasts are handled in hypotonic environment, the plasma membrane often ruptures and disperses as minute particles (see reviews by Weibull, 1956; McQuillen, 1956, 1960; Murray, 1963). In this study, various strains of the obligate thermophile *Bacillus stearothermophilus* were employed. It was noticed that protoplasts are present in water suspensions of cells of this organism ruptured in different ways, and that, unlike corresponding structures from mesophilic organisms, they do not assume a spherical shape. The protoplasts observed were either intact and slightly swollen, maintaining the rod shape of the cells, or nearly intact elongated ghosts. These forms were partly or entirely separated from the cell wall, and could easily be distinguished from it. Also, large fragments of flattened cytoplasmic membrane and interconnected long, narrow, and rounded membranous structures were present in these water suspensions of ruptured cells.

These remarkable properties of the protoplasts and the membranous structures of these thermophilic bacilli served as the basis for this electron microscopic study. In this paper, electron micrographs of negatively stained preparations will be presented. They reveal structural details of protoplasts and cytoplasmic membranes that have not been observed previously in bacteria. Of special interest are the structural units present

on the bacterial cytoplasmic membrane that are similar to the units that have been shown to occur on the inner membrane of mitochondria (Fernández-Morán, 1962; Green, Blair, and Oda, 1963; Parsons, 1963; Sjöstrand, 1963; Smith, 1963; Stoeckenius, 1963; Fernández-Morán et al., 1964). Preliminary reports of parts of this study have been presented on two previous occasions (Abram, 1964*a*, *b*).

MATERIALS AND METHODS

Five strains of *B. stearothermophilus* were used: NCR 2184, Nebraska 10, Purdue CD and FJW, and 194, obtained from H. Sobotka (Mount Sinai Hospital, New York). Cultures were grown at 58 to 60 C on a medium containing 1% Trypticase, 0.2% yeast extract, and 1.5% agar. Unless otherwise mentioned, the cells were washed off the medium with deionized water at room temperature after the cultures had been cooled. The suspensions contained 10^8 to 10^9 cells per milliliter. In the first four strains mentioned, cells in the exponential and stationary phases of growth were harvested after 4 to 6 and 10 to 14 hr of incubation, respectively.

To cause only slight damage to the cells or to accomplish only partial lysis of the cell wall, suspensions of cells in the exponential phase of growth were treated in the following ways: (i) volumes of 1 ml were treated by sonic oscillation for 2 to 30 sec with a model HG-003 ultrasonic generator (Electrosonic System, Inc., Los Angeles, Calif.) set for 1.6 amp output and operating at maximal power; (ii) the suspensions were diluted 100-fold with aqueous solutions of lysozyme to give final concentrations of 0.01 and 0.1 mg/ml of lysozyme, and incubated for 5 to 10 min at 26 C and 10 to 30 min at 4 C, respectively; and (iii) suspensions were also "temperature shocked" by washing the cells from the medium with water (at 18 to 20 C) 1 to 2 min after the cultures had been removed from the incubator and were still at 50 to 55 C.

Also, cells in the stationary phase of growth were examined for autolyzed forms.

Suspensions of *B. stearothermophilus* 194 were prepared after 4 to 5 hr of growth. This strain was phage-infected, and at that stage of growth many cells appeared ruptured.

Specimens for electron microscopy were prepared from suspensions kept at room temperature for no longer than 30 min. They were stained negatively with a 2% aqueous solution of potassium phosphotungstate (*p*H 7.0 to 7.2). Copper specimen grids covered with thin carbon-coated collodion membrane were used. A drop of the specimen suspension placed on the grid was withdrawn with filter paper without drying and was followed with a second drop of the stain. After 5 to 30 sec, the liquid was withdrawn with filter paper to leave a thin film, which dried at room temperature in no longer than 30 sec. The specimens were examined in a Hitachi HU-11A or a Phillips 200 electron microscope with double-condenser illumination at accelerating voltages of 50 and 60 kv, respectively, with a 20-μ objective aperture.

RESULTS

Protoplasts. In suspensions treated by sonic oscillation, only some of the cells were affected. These were damaged to various degrees. To observe protoplasts, cells least damaged were of interest, and a few are illustrated in Fig. 1 to 3. Elongated protoplast ghosts that are nearly intact are seen inside sheared cell walls (Fig. 1 and 2). In Fig. 3 the parts of the cell wall and the protoplast are of similar length. They were probably sheared when the cell was intact, and separated as the specimen was drying on the support membrane of the grid.

In the suspensions incubated in the presence of lysozyme under the conditions described, only partial lysis of the cell wall occurred. Disrupted cells, as a result of partial lysis of the cell wall, appear in many forms, some of which are illustrated in Fig. 4 to 8 and 10. Occasionally, the cell wall appears damaged locally, and a nearly intact, partially swollen protoplast emerges from it, as in Fig. 4. In other cases, elongated, practically intact protoplasts emerge from cell wall that appears to have been digested to a great extent, as in Fig. 5. Often the cell wall appears ruptured in the middle of the cell. Elongated protoplasts, collapsed, as in Fig. 6, or partially swollen, as in Fig. 7, are enclosed in the two parts of the ruptured cell wall. In these cases, it is possible that the cells were dividing and the areas near which new septa are formed are more susceptible to lysozyme digestion. Figure 8 shows a collapsed protoplast that appears as flattened, interconnected membrane fragments enclosed by partially digested cell wall. In this case, the cell wall appears uniformly affected by the lysozyme. Figure 10 shows the membrane of a disrupted protoplast only partially enclosed in the lysozyme-digested cell wall. Intact protoplasts, which are present in "temperature shocked" suspensions, appear partly swollen, and in part retain the rod shape of the intact cell (Fig. 11 and 12). Occasionally, the protoplast and the cell wall separate completely, as in Fig. 11, but, more often, some of the protoplast is enclosed in part of the ruptured cell wall, as in Fig. 12. Elongated, nearly intact ghosts of protoplasts were observed in suspensions prepared from autolyzed cells in the stationary phase of growth (Fig. 9 and 13). In all the cells described thus far, the texture of the protoplast can easily be distinguished from that of the cell wall. The cell wall appears relatively electron-lucid and amorphous,

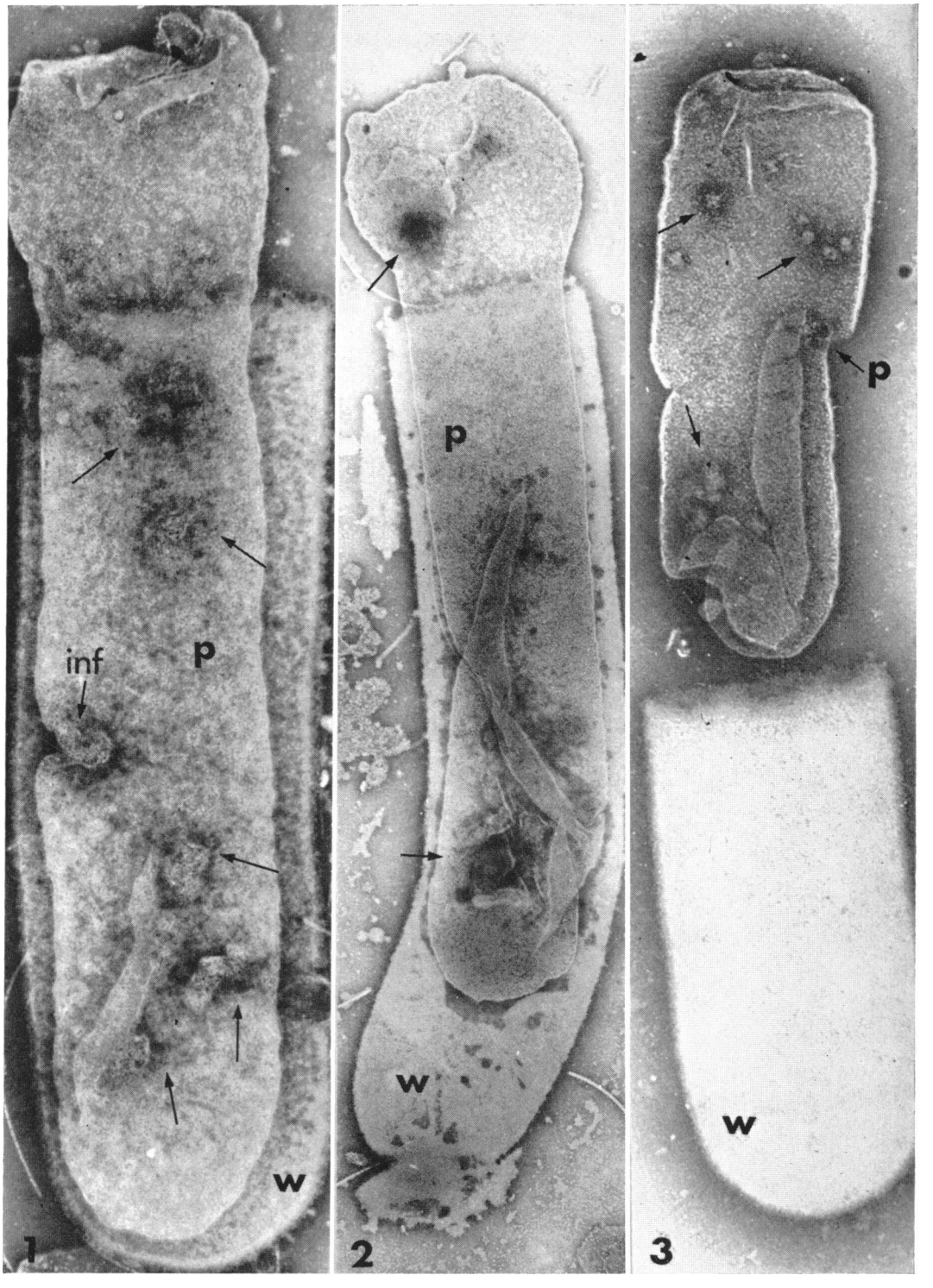


FIG. 1, 2, and 3. *Cells of Bacillus stearothermophilus 10 (Fig. 1) and 2184 (Fig. 2 and 3) damaged slightly by treatment with sonic oscillation. The sheared cell walls (W) are relatively electron-lucid and amorphous, and allow one to observe the parts of the protoplasts (P) enclosed in them. The surface of the protoplast is differentiated, showing electron-lucid particles that are densely and uniformly distributed. Infolding of the cytoplasmic membrane (inf) that appears as an invagination at the edge of the protoplast is seen in Fig. 1. Note the electron-opaque areas on the protoplasts, some of which are differentiated (arrows). Fig. 1, ×46,000; Fig. 2, ×36,000; Fig. 3, ×43,000.*

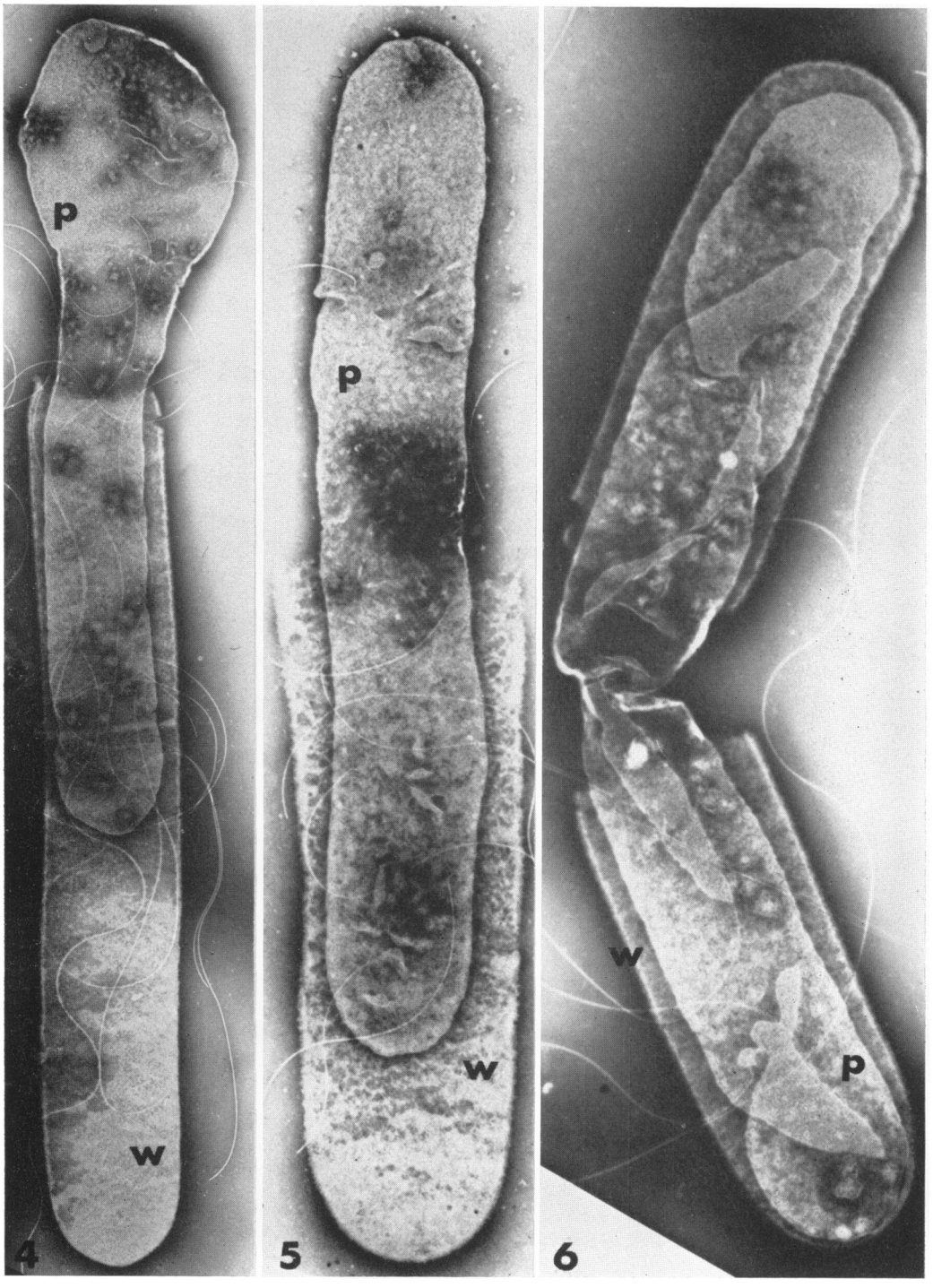


FIG. 4, 5, and 6. *Three different forms of disrupted cells of Bacillus stearothermophilus 10, as a result of partial lysis of the cell wall by lysozyme. The elongated protoplasts (P) are nearly intact. The cell wall (W) is often affected only locally, as in Fig. 4, at the end of the cell, or, as in Fig. 6, in the middle of the cell. In Fig. 5, the cell wall is digested to a greater extent. Note the characteristic texture of the protoplasts, the electron-opaque differentiated areas on the surface of the protoplast, and the flagella attached to them. Fig. 4, ×24,000; Fig. 5, ×27,000; Fig. 6, ×23,000.*

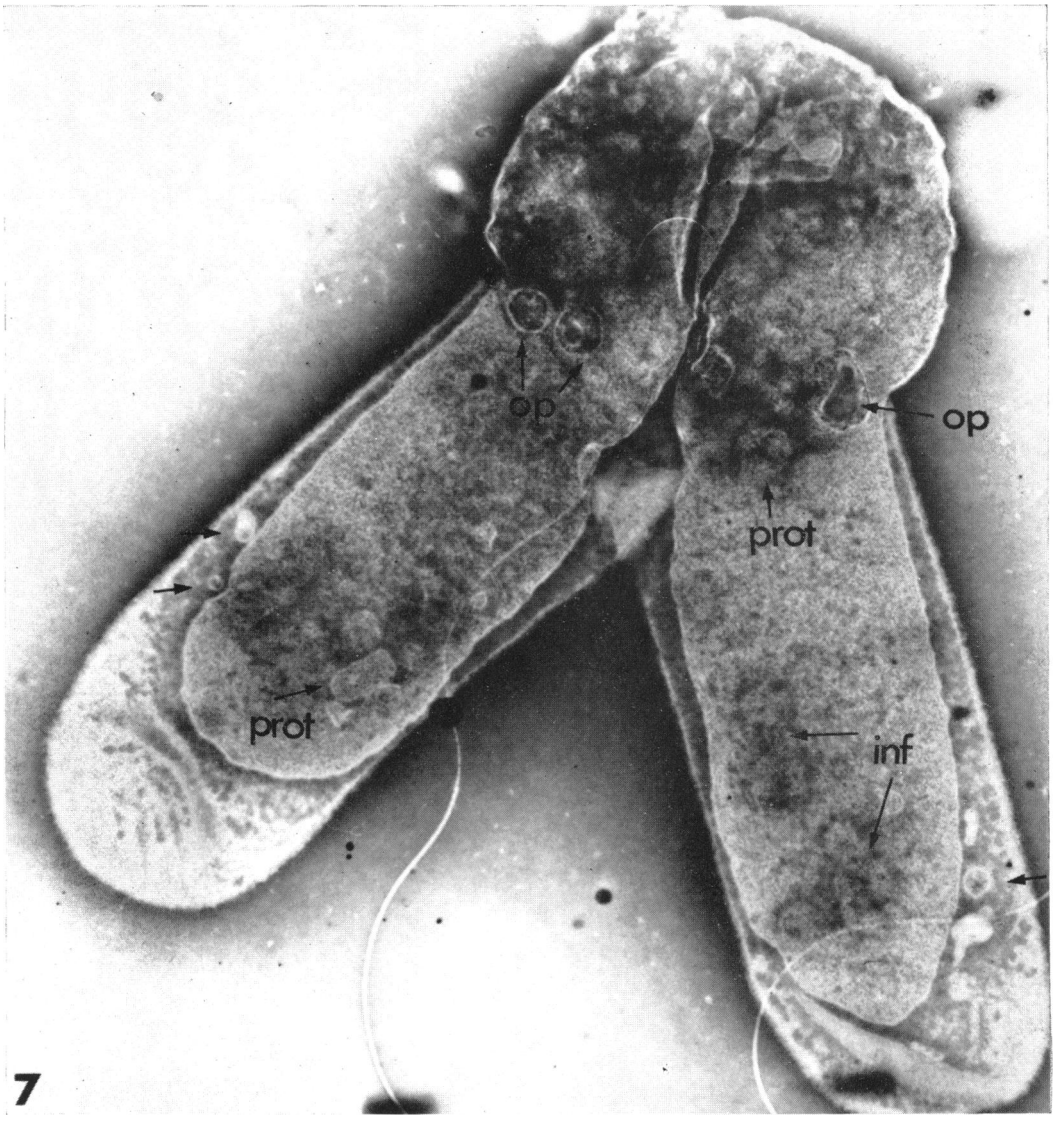


FIG. 7. *Micrograph at a higher magnification of the same preparation shown in Fig. 4 to 6. Note the differentiated electron-opaque areas on the protoplast. The differentiation possibly represents infolds (inf) and protrusions (prot) of the cytoplasmic membrane, or openings on the surface of the intracytoplasmic membranous elements (op). The structures outside the protoplast that have a similar texture to that of the protoplast could have been released from the intracytoplasmic membrane structures (arrows).* ×*46,000.*

and, therefore, it is possible to observe the parts of the protoplast enclosed in it. On the other hand, the surface of the protoplasts is differentiated. Particles which appear more electron-lucid than the background are densely and uniformly distributed on the surface. This appearance is characteristic of protoplasts and their ghosts, and permits their identification. The surface of the intact or nearly intact protoplasts is complex, because one is viewing two superimposed membranes of the collapsed protoplast. Therefore, very little could be learned from these preparations about the fine structure of the cytoplasmic membrane. However, some features of the general structure of the protoplasts could be recognized. Infoldings of the cytoplasmic membrane that appear as invaginations at the edges of the protoplasts can be seen clearly, as in Fig. 1 and 9. They are surrounded by electron-opaque areas, which probably are due to the stain that entered the

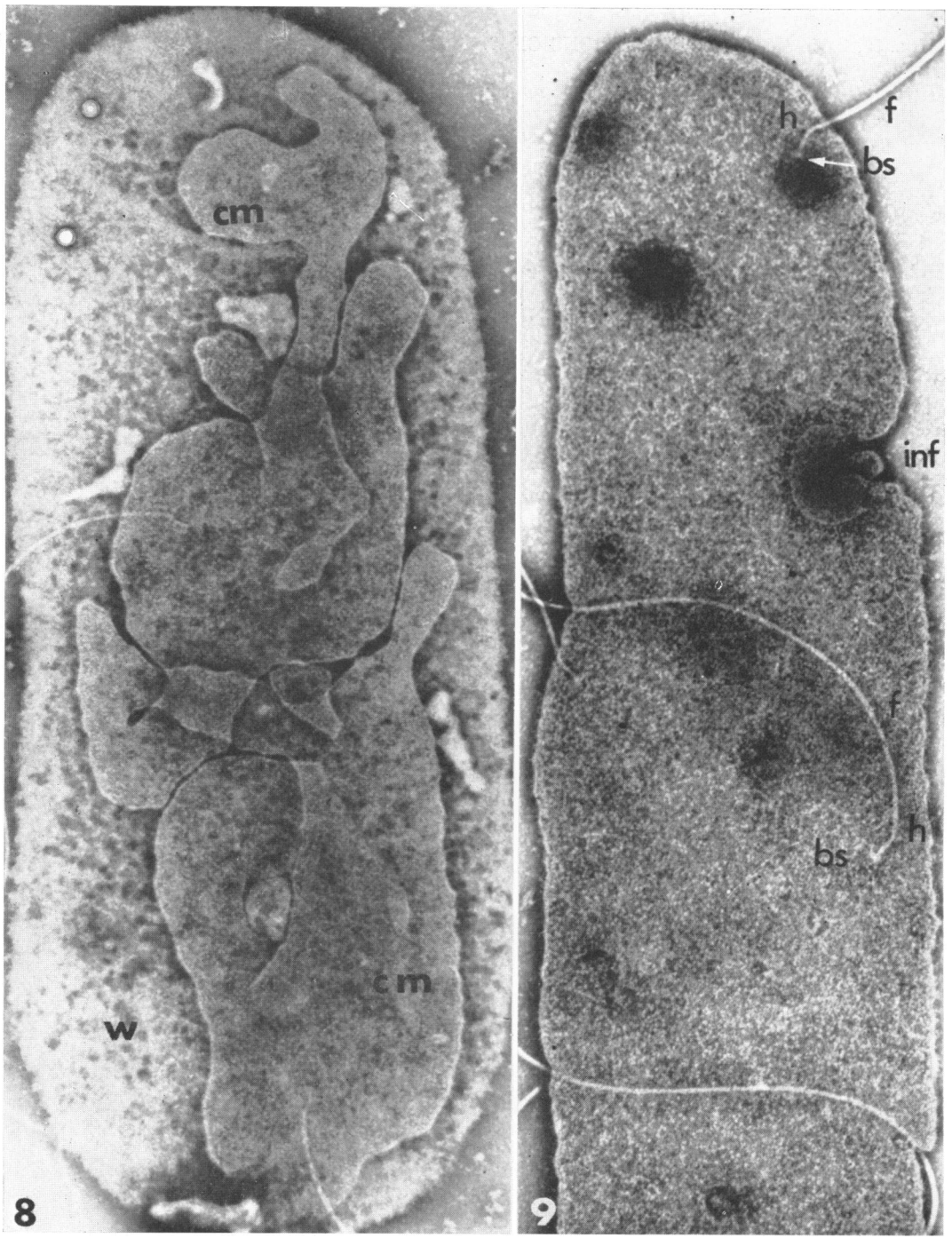


FIG. 8. *Ghost cell of Bacillus stearothermophilus 10 in a lysozyme-treated suspension. The cell wall (W) is digested evenly, and the collapsed protoplast appears as interconnected flattened membrane fragments (CM).* ×*65,000.*

FIG. 9. *Portion of a practically intact elongated protoplast in suspension of autolyzed cells of Bacillus stearothermophilus 10. The differentiated surface is covered with densely, uniformly distributed, electron-lucid particles. An invagination of the cytoplasmic membrane is seen at the edge of the protoplast and is surrounded by an electron-opaque area (inf). Note the electron-opaque areas that possibly correspond to openings on the surface of the protoplast of intracytoplasmic membranous elements. Flagella (f) are seen clearly originating via hooks (h) in "basal structures" (bs) that are electron-lucid differentiated areas associated with the cytoplasmic membrane.* ×*78,000.*

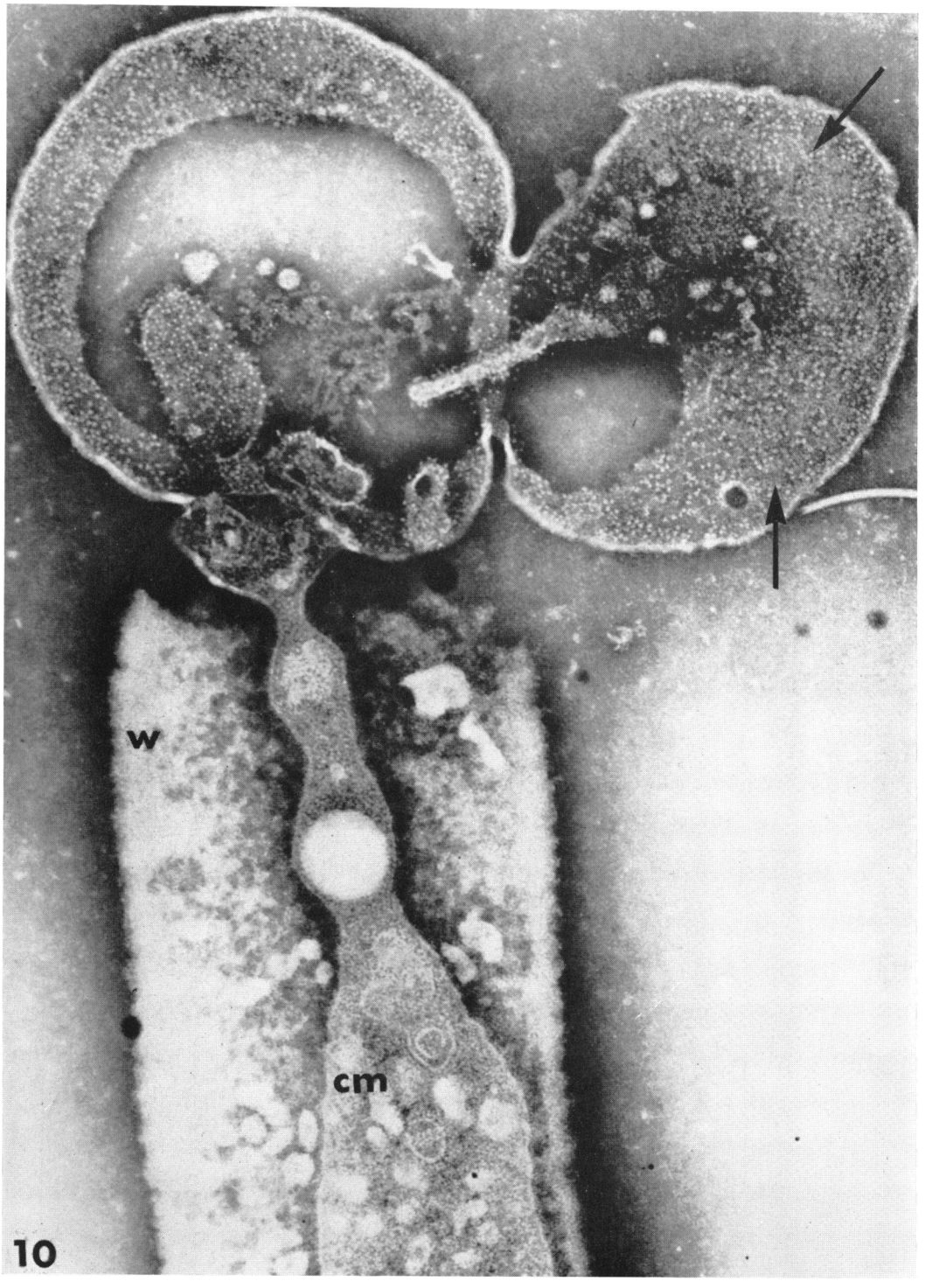


FIG. 10. *Ruptured protoplast of Bacillus stearothermophilus 10 emerging from partially lysozyme-digested cell wall (W). The surface of the cytoplasmic membrane (CM) inside the cell wall has a complex texture due to two superimposed membranes. The surface of the membrane outside the cell is one layer thick and shows details of fine structure. The surface is covered with densely distributed, roughly spherical structural units, 65 to 85 A in diameter. Some of these units are electron-lucid and others have opaque cores (arrows). Along folded edges of the membrane the units are arranged in rows.* ×*90,000.*

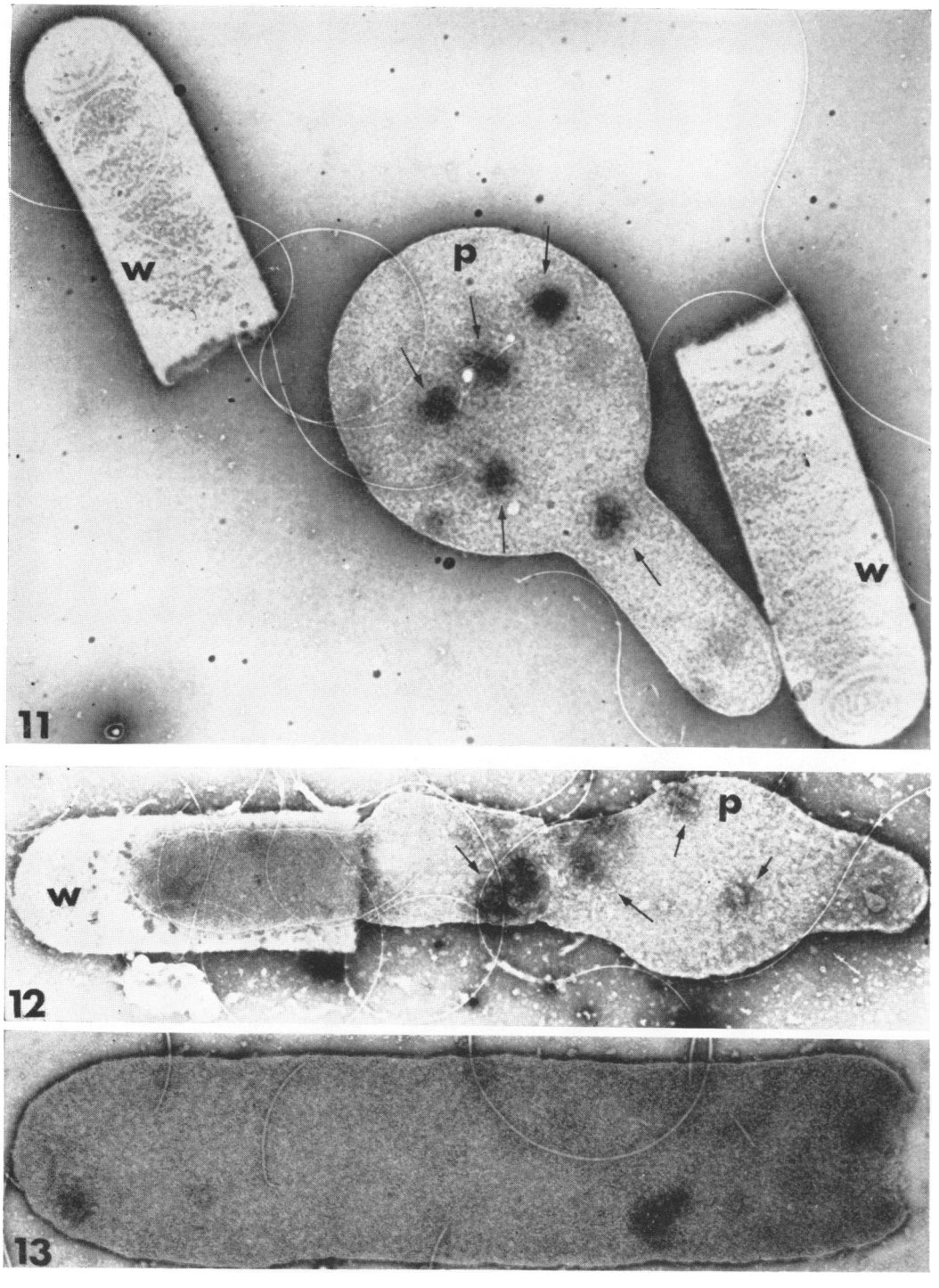


FIG. 11 and 12. *Intact, partly swollen protoplasts (P) in a suspension of cells of Bacillus stearothermophilus 10 after "temperature shock." Note the cell walls (W) and the flagella attached to the protoplasts (P). The electron-opaque areas on the surface of the protoplasts (arrows) are probably openings of intracytoplasmic membranous elements. ×22,000.*

FIG. 13. *Nearly intact elongated protoplast in a suspension of autolyzed cells of Bacillus stearothermophilus 10. The differentiated surface is covered with densely, uniformly distributed electron-lucid particles. ×38,000.*

extracytoplasmic spaces that form by the infoldings of the membrane. Electron-opaque areas of different sizes are seen on practically every protoplast described. These possibly are openings of infoldings of the cytoplasmic membrane that are on the surface. Some of these areas appear differentiated, probably because of the actual infolds or protrusions of the cytoplasmic membrane, or as a result of the extrusion of the intracytoplasmic elements upon protoplasting (Fitz-James, 1964; Ryter and Landman, 1964).

Flagella attached to the protoplasts also are observed (Fig. 4 to 7 and 9, 11, and 12). They originate via hooks in differentiated areas on the surface of the protoplast. These "basal structures" are either part of the protoplast membrane or in close association with it (Abram, Vatter, and Koffler, 1964). They will be described in detail in a future communication.

Intracytoplasmic membrane structures. In negatively stained perparations of intact cells of three of the five strains of *B. stearothermophilus* studied (2184, 10, and CD), differentiated areas that appear more opaque are observed (Fig. 14 and 15). These regions probably correspond to the intracytoplasmic membranous elements that have been observed in thin sections of fixed material of almost all bacteria studied (Stuart, 1959; Glauert and Hopwood, 1959, 1960; Fitz-James, 1960; Giesbrecht, 1960; Robinow, 1962; Imaeda and Ogura, 1963; and others), and recently were observed in negatively stained preparations of *Listeria monocytogenes* (Edwards, 1963) and of a *Eubacterium* sp. (Bladen, Nylen, and Fitzgerald, 1964). In these negatively stained preparations, the stain enters the extracytoplasmic spaces that are associated with the infoldings of the cytoplasmic membrane. The overall appearance of these structures suggests a vesicular or tubular arrangement of the infolded membrane (Fig. 15). These structures are always located in regions of new septa formation in dividing cells, as in Fig. 14*d*. Very often, two interconnected structures are seen on either side of what appears to be a newly formed septum, as in Fig. 14*e*. Their association with the formation of cross walls was previously shown in thin sections (Chapman and Hillier, 1953; Fitz-James, 1960; Glauert, Brieger, and Allen, 1961; Van Iterson, 1962; Imaeda and Ogura, 1963; Edwards and Stevens, 1963).

In suspensions of cells in the exponential phase of growth, these structures are abundant and are seen in every cell; however, they vary in number, shape, and size, being 0.05 to 0.75 μ long (Fig. 14 and 15). The cells usually are embedded in an excess of the stain; therefore, the cell wall can be recognized only occasionally all around the cell. Frequently, the cell wall is seen more clearly at both polar ends of the cell (Fig. 14*c*, *e*). Structural differences associated with the cell wall, responsible for the lack of penetration of the stain, may account for the fact that these structures could not be observed in two of the strains studied (*B. stearothermophilus* 194 and FJW).

Cytoplasmic membrane. The fine structure of the cytoplasmic membrane, which is responsible for the texture observed on the intact and nearly intact protoplasts, can best be seen on flattened membrane fragments, one layer thick. Such fragments were present in the cell suspensions that had been treated by sonic oscillation or lysozyme, as well as in suspensions of autolyzed and phagelysed cells (Fig. 10 and 16 to 24). Membrane fragments were observed in association with partially damaged cells (Fig. 10, 16, and 17) or completely separated from the cell walls (Fig. 18 to 24). The completely separated fragments originate in cells damaged to a greater extent than the ones described in the first part of this paper, and they vary in size and shape. The larger fragments can be sedimented by centrifugation at 3,500 rev/min for 5 to 10 min. Figure 10 shows a ruptured protoplast, part of which is enclosed in a cell wall partially digested by lysozyme. On the flattened cytoplasmic membrane outside the cell wall, a fine granular structure can be observed. The surface of the membrane is covered with densely distributed, roughly spherical structural units, 65 to 85 A in diameter, some of which are electron-lucid, whereas others have opaque cores. The structural units often are arranged in rows along the edges of the ruptured membrane fragments. The membrane edges are more electron-lucid than the surrounding areas; hence, it is probable that they are folded (Fig. 17, 20, and 21). This arrangement of the structural units along the edge of the membrane is never observed on the outside edges of protoplast ghosts. Therefore, it is assumed that these units are present only on one side of the membrane, that is, they face the inside of the cell. If this is the case, the structural units may be arranged in rows along the folded edges of the membrane fragments whenever their inner face, namely, the cytoplasmic side of the membrane, is turned inside out. Occasionally, in autolyzed ghost cells, the fragmented cytoplasmic membrane is retained within the cell wall. Figure 16 illustrates a portion of an autolyzed cell that happened to maintain through its entire length the rod shape of the intact cell. Interconnected, rounded, and flattened membrane fragments are seen through the cell wall, which appears lysed. The structural units that can be recognized on the membrane surfaces are seen standing on end on the membrane fragments, and are arranged in rows along their folded edges (Fig. 17). At higher

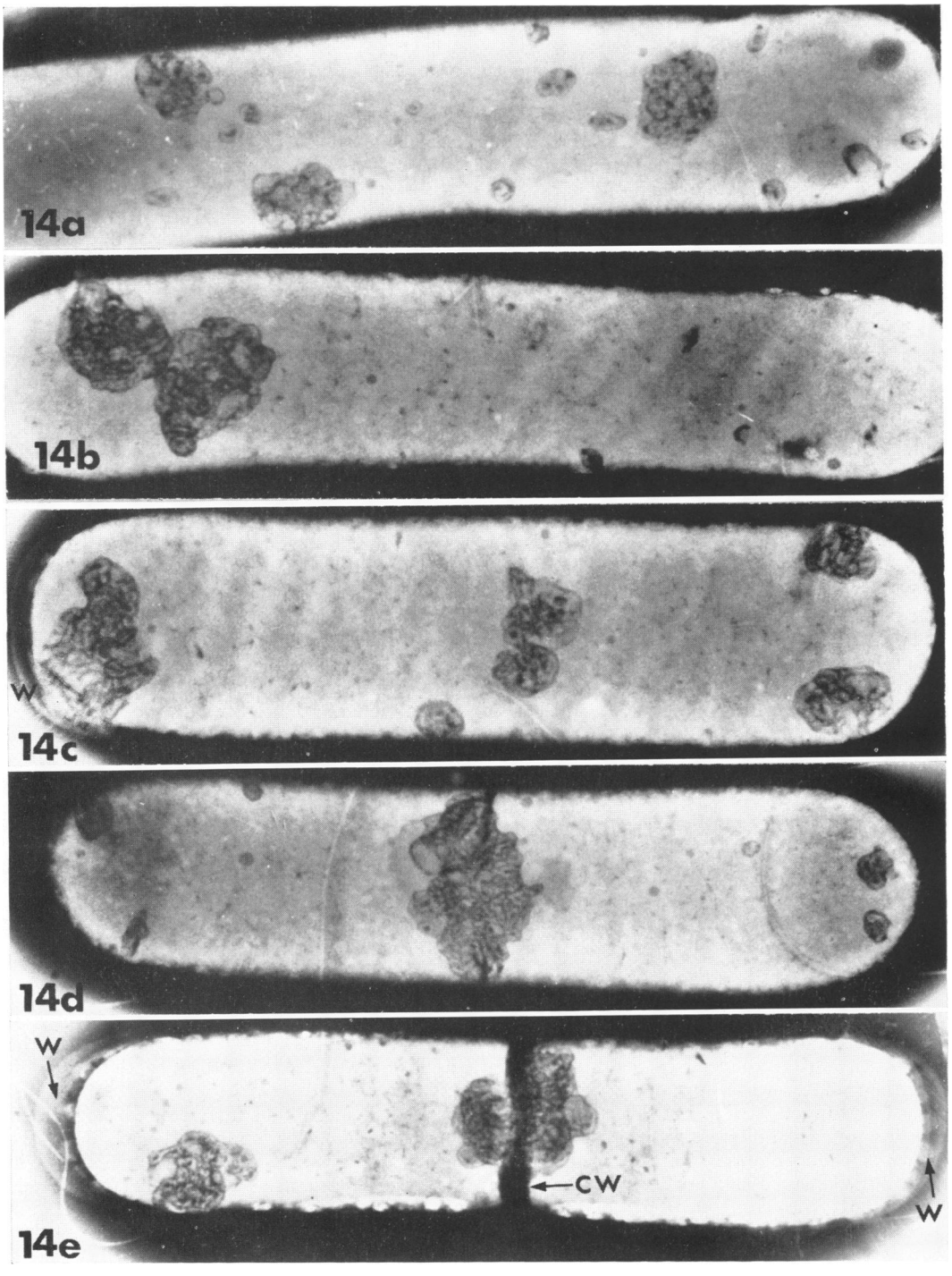
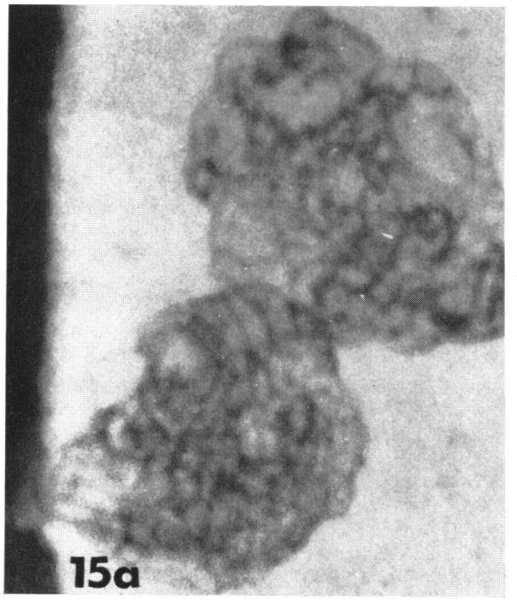
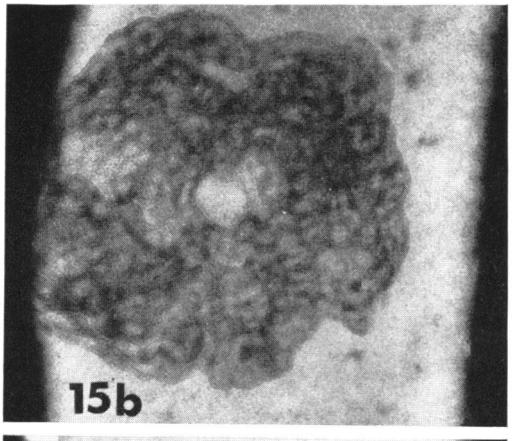
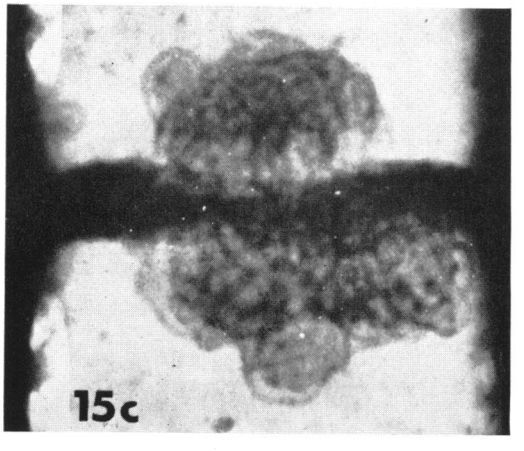


FIG. 14. *Several intact cells of Bacillus stearothermophilus 2184 in the exponential phase of growth. The differentiated electron-opaque areas are the intracytoplasmic membranous elements that are seen through the cell wall. They differ in size, shape, and number in the different cells, as shown in Fig. 14a, b, and c. In the cell seen in Fig. 14d, which is at an early stage of division, a large element is seen at the region of a cross wall formation. In Fig. 14e, two connected elements are seen on either side of what appears to be a newly formed cross wall (CW). In Fig. 14c and e, the cell wall can best be recognized at the polar ends of the cell (W). Fig. 14a, ×47,500; Fig. 14b, ×52,000; Fig. 14c, ×55,000; Fig. 14d, ×52,000; Fig. 14e, ×46,000.*







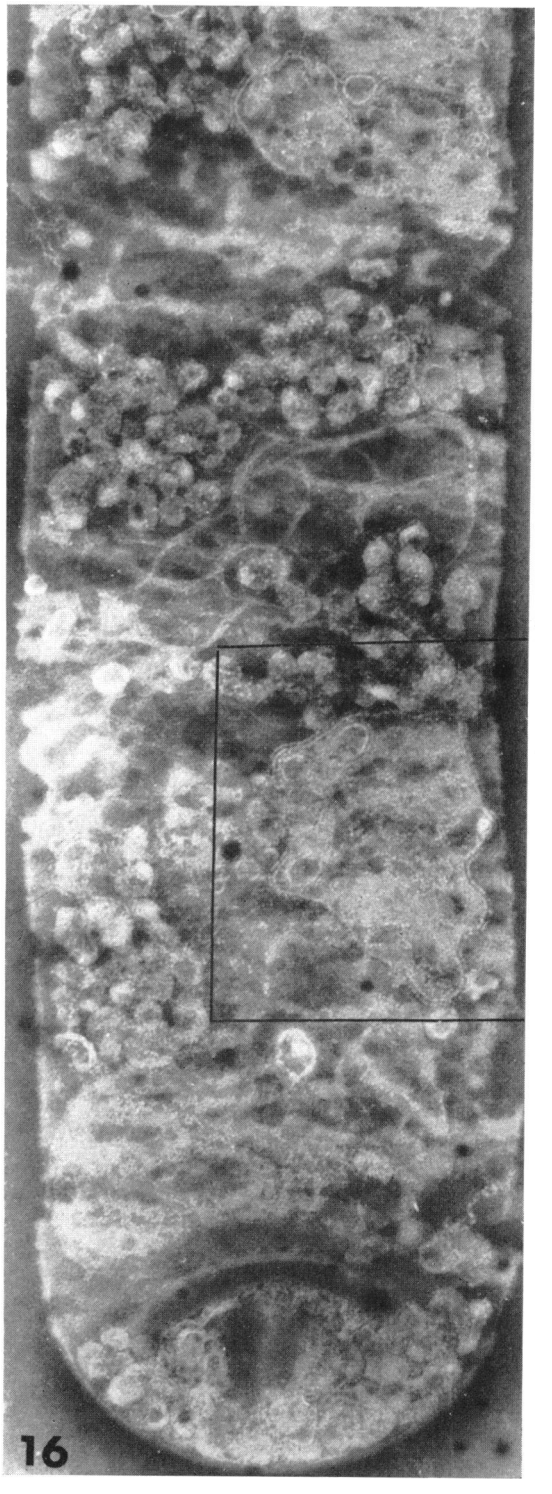


FIG. 15. *Micrographs at a higher magnification of the same specimens shown in Fig. 14. The vesicular arrangement of the intracytoplasmic membranous elements can be recognized. Fig. 15a, ×135,000; Fig. 15b, ×110,000; Fig. 15c, ×108,000.*

FIG. 16. *Portion of an autolyzed ghost cell of Bacillus stearothermophilus FJW. The disrupted cytoplasmic membrane is seen through the lysed cell wall. Interconnected rounded and flattened membrane fragments can be recognized. ×55,000.*

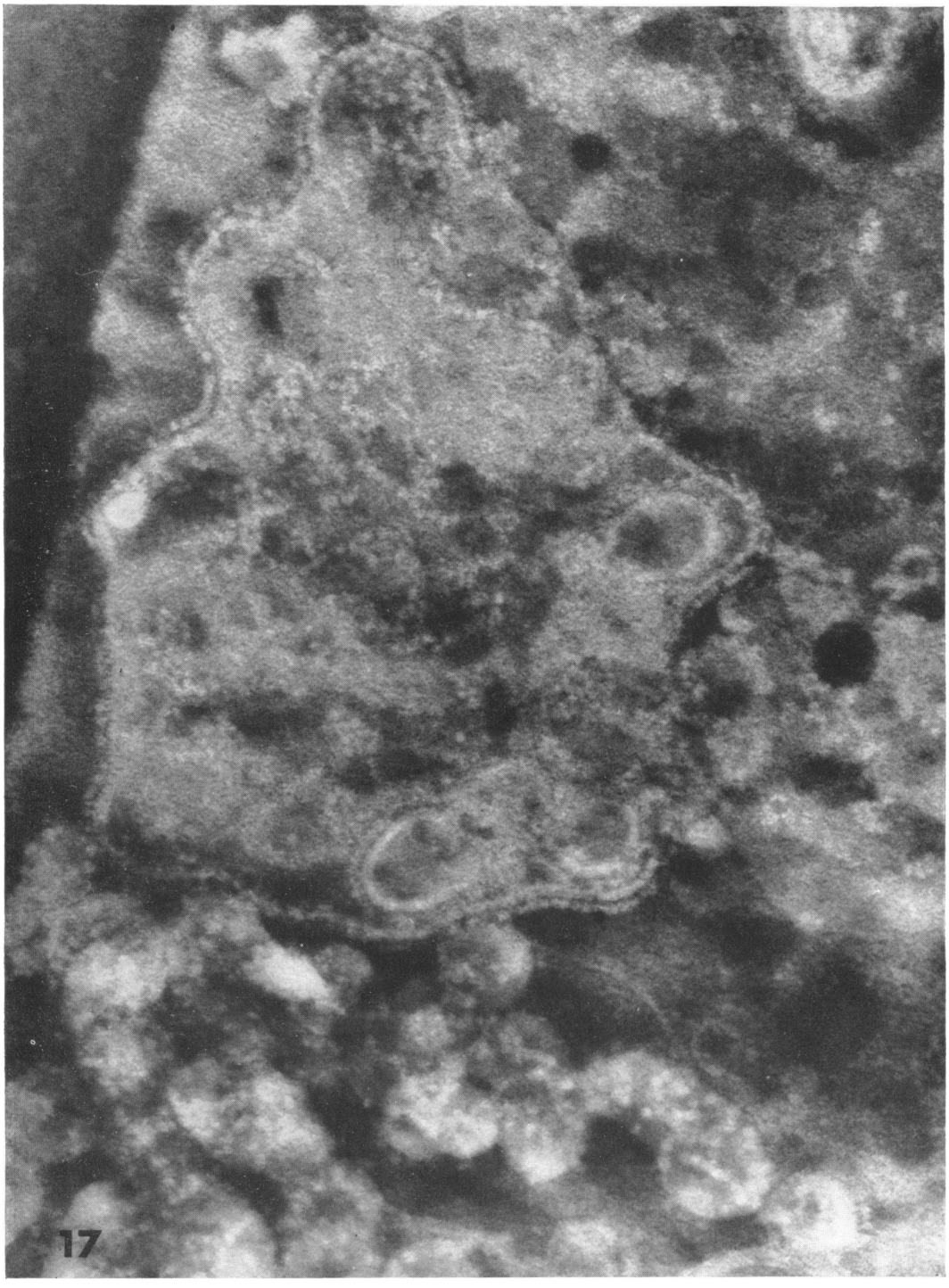


FIG. 17. *Micrograph at a higher magnification of the same specimen shown in Fig. 16. The structural units of the cytoplasmic membrane are recognized through the lysed cell wall. They are seen standing on end on the flattened membrane fragments and arranged in rows along the folded edges of the membrane. The folded edges of the membrane are more electron-lucid than the surrounding flattened membrane.* ×*187,000.*

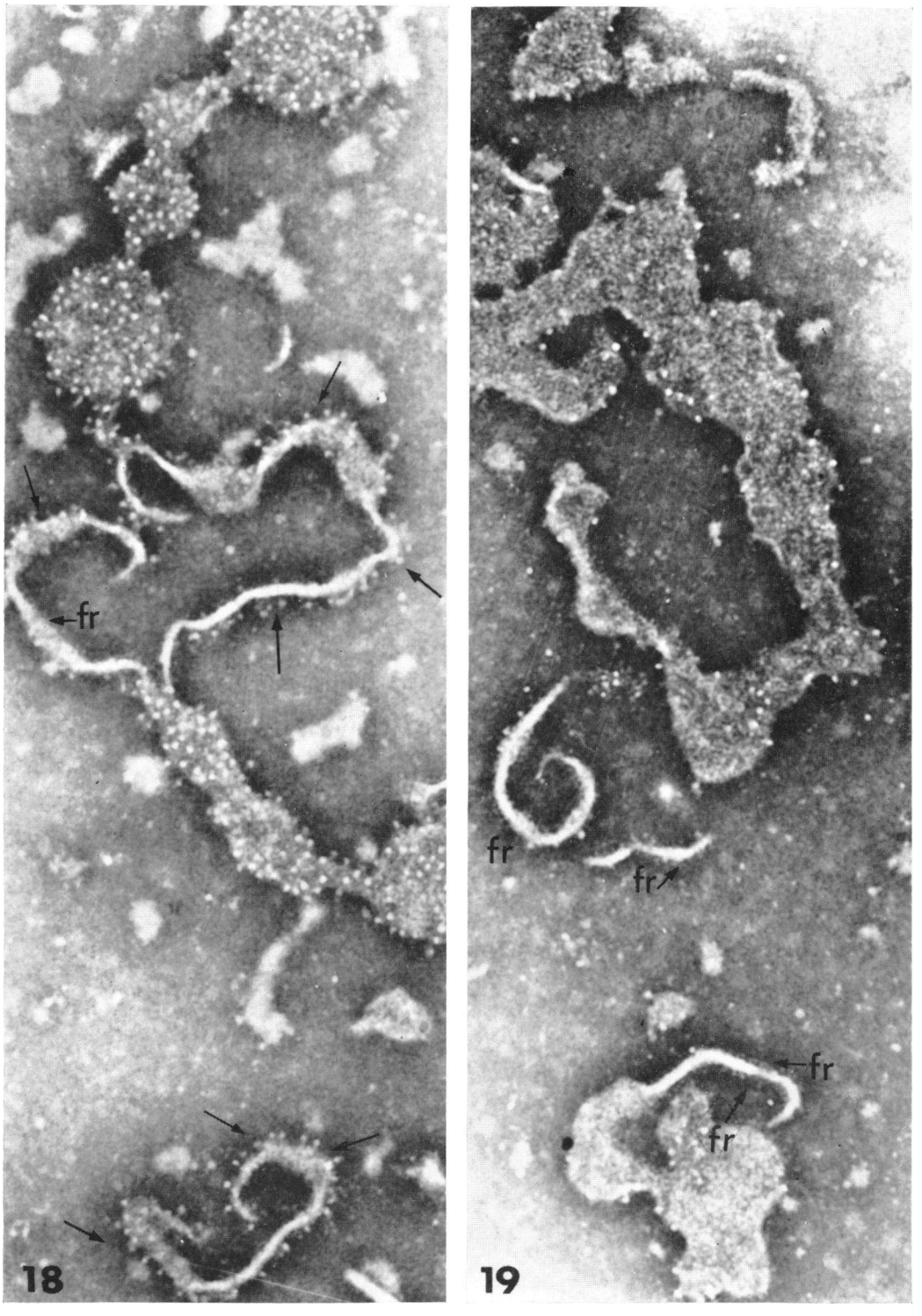


FIG. 18 and 19. *Cytoplasmic membrane fragments in a preparation of phage-lysed cells of Bacillus stearothermophilus 194. In Fig. 18, the interconnected rounded, long, narrow flattened, and the twisted strands of membrane fragments are covered with densely distributed structural units. Along the twisted strands, some of the structural units are attached to the membrane by fine stalks (arrows). In Fig. 19, the membrane fragments are stripped from practically all the structural units and have a differentiated surface. A fringe (fr) can be seen on the narrow twisted strands, which may represent the stalks of the structural units that remained attached to the stripped membrane.* ×*132,000.*

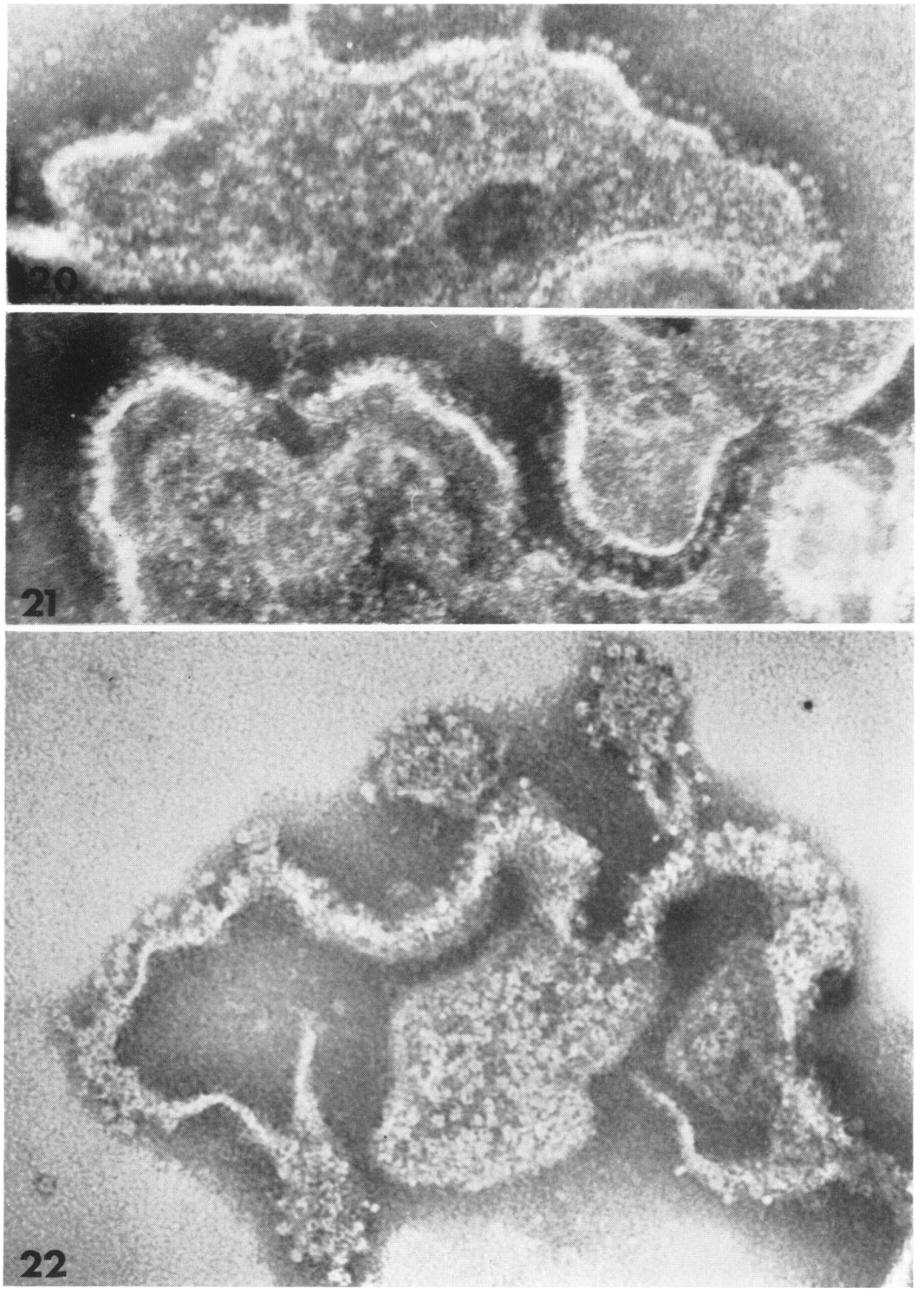


FIG. 20, 21, and 22. *Fragments of cytoplasmic membrane at higher magnifications, showing details of the structural units. In Fig. 20 and 21 (Bacillus stearothermophilus FJW, in suspension of autolyzed cells) the structural units are arranged along folded edges of membrane structures and are attached to the membrane by fine stalks. The folded edges of the membrane fragments are more electron-lucid than the surrounding flattened membrane. In Fig. 22 (B. stearothermophilus 2184 in a suspension of autolyzed cells) the structural units show clearly opaque cores. Fig. 20, ×200,000; Fig. 21, ×160,000; Fig. 22, ×287,000.*

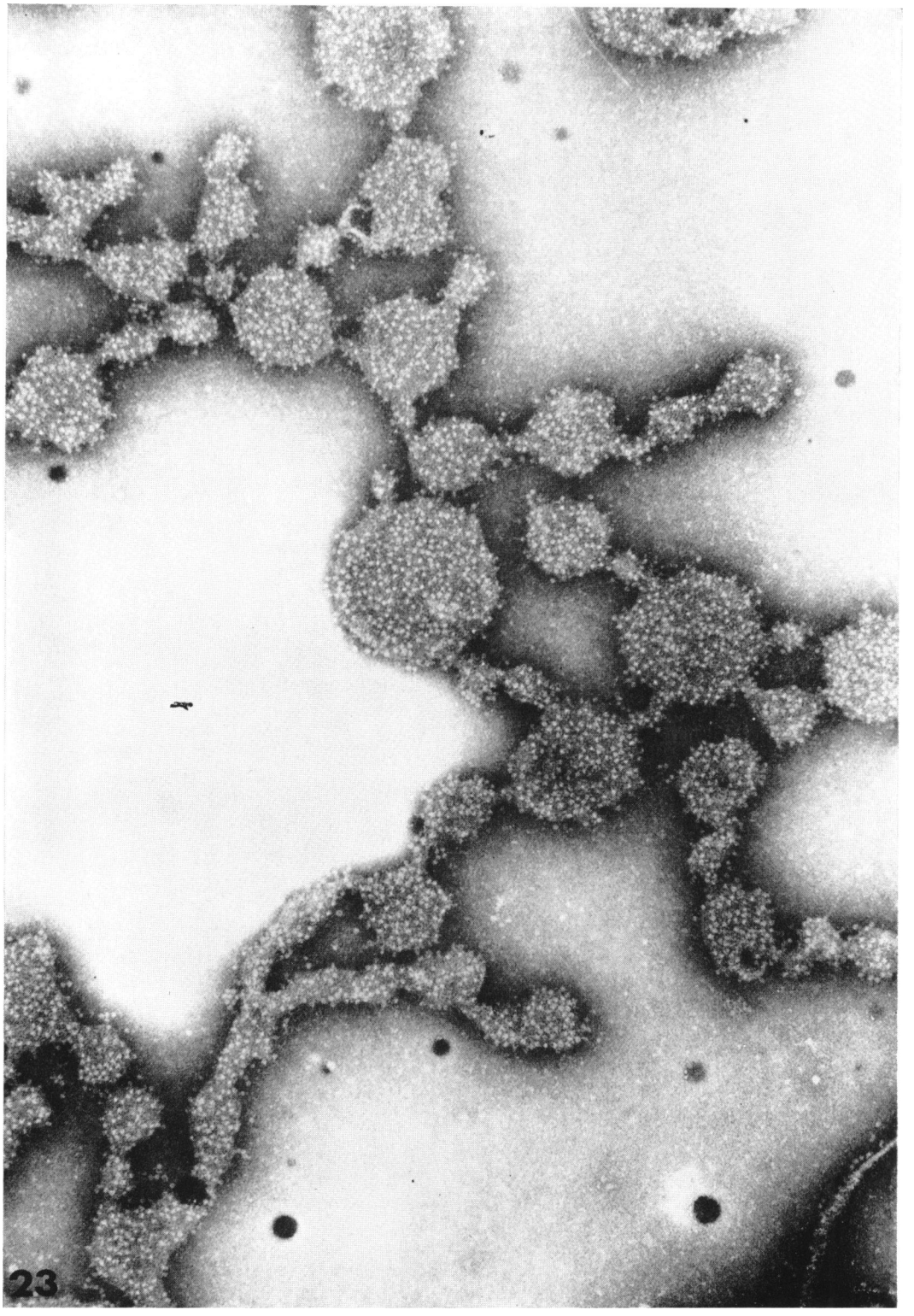


FIG. 23. *Cytoplasmic membrane fragments of Bacillus stearothermophilus FJW in a suspension of autolyzed cells in the stationary phase of growth. A low-magnification view of a typical arrangement of long, narrow, and rounded interconnected membranous structures. Densely distributed structural units cover the membrane, and similar particles can also be recognized in the background.* ×*102,000.*

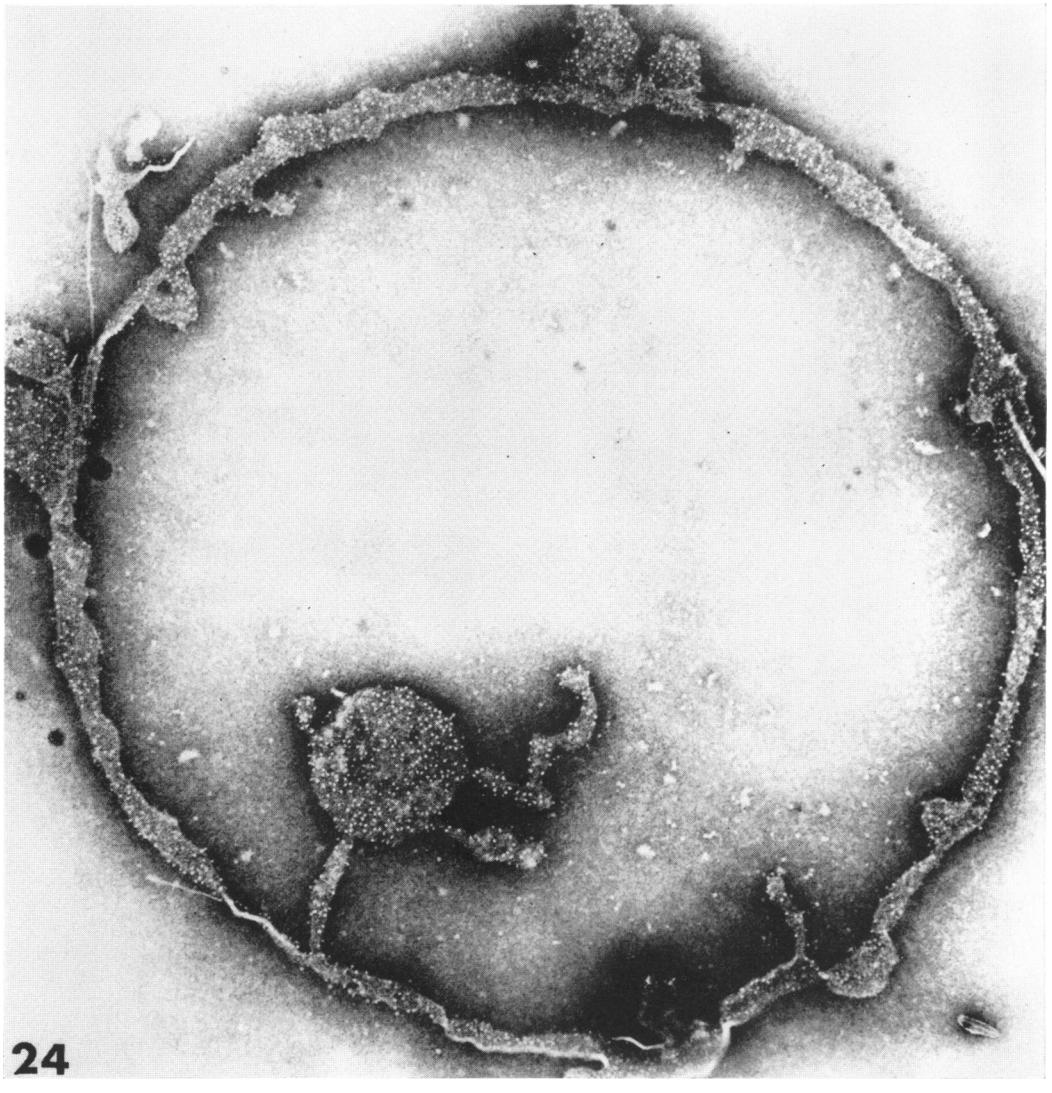


FIG. 24. *Cytoplasmic membrane fragment of Bacillus stearothermophilus 2184 in a suspension of lysozyme-treated cells. A low-magnification view of a narrow membrane fragment that forms a circle. Parts of the membrane are flattened, others are folded, or appear twisted and form narrow strands. The electron-lucid structural units can be recognized even at this low magnification.* ×*69,000.*

magnifications, as in Fig. 18 and 20 to 22, the structural units can be seen attached to folded edges of flattened membrane fragments by fine stalks, 40 to 60 A long. In many preparations, long membranous structures are present, which seem to have twisted and which appear as narrow strands (Fig. 18, 19, and 24). Often, the structural units that are arranged along these strands can be seen attached to them by the fine stalks mentioned (Fig. 18). However, strands of membrane that appear stripped of the structural units frequently exhibit a fringe (Fig. 18 and 19), which may represent the stalks remaining attached to the stripped membrane. The density of the structural units on membrane fragments is not uniform. Not only does the density differ from one preparation to another, but it also varies in the same preparation on different fragments (Fig. 18 and 19). Occasionally, a membrane fragment stripped from almost all of the structural units is observed (Fig. 19), and its surface appears differentiated. In the vicinity of membrane fragments,

one can see particles, some of which appear electron-lucid and others of which show an opaque core (Fig. 18, 19, 23, and 24). They are similar in size and shape to the structural units attached to membrane fragments, and probably correspond to them. It appears that the structural units are responsible for the uniform texture observed on the protoplast ghosts. One may assume that these units are not attached firmly to the membrane, and that they fall off after the membrane is disrupted. Typical arrangements of some membrane fragments are illustrated in Fig. 18, 19, 23, and 24. Narrow and rounded membranous structures can be seen interconnected, as in Fig. 23. The long structures are often seen twisted, as in Fig. 18 and 19. Longer membranous structures frequently form circles, as in Fig. 24. The membrane fragments that are arranged as described above may originate from the vesicular or tubular intracytoplasmic membrane elements. The latter have been shown to be abundant in cells in the exponential phase of growth (Fig. 14).

DISCUSSION

Elongated intact and nearly intact protoplast and large fragments of the cytoplasmic membrane have been observed in water suspensions of ruptured cells of thermophilic bacilli. Under similar conditions, it was not possible to observe corresponding structures in suspensions of mesophilic organisms. Perhaps the cytoplasmic membrane of thermophiles possesses certain structural elements, or their cytoplasm has certain physical properties, that are responsible for the observations described. Whether these have a bearing on biological thermostability warrants a more thorough biochemical and physical study. Regardless of this, the apparently unique properties of the thermophilic bacilli made it possible to study by negative staining the structure of the bacterial cytoplasmic membrane, which previously was examined only in thin sections of fixed material. The observations described in this paper provide additional information on the fine structure of the bacterial cytoplasmic membrane. In thin sections, the bacterial membrane is morphologically similar to the limiting membranes of other cells and organelles; namely, it appears as a "unit" membrane (Robertson, 1959, 1960). In negatively stained preparations, approximately spherical structural units, 65 to 85 A in diameter, were seen attached to the cytoplasmic membrane by fine stalks, 40 to 60 A long. These units seem to be attached to the membrane only on one side, facing the cytoplasm; they are loosely bound and are easily detached from the membrane.

Once the details of this fine structure were established in the thermophiles, successful attempts were made to identify the fine structure of the cytoplasmic membrane of mesophilic organisms. So far, they have been observed on membrane fragments of four mesophilic bacilli (*B. pumilus*, *B. licheniformis*, *B. brevis*, and *B. circulans*) and of three mesophilic gram-negative organisms: *Escherichia coli* C, *Proteus vulgaris*, and *Shigella dysenteriae* Y6R. The techniques employed for preparing the specimens of the mesophilic organisms are more involved than those for the thermophilic ones, and this work is still in progress. However, these observations show that the existence of the structural units described is not restricted to membranes of thermophilic organisms. Yet, it still remains to show whether this fine structure is common to all microorganisms, obligate aerobes as well as obligate anaerobes, and autotrophs as well as heterotrophs. So far, only aerobic heterotrophs have been examined.

Bacteria, as all procaryotic organisms, do not possess any intracytoplasmic membranous organelles that are characteristic for eucaryotic organisms. However, in almost all the bacteria examined, membranous elements, vesicular, tubular, or lamellar, have been observed (see review: Murray, 1963). These elements are infoldings of the cytoplasmic membrane and are difficult to isolate or separate physically from the membrane proper. Such elements have been observed in abundance in the thermophiles studied here. Because, in the preparations examined, all the membranous structures show similar structural details, it is assumed that the structural units described are present on the cytoplasmic membrane proper, as well as on its infoldings.

The similarity between the structural units that have been seen in the last few years on the inner membrane of mitochondria of eucaryotic organisms (Fernández-Morán, 1962; Green, Blair, and Oda, 1963; Parsons, 1963; Sjöstrand, 1963; Smith, 1963; Stoeckenius, 1963; Fernández-Morán et al., 1964) and those described in this paper on bacterial cytoplasmic membrane is very striking. However, this similarity is not astonishing in light of the fact that the same enzyme machinery that is built into the inner membrane of mitochondria appears to be localized in the cytoplasmic membrane of microorganisms. The respiratory enzymes and the cytochrome-linked electron transport system have been shown in preparations of the cytoplasmic membrane of aerobic microorganisms. Oxidative phosphorylation was demonstrated in particulate fractions obtained from whole cells containing both cell

wall and cytoplasmic membrane fragments. Also, adenosine triphosphatase activity was shown to be bound to membranes from most organisms so far examined (see review by Hughes, 1962). Recently, the structural units on the inner membrane of mitochondria have been shown to be the site of part of the mitochondrial adenosine triphosphatase (Parsons, Escoffery, and Verboon, 1964; Racker, Chance, and Parsons, 1964). It still remains to identify the function of the similar units in bacteria.

The bacterial cytoplasmic membrane is an integral part of the cell and is present in all cells, irrespective of growth conditions. Yeast mitochondria, on the other hand, do not form under anaerobic conditions of growth (Linnane, Vitols, and Nowland, 1962), or, according to Yotsuyanagi (*see* Gibor and Granich, 1964), exist under these conditions only as small promitochondria. Unlike in mitochondria, the respiratory electron systems in bacteria show great variations. There are aerobic and anaerobic electron transport systems, and it appears that there is no direct correlation between the respiratory activity and the cytochrome content. Also, the respiratory chain changes with environmental conditions (Dolin, 1961*a*, *b*; Newton and Kamen, 1961; Smith, 1961). In addition to that, some enzymes that are located in the membrane are inducible, such as the nicotinic acid hydroxylase system (Hunt, Rogers, and Hughes, 1959), and other membrane-bound enzymes, such as many polyoldehydrogenases, occur exclusively in microorganisms (Hughes, 1962). Therefore, with more information on the fine structure of the bacterial cytoplasmic membrane, it should become possible to correlate a wide spectrum of functional capacities with their structural bases and to locate these activities within the membrane.

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