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# Symposium on the Fine Structure and Replication of Bacteria and Their Parts

## II. Bacterial Cytoplasm[1]

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### INTRODUCTION

One of the deeper insights into the organization of living matter gained today is the recognition, almost down to the molecular level, of a basic organizational pattern common to all cells. Kluyver's theory of the "unity of biochemistry" is becoming balanced by knowledge of a "unity of organizational pattern on the ultra-structural level." In the tissue cells of plants and animals, this pattern may be complicated by morphological specialization related with function; yet comparable physiological functions are integrated in similar morphological entities. Oxidative phosphorylation takes place in mitochondria, for example, and protein synthesis on ribosomes.

Since, chronologically, most of the knowledge of the ultrastructural pattern of the "higher" cells was obtained before that of the microbial cells, and because, in the former cells, this pattern is more differentiated than in the latter, it seems logical to summarize first the main features of cytoplasmic fine structure of cells in general before passing on to an interpretation of the fine structure of the microbial cell.

The present treatment of the cytoplasmic structure in the bacteria is not exhaustive, and is based mainly on observations made in the author's laboratory in Amsterdam.

### OUTLINE OF THE CYTOPLASMIC STRUCTURE OF THE "CELL IN GENERAL"

One of the main features of the cytoplasmic area, situated between the outer cell integument and the nucleus, is its intersection by numerous membranous profiles (Diagram 1). These membranes fall into various classes. There is, generally, an elaborate reticular system coinciding with the chromidial, or basophilic, part of the cell (ergastoplasm), which is referred to as "endoplasmic reticulum" (ER) by Porter (64) or "cytomembranes" by Sjöstrand (80). The true existence of this system was confirmed by observations in the living state (45); the electron microscope reveals that it consists of clusters or stacks of thin membrane-limited vesicles (cisternae) with numerous small particles attached to their surfaces (the rough form of the ER), and also of an extensive network of tubules with apparently no association of particles (the smooth form of the ER). The particles are made up of ribonucleic acid (RNA) and protein and are the ribosomes responsible for the basophilia of the cell. The configuration and extension of the ER varies with the cell type in which it occurs and with the latter's physiological condition. For example, association of the ribosomes with the ER membranes is very frequently encountered in cells engaged in the synthesis of protein for export, such as in secretory cells. The elements of the smooth form of the ER are supposed to be involved in the segregation of all products other than protein. The ER is assumed to represent an intracellular transport system, and to have a role in (i) the amino acid incorporation by the protein-synthesizing ribosomes on its surface (see below), (ii) the carrying of enzymes and metabolites and hormones important in physiological events taking place in certain cytoplasmic regions (62), (iii) intracellular pulse conduction, and (iv) shifting between different substructural states with different phases of function which could be the basis for protoplasmic streaming (46).

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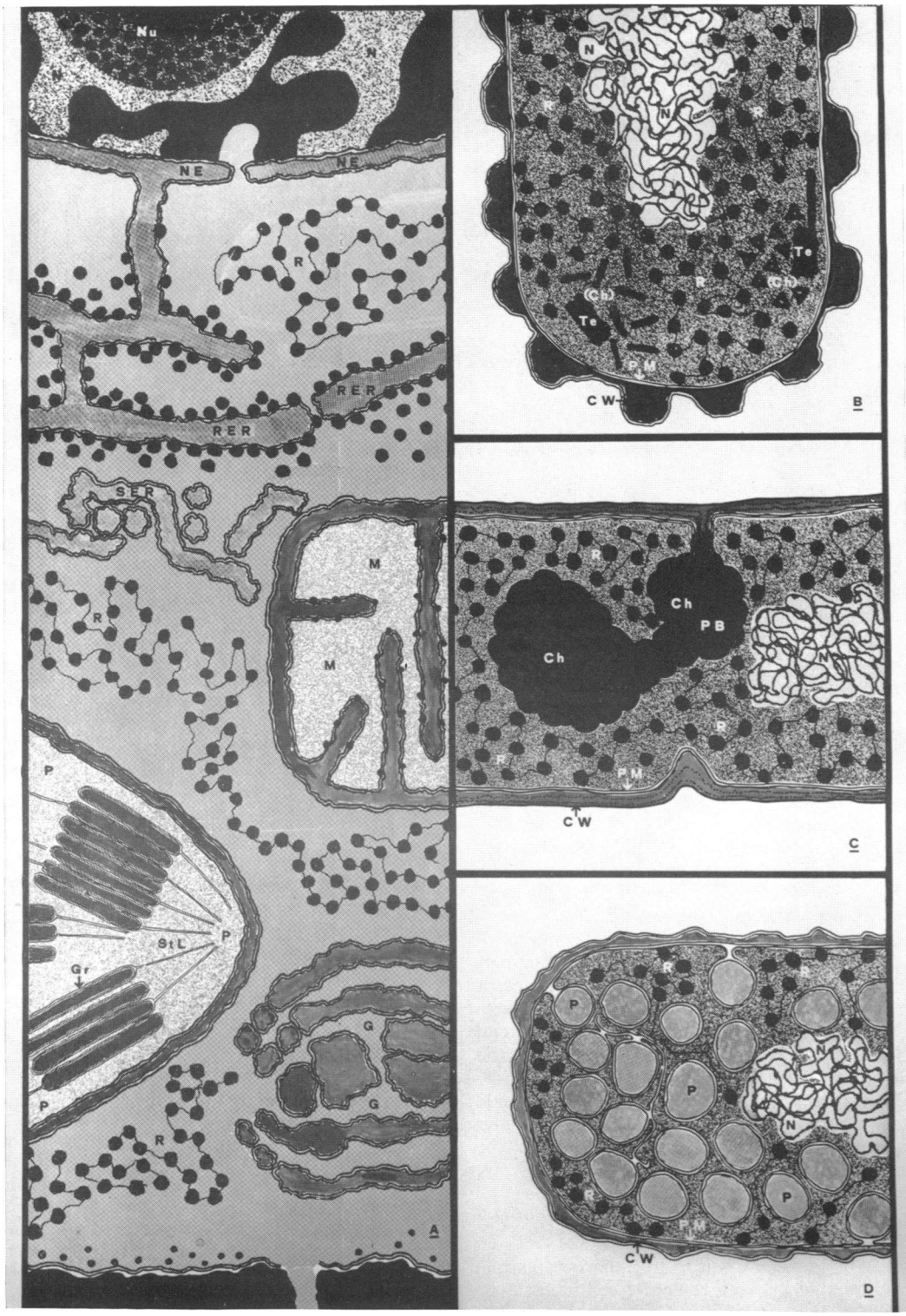



DIAGRAM 1. *Diagrams of the "cell in general" (A), a gram-negative bacterium (treated with tellurite; see text; B), a gram-positive bacterium (C), and a photosynthetic bacterium (D). To facilitate comparison of the fine structural detail in these different cell types, the drawings were made more or less to scale. Symbols: (A) RER, rough form of ER; SER, smooth form of ER; R, ribosomes; G, Golgi complex; N, nucleus; NE, nuclear envelope; Nu, nucleolus; M, mitochondrion; P, plastid (chloroplast); Gr, granum disc; StL, stroma lamellae. (B, C, D) PM, plasma membrane; CW, cell wall; P, plastid (chromatophore); Ch, chondrioid; PB, peripheral body; N, nucleoid; Te, reduced tellurite; R, ribosomes.*

Distinct from the ER, but intermittently continuous with the rough-surfaced cisternae, is a compact system of slender membranous sacs and associated vesicles, the Golgi system, often referred to as dictyosomes. It is regarded as a "special system set aside for packaging materials prior to their secretion" (64). Thus, enzymes synthesized by the ribosomes are first segregated in the cisternae of the ER and then enter the vesicles of the Golgi complex. In the Golgi system, these proteins are stored as droplets or granules and, when increasing in volume, they may become the bodies described to occur in some secretory cells similar to the zymogen granules in the pancreas.

In many cells there are other membrane-limited elements, such as the lysosomes (which contain hydrolytic enzymes), the microbodies, and related particles (61).

Even in the "higher" cells, the ER is not completely independent of other cell membranes. Connections have been observed between membranes limiting vesicular elements of the ER and the outer one of the two membranes covering the nucleus. For this reason, Porter regards the cytoplasmic reticulum as an extension of the nuclear envelope. Robertson (67) suggested that the ER represents an elaboration of the cell's surface and included in his hypothesis even the mitochondria, which he considers to have originated from infoldings of that membrane. This latter idea is not founded upon observation; however, in more primitive organisms such as some fungi, McAlear and Edwards (53) observed continuity of the plasma membrane with the endomembrane system and the outer nuclear membrane. In this connection it may be recalled that, in the yeast *Pichia membranaefaciens*, I observed that the mitochondrial boundary forms a continuum with the nuclear envelope (42). This fact would make it possible to define such a mitochondrial boundary as belonging to the ER. Recently, however, considerable doubt has been cast on the unitary concept of membrane structure, in which all intracellular membranes are regarded as essentially one system in which some interconnections are lost in the course of evolution. Robertson's hypothesis was based on the argument that all membranes would essentially be of similar structure, i.e., a bimolecular leaflet of lipid molecules sandwiched between two protein layers. Data are now rapidly accumulating which show that, even in the same cell type, the chemical composition and the ultrastructure of membranes of the various cell constituents vary significantly (7, 80).

All the membrane systems mentioned above have in common that a single membrane envelops their elements. It is of significance that the organelles bearing the enzymatic system for respiratory electron transport, i.e., the mitochondria, and those bearing the enzymatic system and pigments for the photosynthetic electron transport, i.e., the plastids, are typically built with two parallel membranes. As is widely known, the mitochondrium is bounded by a wall composed of two membranes, the innermost of which is folded as the parallel *cristae mitochondriales* into a homogeneous matrix in the mitochondrial cavity. [At present a great deal of activity is centered around ultrastructural investigation of the mitochondrial membranes carrying electron-transport particles; provocative observations have been made on negatively and positively stained material by Férnandez-Morán (15), Parssons (63), Stoeckenius (83), and Sjöstrand (79).] There are cases, however, in which the arrangement of the cristae may be different, e.g., as concentric membranes in spermatides (2), or as tubules in some protozoa (74, 96). Likewise, the chloroplast is bounded by two membranes and encloses lamellae consisting of two membranes which, in the grana regions, are often a little further apart than in the stroma region, thus forming the granum disc (30).

Little is known of the origin and development of most cell components. The kinetosomes, the centrioles and basal bodies at the base of each cilium or flagellum, have had the reputation for a long time of being self-perpetuating particles in the cytoplasm. Similar ideas have been put forward about the mitochondria and plastids; therefore, it is intriguing that data are accumulating that both are provided with small quantities of deoxyribonucleic acid (DNA; 58, 59, 66).

In the space between the membrane-limited elements, ribosomes are often the only structures that can be resolved. Fibrous elements such as the myofilaments of muscle fibers, the tonofibrils of epithelial cells, or fibroglial fibers of motile cells should, of course, be regarded as specializations. The empty spaces that become visible at sufficiently high electron-microscopical resolution may account for the high water content of the cytoplasm in its original, fresh state. In many cells, part, or even all, of the ribosomes do not appear attached to ER membrane surfaces. In vivo experiments with guinea-pig pancreas cells (77, 78) and with rat-liver (36) cytoplasm suggest that the membrane-bound ribosomes are the major site of the protein synthesis. According to Henshaw et al. (36), the "free" or "unattached" ribosomes would be almost completely inert. This would seem rather contradictory since these latter ribosomes are characteristic of embryonic cells. It has been suggested that the

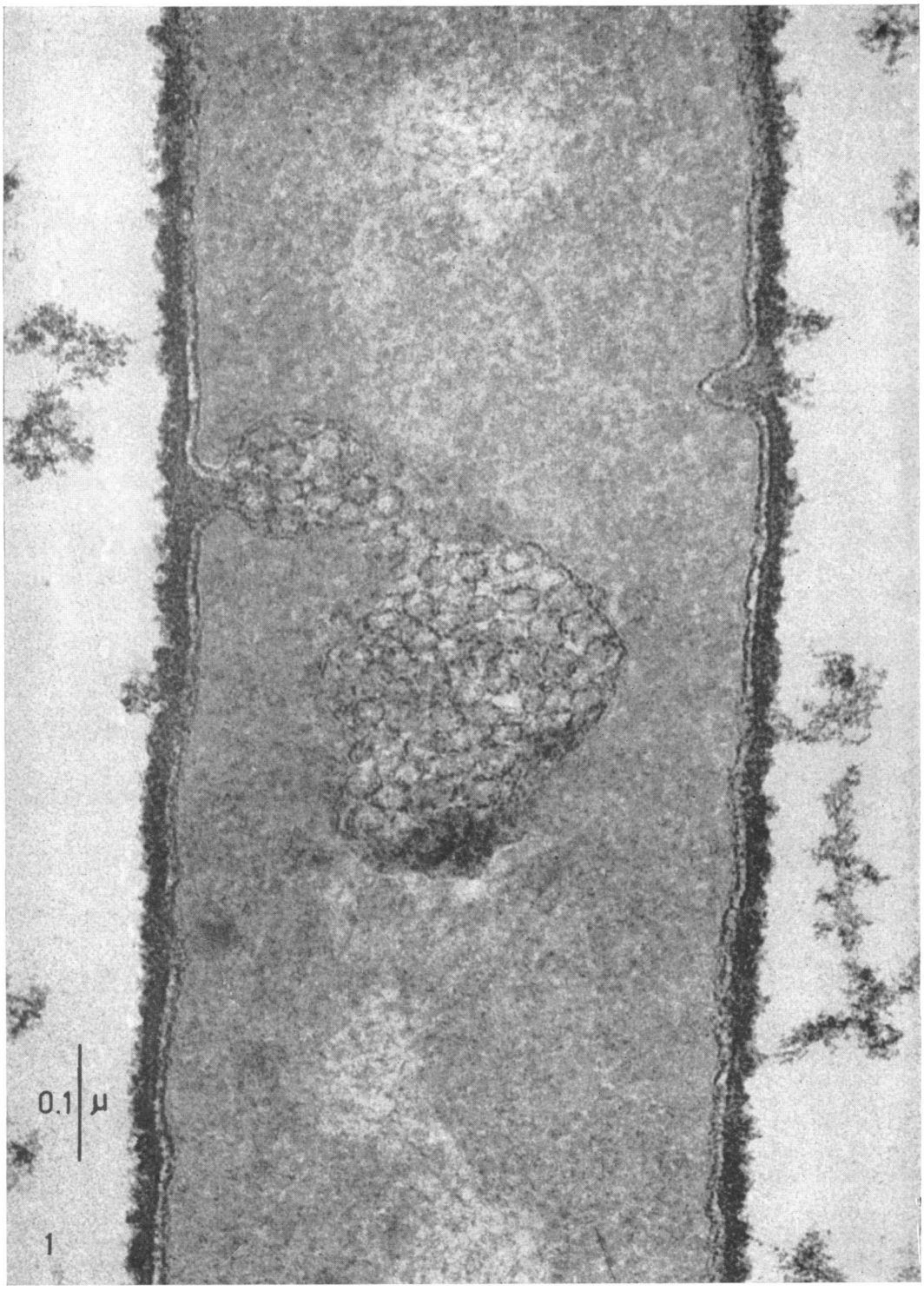



FIG. 1. *Bacillus subtilis as an example of the gram-positive cell type in which the thick wall and the plasma membrane are united along their entire length. The cell division in the Bacillus is effected through centripetal growth of a cross wall, in the formation of which the vesiculated organelle ("peripheral body," "chondrioid") may be involved. More centrally the vesicle cluster is in contact with nucleoplasm.*

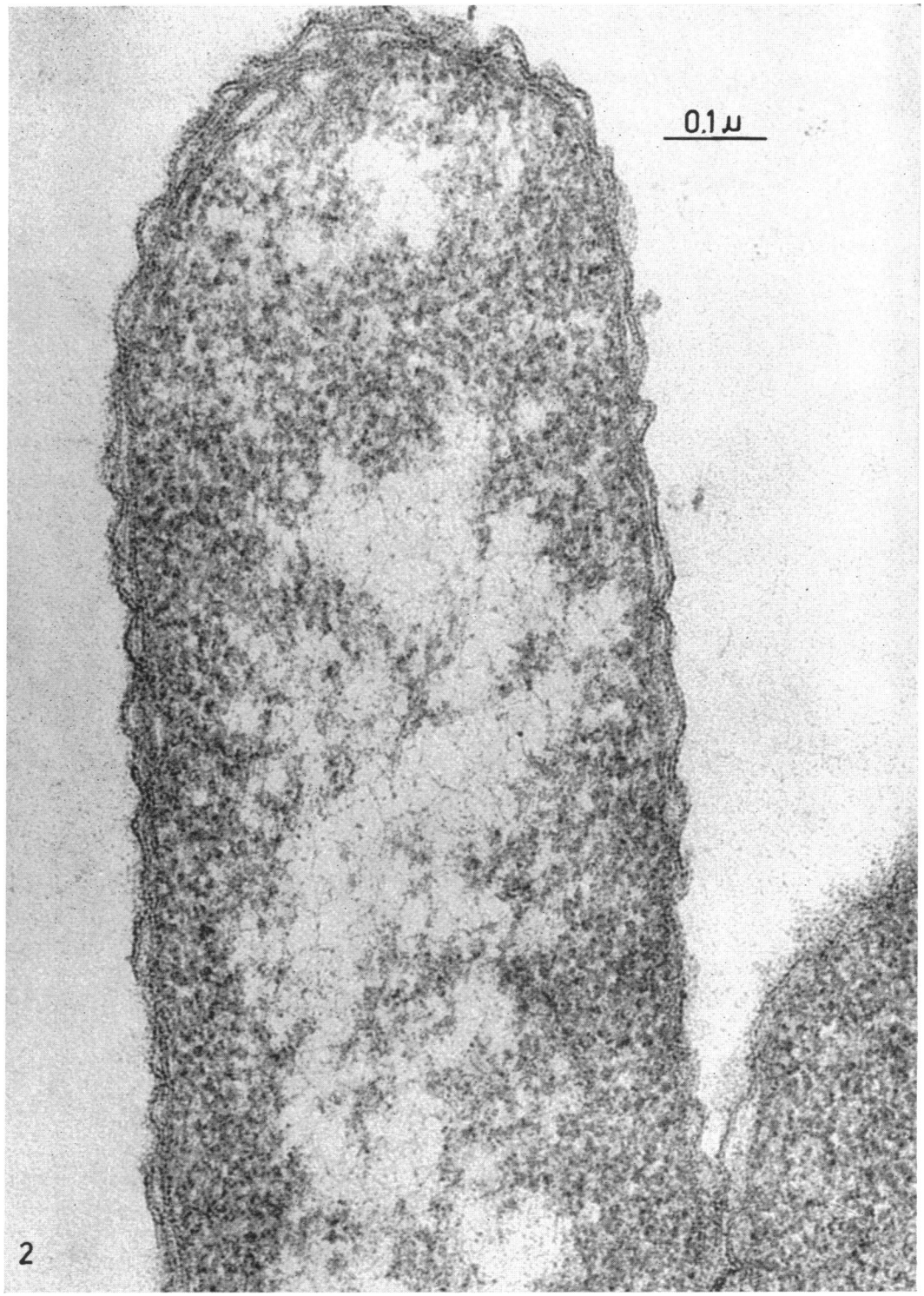



FIG. 2. *Escherichia coli B as an example of the gram-negative cell type in which the cell wall is sinuous and not parallel to the plasma membrane. At the cell tip, the plasma membrane has additional layers and infoldings into the cytoplasm. Note the fine structure of the cytoplasm.*

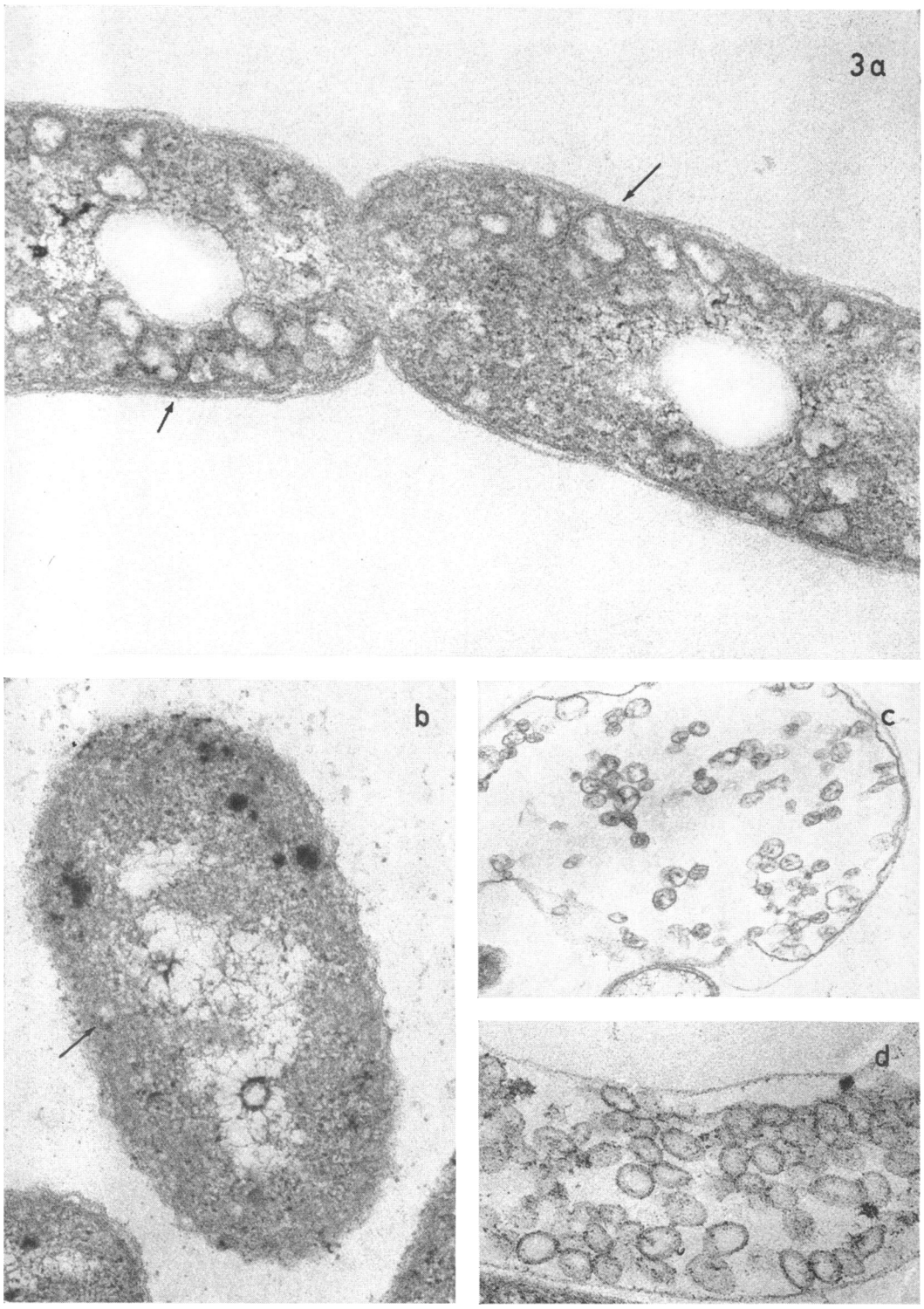
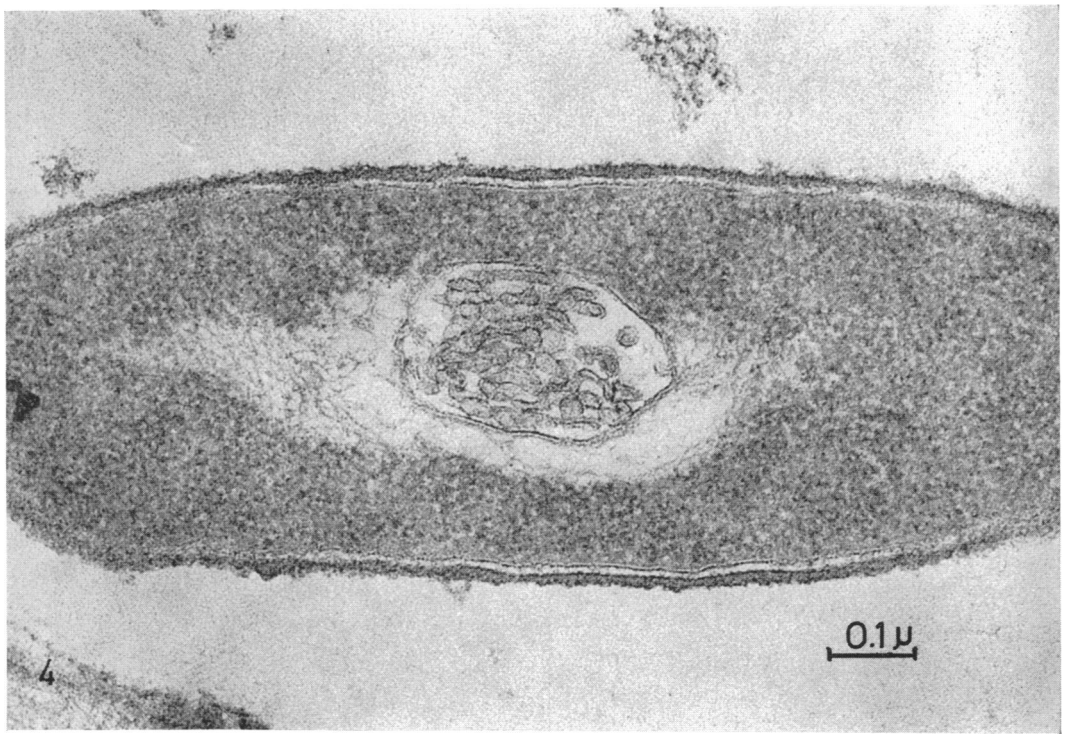



FIG. 3. *Rhodospirillum rubrum. When Rhodospirillum cells grown aerobically in the dark are cultured anaerobically in light, photosynthetic vesicles slowly appear. (a) A cell from a 36-hr-old culture grown under moderately strong illumination. The plastids (chromatophores) are vesicles bounded by a single membrane, comparable to the plasma membrane with which they are occasionally seen to be connected (at arrows). The big holes were originally occupied by poly-β-hydroxybutyric acid. (b) Cell from a culture exposed 6 hr to light. At this stage, there are few membranous profiles, but they are definitely present (arrow). The dark granules may be polyphosphate. (c and d) In autolyzed cells, it can more easily be seen than in intact cells that there is actually a reticulum of interconnected vesicles continuous with the plasma membrane.*



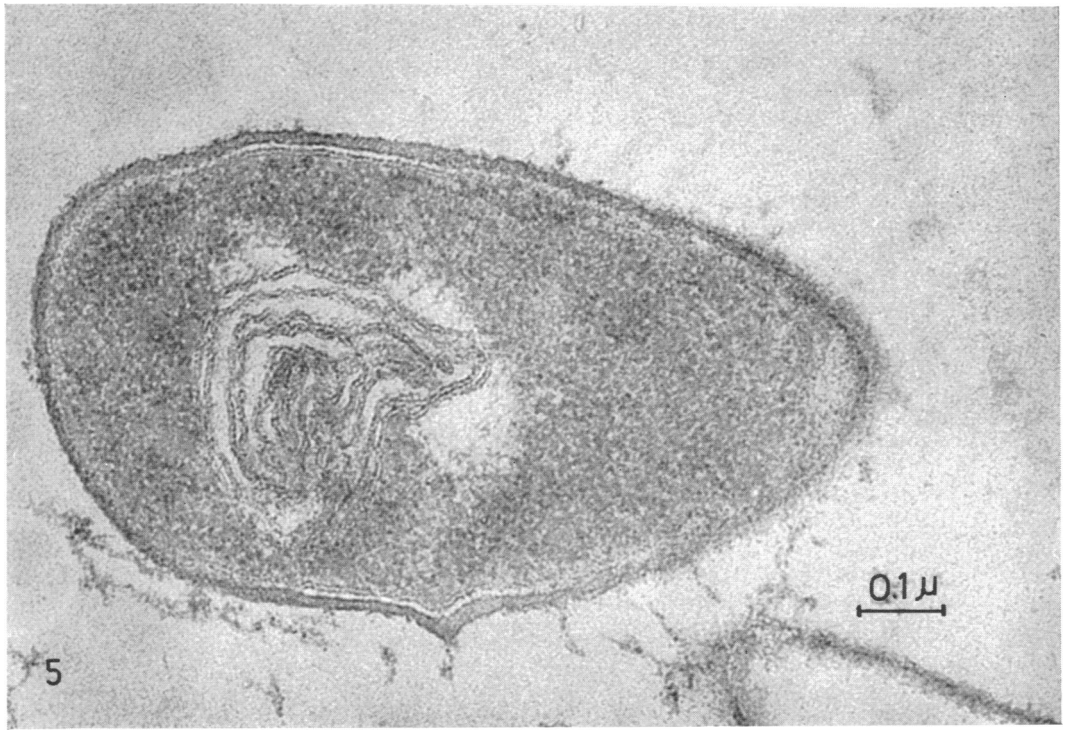




FIG. 4. *Bacillus subtilis. Chondrioid of vesiculo-tubular structure in contact with nucleoplasm. Note that the borders of the vesicles and tubules, the membrane surrounding the chondrioid, and the plasma membrane have about the same electron density.*

FIG. 5. *Bacillus subtilis. The chondrioid is here a whorl of membranes of about the same electron density as the plasma membrane.*

"free" ribosomes are associated with processes of growth and differentiation in which the products of synthesis are retained. In the cytoplasm of embryonic or differentiating cells, the "free" ribosomes appear sometimes to be arranged in helices (6, 88), and much emphasis is being placed on the agglomerations of ribosomes, called polyribosomes, which are considered to be the operative units in the synthesis of, for instance, hemoglobin.

## OUTLINE OF THE CYTOPLASMIC FINE STRUCTURE IN GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Comparable with the cytoplasm in all known cells, the bacterial cytoplasm is bounded by a plasma membrane or plasmalemma of triple-layered structure (Fig. 1, 2, 4, 5). After successful Ryter-Kellenberger fixation, this plasma membrane in gram-positive bacteria is covered smoothly by a thick wall (Fig. 1). On the other hand, in the gram-negative organisms investigated so far, the plasma membrane seems to be, along most of its length, separate from the thin sinuous cell wall (Fig. 2). I believe that, in gram-positive cells, both integuments are naturally united (Fig. 1), which explains why generally these bacteria are more difficult to plasmolyze than the gram-negative ones (16).

A very important difference from most other cells is that, in the bacterial cell, any delimitation by means of a membrane between the cytoplasm and the nucleoplasm is nonexistent. Some structural consequences of this phenomenon will be dealt with in the section on the ribonucleoprotein in the cell.

In sufficiently thin sections, almost the whole cytoplasmic area appears in the electron microscope as a delicate mosaic that can only be studied effectively at high resolution (see section on ribonucleoprotein). In certain bacteria, but definitely not in all, membranous profiles can be observed. In thin sections of *Escherichia coli* (Fig. 2), *Haemophilus influenzae*, and *Proteus vulgaris*, frequently none of these can be seen; if profiles are occasionally present, they are primarily single and enclose some cytoplasm or an empty-looking space. The most pronounced development of "empty" membrane-bounded spaces that I have noticed is in the *Proteus* L-form, L-9. Often, additional membranous profiles in these spaces enclose isolated quantities of cell material ("minimal reproductive units"?). Membranous elements of a comparatively simple kind can be seen in *Spirillum serpens*. [The interesting "polar membrane complex" close to the flagella bundles described for *S. serpens* by Murray and Birch-Anderson (57) need not be considered here.] In *Azotobacter agilis* (42) and *Rhodospirillum rubrum* (42, 11, 8), the cells can be filled up with vesicles appearing as rounded profiles of single membranes, a phenomenon that appears to be connected with their respective processes of nitrogen fixation and photosynthesis. In *R. rubrum* (Fig. 3), indications were found that the vesicles are interconnected and connected with the plasma membrane (42). Each of them is like the granum disc bounded by a single membrane. All the bacteria mentioned so far are gram-negative.

In gram-positive bacteria, the situation of membranous profiles is very different from that in the gram-negative species; the structures are more complex. Systems of such membranes have been described for several *Actinomyces* species (23 to 26, 13), in which they are the most elaborate so far found, for mycobacteria (25, 12, 76, 49, 38), *Corynebacterium* (25), *Micrococcus* (40, 56, 71), *Staphylococcus* (28, 84), *Lactobacillus* (28), *Bacillus* (40, 17, 20, 27), and *Listeria* (14, 47). One explanation—but definitely not the only one—for the occurrence of particular membranous organelles in gram-positive bacteria is their relation to the process of division. Differences in the mode of division have been analyzed with the light microscope by Robinow (68). In the gram-positive bacteria, the division is initiated by the inward growth of a cross wall, whereas in the gram-negative cells a constriction of the middle of the cell precedes the separation of the two daughter cells (42). Favorable sections of gram-positive species (Fig. 1 of *B. subtilis*) show a so-called peripheral body (9) in connection with the ingrowing septum. In Fig. 1 the body appears to be integral with this new wall; it is composed of vesicles, each bounded by one dense line. Both the peripheral body and the developing cross wall have a continuous bound-

FIG. 6. *Bacillus subtilis treated with potassium tellurite. Series of four sections through a chondrioid showing the latter's connection to the cell envelope. (a) On the lower side of the picture, a little underneath the electron-opaque deposit of reduced tellurite on the left, is the lower part of the tube connecting the chondrioid with the cell envelope. (b) The vesiculo-tubular structures in the chondrioid are connected to the cell periphery by a tube. (c) In the original print it can be observed that the chondrioid is enveloped by a continuation of the plasma membrane, and furthermore, that the tube within the lower space of the chondrioid protrudes towards the cell wall through the empty space between the cell wall and the plasma membrane caused by the latter's retraction. The empty-looking space inside the chondrioid and the space between the plasma membrane and the cell wall are thus interconnected. (d) In the chondrioid are concentric membranes which accumulated reduced tellurite.*

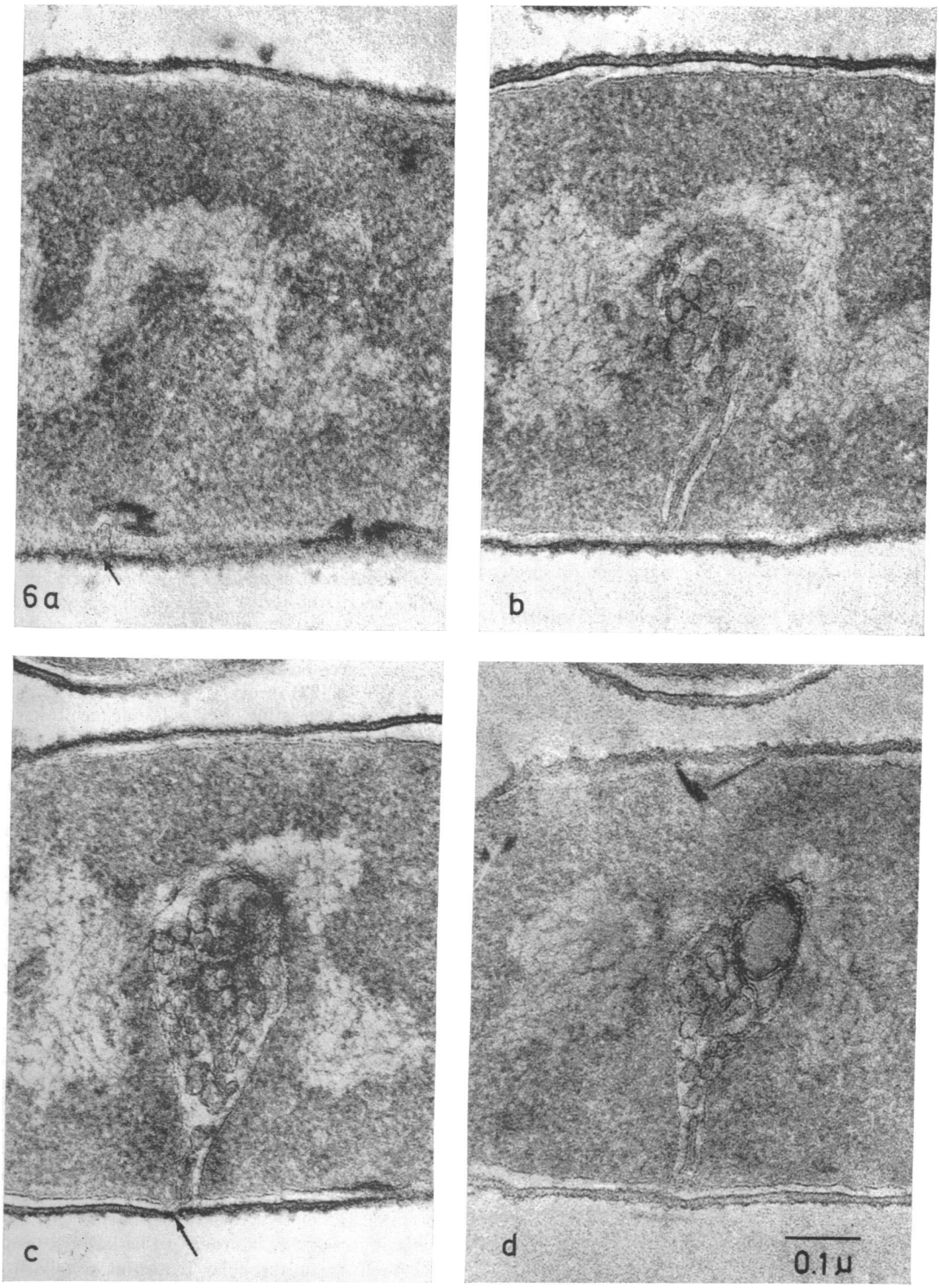



FIG. 6

ary, seen as a delicate line. The fine structure of the cell wall appears to continue in the basal part of the peripheral body, suggesting that this organelle is functional in the synthesis of the new septum (9, 40, 26, 41). In the cell center, the vesicle cluster appears in contact with the nucleoplasm.

Fitz-James (17, 18) deduced from his micrographs of bacilli that similar bodies may play a role in the formation of the spore membrane. However, the presence of the membranous structures is not restricted in cells to sites where new walls or spores are being formed. In single sections, these organelles can be seen to occur in the nuclear area (Figs. 4, 5), in the cytoplasm, or in the periphery of the cell (41). Their fine structure is also variable; they can appear as clusters of vesicles and tubules (Fig. 1 and 4), as membranous whorls (Fig. 5), or as combinations of these two types of structures (Fig. 6d).

Fitz-James (17) introduced the word "mesosome" for these membranous structures, a term based on Robertson's (67) suggestion for electron microscopy "that 'meso' is either a double membrane, leading from some included structure to the outside" or might be used for "cytoplasmic structures connected to the outside by a double membrane." The composite structures shown here, however, appear wrapped in a unit membrane sometimes seem to be continuous with the plasma membrane (Fig. 6a to d), and it seems quite possible that complete series of sections of whole bacteria will eventually prove that it is a regular feature of the membranous structures in all gram-positive bacteria to have a connection somewhere with the outer envelope (70). It appears preferable not to coin a term based on a morphological feature, but to wait until a name can be suggested which qualifies the most important function (see below). In some micrographs (Fig. 6a to d; 43), it can be observed that an element, as well as the space in the tube connecting the membranous organelle with the cell envelope, is in open connection with the cell wall proper. Such a construction seems to imply that a separately enclosed system within the cytoplasm and nucleoplasm may allow exchange of solutes between the cell interior and the environment without the necessity of passing the cytoplasm proper. In case such functions are likely to occur, it may be possible to relate the membranous organelles to the endoplasmic reticulum (ER) and to the Golgi system of the more highly differentiated cell. But, as yet, such speculations are premature. I investigated the possibility that these membrane systems might harbor the respiratory enzyme chain comparable with that in the mitochondria of the ordinary plant or animal cell.

### CHONDRIOIDS

Some authors identified the membranous structures in the gram-positive bacteria with mitochondria on morphological (10, 12, 20) and physiological (76) grounds. This appears premature because, for morphological identity, it must be demonstrated that the structures are built according to the same patterns and principle as true mitochondria. Mitochondria are closed systems based on double membranes, whereas the structures in bacteria are perhaps open towards the cell wall (Fig. 6a to d), are surrounded by one unit membrane only, and have interior structures that are unit membranes or are bordered by an even thinner layer. For physiological identity, it should actually be proved biochemically that, in the structures in the bacteria, the same pathways of oxidative phosphorylation occur as in the mitochondria. Also, few authors appear puzzled by the fact that the membrane systems are typical of gram-positive bacteria mainly, so that the gram-negative organisms would be devoid of analogous mitochondria.

The results of earlier work on the location of the respiratory enzyme chain in particulate "bacterial mitochondria," in particular by Mudd (54, 55), have been doubted on account of Weibull's (91, 92, 93) recovery of important enzymes in a fraction consisting of cytoplasmic membranes but associated with some granular material. There are two approaches to the problem: "direct cytochemistry" (52), i.e., the biochemical study of cell fractions, and "analytical morphology" (52), i.e., location of the sites of enzyme activity in the intact cell. Recently, Salton and Chapman (71) found that it is not very feasible to separate intracytoplasmic membranes and plasma membranes. I considered it desirable to work out, in the first place, a method that would convincingly reveal the location of the respiratory enzymes in thin sections of the complete gram-positive or gram-negative bacterium.

The method chosen was to localize the sites of reductive activity cytochemically with the help of a salt of tellurite. Since not as much is known about this reaction as about the reduction of tetrazolium compounds to formazans, an experiment was also made with one of the newer tetrazolium compounds, tetranitro-blue tetrazolium (69, 75). This method may be more convincing because it is more conventional, but, in the bacteria investigated, it proved to be less spectacular than the intracellular reduction of tellurite. The advantages of the tellurite procedure for bacteria (43) are: (i) potassium tellurite (0.05%) can conveniently be added to the normal culture medium, (ii) the precipitate of the reduced tellurite has a much greater electron opacity than

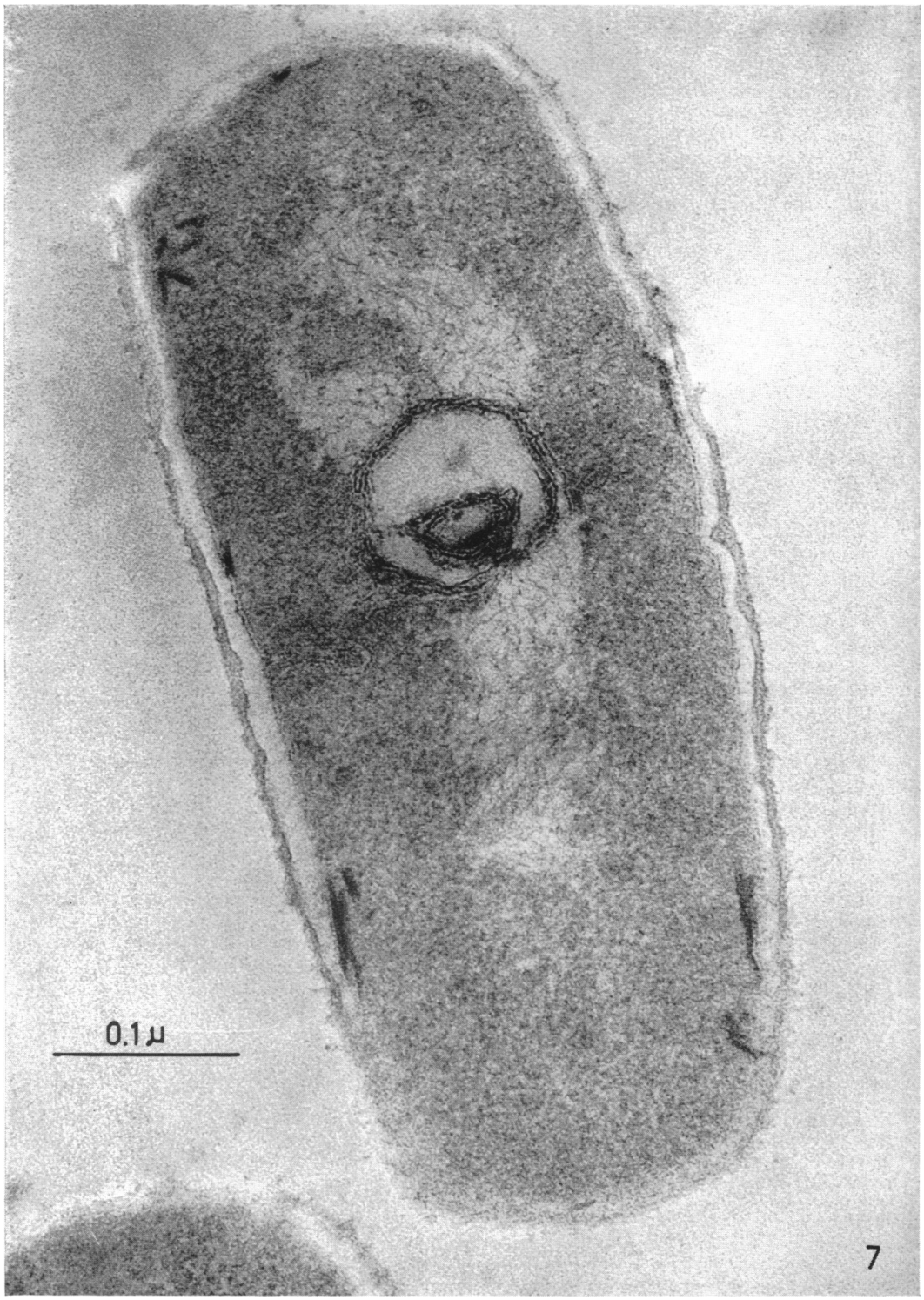



FIG. 7. *Bacillus subtilis cell grown for an extra hour with potassium tellurite in the culture medium. The reduced product appears deposited in, or on, the membranes of the chondrioid, and in slender rodlike structures in the cell periphery. These rodlike structures have not been noticed in untreated cells. In some of the rods, there is a suggestion of structural details, but it may not be completely ruled out that this is influenced by overlapping cytoplasmic structures.*

organic matter, and (iii) the precipitate may be expected to occur close to the site of its formation, since it is insoluble. After one to several additional hours of incubation, in which aeration by agitation was stopped, the bacteria were spun down and prepared for electron microscopy. The sediment of the cells tested (*B. subtilis* and *P. vulgaris*) had then turned black, whereas the supernatant fluid was clear. In thin sections of *B. subtilis*, the reduced product was clearly visible at two distinct sites. Whereas the plasma membrane did not gain in electron opacity by deposition of the reduced product, the reduction product was found on or in the membranes of the particular organelles or "mesosomes" (Fig. 7 with the controls of the same experiment in Fig. 4 and 5). Thus, possibly they may be regarded as the mitochondrial equivalents or chondrioids (48) in this gram-positive bacterium. A second location was in thin rodlike elements at the cell periphery (Fig. 6a, c, d, and 7). The effect of the reaction in the membranes of the chondrioids can be appreciated in particular when their electron opacity is compared with that of the plasma membrane in the same cell. In the control cells (Fig. 4 and 5), these opacities appear quite comparable, in contradistinction to the tellurite-treated cells. When *B. subtilis* was treated with tetranitro-blue tetrazolium in phosphate-buffered succinate solution, a positive reaction of the chondrioids was definitely obtained (Fig. 8), but the formazan formed is not so sharply delimited along the membranes and is far less opaque than the reduced tellurite. Moreover, in these experiments, subtle changes occurred in the cell structure: the details in the cytoplasm became vague, the plasma membrane was difficult to distinguish, and the contrast between cell wall and cytoplasm increased. It is of some interest that, with this method, the rodlike structures in the periphery of the cell are not made visible.

To obtain unequivocal proof of the presence of the reduced tellurite inside the *B. subtilis* cell, electron micrographs were made of tellurite-treated cells subsequently fixed in glutaraldehyde. Such electron micrographs show the various density gradations reflecting the natural mass distribution free from heavy metals introduced during $OsO_4$ fixation and post-treatment with uranyl acetate. Figure 9 reveals clearly the deposits of the reduced tellurite in the center and in the periphery of the cell, although the fine structural details are disturbed.

The slender rods in the cell periphery (Fig. 6 and 7) could perhaps be at the bases of the flagella. With the aid of shadowed whole cells, this possibility was investigated further. Figure 11 indeed conveys the impression that the flagella might emerge from bundles of these dense structures.

A similar study of *P. vulgaris* revealed that in this gram-negative organism the reduced tellurite was deposited in bodies contiguous with the plasma membrane and of a structure very different from that in *B. subtilis* (44). In shadowed whole-cell preparations (Fig. 12), these bodies which incorporate the reduced tellurite appear to be opaque, somewhat rosettelike structures, and there are indications that the flagella might emerge from them. The details of the chondrioids in *Proteus* were studied in serial sections (44). They appeared to have a complex structure consisting of a very fine granular "matrix" of low electron density, dense rodlike structures, and a mass of seemingly entangled, very delicate, filamentous or lamellar structures, partly apposed to the plasma membrane. A limiting membrane was not observed around these complexes (Fig. 13 to 15).

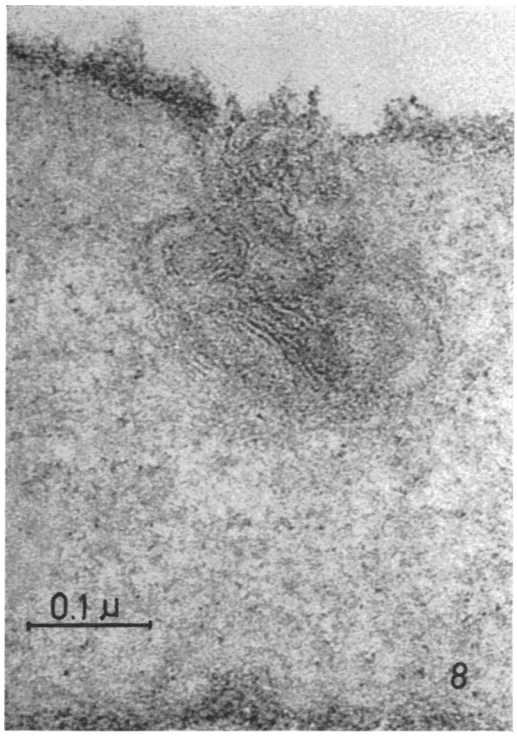
*Proteus* cells fixed in glutaraldehyde, like those of *B. subtilis*, convincingly demonstrate that the plasma membrane has not accumulated any reduced tellurite (Fig. 10), since it is not even visible unless "stained" with a uranyl salt (44).

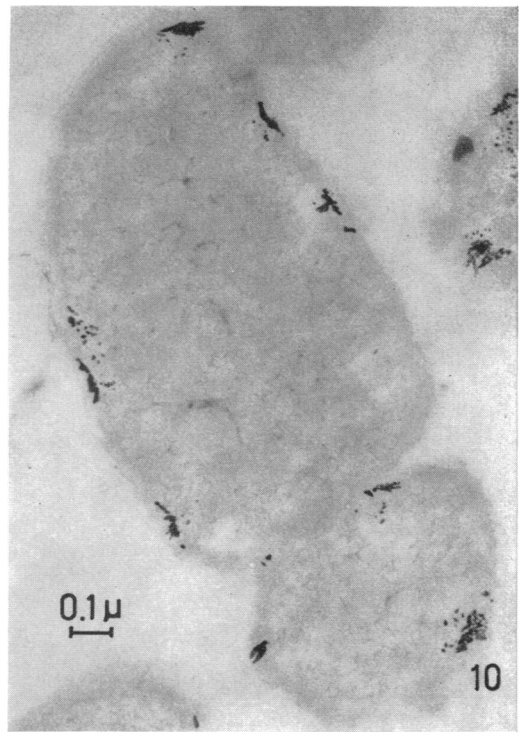
The reaction of the tellurite in these two bacteria is more or less analogous to what Barrnett and Palade (4) observed in the mitochondria of animal tissues. This deposition of reduced product in the mitochondria is explained by these authors

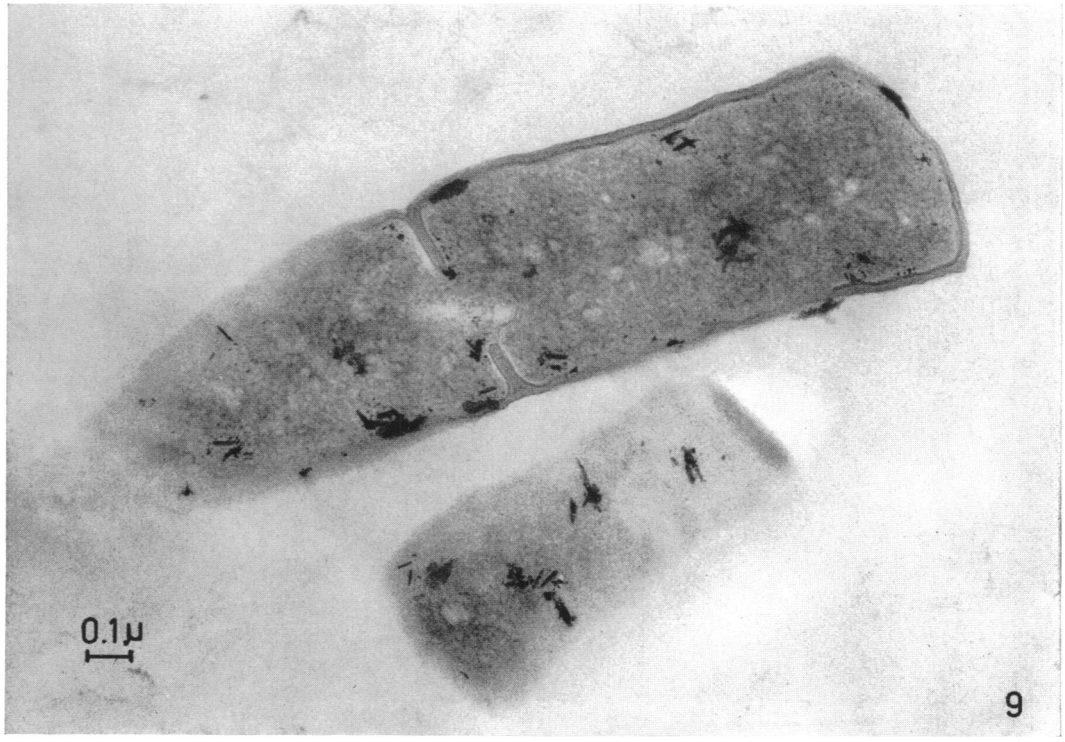
FIG. 8. *Bacillus subtilis cell treated with tetranitro-blue tetrazolium in phosphate-buffered succinate solution. The formazan formed is not so sharply deposited along the membranes of the chondrioid as the tellurite in Fig. 7. The contrast between the cell wall and the cytoplasm appears increased, and the plasma membrane is not easily seen.*

FIG. 9. *Bacillus subtilis cell treated with tellurite and fixed merely in glutaraldehyde. To give unequivocal proof of the presence of reduced tellurite inside the cell, introduction of heavy metals during the preparation was avoided here. The distribution of the electron densities is as nearly natural as could possibly be obtained. In this rather thick section, the reduced tellurite is seen in the cell center, in the periphery, and along the new cross wall. The bacterium on the lower side was sectioned close to its surface.*

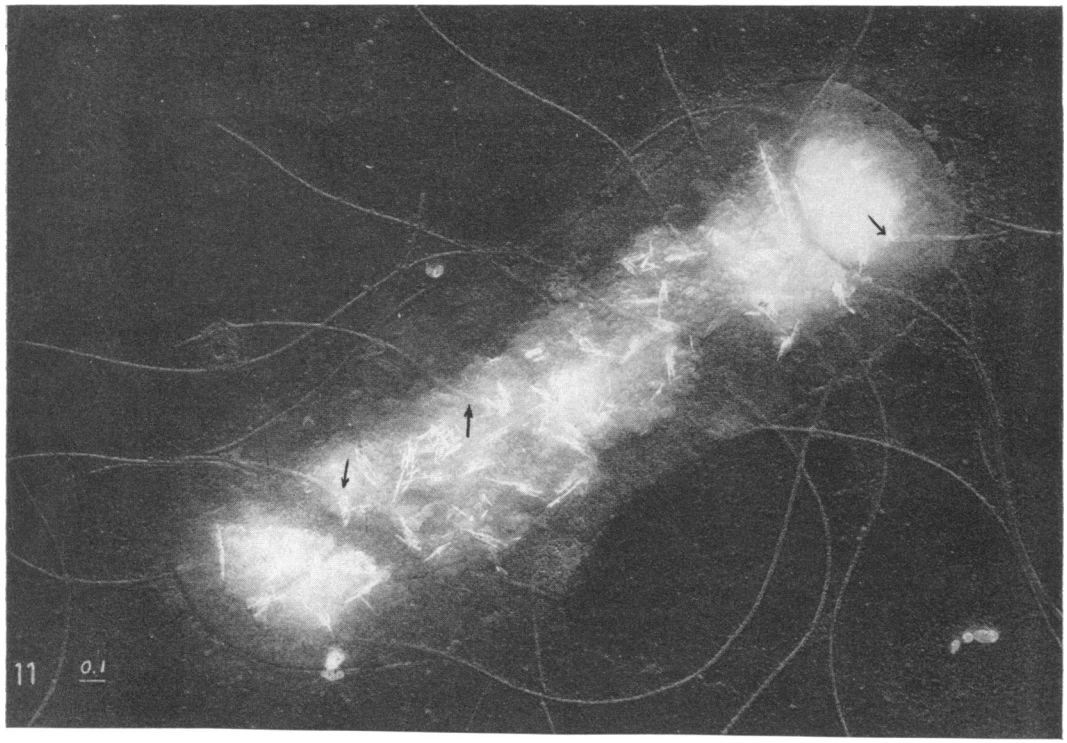
FIG. 10. *Proteus vulgaris cell treated with tellurite. Fixation was effected with 6.5% glutaraldehyde instead of with $OsO_4$, and treatment with uranyl acetate was omitted. The accumulations of reduced product are in the periphery of the cell, very close to the plasma membrane, and at the location at which constriction takes place in preparation for cell division. In this preparation of very poor contrast, the plasma membrane itself is not visible.*




FIGS. 8–10



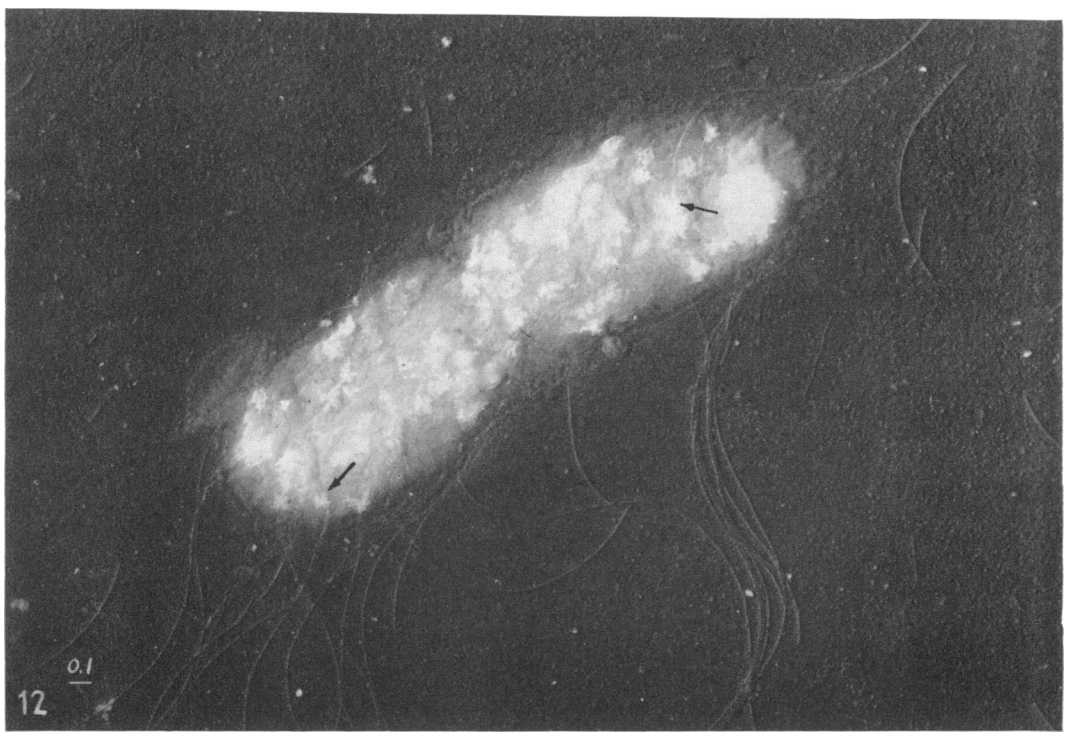




FIG. 11. *Bacillus subtilis treated with tellurite. In this shadowed whole cell, indications are that the flagella could emerge from the opaque accumulations of the reduced tellurite. (Printed in reverse.)*

FIG. 12. *Proteus vulgaris grown for 4 hr with potassium tellurite in the culture medium. In the flattened whole cell, shadowed with platinum, the somewhat rosette-like structures are the bodies which incorporated the reduced tellurite. Indications can be found that the flagella could emerge from these bodies. (Printed in reverse.)*

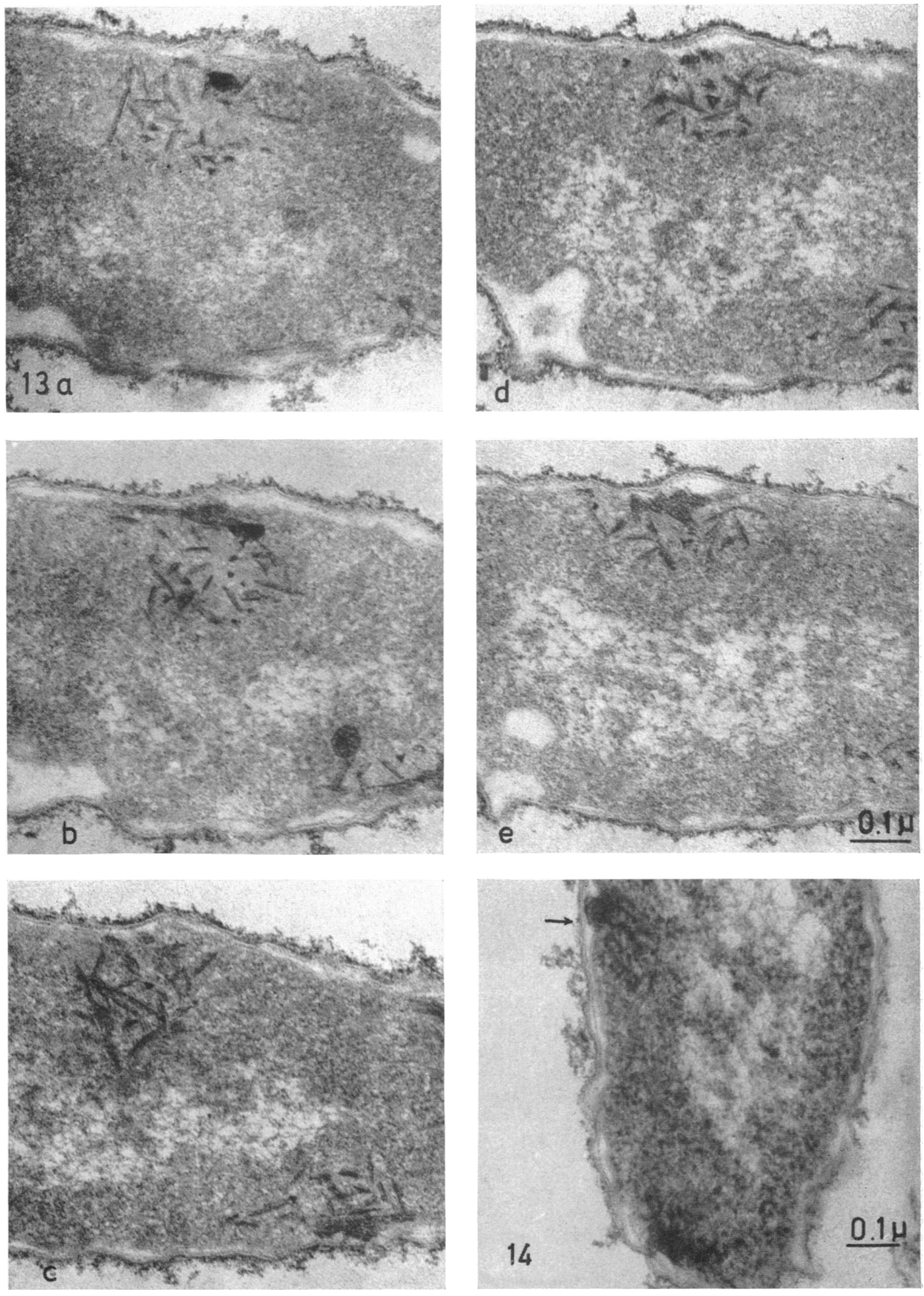



FIG. 13. *Proteus vulgaris. Five serial sections of part of a chondrioid, i.e., of a conglomerate of the elements containing the reduced tellurite. (a) There is a fine granular "matrix" between rodlike elements, in which there is reduced tellurite. In one of the rods is an indication of a periodic fine structure. On the upper side of the chondrioid is a rounded structure, visible also in the next section. (b) Conglomerate of rods around "matrix," a rounded structure, and some opaque material close to the plasma membrane. (c) Rodlike structures. (d) A triangular-shaped cross-section of one of the rods is visible. (e) Characteristic of the chondrioid structure is the dense mass apposed to the plasma membrane. Furthermore, rodlike structures are present.*

FIG. 14. *Proteus vulgaris. In this preparation, the reduced tellurite appears incorporated in very delicate lamellae.*

as indirect acceptance of electrons from respiratory enzyme systems by tellurite. They admit, however, that the exact chemical nature of the final product is unknown; it could be tellurium oxide (TeO) or tellurium (Te), or both. I was unable to obtain an electron diffraction pattern indicative of the presence of metal or metal oxide and, therefore, wonder whether the reduced tellurite might be present in organic compounds.

It remains to be proved whether my observations on *Bacillus* and *Proteus* can be generalized in a simple manner for all gram-positive and gram-negative bacteria. There are bacteria (*Corynebacterium* for instance) that can tolerate a considerable quantity of tellurite and store a large amount of product in their cytoplasm (95). The significance and mechanism of this phenomenon is in need of further investigation.

This study seems to suggest, however, that the plasma membrane itself does not play an important role in the respiratory enzyme chain, but it may, in the case of *Bacillus*, give rise to membranous organelles functioning as mitochondrial equivalents. The bodies close to the plasma membrane described for *P. vulgaris* possibly combine two properties: being the analogons of the membranous chondrioids and being the bases of the flagella in the gram-negative bacterium.

A preliminary account of these observations on the deposition of the reduced tellurite was communicated at the VIIIth International Congress for Microbiology (42). Since then, Vander-Winkel and Murray (87), working with 2,3,5-triphenyltetrazolium, which produces a soluble formazan, reached conclusions which, fundamentally, do not differ from the present ones.

The results indicate that bacterial chondrioids are structurally different from the ordinary mitochondria, and that they are not free in the cytoplasm but are still united with the plasma membrane.

## ORGANIZATION OF RIBONUCLEOPROTEIN IN THE CELL

In recent years, numerous reports have appeared dealing with ribonucleoprotein particles, i.e., ribosomes, that can be isolated after cells have been broken up. Throughout the cytoplasm of all living cells, comparable ribosomes are present and are considered to be the chief manufacturers of the various proteins. These ribosomes are generally characterized by their behavior in the ultracentrifuge. The basic unit of the ribosomal particle in all cells examined is about uniform, with a sedimentation constant of 70 to 85*S*, and is composed of two relatively stable subunits of unequal size, corresponding to about 50 and 30*S* in bacteria and 60 and 40*S* in mammalian ribosomes. The 50 and 30*S* subunits each contain approximately 65% RNA and 35% protein. In *E. coli* dividing every 30 min, the ribonucleoprotein has been determined to account for 30% of the cell's dry weight, and RNA for as much as 25% (86). Therefore, when studying bacterial cytoplasm in the electron microscope, we may expect that a considerable amount of the visible fine structure will be made up of RNA.

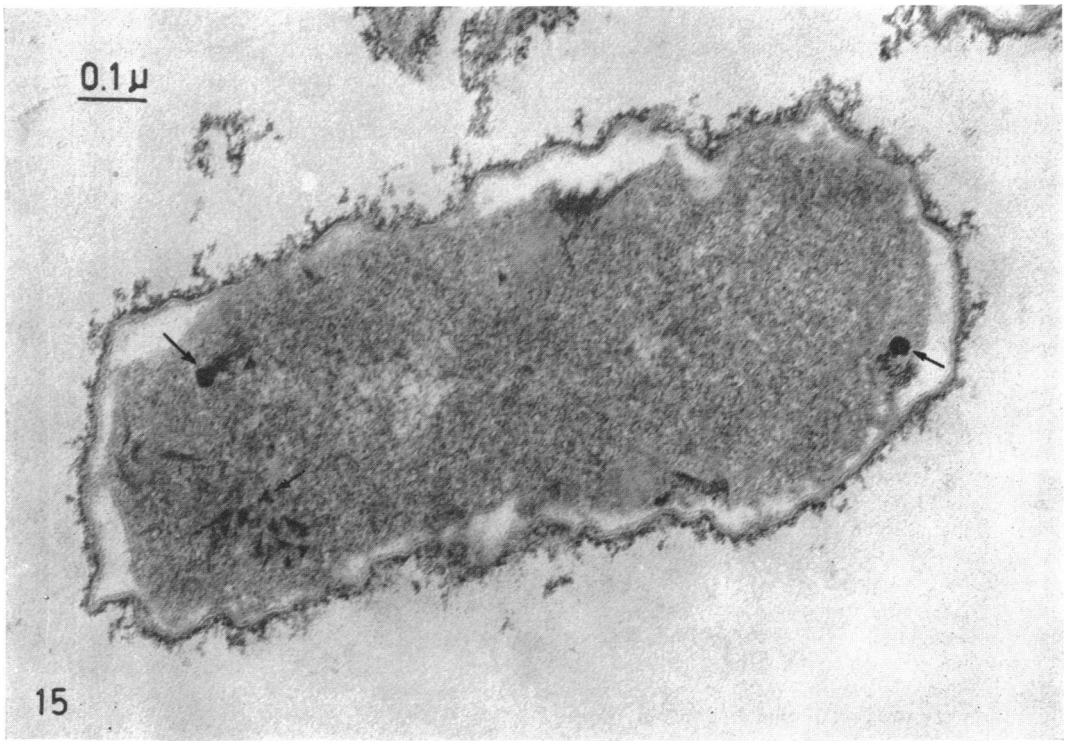
Values for the dimensions of the ribosomes have been given by Hall and Slayter (31) and Huxley and Zubay (37). The latter authors stained purified *E. coli* ribosomes negatively and positively after a fixation in formalin, whereas Hall and Slayter shadowed them with platinum. If the width of the particle is taken to be about 140 to 170 A, the length of the 70*S* particle will be 190 A, and the length of the 100*S* particle 380 A. Hart (32) has given values for the 50*S* ribosomes in particular. In purified extracts, the state of aggregation of the ribosomes is sensitive to the concentration of divalent cations ($Mg^{++}$) and the ionic strength of the medium (97). At low $Mg^{++}$ concentration, the 30 and 50*S* com-

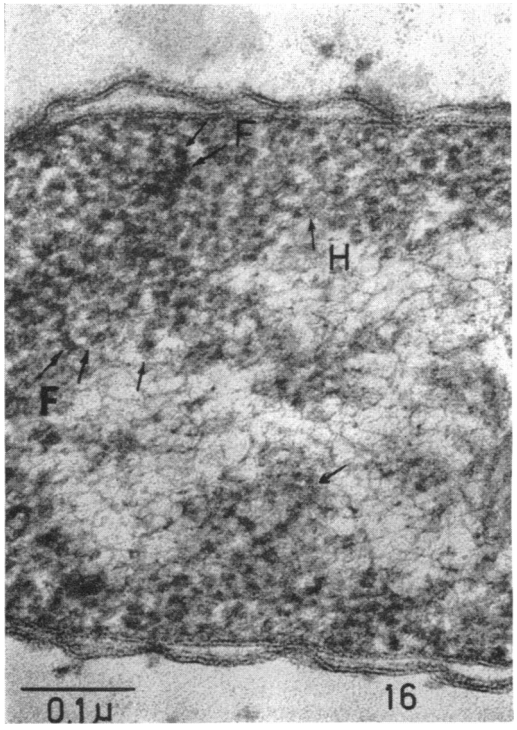
FIG. 15. *Proteus vulgaris cell grown for 4 hr with tellurite in the medium. The reduced product is deposited in several conglomerates of dense elements close to the plasma membrane. These conglomerates are considered to represent the chondrioids of this gram-negative organism. An impression of their fine structure can be obtained from Fig. 13a to e. Here, the following additional details can be seen: rounded structures with delicate fibers or sheets attached to them and triangular cross-sections of rods.*

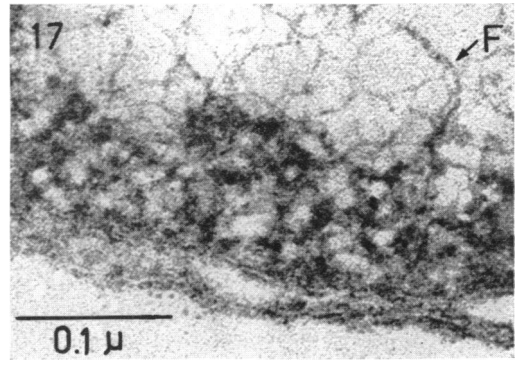
FIG. 16. *Escherichia coli B from exponential phase of growth. There are no independent rounded ribosomes, but the cytoplasm appears rather like a network in which there are denser areas corresponding presumably with ribosomes. Since these denser areas are interconnected, the cytoplasm could be considered to be essentially of polyribosomal structure. The cytoplasmic material is presumably in linear arrays (arrows) stretching from the nuclear area towards the plasma membrane. In the nuclear area, there is contact between the fibrils and the cytoplasmic arrays. In the original exposures in the cytoplasm, opaque fibrils (F) of complicated pattern, and less opaque, more parallel fibrils with helical structure (H), are visible.*

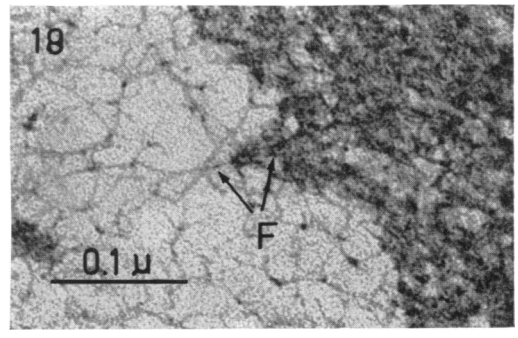
FIG. 17. *Proteus vulgaris. The rigidity of the cell wall was somewhat released by means of penicillin in the presence of sucrose in the medium. Fibrils (arrows F) from the nuclear area enter the cytoplasm and take part in its fine structure.*

FIG. 18. *Proteus vulgaris treated with penicillin. Note the continuation of a long fibril from the nuclear area over a considerable length in the cytoplasm (arrow F).*




FIGS. 15–18

ponents exist separately, whereas without $Mg^{++}$ these components are broken down irreversibly; at 0.01 M magnesium acetate, the 70*S* particles are predominant, and at still higher $Mg^{++}$ concentration two 70*S* particles aggregate to form the 100*S* ribosome.

Until quite recently (35, 1), only a few attempts have been made to correlate adequately the information collected by biochemists with the morphological picture obtained by use of electron microscopy. It happens that, ordinarily, I fix our bacteria from cultures in the exponential phase of growth and in a medium containing 0.01 M $Mg^{++}$, but for years I have wondered whether particles can be distinguished in the cytoplasm of these cells that are really of the required dimensions (see above). In the thin sections of the bacteria, the "granules" in the cytoplasm are usually so densely packed that neither their dimensions nor their shape can be adequately visualized. Therefore, in many cases it seems desirable for the study of cytoplasmic fine structure first to loosen the cytoplasm by relaxing the rigidity of the cell wall. This was done by treating *P. vulgaris* for 4 hr with 2,000 units of penicillin per ml and 0.25 M sucrose in the normal medium (Fig. 17 and 18), and by removing the cell wall of *B. subtilis* with 0.1% lysozyme in phosphate buffer with 0.25 M sucrose (Fig. 19 to 21). In addition, the cytoplasm was studied of a stable L-form of *Proteus*, L-9, kindly given to us by E. Klieneberger-Nobel (Fig. 22).

The problem of whether discrete, rounded, ribosomal particles exist as such in the cytoplasm of bacteria was approached in two experimental ways: (i) by taking micrographs of thinner sections than usual at higher magnifications than applied formerly, and (ii) by taking micrographs of ribosomes fixed immediately after having been released by osmotic shock of the protoplasts. A description will be given of these observations which has not been published before.

The cytoplasm of *E. coli* B is, after Ryter-Kellenberger fixation and embedding in Vestopal W, loose enough by itself to enable fine structural detail to be studied in thin sections. As seen in Fig. 2 and 16, the cytoplasm in these *E. coli* cells does not appear to be composed of independent rounded particles, but rather of a complicated network in which there are denser areas, whose width (100 to 150 A) is difficult to measure. If these more compact areas correspond to ribosomes, the cytoplasm apparently consists of interconnected ribosomes, i.e., of polyribosomes (see below). Occasionally, linear arrangements can be observed in the cytoplasm (Fig. 16, arrows). Some of these rows of cytoplasmic material have been traced from their origins at the plasma membrane towards the nuclear area, where they seem to end in fibrils from the nucleoplasm. In the original photographic prints, a fine fibrillar substructure is just visible in these linear cytoplasmic aggregates. Some of these fibrils are opaque to the electron beam and are arranged in a complicated pattern, whereas others, following a more or less parallel course, are less opaque and are often actually seen to possess a helical structure (arrow H in Fig. 16).

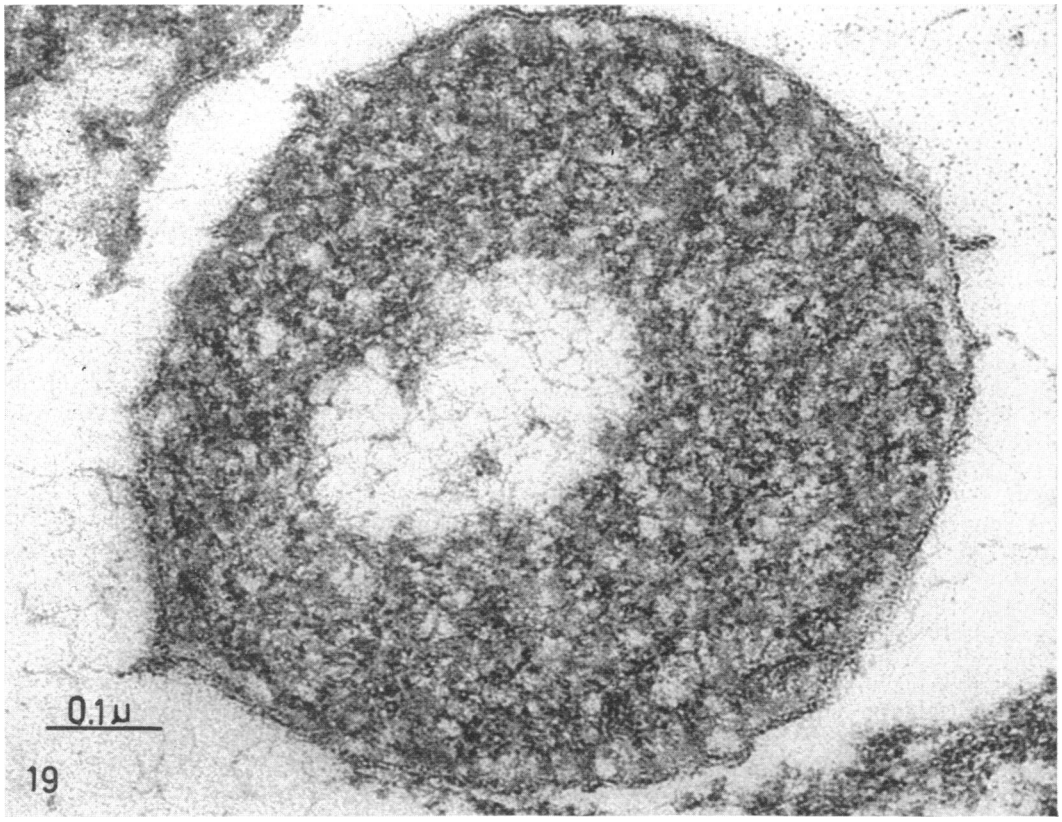
In the best micrographs of the cytoplasmic material of various bacteria, it can be seen that the nuclear fibrils are often composite and helical. An important feature to be derived from all these high-resolution micrographs is that in many places fibrils from the nucleoplasm enter the cytoplasm and appear to take part in its netlike fine structure (arrow F in Fig. 16 to 18, Fig. 20 to 22).

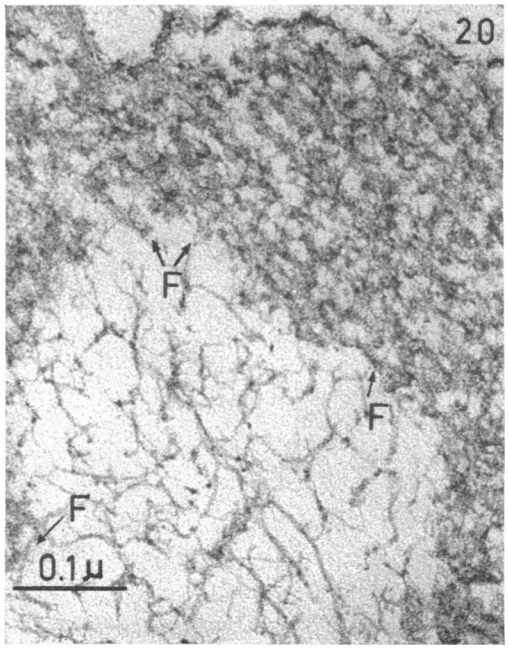
To compare directly the cytoplasmic fine structure in thin sections with ribosomal material obtained from the same cytoplasm, protoplasts were made from *B. subtilis* with lysozyme (see above). These protoplasts were spun down and treated in two ways. Some of them, left for 1 hr in the Ryter-Kellenberger fixative, were subsequently treated in the usual way for 90 min in uranyl acetate and then embedded. Sections of this material stained with lead citrate according to the method of Reynolds (65) can be seen in Fig. 19 to 21. Other protoplasts were broken with osmotic shock by mixing small quantities in drops of distilled water on paraffin wax, the water containing 0.01 M magnesium acetate and 2% uranyl acetate at *p*H 5, or by immersing

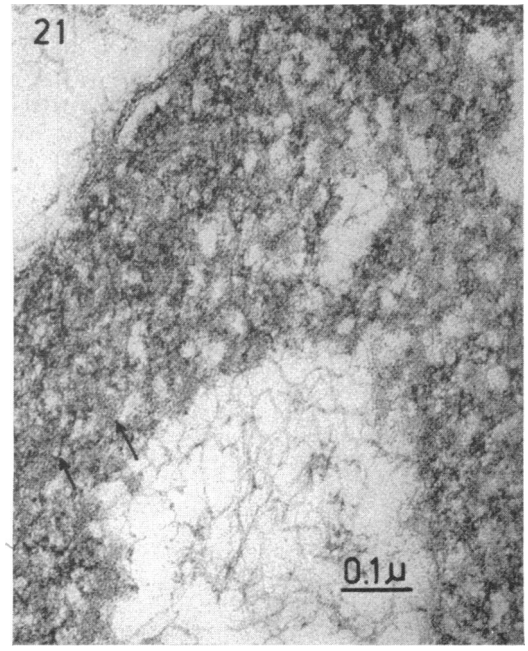
FIG. 19. *Bacillus subtilis cell from which the cell wall has been lysed away in cross section. There are short invaginations of the plasma membrane into the cytoplast. The cytoplasm appears to consist of a complicated network of very dense fibrils and of less dense material.*

FIG. 20. *Bacillus subtilis protoplast made with lysozyme and sucrose in the medium. It seems impossible in the expanded cytoplasm to make out the outlines of the ribosomes. In this tangential section, the cytoplasm appears rather in ramified linear arrays with fibrils of various grades of density. The fibrils in the nucleoplasm appear to branch, and in several of the original exposures a helical structure can be seen. Fibrils from the nucleoplasm take part in the fine structure of the cytoplasm (arrows F).*

FIG. 21. *Bacillus subtilis protoplast. This picture shows more clearly than the preceding one that, in the cytoplasm, there are very opaque fibrils in complicated fabric. In the cytoplasm on the left, such opaque fibrils can more or less be traced towards their ends at the plasma membrane.*




FIGS. 19–21

them in the Ryter-Kellenberger fixative diluted six times; this latter material was then stained in uranyl acetate for 90 min. The cell contents released in the drops on the paraffin were picked up on carbon-coated grids and studied directly in the electron microscope. The purpose of this procedure was to obtain unpurified ribosomal material in a state that can in some ways be compared with the cytoplasm in the thin sections. Some of the results are presented in Fig. 24 to 28. The material in Fig. 29 was obtained by shocking the protoplasts in the diluted Ryter-Kellenberger fixative. The result in the latter case appears to be essentially the same as in the uranyl-fixed samples (Fig. 24 to 28), although released particles may be more irregular.

In thin section, the cytoplasmic material of *B. subtilis*, from which the cell wall had been lysed away (Fig. 19 to 21), is similar to that in the preceding figures of cytoplasmic material (Fig. 16 to 18). In these *B. subtilis* protoplasts, however, it is even more difficult to distinguish the areas that should correspond with separate ribosomal entities. It is not likely that this should be entirely due to application of the Ryter-Kellenberger procedure, which is borne out by other material, e.g., liver or onion root-tip cells or the autolyzed yeast cell in Fig. 23; however, all such ribosomes never appear completely separate, they are always held together by a material of lower density. In the case of the *B. subtilis* protoplasts, the cytoplasm material has perhaps hydrated and expanded so much after removal of the cell wall that the outlines of the particles are no longer apparent. The cytoplasm appears as a complicated three-dimensional network stretching from the plasma membrane to the nuclear area (Fig. 19 to 21). Fibrils are apparently an important component of this cytoplasm. Particularly in Fig. 20 and 21, in which the material was sectioned more tangentially than in Fig. 19, the cytoplasm appears to be in ramifying linear arrays. These arrays contain fibrils of various widths and densities, the thinnest of which approach the limits of resolution of the microscope, i.e., close to 10 A. In the nuclear area, the fibrils appear often to branch and to be considerably thicker than the thinnest of the fibrils in the cytoplasm (Fig. 20).

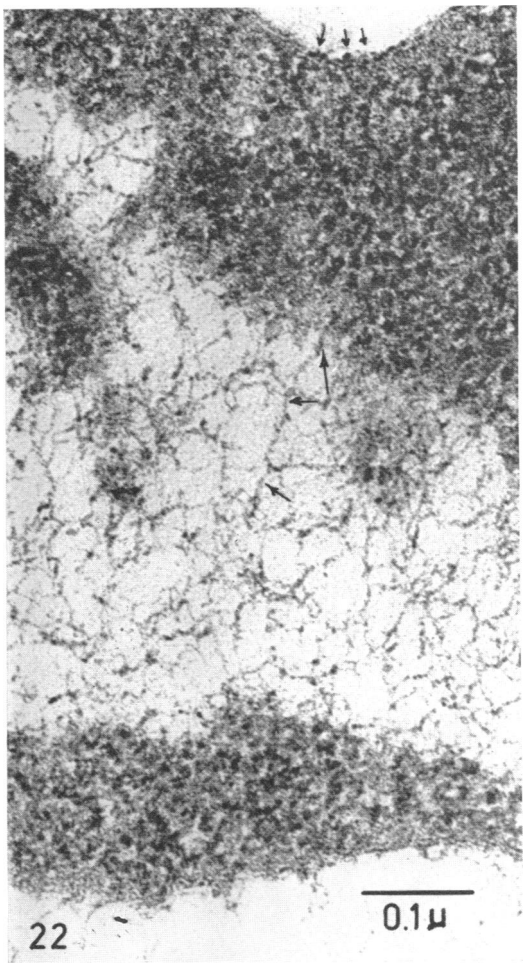
The released cytoplasmic material (the free particles and clusters, Fig. 24 to 28), fixed and stained in uranyl acetate, resembles closely enough the purified ribosomes in electron micrographs found in the literature (31, 37, 32, 81, 5, 34) to be considered as representing ribosomes and polyribosomes. Polyribosomes (polysomes), described first by Warner, Rich, and Hall (89), or ergosomes, as described by Wettstein et al. (94), are interconnected ribosomes which, as has now been established, are active in protein synthesis (88, 19, 21, 22). However, in the present presumed multiple ribosomal complexes released from the *B. subtilis* protoplasts, individual particles are often not distinguishable (Fig. 24 to 28). This is in contradistinction, for instance, to the appearance of purified reticulocyte polysomes, in which it was seen by electron microscopy that a thread of messenger RNA connected the separate ribosomal particles (89, 81). In Fig. 24, thin threads seem to fray off from the polysomes. Characteristic of the released ribosomes, particularly of the larger clumps, is a substructure of electron-dense threads which appears to be continuous over a whole row of these particles as a complicated fabric (Fig. 25 and 27). In Fig. 28 there is visible at high magnification part of an emptied plasma membrane with some cytoplasmic material still attached to it. The arrow points to a kind of ribbon of fibrils ending in two parallel fibrils, in association with which there are a few ribosomal particles. From some of these ribosomes, fibrils stick out as if other parts had been broken off. Figure 27 should perhaps be interpreted as representing a coagulated strand of cytoplasm. All such observations convey the impression that, in the original state, the ribosomes are not separate but are integrated in much larger complexes in which fibrillar structures play an important part. The electron-dense fabric of fibrils in the released material would seem to be identical with the complicated fibrillar structures in the thin sections (Fig. 16 to 22,

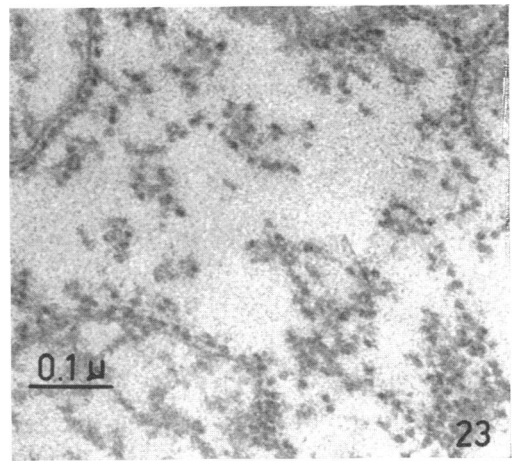
FIG. 22. *Proteus L-form, L-9. Note the fibril from the nucleoplasm entering the cytoplasm and reaching the plasma membrane. The more opaque areas on this linear cytoplasmic array should be identical with the ribosomes. The cytoplasm apparently consists of interconnected arrays stretching from the nucleoplasm towards the plasma membrane.*

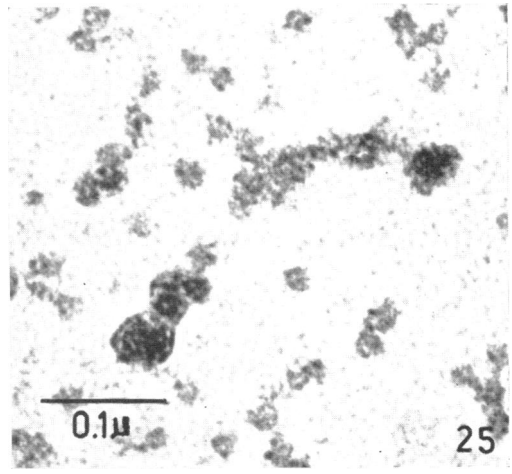
FIG. 23. *Pichia membranaefaciens. Area from an autolyzed yeast cell to demonstrate that, with the Ryter-Kellenberger method, it is quite possible to preserve ribosomal particles. However, here too the particles do not appear completely free but are interconnected by material of lower contrast in which occasionally fine fibrils can be discerned.*

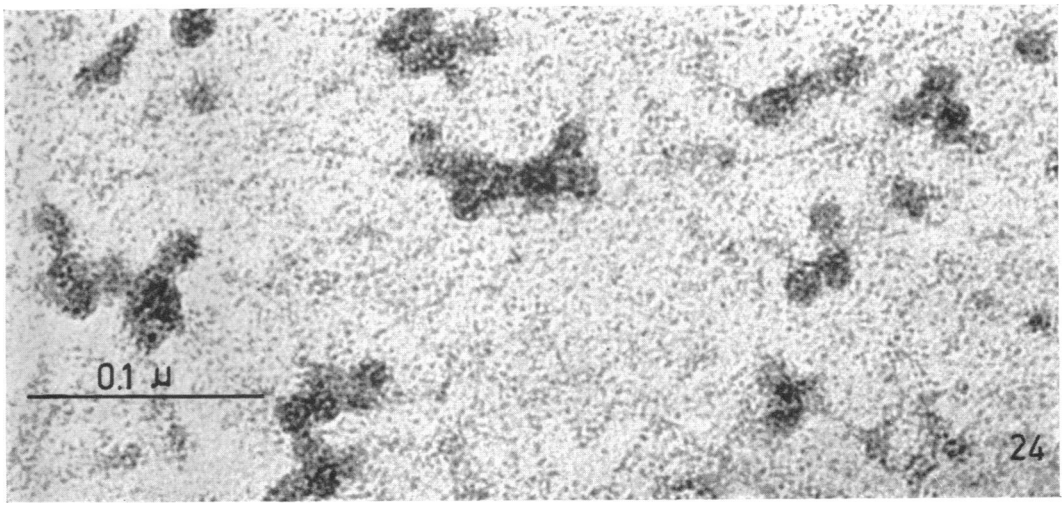
FIG. 24. *Cytoplasmic material released from Bacillus subtilis by osmotic shock in 2% uranyl acetate and 0.01 M $Mg^{++}$ in distilled water. Note that delicate fibrils stick out from the clumps of cytoplasm that must be identical with the polyribosomes described in the literature. These cytoplasmic fragments show a substructure of electron-dense threads appearing as a complicated fabric.*

FIG. 25. *Released cytoplasmic material as in the previous figure. Note small particles and linear array of material with electron-dense substructure.*

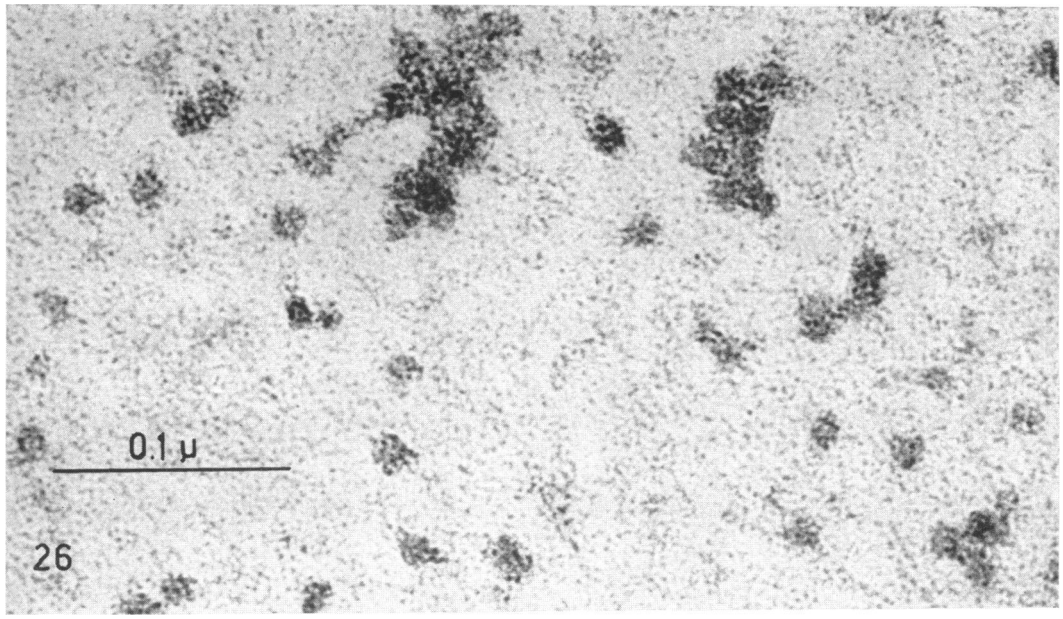



FIGS. 22–25



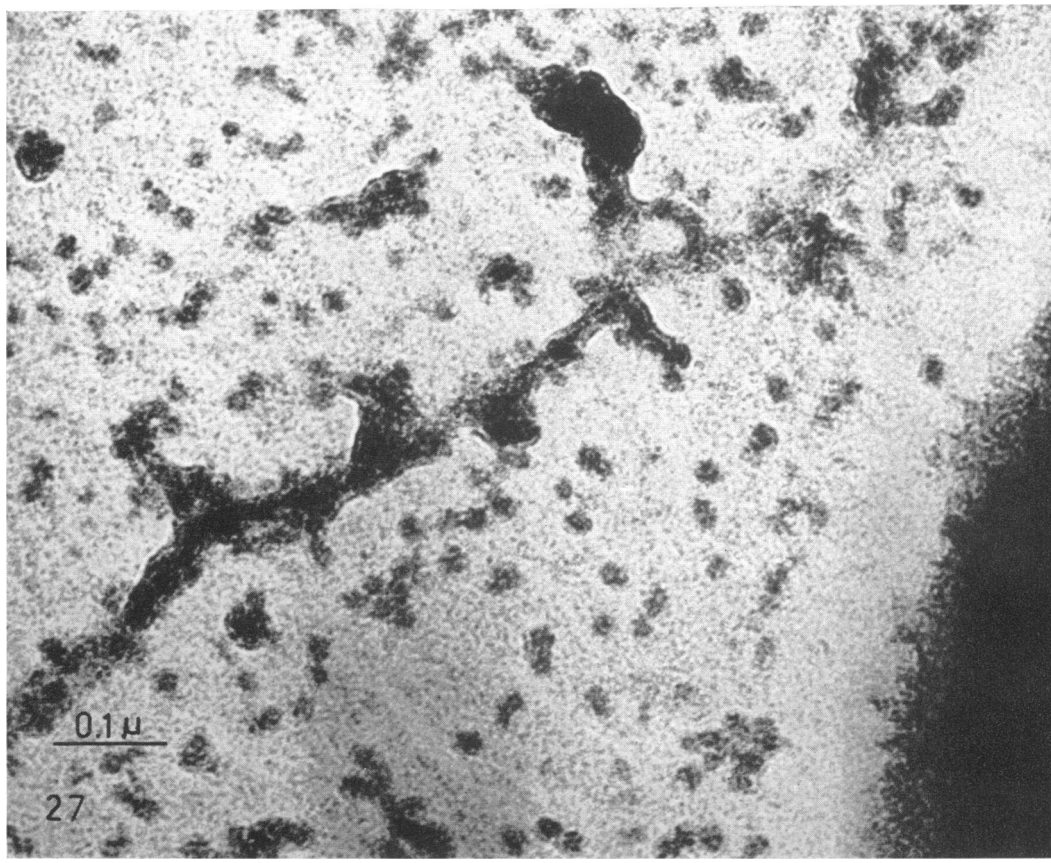
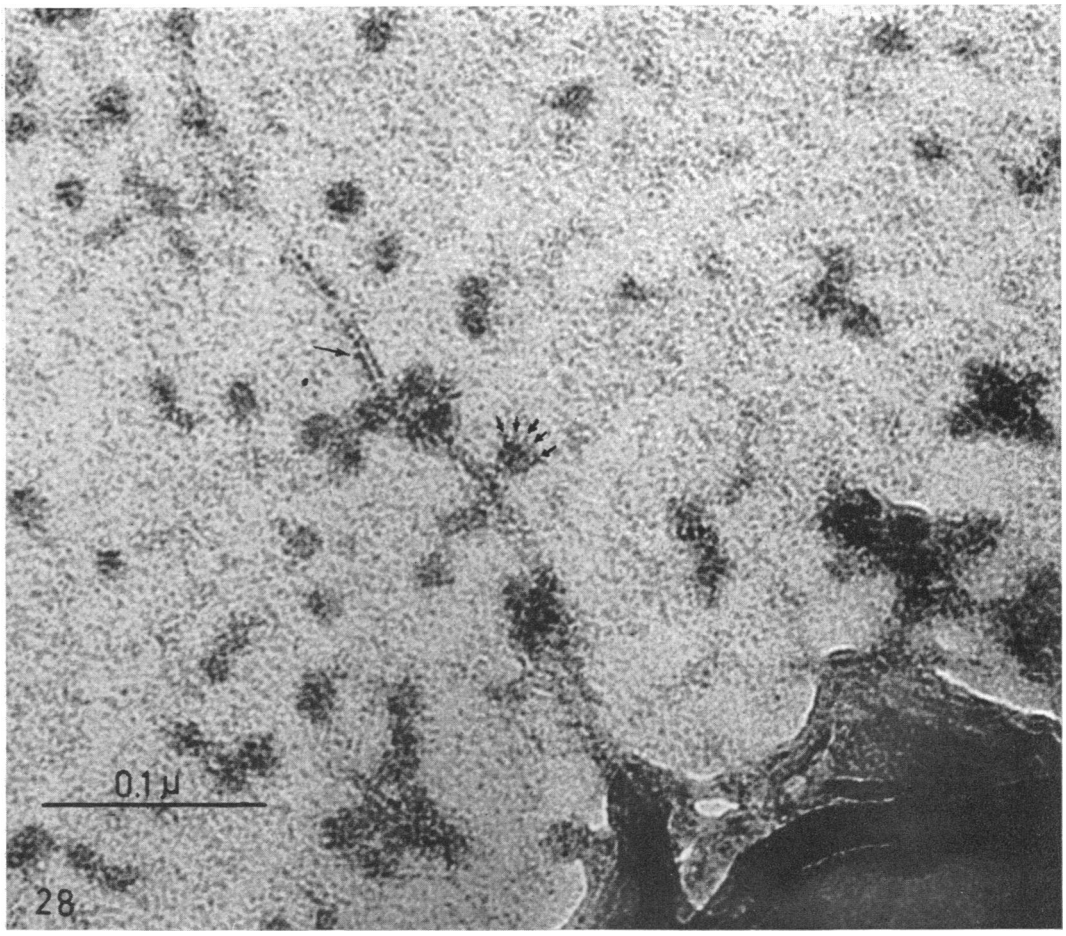




FIG. 26. *Released cytoplasmic material as in the previous figures. The small particles are presumably ribosomes. The larger clumps must be clusters of ribosomes, i.e., polyribosomes. The substructure appears to be continuous over such clusters.*

FIG. 27. *Released cytoplasmic material as in previous figures. The long structure must be a coagulated "strand" of cytoplasm. Note the "free" ribosomal particles, often still interconnected.*



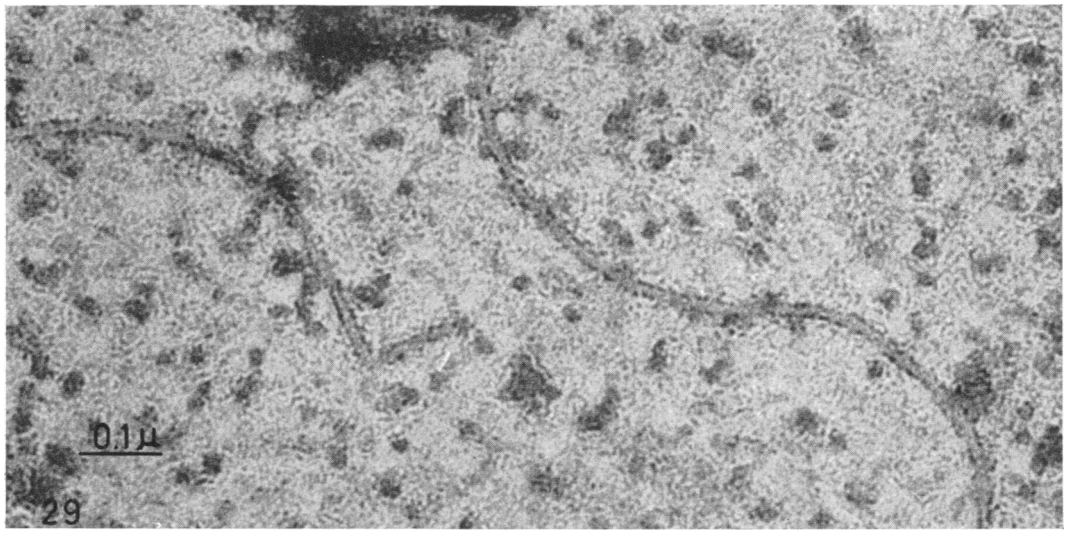




FIG. 28. *Part of the plasma membrane (left side of figure) from the Bacillus subtilis protoplast from which the cytoplasm was released through osmotic shock. The arrow points at a kind of ribbon of fibrils ending in two parallel fibrils. Associated with these fibrils are ribosomal particles; fibrils, belonging to the electron-dense substructure, stick out from some of these particles.*

FIG. 29. *Similar preparation of cytoplasmic material released in the Ryter-Kellenberger fixative diluted six times and stained with uranyl acetate. The ribosomal material is mixed with some flagella.*

arrow F). Low-angle diffraction experiments performed by Langridge (50, 51) indicate that concentrated gels of *E. coli* ribosomes form a linear polymer in which part of the RNA could be in the form of four or five double helices 45 to 50 A apart. Langridge suggests "the possibility that the dispersed state observed in the analytical centrifuge and electron microscope (what is meant is e.m. of free, purified, particles) may not correspond to the physiological significant state within the cell" (50). The image of the cytoplasm in thin sections, as well as that of released coagulated cytoplasm, seems to support the idea that, in the integrated state, the ribonucleoprotein is not arranged in separate rounded particles, but in linear arrays with numerous anastomoses. These arrays are contiguous with the plasma membrane and possibly with intracytoplasmic membranes when these are present.

## SYNOPSIS

Comparison of the fine structure of the cytoplasm of the bacterial cell with that of the "cell in general" reveals important differences leading to the conclusion that, in the bacterial cytoplasm, there is little differentiation into separate functional entities. There is no nuclear membrane, and fine fibrils appear to extend from the nucleoplasm into the cytoplasm where they may expand in all directions. The cytoplasm is not necessarily intersected by membranous profiles of special configuration, as is borne out by *E. coli* B and other gram-negative organisms. When membranous profiles of special configuration are present, as in the gram-positive *B. subtilis*, these do not appear differentiated into clearly definable endoplasmic reticulum, Golgi substance, microbodies, etc., and mitochondria. As for kinetosomes, the presence of centrioles is perhaps not very likely. The experiments in which tellurite is reduced in the cells suggest that there are structures at the base of the flagella with reductive capacity. However, upon application of the tetranitro-blue tetrazolium technique to *B. subtilis*, these are not stained. They are presumably identical with basal granules (39).

The membranous structures in *B. subtilis* are called chondrioids (48) by us because probably one of their functions is that they are the sites of the respiratory enzyme chain. In the gram-negative *P. vulgaris*, the probable site of the respiratory chain has a structure very different from that in the *Bacillus*; it is not clear whether membranes are involved in their structure. Morphologically, even in *B. subtilis* chondrioids differ fundamentally from the mitochondria in that they are not built up from composite membranes, they are extensions of the plasma membrane, and they may not be completely closed systems. Regarding this last point, it was observed (Fig. 6a to d) that chondrioids may possibly stand in open connection with the cell's environment, since its lumen is not separated from the cell wall by a plasma membrane. Although as yet nothing is known about this, it does not seem to be precluded that these membranous organelles may function also as intracellular transport systems. Contrary to Porter's ideas about ER, the chondrioids are not extensions of a nuclear envelope, but of the plasma membrane. (For further discussion see 14). On the other hand, they are in the nuclear area, in direct contact with the nucleoplasm, and therefore, perhaps, they need not carry in their interior some DNA, as is now becoming known for ordinary mitochondria.

The chromatophore vesicle is of a simple structure and in connection with the plasma membrane. It is covered by a single membrane and thus resembles the granum disc, but it is much smaller.

In tissue cells, an important function of the ER is the support of protein synthesis by ribosomes bound to their surfaces. Evidence is now accumulating that the active complexes for polypeptide synthesis in bacteria are also membrane-bound (73, 29, 33, 34, 85, 1) and are series of 70*S* ribosomes using the same messenger RNA molecule (3, 82 21, 72). It remains a contradictory situation that the particles obtained here through release of cytoplasm from protoplasts in diluted, rather crude fixing media, and identified as ribosomes, could not be recognized as such in thin sections of the Ryter-Kellenberger fixed protoplasts. This point is much in need of clarification. But even if the cytoplasm in the thin sections of the protoplasts is in a state of dispersion brought about artificially, it is still apparent from the various micrographs that basically the cytoplasm is a network of presumably linear arrays, with many interconnections due to fibrillar systems. Fibrils from the nucleoplasm penetrate deeply into the cytoplasm. The presence of fine fibrils of various thicknesses, electron densities, and orientations makes high-resolution study of the cytoplasmic details an intriguing prospect from which much fundamental information can be gained.

The organizational pattern on the ultrastructural level in bacteria reveals fundamental similarities and divergencies from that in the "cell in general."

## ACKNOWLEDGMENTS

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ADDENDUM IN PROOF

Since the termination of this manuscript, new ideas have been developed and others have been modified. An important new element, discovered in many types of cells, is the microtubule. To the best of my knowledge, the equivalent of this structure has not yet been discovered in any type of bacterial cell.

LITERATURE CITED


1. ABRAMS, A., L. NIELSEN, AND J. THAEMERT. 1964. Rapidly synthesized ribonucleic acid in membrane ghosts from *Streptococcus fecalis* protoplasts. Biochim. Biophys. Acta **80**:325–337.
2. ANDRÉ, J. 1962. Contribution à la connaissance du chondriome. Étude de ses modifications ultrastructurales pendant la spermatogénèse. J. Ultrastruct. Res. Suppl. **3**:390–406.
3. BARONDES, S. H., AND M. W. NIRENBERG. 1962. Fate of a synthetic polynucleotide directing cell-free protein synthesis. II. Association in the ribosomes. Science **138**: 813–816.
4. BARRNETT, R. J., AND G. E. PALADE. 1957. Histochemical demonstration of the sites of activity of dehydrogenase systems with the electron microscope. J. Biophys. Biochem. Cytol. **3**:577–588.
5. BAYLEY, S. T. 1964. Physical studies on ribosomes from pea seedlings. J. Mol. Biol. **8**: 231–238.
6. BEHNKE, O. 1963. Helical arrangement of ribosomes in the cytoplasm of differentiating cells of the small intestine of the rat foetuses. Exptl. Cell Res. **30**:597–598.
7. BENEDETTI, E. L., AND B. BERTOLINI. 1963. The use of the phosphotungstic acid (PTA) as a stain for the plasma membrane. J. Roy. Microscop. Soc. **81**:219–222.
8. BOATMAN E. S. 1964. Observations on the fine structure of spheroplasts of *Rhodospirillum rubrum*. J. Cell Biol. **20**:297–311.
9. 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.
10. CHAPMAN, G. B., J. H. HANKS, AND J. H. WALLACE. 1959. An electron microscope study of the disposition and fine structure of *Mycobacterium lepraemurium* in mouse spleen. J. Bacteriol. **77**:205–211.
11. COHEN-BAZIRE, G., AND R. KUNISAWA. 1963. The fine structure of *Rhodospirillum rubrum*. J. Cell Biol. **16**:401–419.
12. DREWS, G. 1960. Elektronenmikroskopische Untersuchungen an *Mycobacterium phlei*. Arch. Mikrobiol. **35**:53–62.
13. EDWARDS, M. R., AND M. A. GORDON. 1962. Membrane systems of *Actinomyces bovis*, p. UU-3. *In* S. S. Breese, Jr. [ed.], Electron microscopy, vol. 2. Academic Press, Inc., New York.
14. EDWARDS, M. R., AND R. W. STEVENS. 1963. Fine structure of *Listeria monocytogenes*. J. Bacteriol. **86**:414–428.
15. FERNÁNDEZ-MORÁN, H. 1962. New approaches in the study of biological ultrastructure by high-resolution electron microscopy, p. 411–427. *In* R. J. C. Harris [ed.], The interpretation of ultrastructure. Academic Press, Inc., New York.
16. FISCHER, A. 1903. Vorlesungen über Bakterien. Gustav Fischer Verlag, Jena, Germany.
17. FITZ-JAMES, P. C. 1960. Participation of the cytoplasmic membrane in the growth and spore formation of bacilli. J. Biophys. Biochem. Cytol. **8**:507–528.
18. FITZ-JAMES, P. C. 1962. Morphology of spore development in *Clostridium pectinovorum*. J. Bacteriol. **84**:104–114.
19. GIERER, A. 1963. Function of aggregated reticulocyte ribosomes in protein synthesis. J. Mol. Biol. **6**:148–157.
20. GIESBRECHT, P. 1960. Über organisierte Mitochondrien und andere Beinstrukture von *Bacillus megaterium*. Zentr. Bakteriol Parasitenk. Abt. I Orig. **179**:538–582.
21. GILBERT, W. 1963. Polypeptide synthesis in *Escherichia coli*. I. Ribosomes and the active complex. J. Mol. Biol. **6**:374–388.
22. GILBERT, W. 1963. Polypeptide synthesis in *Escherichia coli*. II. The polypeptide chain and S-RNA. J. Mol. Biol. **6**:389–403.
23. GLAUERT, A. M., AND D. A. HOPWOOD. 1959. A membranous component of the cytoplasm in *Streptomyces coelicolor*. J. Biophys. Biochem. Cytol. **6**:515–516.
24. GLAUERT, A. M., AND D. A. HOPWOOD. 1960. The fine structure of *Streptomyces coelicolor*. I. The cytoplasmic membrane system. J. Biophys. Biochem. Cytol. **7**:479–488.
25. GLAUERT, A. M., AND D. A. HOPWOOD. 1960. Membrane systems in the cytoplasm of bacteria. Proc. European Regional Conf. Electron Microscopy, Delft, 1960, p. 759–762.
26. GLAUERT, A. M., AND D. A. HOPWOOD. 1961. The fine structure of *Streptomyces violaceoruber* (*S. coelicolor*). III. The walls of the mycelium and spores. J. Biophys. Biochem. Cytol. **10**:505–516.
27. GLAUERT, A. M., E. M. BRIEGER, AND J. F. ALLEN. 1961. The fine structures of vegetative cells of *Bacillus subtilis*. Exptl. Cell Res. **22**:73–85.
28. GLAUERT, A. M. 1962. The fine structure of bacteria. Brit. Med. Bull. **18**:245–250.
29. GODSON, G. N., G. D. HUNTER, AND J. A. V. BUTLER. 1961. Cellular components of *Bacillus megaterium* and their role in protein biosynthesis. Biochem. J. **81**:59–68.
30. GRANICK, S. 1961. The chloroplasts: inheritance, structure and function, p. 499–602. *In* J. Brachet and A. E. Mirsky [ed.], The cell: biochemistry, physiology, morphology, vol. 2. Academic Press, Inc., New York.
31. HALL, C. E., AND H. S. SLAYTER. 1959. Electron microscopy of ribonucleoprotein particles from *E. coli*. J. Mol. Biol. **1**:329–332.
32. HART, R. G. 1962. Electron microscopy of the 50-S ribosomes of *Escherichia coli*. Biochim. Biophys. Acta **60**:629–637.

33. HENDLER, R. W. 1962. A model for protein synthesis. Nature **193**:821–823.
34. HENDLER, R. W., AND J. TANI. 1964. On the cytological unit for protein synthesis *in vivo* in *Escherichia coli*. II. Studies with intact cells of type B. Biochim. Biophys. Acta **80**:294–306.
35. HENDLER, R. W., W. G. BANFIELD, J. TANI, AND E. L. KUFF. 1964. On the cytological unit for protein synthesis *in vivo* in *E. coli*. III. Electron microscopic and ultracentrifugal examination of intact cells and fractions. Biochim. Biophys. Acta **80**:307–314.
36. HENSHAW, E. C., T. B. BOJARSKI, AND H. H. HIATT. 1963. Protein synthesis by free and bound rat liver ribosomes *in vivo* and *in vitro*. J. Mol. Biol. **7**:122–129.
37. HUXLEY, H. E., AND G. ZUBAY. 1960. Electron microscope observations on the structure of microsomal particles from *Escherichia coli*. J. Mol. Biol. **2**:10–18.
38. IMAEDA, T., AND J. CONVIT. 1962. Electron microscope study of *Mycobacterium leprae* and its environments in a vesicular leprous lesion. J. Bacteriol. **83**:43–52.
39. ITERSON, W. VAN. 1953. Some remarks on the present state of our knowledge of bacterial flagellation. Symp. Citologia Batteria, Rome, p. 24–38.
40. ITERSON, W. VAN. 1960. Membranes, particular organelles and peripheral bodies in bacteria. Proc. European Regional Conf. Electron Microscopy, Delft, 1960, p. 763–768.
41. ITERSON, W. VAN. 1961. Some features of a remarkable organelle in *Bacillus subtilis*. J. Biophys. Biochem. Cytol. **9**:183–192.
42. ITERSON, W. VAN. 1962. Membranous structures in micro-organisms, p. 14–22. *In* N. E. Gibbons [ed.], Recent progress in microbiology (Intern. Congr. Microbiol., 8th, Montreal). University of Toronto Press.
43. ITERSON, W. VAN, AND W. LEENE. 1964. A cytochemical localization of reductive sites in a Gram-positive bacterium. Tellurite reduction in *Bacillus subtilis*. J. Cell Biol. **20**:361–375.
44. ITERSON, W. VAN, AND W. LEENE. 1964. A cytochemical localization of reductive sites in a Gram-negative bacterium. Tellurite reduction in *Proteus vulgaris*. J. Cell Biol. **20**:377–387.
45. ITO, S. 1962. Light and electron microscopic study of membranous cytoplasmic organelles, p. 129–148. *In* R. J. C. Harris [ed.], The interpretation of ultrastructure. Academic Press, Inc., New York.
46. KAVANAU, L. 1963. Structure and function of biological membranes. Nature **198**:525–530.
47. KAWATA, T. 1963. Fine structure of intracytoplasmic membrane system in *Listeria monocytogenes*. J. Gen. Appl. Microbiol. (Tokyo) **9**:1–13.
48. KELLENBERGER, E., AND L. HUBER. 1953. Contribution à l'étude des équivalents des mitochondries dans les bactéries. Experientia **9**:289–291.
49. KOIKE, M., AND K. TAKEYA. 1961. Fine structures of intracytoplasmic organelles of Mycobacteria. J. Biophys. Biochem. Cytol. **9**:597–608.
50. LANGRIDGE, R. 1962. X-ray diffraction studies of concentrated gels of ribosomes from *E. coli*. J. Mol. Biol. **5**:611–617.
51. LANGRIDGE, R. 1963. Ribosomes: a common structural feature. Science **140**:1000.
52. MARR, A. G. 1960. Localization of enzymes in bacteria, p. 443–468. *In* I. C. Gunsalus and R. Y. Stanier [ed.], The bacteria, vol. 1. Academic Press, Inc., New York.
53. MCALEAR, J. H., AND G. A. EDWARDS. 1959. Continuity of plasma membrane and nuclear membrane. Exptl. Cell Res. **16**:689–692.
54. MUDD, S. 1954. Cytology of bacteria. I. The bacterial cell. Ann. Rev. Microbiol. **8**:1–22.
55. MUDD, S. 1956. Cellular organization in relation to function. Bacteriol. Rev. **20**:268–271.
56. MURRAY, R. G. E. 1961. The structure of microbial cytoplasm and the development of membrane systems, p. 1–21. *In* Lectures on theoretical and applied aspects of modern microbiology. Sponsored jointly by the American Cyanamid Co., Chas. Pfizer & Co., Inc., and Merck & Co., Inc.
57. MURRAY, R. G. E., and A. BIRCH-ANDERSON. 1963. Specialized structure in the region of the flagella tuft in *Spirillum serpens*. Can. J. Microbiol. **9**:393–402.
58. NASS, M. M. K., AND S. NASS. 1963. Intramitochondrial fibers with DNA characteristics. I. Fixation and electron staining reactions. J. Cell Biol. **19**:593–611.
59. NASS, S., AND M. M. K. NASS. 1963. Intramitochondrial fibers with DNA characteristics. II. Enzymatic and other hydrolytic treatments. J. Cell Biol. **19**:613–629.
60. NOVIKOFF, A. B. 1961. Mitochondria (chondriosomes), p. 299–421. *In* J. Brachet and A. E. Mirsky [ed.], The cell: biochemistry, physiology, morphology, vol. 2. Academic Press, Inc., New York.
61. NOVIKOFF, A. B. 1961. Lysosomes and related particles, p. 423–488. *In* J. Brachet and A. E. Mirsky [ed.], The cell: biochemistry, physiology, morphology, vol. 2. Academic Press, Inc., New York.
62. NOVIKOFF, A. B., E. ESSNER, S. GOLDFISCHER, AND M. HEUS. 1962. Nucleoside phosphatase activities of cytomembranes, p. 149–192. *In* R. C. J. Harris [ed.], The interpretation of ultrastructure. Academic Press, Inc., New York.
63. PARSSONS, D. F. 1963. Mitochondrial structure: two types of subunits on negatively stained mitochondrial membranes. Science **140**:985–987.
64. PORTER, K. R., AND M. A. BONNEVILLE. 1963. An introduction to the fine structure of cells and tissues. Lee & Febiger, Philadelphia.
65. REYNOLDS, E. S. 1963. The use of lead citrate

at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**:208–212.
66. RIS, H. 1962. Ultrastructure of certain self-dependent cytoplasmic organelles, p. XX–1. *In* S. S. Breese Jr. [ed.], Electron microscopy, vol. 2. Academic Press, Inc., New York.
67. ROBERTSON, J. D. 1959. The ultrastructure of cell membranes and their derivatives. Biochem. Soc. Symp. (Cambridge, Engl.) **16**:3–43.
68. ROBINOW, C. F. 1947. Nuclear apparatus and cell structure of rod-shaped bacteria, p. 355–377. *In* R. J. Dubos [ed.], The bacterial cell. Harvard University Press, Cambridge, Mass.
69. ROSA, C. G., AND K. C. TSOU. 1963. The use of tetranitro-blue tetrazolium for the cytochemical localization of succinic dehydrogenase. J. Cell Biol. **16**:445–454.
70. RYTER, A., AND M. F. JACOB. 1963. Étude au microscope électronique des relations entre mésosomes et noyaux chez *Bacillus subtilis*. Compt. Rend. **257**:3060–3063.
71. SALTON, M. R. J., AND J. A. CHAPMAN. 1962. Isolation of the membrane-mesosome structures from *Micrococcus lysodeikticus*. J. Ultrastruct. Res. **6**:489–498.
72. SCHAECHTER, M. 1963. Bacterial polyribosomes and their participation in protein synthesis *in vivo*. J. Mol. Biol. **7**:561–568.
73. SCHLESSINGER, D. 1963. Protein synthesis by polyribosomes on protoplast membranes of *B. megaterium*. J. Mol. Biol. **7**:569–582.
74. SEDAR, A. W., AND M. A. RUDZINSKA. 1956. Mitochondria of protozoa. J. Biophys. Biochem. Cytol. **2**:331–336.
75. SEDAR, A. W., C. G. ROSA, AND K. C. TSOU. 1962. Tetranitro-blue tetrazolium and the electron histochemistry of succinic dehydrogenase. J. Histochem. Cytochem. **10**: 506–508.
76. SHINOHARA, C., K. FUKUSHI, AND J. SUZUKI. 1958. Mitochondrial structure of *Mycobacterium tuberculosis* relating to its function. J. Electronmicroscopy (Tokyo) **6**:47–52.
77. SIEKEVITZ, P., AND G. E. PALADE. 1960. A cytochemical study on the pancreas of the guinea pig. V. *In vivo* incorporation of leucine-1-$C^{14}$ into chymotrypsinogen of various cell fractions. J. Biophys. Biochem. Cytol. **7**:619–630.
78. SIEKEVITZ, P., AND G. E. PALADE. 1960. A cytochemical study on the pancreas of the guinea pig. VI. Release of enzymes and ribonucleic acid from ribonucleoprotein particles. J. Biophys. Biochem. Cytol. **7**:631–644.
79. SJÖSTRAND, F. S. 1963. A new ultrastructural element of the membranes in mitochondria and some cytoplasmic membranes. J. Ultrastruct. Res. **9**:340–361.
80. SJÖSTRAND, F. S. 1963. A comparison of plasma membrane, cytomembranes, and mitochondrial membrane elements with respect to ultrastructural features. J. Ultrastruct. Res. **9**:561–580.
81. SLAYTER, H. S., J. R. WARNER, A. RICH, AND E. C. HALL. 1963. The visualization of polyribosomal structure. J. Mol. Biol. **7**:652–657.
82. SPYRIDES, G. J., AND F. LIPMAN. 1962. Polypeptide synthesis with sucrose gradient fractions of *E. coli* ribosomes. Proc. Natl. Acad. Sci. U.S. **48**:1977–1983.
83. STOECKENIUS, W. 1963. Some observations on negatively stained mitochondria. J. Cell Biol. **17**:443–454.
84. SUGANUMA, A. 1963. Some features of membranous structures in *Staphylococcus aureus*. J. Infect. Diseases **113**:179–185.
85. TANI, J. AND R. W. HENDLER. 1964. On the cytological unit for protein synthesis *in vivo* in *Escherichia coli*. I. Studies with spheroplasts of type K 12. Biochim. Biophys. Acta **80**:279–293.
86. TISSIÈRES, A., J. D. WATSON, D. SCHLESSINGER, AND B. R. HOLLINGWORTH. 1959. Ribonucleoprotein particles from *Escherichia coli*. J. Mol. Biol. **1**:221–233.
87. VANDERWINKEL, E., AND R. G. E. MURRAY. 1962. Organelles intracytoplasmiques bactériens et site d'activité oxydo-réductrice. J. Ultrastruct. Res. **7**:185–199.
88. WADDINGTON, C. H., AND M. M. PERRY. 1963. Helical arrangement of ribosomes in differentiating muscle cells. Exptl. Cell Res. **30**: 599–600.
89. WARNER, J. R., A. RICH, AND C. E. HALL. 1962. Electron microscope studies of ribosomal clusters synthesizing hemoglobin. Science **138**:1399–1403.
90. WARNER, J. R., P. M. KNOPF, AND A. RICH. 1963. A multiple ribosomal structure in protein synthesis. Proc. Natl. Acad. Sci. U.S. **49**:122–129.
91. WEIBULL, C. 1953. Characterization of the protoplasmic constituents of *Bacillus megaterium*. J. Bacteriol. **66**:696–701.
92. WEIBULL, C., AND K. G. THORSSON. 1956. Comparative studies on sections of intact cells, protoplasts and "ghosts" of a *Bacillus* species, p. 266–267. *In* F. S. Sjöstrand and J. Rhodin [ed.], Electron microscopy. Almquist & Wiksell, Stockholm.
93. WEIBULL, C., H. BECKMAN, AND L. BERGSTRÖM. 1959. Localization of enzymes in *Bacillus megaterium*, strain M. J. Gen. Microbiol. **20**:519–531.
94. WETTSTEIN, F. O., T. STECHELIN, AND H. NOLL. 1963. Ribosomal aggregate engaged in protein synthesis: characterization of the ergosome. Nature **197**:430–435.
95. WINKLER, A. 1958. Feinstruktur des Bakterienzellinhaltes. Zentr. Bakteriol. Parasitenk. Abt. I Orig. **173**:369–375.
96. WOHLFARTH-BOTTERMAN, K. E. 1963. Grundelemente der Zellstruktur. Die Naturwissenschaften **7**:237–249.
97. ZUBAY, G. 1962. Physical and chemical properties of particles, p. 65–79. *In* N. E. Gibbons [ed.], Recent progress in microbiology (Intern. Congr. Microbiol., 8th, Montreal), University of Toronto Press.