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ULTRASTRUCTURE OF *MYCOPLASMA* SPECIES

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ABSTRACT

DOMERMUTH, C. H. (Statens Seruminstitut, Copenhagen, Denmark), M. H. NIELSEN, E. A. FREUNDT, AND A. BIRCH-ANDERSEN. Ultrastructure of *Mycoplasma* species. J. Bacteriol. **88**:727-744. 1964.—The ultrastructure of 19 strains (15 species) of Mycoplasmatales grown on solid medium was studied with the aid of an electron microscope. The cells possessed a triple-layered limiting membrane 75 to 100 A thick. This membrane appeared to be symmetrical in some strains and asymmetrical in others. An electron-dense material found in close contact with the cell surface was tentatively interpreted to be a capsular substance. Ribosomes and strands of nuclear material were observed in the cytoplasm of cells of all strains. Ribosomes observed in the JA strain of *M. gallisepticum* were frequently arranged in a regular geometric pattern of characteristic appearance. Dense inclusions sometimes limited by triple-layered membranes (possibly developing elementary bodies), as well as membrane-surrounded vesicles, were observed in the cytoplasm of cells of some strains.

The ubiquity, importance, and uniqueness of members of bacterial order X, the Mycoplasmatales, is becoming increasingly evident. Certain unique features of these organisms have been revealed only in electron micrographs of thin sections of a limited number of species and strains (Edwards and Fogh, 1960; Freundt, 1960; Ruys and Van Iterson, 1961; Sharp, 1960; Van Iterson and Ruys, 1960*a*, *b*). The present study of 15 of the better known *Mycoplasma* species was made in an effort to study more extensively the fine structure of these organisms.

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MATERIALS AND METHODS

Strains examined. *M. agalactiae* PG 2, *M. arthriditis* R 3, *M. canis* c 55 β, *M. fermentans* S 38, *M. gallinarum* PG 16, *M. gallisepticum* JA and W, *M. hominis* type 1 (strain PG 21), *M. hominis* type 2 (strain campo), *M. hyorhinis* PG 29, *M. laidlawii* types A and B, *M. mycoides* var. *mycoides* (strain Institut Pasteur), *M. mycoides* var. *capri* (strain PG 3), *M. neurolyticum* L 5, *M. pneumoniae* (strain Mac), *M. pulmonis* (strain Ash), *M. salivarium* PG 20, and *M. spumans* (strain c 48 α) were examined.

Except for *M. gallisepticum*, all strains were propagated on ox-heart infusion broth. [Equal amounts (w/w) of ground ox heart and tap water were boiled for 20 min, the resulting liquid was poured off and retained, fresh tap water was added, and the process was repeated. The resulting liquid was combined, 1% peptone (Difco) and 0.5% NaCl (w/w) were added, and the pH was adjusted to 7.7 with 5 N NaOH]. The ox-heart infusion broth contained 1.4% agar, 2% Tryptose (Difco), 20% horse or swine serum (swine serum was used to propagate *M. pneumoniae*), 1:2,000 parts thallium acetate, 100 international units of penicillin per ml, and 3% yeast autolysate. The final pH of the medium was 7.8.

The yeast autolysate was prepared as follows. Dry baker's yeast (120 g) in 200 ml of distilled water was incubated at 56 C for 48 hr, and was centrifuged at 3,000 $\times$ *g* for 30 min; the supernatant fluid was filter-sterilized and retained.

M. gallisepticum (strains JA and W) was propagated on the same medium less ox-heart infusion broth and peptone, with 10% turkey serum substituted for horse serum plus 2.1% Difco PPLO broth without crystal violet.

Preparation of specimens. *Mycoplasma* species were grown on agar at 37 C until colonies were large enough to be clearly seen with the aid of a microscope (magnification, $\times$150). They were

transferred by cutting out and streaking agar blocks containing colonies over the surface of uninoculated agar. Areas of almost confluent growth were chosen for electron microscopy whenever such luxuriant growth could be obtained. Strains which did not grow luxuriously were used with the quantity of growth which could be obtained. After selection, the colonies were fixed *in situ* in fumes from a 40% formaldehyde solution for 1 hr at room temperature. The specimens were then covered with a thin layer of melted agar [physiological saline, 1.5% agar, 1% neutral formalin (0.4% formaldehyde)] which had been cooled to 45 C. Phenol red was added to this agar layer to aid in specimen orientation.

Agar blocks (about 1 by 1 by 2 mm) were cut from these preparations. They were fixed for 24 hr at room temperature with 1% osmium tetroxide in Veronal acetate buffer containing 0.01 M calcium chloride, pH 6.1 (Ryter and Kellenberger, 1958); 10% of a yeast extract-sodium acetate-peptone medium (0.3, 0.05, and 0.3%, respectively of the Difco products plus 0.005% cystine) was added to the fixative.

After fixation, the blocks were treated for 1 hr with 2% uranyl acetate in the Veronal acetate buffer (Ryter and Kellenberger, 1958) to obtain good preservation of nuclear regions.

Dehydration was carried out in a series of increasing concentrations of acetone through 100% dry acetone. The blocks were then transferred to a 3:1 mixture of dry acetone and Vestopal W (obtained from Martin Jaeger, Vésenaz/Geneva, Switzerland) plus initiator and accelerator [1% (v/v) of each] and stored at 30 C in unstoppered vials overnight. During this period of time most of the acetone evaporated.

The blocks were then resuspended in 100% Vestopal W containing initiator and accelerator. They were left in this solution at 30 C for at least 24 hr. To reduce the rate of polymerization during this period of time, they were twice transferred to freshly prepared Vestopal W containing the proper concentrations of initiator and accelerator. Finally, the blocks were transferred to gelatin capsules filled with Vestopal W with initiator and accelerator and oriented so that the layers of agar containing colonies were placed paralled to the long axis of the capsules.

The blocks were cured first at 40 C for 24 hr and then at 60 C for 48 hr. Caps were never used on specimen containing capsules to facilitate the evaporation of acetone at each temperature level used.

Sections were cut with glass knives on an LKB Ultrotome microtome. Electron microscopy was performed with a Philips EM 100 B equipped with an objective polepiece with a 1.8-mm bore and an electrostatic stigmator. Fields of view were taken at primary magnifications of 1,500 or 9,400 ×. Photographic printing was generally performed by using an enlargement of ten times. About 500 electron micrographs were evaluated.

RESULTS

Gross morphology. The morphology of the colonies of the various strains studied by electron microscopy was similar. In vertical section, the colonies appeared conical in shape and grew into the agar with varied, although mainly sparse, amounts of growth on the surface (Fig. 1).

The colonies were composed of individual small-to-large, spherical-to-pleomorphic cells. Filamentous structures (Fig. 8, 16, and 17) and short rows of connected cells (Fig. 4) were sometimes observed.

The majority of colonies showed a wide variation in size of individual cells. Larger cells were most frequently found on or near the surface of the colony, whereas smaller denser cells were more frequently found further down in the agar (Fig. 1). Diameters of individual cells of all of the strains studied are presented in Table 1. All measurements were made of cells showing definite limiting membranes.

Ultrastructure of cells: limiting membranes. In all strains examined, individual cells possessed limiting membranes composed of two electron-dense layers with a less-dense intermediate layer (Fig. 7). The minimal total thickness of these three layers was about 75 A for all strains except *M. neurolyticum*. The membrane of this strain was found to be approximately 100 A thick (Fig. 20).

In 11 of the strains studied, the outer layer of the limiting membrane was found to be more distinct than the inner layer (Fig. 2, 22). In the remaining seven strains, the density and thickness of the two electron dense layers were identical, making the entire membrane appear symmetrical (Table 1; Fig. 7, 18, 19). It should be noted, however, that the large empty cells generally had membranes of the symmetrical type, both in cultures classified as possessing asymmetric and in cultures classified as possessing symmetric

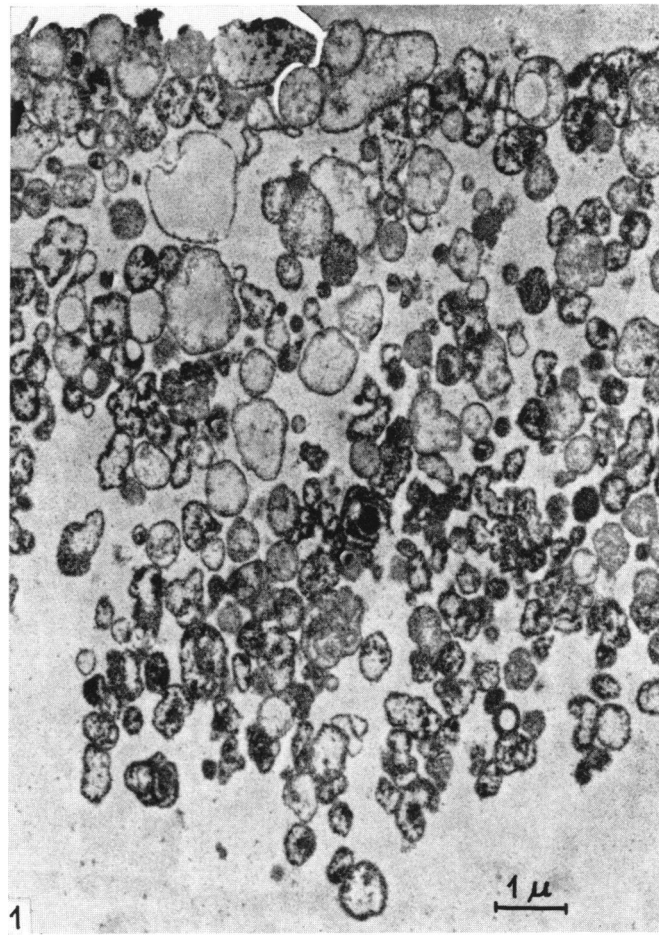


FIG. 1. *Mycoplasma canis: 48-hr culture. Vertical section through the central part of a colony. Conically shaped growth into the agar. Higher proportion of large, empty cells near the surface of the colony. 15,000* ×.

limiting membranes (Fig. 3). With the exception of *M. hominis* type 1 and 2 (Fig. 12, 13), all strains or types of the same species exhibited similar membrane symmetry.

In all strains examined, electron-dense material was found close to the cell membrane. This layer varied in thickness and density, and seemed to consist of small floccules in direct contact with the cell surface (Fig. 2, 5, 8, 9). Often a layer or layers of this substance were observed on limited regions of the cell surface, which sometimes gave the impression of additional membranes outside the limiting membrane of the cell (Fig. 5, 14, 22).

Cytoplasm. Generally cytoplasm occupied most of the interior of the cells. Often a less-dense region of cytoplasm was found more or less centrally located within the cells (Fig. 12, 14). The ratio of less-dense cytoplasm to the denser surrounding cytoplasm varied with the size of the individual cells. Smaller cells, "elementary bodies," were generally completely filled with dense material (Fig. 5, 7, 11, 17, 20, 24), whereas larger cells had more extensive areas of lesser density (Fig. 5, 11, 20, 24). Delicate filaments similar to those found in the nuclear regions of other bacteria were located in this less-dense region (Fig. 12, 18, 19, 21, 22).

The dense regions of the larger cells were made up of ribosomes 20 mμ in diameter in a homogenous matrix (Fig. 2, 15, 18, 22). The ribosomes were usually randomly distributed near the periphery of the cells; however, a uniform arrangement of ribosomes was observed in *M. gallisepticum* strain JA. In this strain, certain of the ribosomes of the cells were arranged in closely packed square groups of four, which were them-

TABLE 1. *Comparative structural features of Mycoplasma species*

Mycoplasma species studied	Culture age	Cell diameter in mμ			Symmetry (S)* or asymmetry (A) of limiting membrane	Electron-dense lines outside limiting membrane	Cytoplasmic inclusions	
		Elementary bodies	Mature cells				Dense bodies†	Vacuoles
			Maximum	Avg				
	days							
M. agalactiae	¾	210 by 210	750 by 1,100	650 by 900	A	+	−	+
M. arthritidis	2	120 by 140	850 by 1,200	490 by 750	A	+	+	−
M. canis	2	160 by 210	750 by 1,300	560 by 750	A	+	+	+
M. fermentans	1	130 by 140	600 by 900	410 by 590	S	−	+	+
M. gallinarum	2	130 by 140	850 by 850	460 by 720	A	+	+	+
M. gallisepticum strain JA	4	170 by 190	900 by 1,400	510 by 720	A	+	+	−
M. gallisepticum strain W	4	120 by 130	850 by 1,900	610 by 790	A	+	+	−
M. hominis typ. hominis	3	95 by 130	750 by 1,200	430 by 750	S	−	−	−
M. hominis typ. campo.	2	150 by 170	690 by 950	550 by 720	A	+	+	−
M. hyorhinis	3	130 by 140	690 by 1,420	510 by 850	A	−	+	−
M. laidlawii type A	1	130 by 160	690 by 1,300	470 by 720	S	−	+	+
M. laidlawii type B	1	120 by 140‡	650 by 900	500 by 650	S	−	−	+
M. mycoides var. *mycoides*	2	140 by 170	850 by 1,000	480 by 690	S	−	+	+
M. mycoides var. *capri*	1	—	600 by 1,000	460 by 670	S	−	−	+
M. neurolyticum	4	75 by 105	1,400 by 1,900	850 by 108	S	+	−	−
M. pneumoniae	7	105 by 120	690 by 750	440 by 590	A	+	+	−
M. pulmonis	1	105 by 130	1,200 by 1,400	700 by 940	A	−	+	−
M. salivarium	4	150 by 190	430 by 1,200	650 by 940	S	+	+	+
M. spumans	14	130 by 130	1,050 by 1,250	690 by 1,000	A	−	+	−
Avg of cell sizes		132 by 153	802 by 1,206	548 by 729				

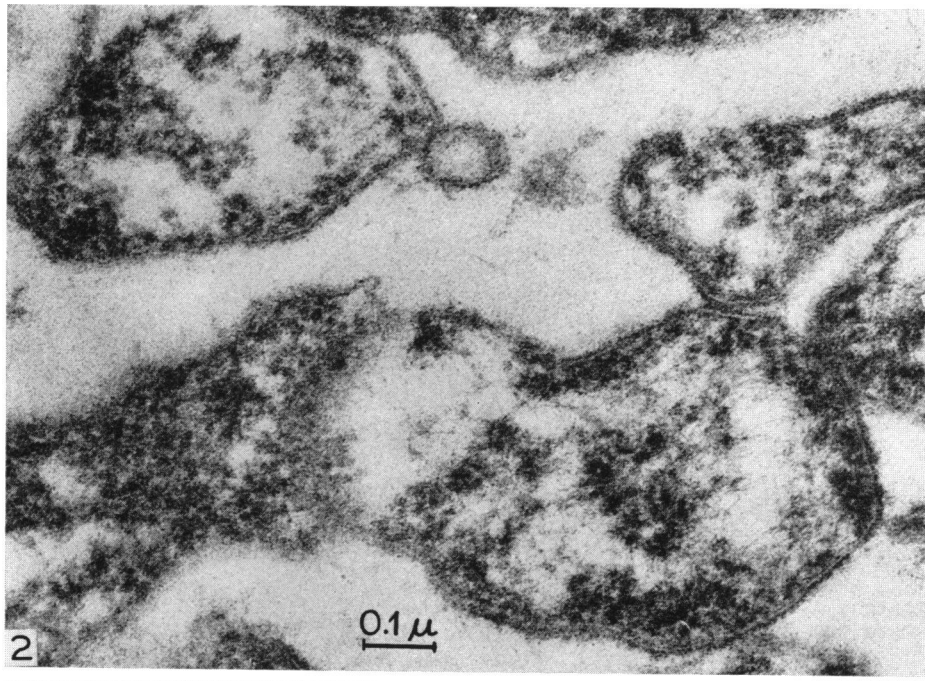
* Outer and inner layer of triple-layered membrane of different thicknesses.
† Possible developing elementary bodies.
‡ From 2-day-old culture.

selves arranged adjacent to each other to form columns resembling corncobs which often were more than 25 ribosomes in length (100+ ribosomes per column). The long axis of these columns was parallel to the limiting membrane of the cell (Fig. 10a).

Homogenous spherical condensations 100 to 200 mμ in diameter with no limiting membranes were often observed. These condensations were usually located at the pheriphery of the cells and frequently in or adjacent to protrusions of limiting membranes (Fig. 8, 10). A common finding in *M. gallisepticum* strain W was one such spherical and two to three semilunar bodies lined up within and adjacent to a cell-membrane protrusion (Fig. 11). In *M. pneumoniae* these bodies were composed of electron-dense rod-shaped structures surrounded by less-dense cytoplasm the diameter of which corresponded to the diameter of the spherical bodies generally observed (Fig. 21).

In several large swollen cells of some of the strains, remnants of cytoplasmic material were located as rather homogenous electron-dense accumulations in intimate contact with the limiting membranes (Fig. 23, 24).

In addition to these cytoplasmic elements, two different types of membrane-bounded structures were seen. One was a seemingly empty round or elongated vesicle limited by a triple-layered membrane of the same width as the limiting membrane of the cell (Fig. 9, 15). The other was a dense spherical body 100 to 200 mμ in diameter surrounded by the same type of membrane (Fig. 6, 8, 23). With regard to symmetry, the intracel-



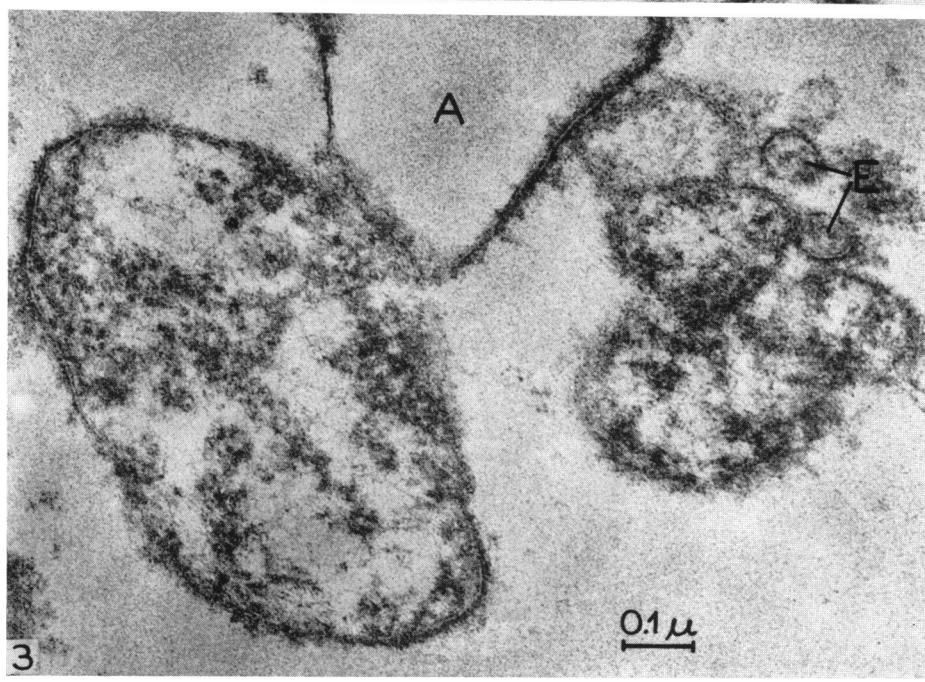


FIG. 2. *Mycoplasma agalactiae: 18-hr culture. Pleomorphic cells limited by asymmetric membranes; i.e., the outer electron-dense layer is more distinct than the inner. Small floccules of electron-dense material are in close contact with the outer layer. Ribosomes are distributed in the cytoplasm.*

FIG. 3. *Mycoplasma arthritidis: 48-hr culture. Cells of varying size. Elementary bodies (E), larger and supposedly fully viable cells and a part of a more autolyzed cell (A). The limiting membrane of the elementary bodies and of the larger viable cell is of the asymmetric type, whereas the autolyzed cell has a symmetrical limiting membrane. 94,000 ×.*

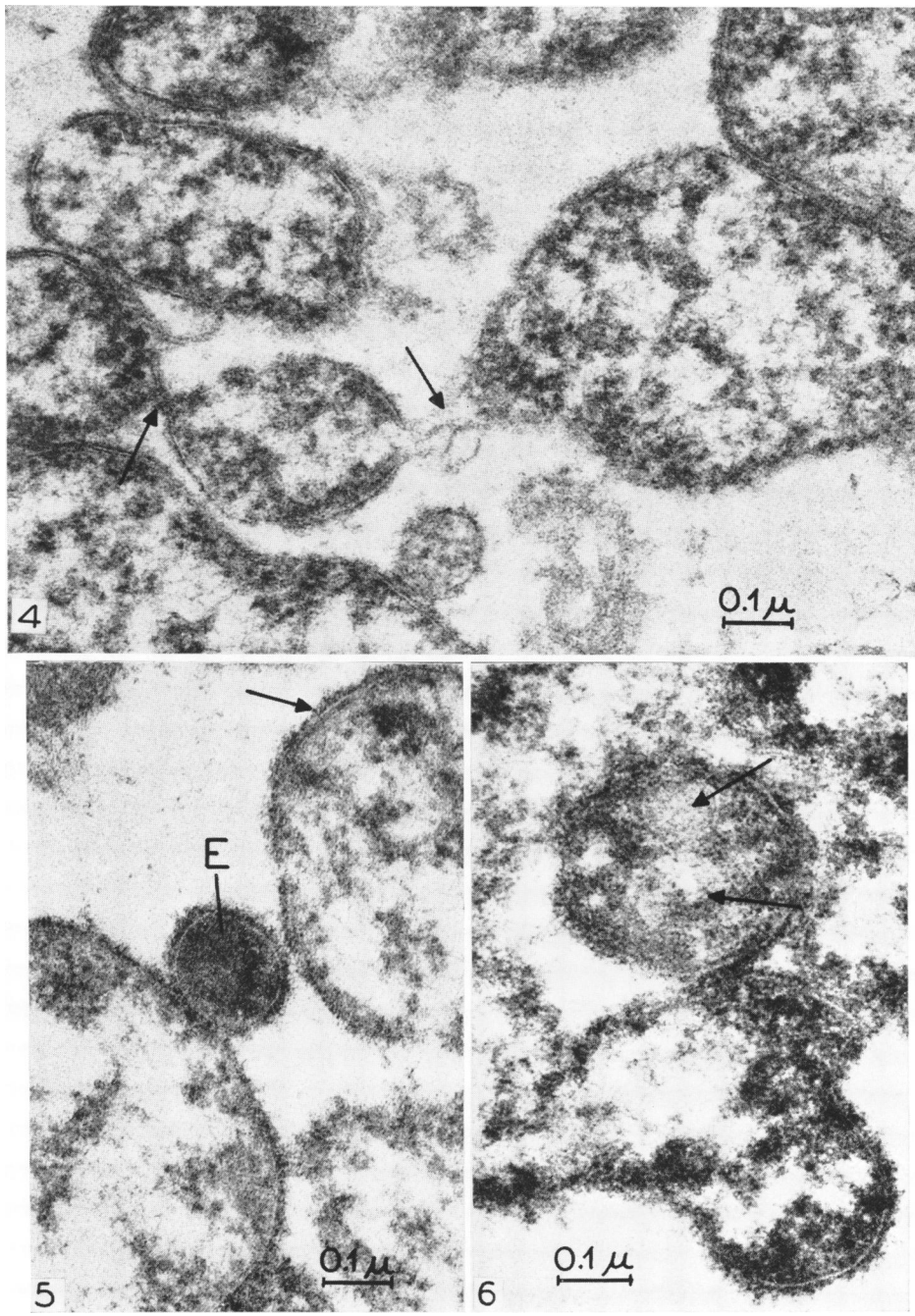


FIG. 4. *Mycoplasma arthritidis: 48-hr culture. Three connected cells (arrows). 94,000 ×.*

FIG. 5. *Mycoplasma canis: 48-hr culture. One small and parts of three larger cells. The difference in electron density of the cytoplasm of individual cells should be noted. The elementary body marked E is particularly dense. Ribosomes are also visible. Close to the outer layer of the limiting membrane, floccules of electron-dense material are seen. The arrow points at two dense lines in the substance on the surface of the limiting membrane. 94,000 ×.*

FIG. 6. *Mycoplasma canis: 48-hr culture. A cell containing one or possibly two membrane-covered spherical bodies in the cytoplasm (arrows). These structures are probably developing elementary bodies. 94,000 ×.*

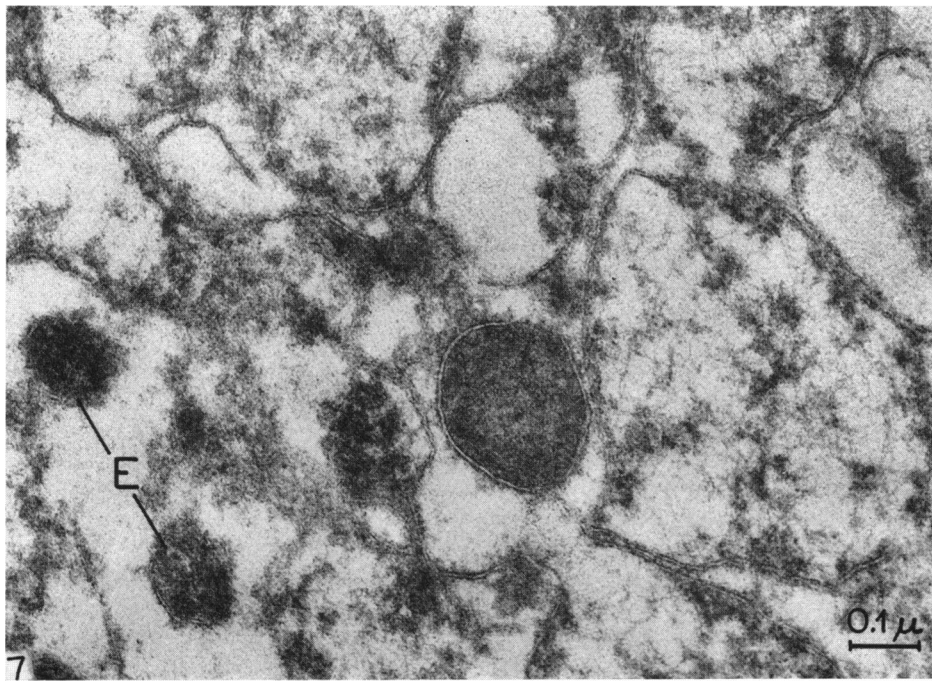


FIG. 7. *Mycoplasma fermentans: 22-hr culture. Individual cells show varying density of cytoplasm. The small dense cells are considered to be elementary bodies (E). All cells are limited by a triple-layered membrane of the symmetric type, i.e., the outer and the inner electron-dense layer being of the same width and density. 94,000 ×.*

lular membranes were always of the same type as the cell membrane.

Areas of an electron-dense substance were sometimes found in the interstices between cells (Fig. 20, 22, 24). This substance resembled that described as being in direct contact with the outer layer of the cell membrane. The amount of this material varied considerably from strain to strain.

DISCUSSION

It should be emphasized when discussing the results that all strains with but two exceptions (*M. gallisepticum* and *M. pneumoniae*) were grown on the same medium. This medium generally affords excellent growth conditions for several mycoplasmas, but it cannot be considered optimal for all of the strains examined. This is indicated, for example, by *M. spumans* requirement of a 2-week growth period to produce detectable microcolonies. This slow growth of *M. spumans* and other strains may be due to the fact that a serum which stimulates the growth of some strains may in the same concentration be inhibitory to others (Freundt, 1958). Although previous experiments clearly proved that the general morphology of the mycoplasmas depends on growth conditions (Freundt, 1958), nothing is known of the influence of growth conditions on their ultrastructure. Therefore, some reservation is needed in evaluating the significance of the ultrastructural differences observed among the various strains included in this study. Direct comparisons are made difficult, moreover, by the fact that the different growth rates which characterize the various strains precluded a systematic examination of cultures of approximately the same physiological age.

Colonies grown on solid media were studied instead of liquid cultures because the cells naturally embedded in the agar medium as a result of growth were considered less susceptible to changes in size and shape during preparation for electron microscopy. Another advantage of studying colonies sectioned *in situ* is the possibility of distinguishing older from younger (more dense) cells within the colony.

Since the primary purpose of this study was to

examine the ultrastructure of the mycoplasma cell, embedding and sectioning techniques were used in preparing specimens. These techniques not only have the advantage of revealing ultrastructure but also preserve the shape of the cell so that accurate whole-cell dimensions can be determined. Owing to the age of the cultures and to the method of specimen preparation, it is not surprising that only relatively few examples of filament formation (mode of multiplication) were observed. Shadow-cast specimens of whole cells proved to be better for the study of this phenomenon.

The dimensions of the external elementary bodies observed in this study fall approximately within the size range repeatedly reported in the literature (summarized by Breed, Murray, and Smith, 1957). Since this is apparently the first study in which direct comparative measurements have been made on representative strains of most of the *Mycoplasma* spp. established so far, it is interesting to note that the size of the cell types of all of these strains is fairly uniform.

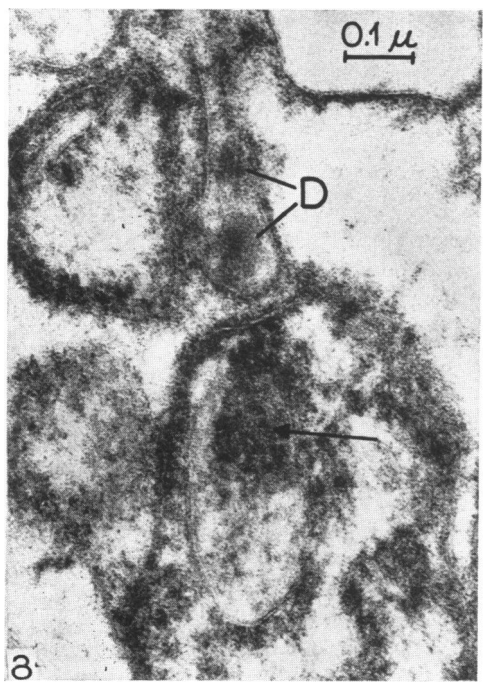
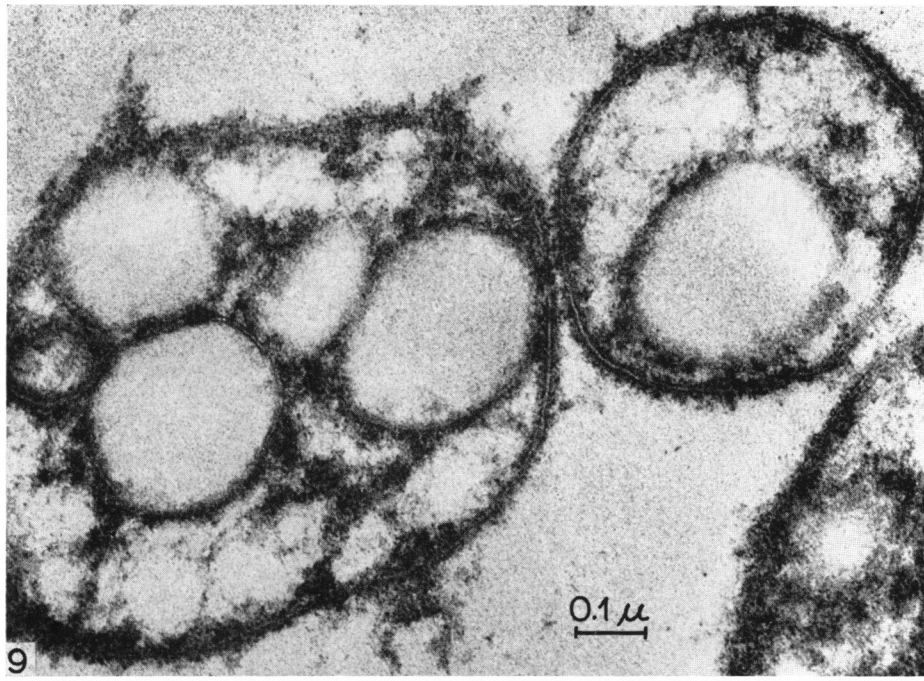


FIG. 8. *Mycoplasma gallinarum: 48-hr culture. Filamentous structure with spherical electron-dense condensations (D) in cytoplasm. An intracellular membrane-covered structure which is probably a developing elementary body (arrow). Floccules of electron-dense material are present on the outer surfaces of limiting membranes. 94,000 ×.*

It should be mentioned also that size of the cells as measured on electron micrographs (about 500 to 700 mμ) places the "average mycoplasma cell" within the same size range as other small bacteria such as certain *Serratia* or even *Streptococcus* spp. The external elementary bodies with an average size of only about 130 by 150 mμ still distinguishes the mycoplasmas as the smallest known organisms capable of growing on cell-free media. Here it must be mentioned that the terms "average" and "maximal size" mean little until the general appearance of the cells is considered. A fairly large proportion of the larger and largest cells appear more or less empty, and probably represent nonviable involution forms which are of little interest. Greater importance must undoubtedly be attached to cells of average size such as the "corncob" containing cells of *M. gallisepticum*, the ribosomal density and highly organized configuration of which provide strong evidence of normal physiological activity. Based on these criteria, the average diameter of the largest viable cell is about 550 by 700 mμ.

Interesting and sometimes unique details were revealed in the ultrastructure of the mycoplasma cell. In all strains examined, a triple-layered cell membrane of 75 to 100 A thickness surrounded spherical cells as well as filaments. These findings are in agreement with observations made by Van Iterson and Ruys (1960*a*, *b*) and Ruys and Van Iterson (1961) on strains of human and avian sources. Our additional observation of a distinct variation in the relative thickness of the two electron-dense layers of the membrane would seem to merit particular attention. There is evidence that structural symmetry versus asymmetry of the cell membrane, as well as of the intracytoplasmic membranes, does not depend exclusively on the age of the cell. However, final proof of the theory that the molecular structure of the membrane system of the mycoplasmas is a species-specific characteristic must await future studies based on time-lapse experiments with strains representing each of the two provisional groups.

Even greater significance may perhaps be attributed to the amorphous or floccular substance which was consistently, although in variable amounts, found in close connection with the surface of the cell membrane of every strain. Although the exact nature of this substance



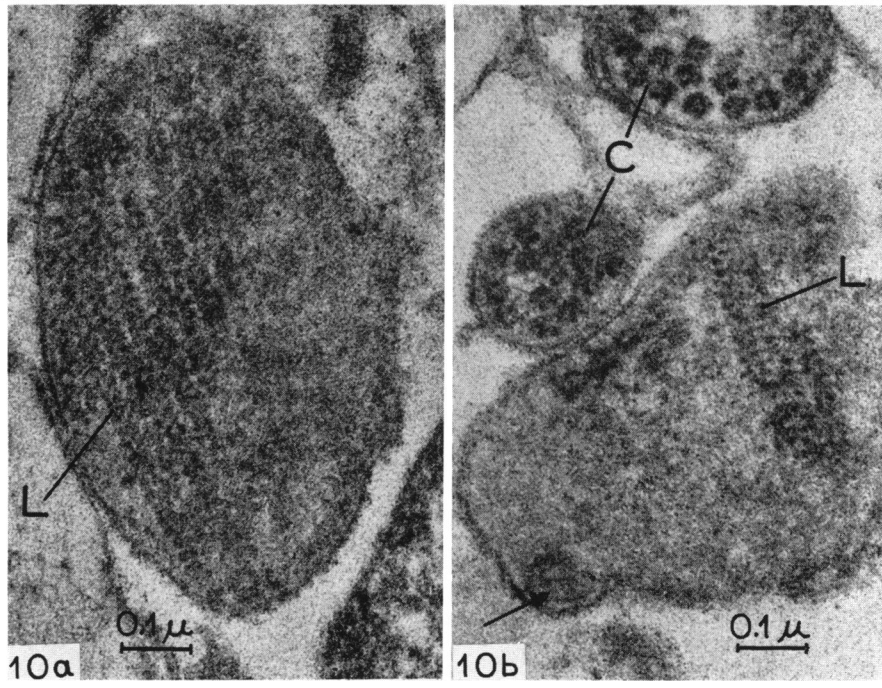


FIG. 9. *Mycoplasma gallinarum: 48-hr culture. Cells with several membrane-bounded vesicles in the cytoplasm. The vesicle membrane seems to be morphologically identical with the cell membrane. An appreciable amount of dense material is present on the surface of the cells. 94,000 ×.*

FIG. 10. *Mycoplasma gallisepticum strain JA: 96-hr culture. Cells with ribosomes arranged in "corncob-like" patterns. Longitudinal and cross sections of these structures are marked L and C, respectively. A spherical cytoplasmic condensation lacking a limiting membrane is seen in one of the cells (arrow). It is located within a protrusion of the cell membrane. 94,000 ×.*

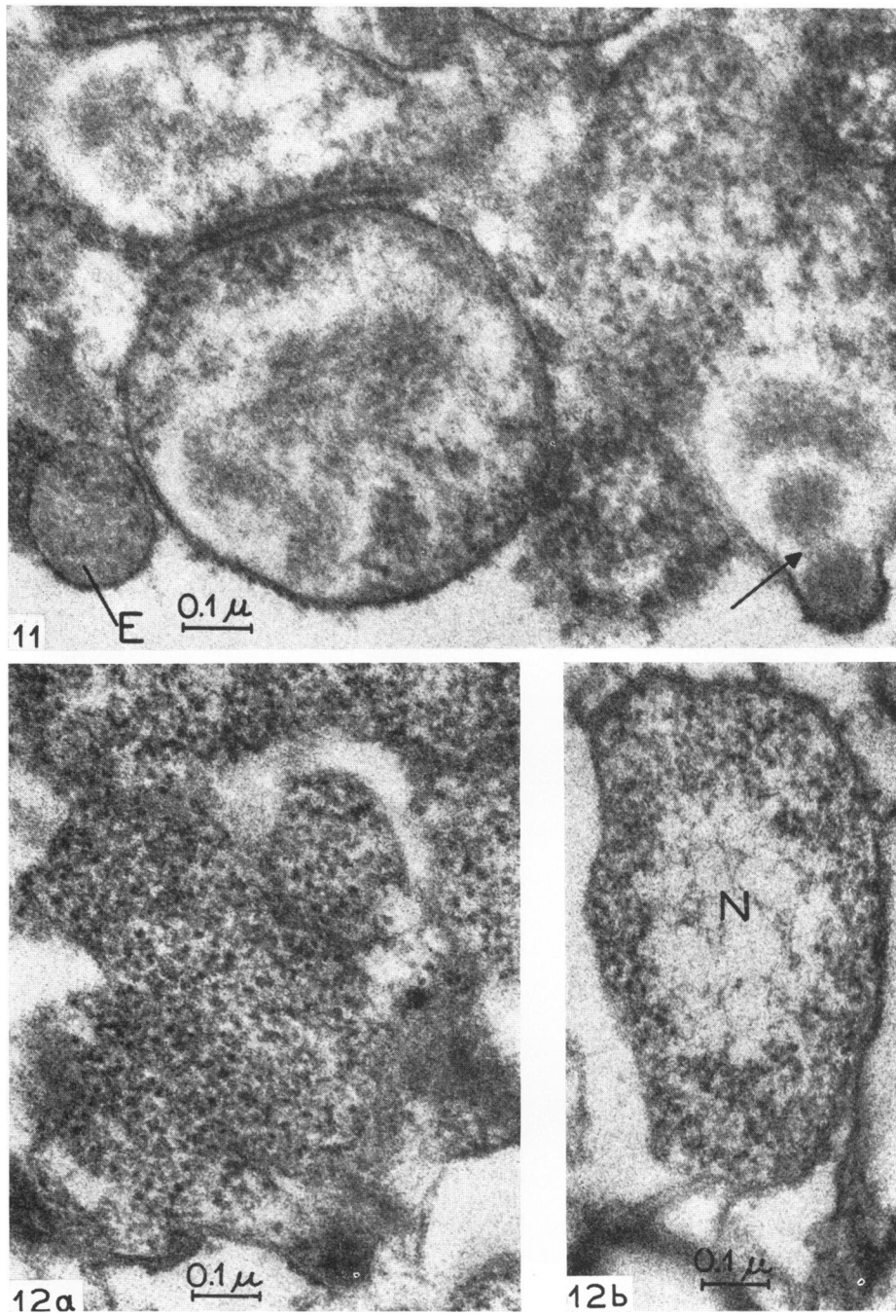


FIG. 11. *Mycoplasma gallisepticum strain W: 96-hr culture. The elementary body (E) contains cytoplasm of marked electron density. One semilunar and two spherical cytoplasmic condensations are located within a protrusion of the cell membrane (arrow). 94,000 ×.*

FIG. 12. *Mycoplasma hominis type 1: 72-hr culture. Cells with distinct ribosomes. The nuclear area (N) is centrally located in the cell. The cell membrane is of the symmetric type. 94,000 ×.*

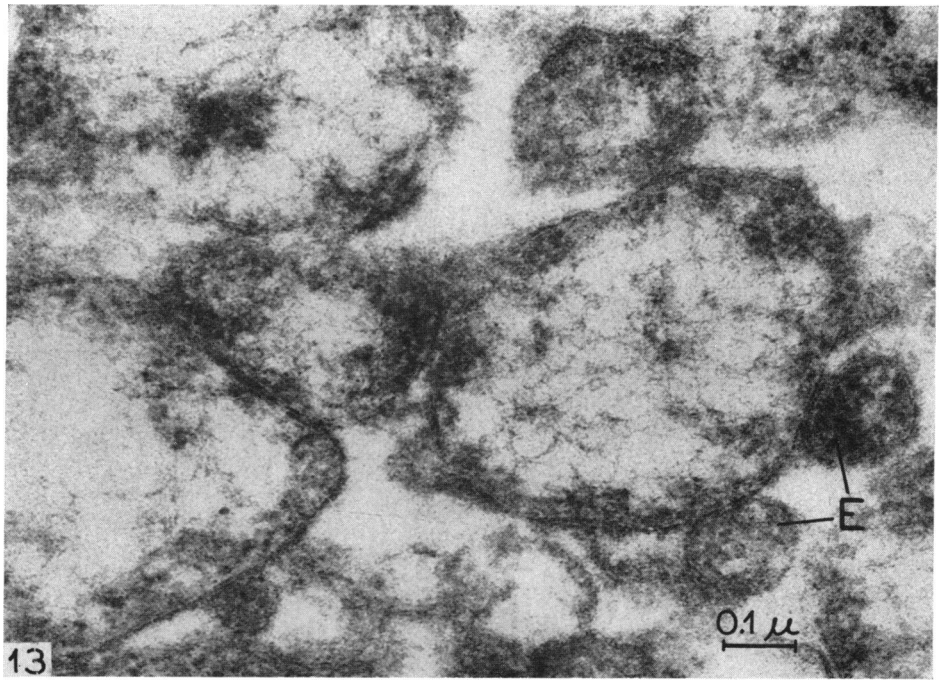


FIG. 13. *Mycoplasma hominis type 2: 48-hr culture. Cells of varying size. At E are elementary bodies. The limiting membrane is of the asymmetric type. 94,000 ×.*

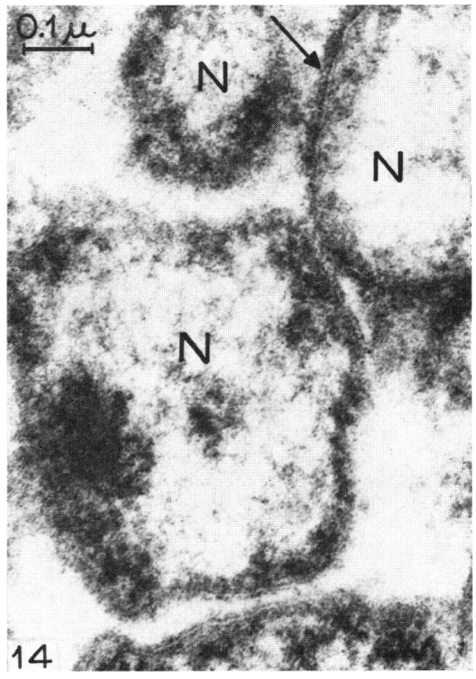
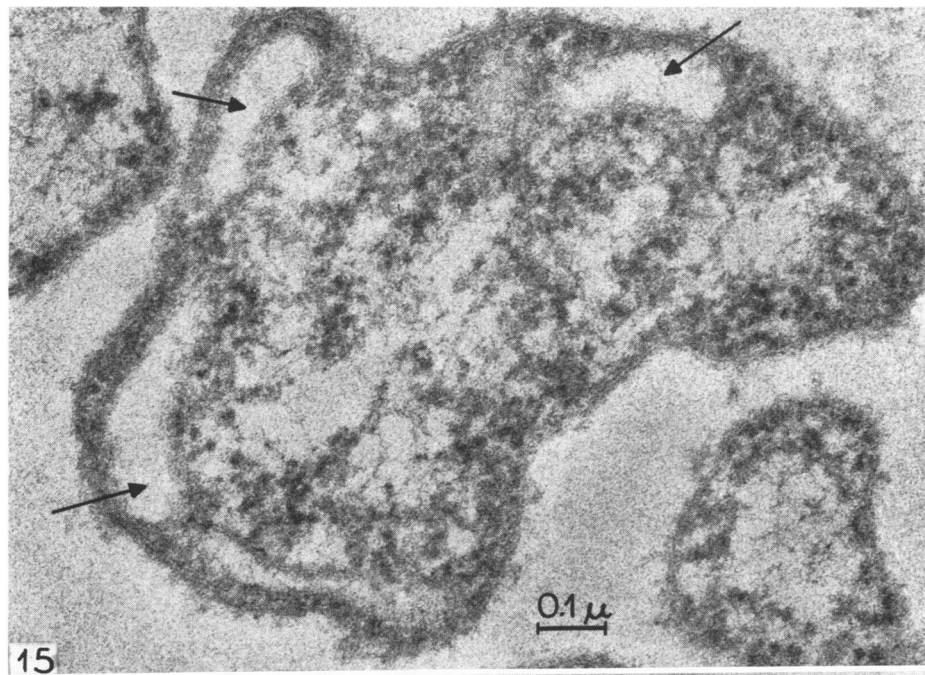


FIG. 14. *Mycoplasma hyorhinis: 72-hr culture. The limiting membranes of the cells are of the asymmetric type. An extra-dense line is visible in the floccular material on the surface of the cell (arrow). Centrally located within the cells are less-dense regions with delicate strands of nuclear material (N). 94,000 ×.*



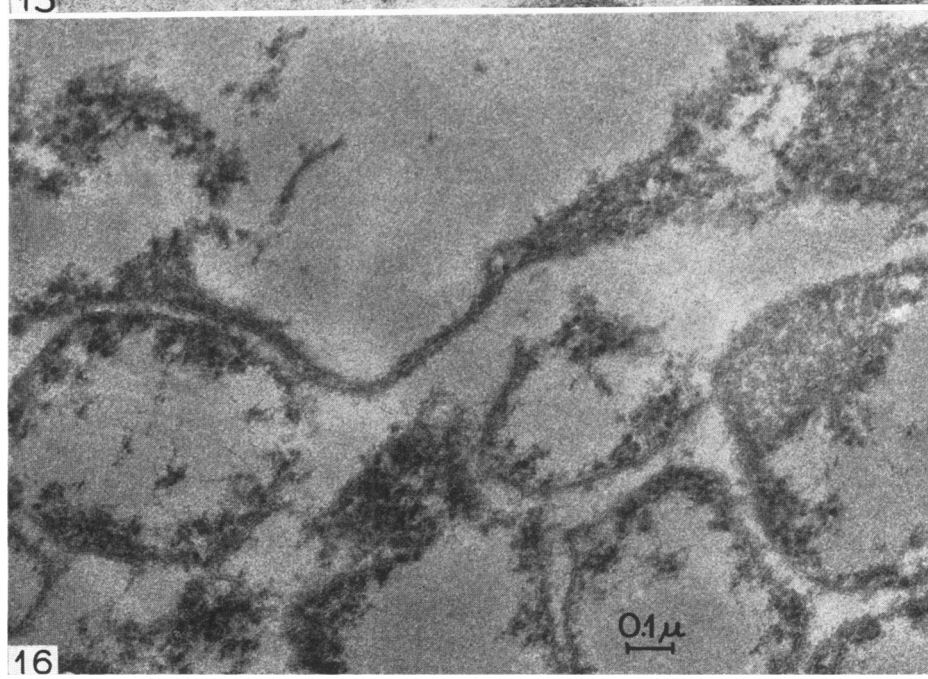


FIG. 15. *Mycoplasma laidlawii type A: 24-hr culture. Membrane-bounded vesicles are located at the cell periphery (arrows). The vesicular membrane and the cell membrane are both of the symmetric type. 94,000 ×.*

FIG. 16. *Mycoplasma laidlawii type B: 24-hr culture. This micrograph shows a longitudinal section of a filamentous structure. The limiting membranes are of the symmetric type. 94,000 ×.*

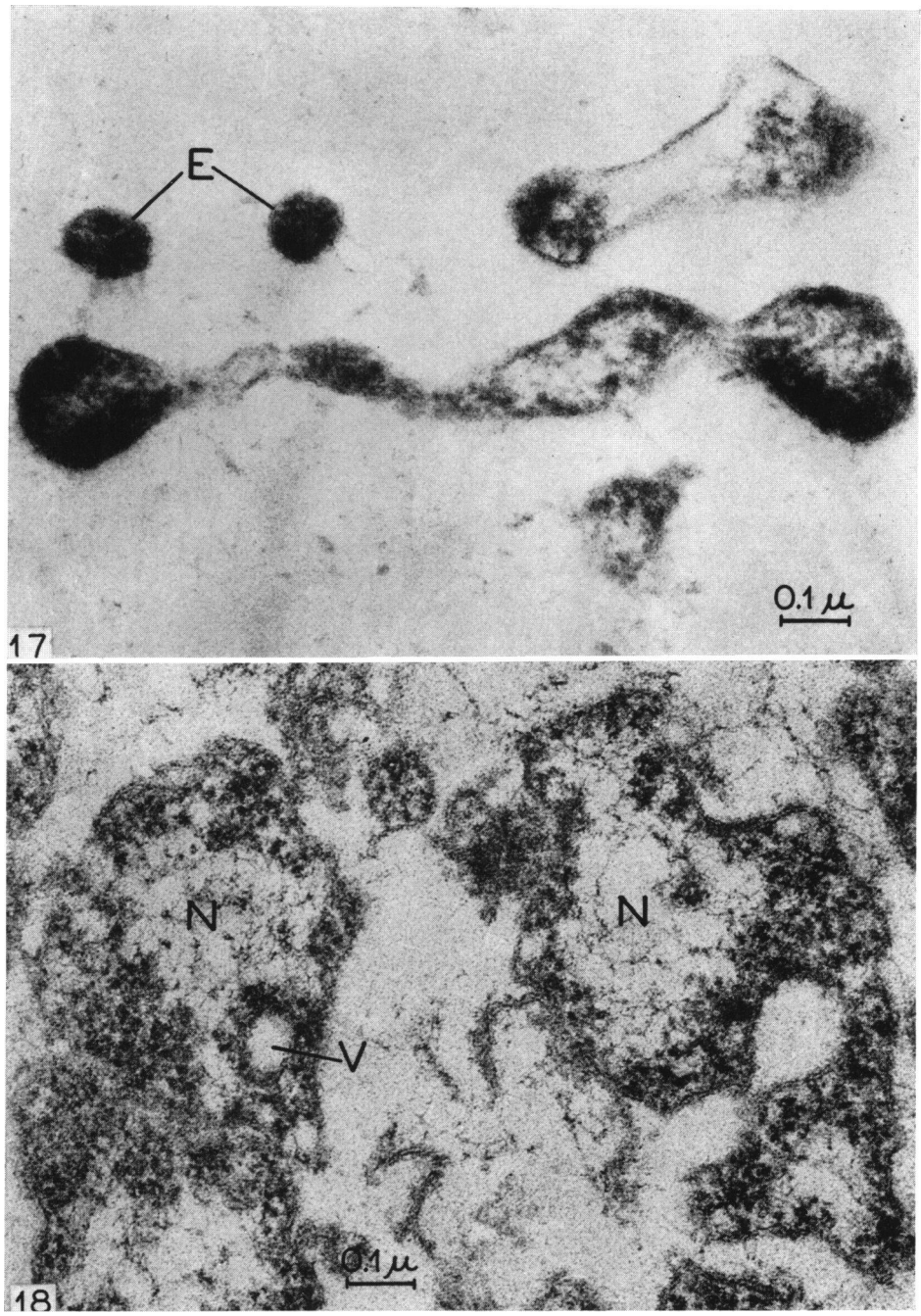
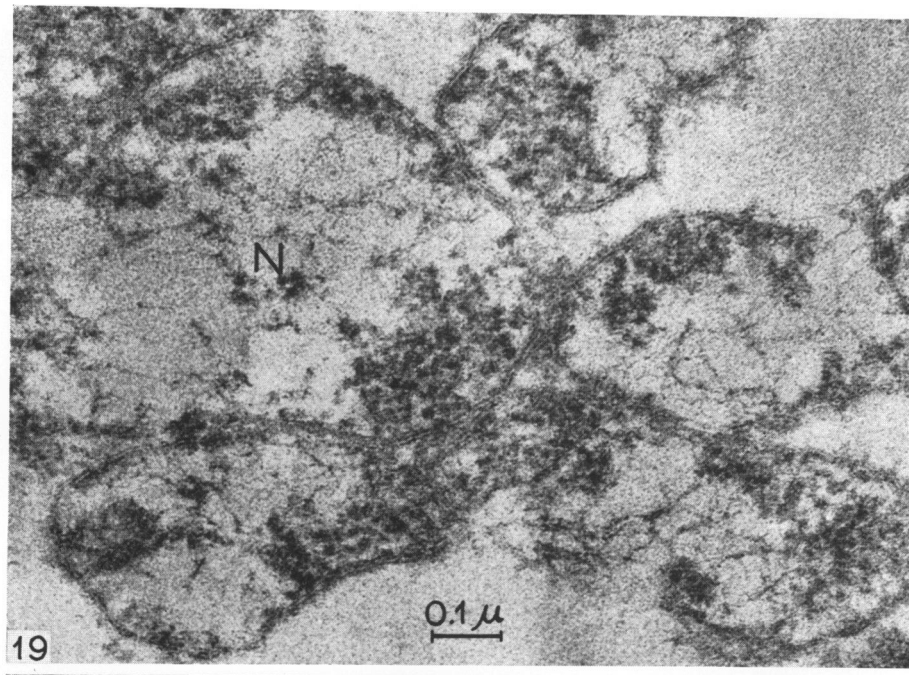


FIG. 17. *Mycoplasma mycoides var. mycoides: 12-hr culture. Longitudinal section of a filament. Elementary bodies (E) with electron-dense cytoplasm are also present. Cell membranes are of the symmetric type. 94,000 ×.*

FIG. 18. *Mycoplasma mycoides var. mycoides: 48-hr culture. Pleomorphic cells with symmetrical limiting membranes. One of the cells contains a vacuole (V) limited by a triple-layered membrane. Nuclear areas (N) with delicate strands are apparent. The ribosomes are located mainly at the cell periphery. 94,000 ×.*



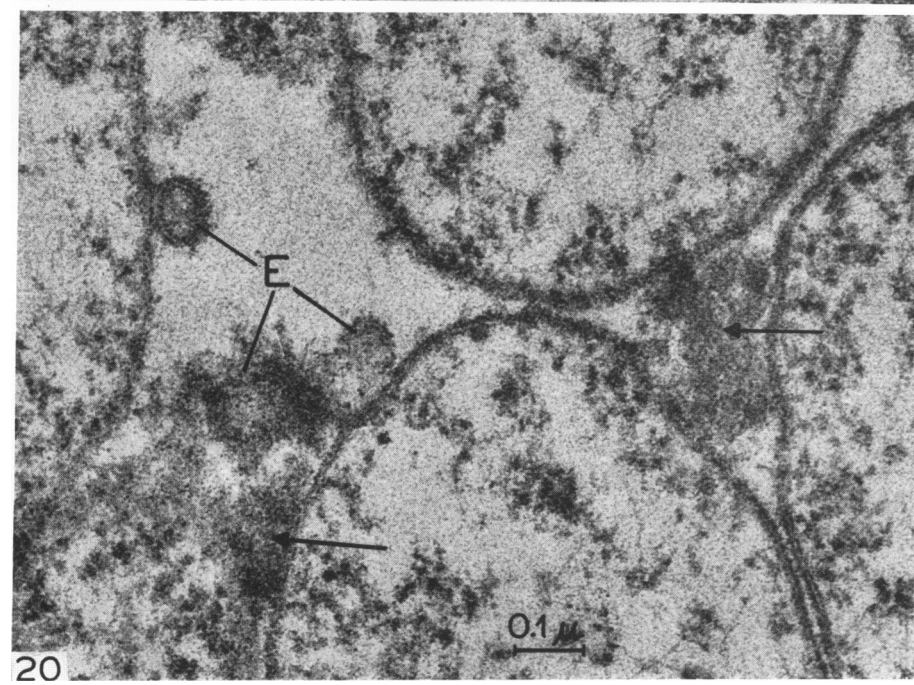


FIG. 19. *Mycoplasma mycoides var. capri: 24-hr culture. Pleomorphic cells with distinct ribosomes and electron-dense filaments in the nuclear region (N). The limiting membrane is of the symmetric type. 94,000 ×.*

FIG. 20. *Mycoplasma neurolyticum: 96-hr culture. The cells are limited by triple-layered membranes of the symmetric type. The total thickness of the three layers is approximately 100 A. Elementary bodies are also present (E). Areas of electron-dense intercellular material at arrows. 94,000 ×.*

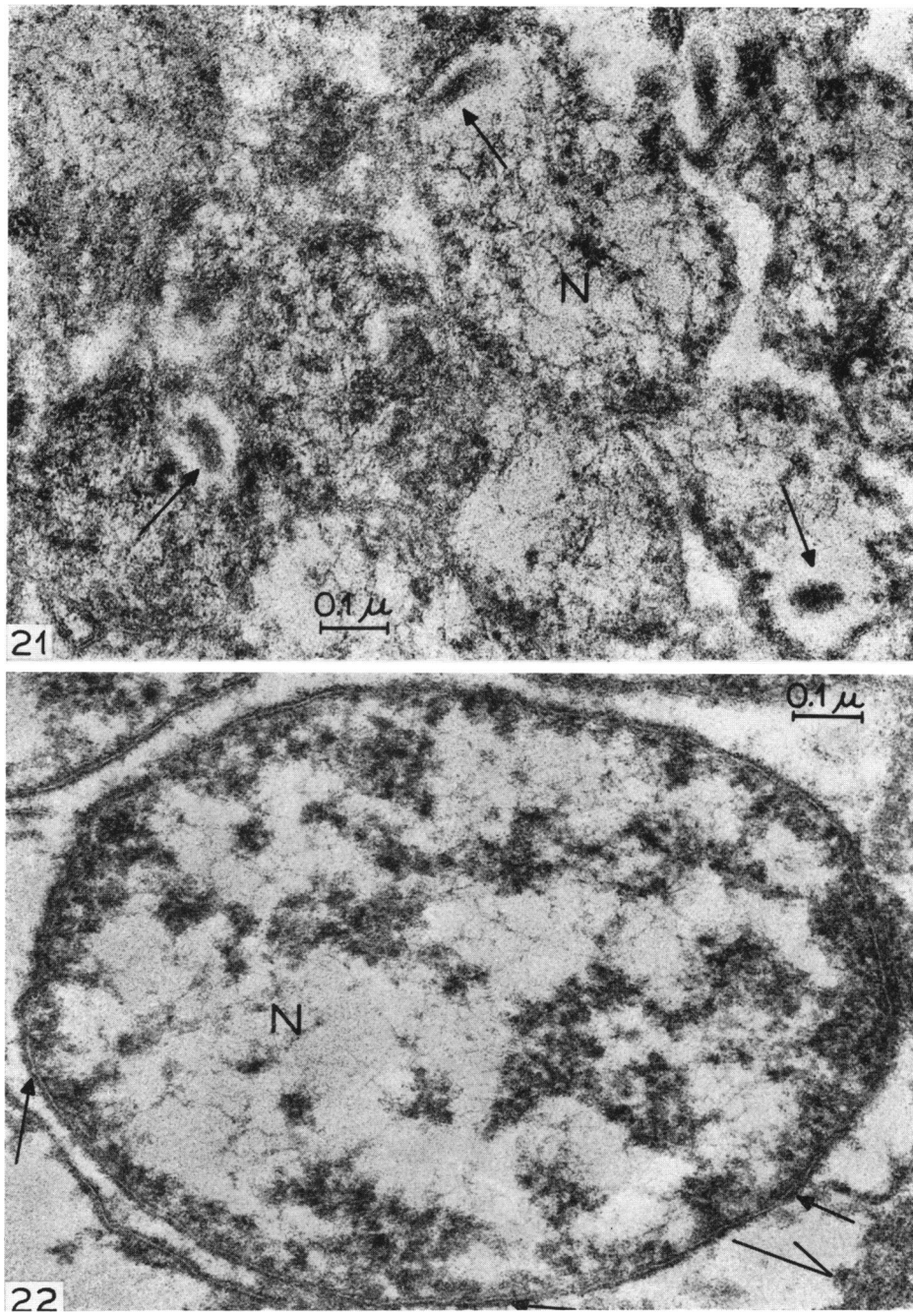


FIG. 21. *Mycoplasma pneumoniae: 7-day culture. Closely packed cells with cytoplasmic electron-dense rod-shaped condensation surrounded by a less-dense region (arrows). These structures are located at the cell periphery. Nuclear areas (N) are more or less centrally located within the cell. 94,000 ×.*

FIG. 22. *Mycoplasma pulmonis: 24-hr culture. Cell with distinct ribosomes and a large nuclear region (N) with delicate fibrils. The limiting membrane of the cell is of the asymmetric type. In the floccular substance on the outer layer of the cell membrane, an additional dense line can be observed (arrows). Some electron-dense intercellular substance is also present (hook). 94,000 ×.*

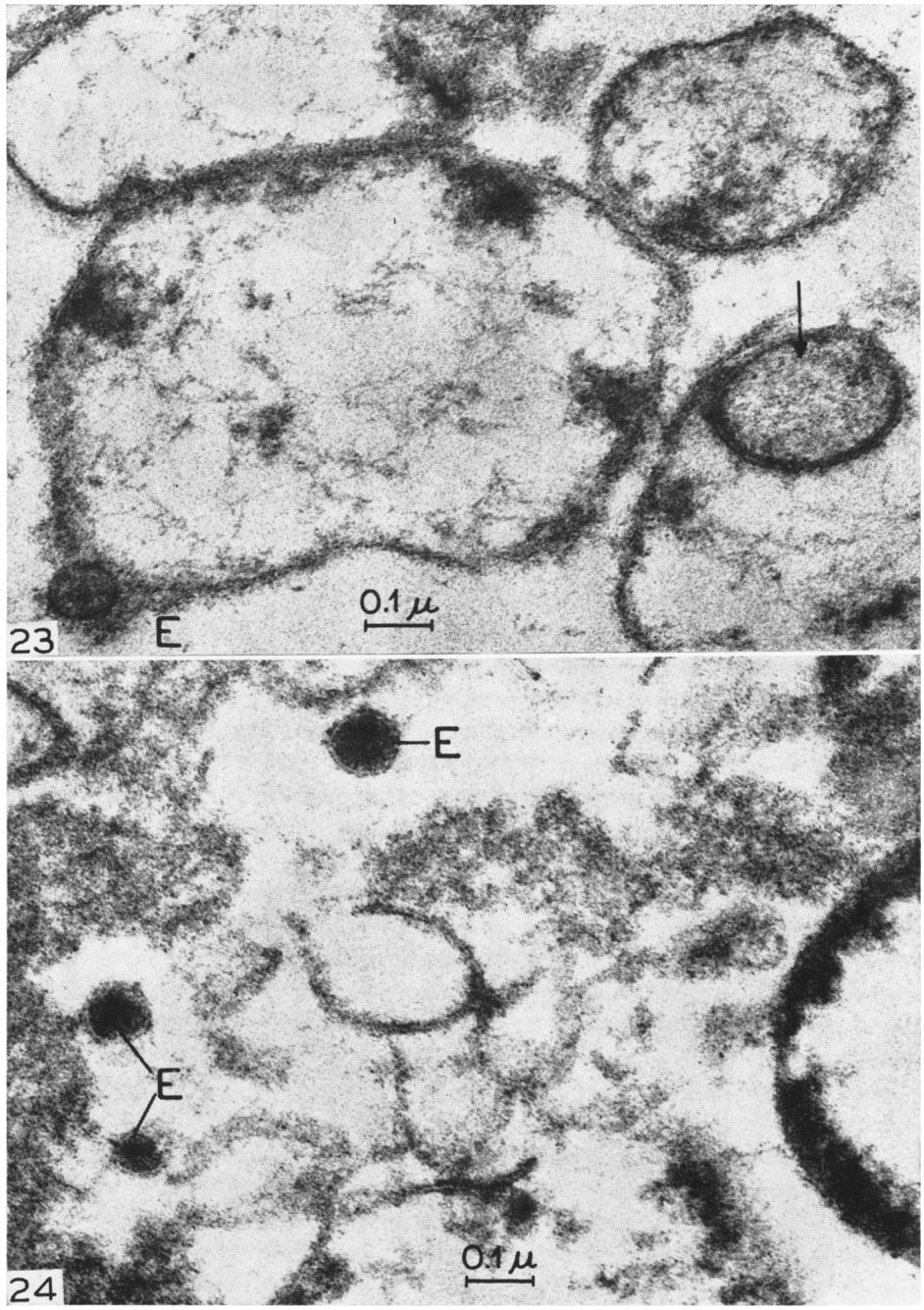


FIG. 23. *Mycoplasma salivarium: 96-hr culture. Large cells almost completely lacking ribosomes. Electron-dense accumulations are found in contact with the innermost layer of the cell membrane. E is probably an elementary body. The structure indicated by an arrow is an electron-dense membrane-bounded cytoplasmic condensation. 94,000 ×.*

FIG. 24. *Mycoplasma spumans: 14-day culture. The elementary bodies (E) contain dense cytoplasm. Part of a larger cell contains an extensive cytoplasmic area of lesser density and accumulations of rather homogeneous electron-dense material in contact with the inner surface of the limiting membrane. Intercellular electron-dense material as well as some membranous debris are also present. 94,000 ×.*

remains obscure so far, there are two possible explanations concerning its origin. It is either a precipitate from the culture medium or a substance secreted by the organism. The fairly frequent occurrence of one or more condensed lines similar to the dense layers of the limiting membrane itself seems to be consistent with the latter explanation. Thus, the extracellular substance could be a capsule or slime layer acting as an additional structural support or as an additional osmotic barrier, or both, which might be useful to free-living microorganisms lacking a regular rigid cell wall. The existence of such a capsular substance was recently suggested by Plackett, Buttery, and Gottew (1962) from their finding of polysaccharides of unusual structure in *M. mycoides* and related strains.

The general appearance and arrangement in the mycoplasma cell of familiar cytoplasmic components, such as ribosomes and nuclear areas with DNA-like strands, is not unlike what is found in other bacteria. The unique pattern, resembling corncobs, so frequently formed by the ribosomes of the JA strain of *M. gallisepticum* is a remarkable exception to the rule. Further studies are needed to determine whether this phenomenon is restricted to one or perhaps a few particular strains, or whether it is found in other mycoplasmas as well. This configuration of the ribosomes could be linked, for example, with a certain growth phase or with certain growth conditions not encountered so far with the remainder of the strains examined.

Among the dense inclusions which could be demonstrated in a high proportion of the strains, those surrounded by a membrane are similar to the inclusion observed by Van Iterson and Ruys (1960*a*) in *M. fermentans* and described as "a structure of unknown significance." We believe that the more or less spherical condensations of varying density, and the membrane-bounded dense inclusions, represent various developmental stages of an "assembly line" leading to the formation of the minimal reproductive units, the "elementary bodies." Suggestive evidence in support of this postulate is provided by the fact that the inclusions are of the same order of size (100 to 200 mμ) as the external elementary bodies, and, when surrounded by a membrane, are virtually indistinguishable from them. Also, in some instances (*M. gallinarum* and *M. canis*), the structural type (symmetrical or asymmetrical) of the limiting membrane of external elementary bodies, larger mature cells, and the intracellular inclusions was the same. Moreover, the frequent location of one or more of the inclusions in or adjacent to protrusions of the cell, and arranged similar to the developing elementary bodies within filaments of *M. mycoides* (e.g., Freundt, 1958), also seems in our opinion to be in favor of the proposed hypothesis. A more detailed discussion of these observations will be presented in a subsequent paper.

The interpretation of the intracellular membranous vesicles imposes a more severe problem on the combined imagination of the authors. Though the apparently empty vesicles are reminiscent of the membranous "organelles" that have been demonstrated in *Azotobacter agilis* and certain other bacteria (Van Iterson, 1962), we prefer at present suspending any further discussion of the significance and possible function of these structures.

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