BACTERIOLOGICAL REVIEWS, Sept. 1972, p. 263-290
Vol. 36, No. 3

Cell Biology of the Mycoplasmas

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INTRODUCTION

The term mycoplasma is the name generally used for a group of microorganisms (order *Mycoplasmatales*), earlier called the pleuropneumonia-like organisms, or PPLO. This group lacks cell walls and includes the genus *Mycoplasma* (which requires sterol), the genus *Acholeplasma* (which does not require sterol), and the T strains (which require urea). A recent thermophilic, acidophilic isolate has been proposed as a new genus, *Thermoplasma* (29), but since this group has not been extensively characterized, it will not be considered further here.

The extensive data concerning the mycoplasmas, which have been reported over the past few years, is now sufficient to allow us to contruct an overview of the cellular and molecular biology of these organisms. Therefore, this paper will be critical rather than exhaustive, since more encyclopedic reviews are available (e.g., 57, 126, 156, 166) and it is now recognized that many earlier published results reflect serious preparation artifacts.

Several generalizations can be derived from a critique of the existing literature in terms of current criteria of validity, particularly the necessity for monitoring the viability of the culture being studied to make sure that biochemical and cytological data are collected on exponentially growing cells and not on the debris found in older, dead cultures.

The first generalization is that the mycoplasmas are a sufficiently heterogeneous group of organisms that various species cannot be expected to be described by a single type of morphology. In fact, since *the mycoplasmas are a group of small procaryotic cells bounded only by a single lipoprotein ("unit") membrane*, they may well represent a group of microorganisms as diverse as the bacteria. The second generalization is that, under defined culture conditions, each species has a characteristic morphology. In cytological preparations, the normal plasticity (polymorphism) arising from the absence of a rigid surface envelope should not be confused with pleomorphism, which would necessarily imply the exist-

ence of more than one distinct cellular form during the organism's life cycle. The third generalization is that the smallest known viable mycoplasmas (single cells capable of forming a clone) are about 0.33 μm in diameter (85). These are the smallest reported living cells. It would be very interesting if smaller cells are found some day, but the smallest mycoplasms are within an order of magnitude of the 0.1 μm theoretical minimal cell (100, 101). Recent reevaluation of the theoretical minimal cell (*discussed below*) raises this postulated value to about 0.15 μm. The fourth generalization is that most mycoplasmas replicate by a binary division (i.e., one cell forms two daughter cells), while some filamentous species are probably able to break into a small number of viable filamentous daughter cells. More elaborate life cycles have previously been postulated for the mycoplasmas. In the most widely known of these it was assumed that an individual cell enlarged giving rise to a "large body." Within this structure "elementary bodies" formed. These "elementary bodies" were believed to be membrane-bounded virus-like particles of dimensions between 0.10 and 0.15 μm. The large cell was assumed to rupture, releasing the "elementary bodies" each of which proceeded to grow into cells which could then either replicate by binary fission or mature into "large bodies" (40, 41, 42, 74, 107). No currently acceptable data support such a theory and no viable "elementary body" has ever been isolated. The fifth generalization is that the biochemistry of the mycoplasmas is similar to the biochemistry of other cellular systems; thus the unique molecular properties in terrestrial biology that biologists had originally hoped to find in these small cells have not been found (e.g., 100, 101, 115, 116). The possible significance of this point will be discussed below.

CYTOLOGY

The lack of viability data in many references makes it impossible to know the growth phase of most of the cultures that have been studied. This is important because, as mycoplasma cultures age, there are morphological changes (13, 68, 87), loss of enzyme and transport activities (126), decrease in membrane fluidity (152), increase in osmotic fragility (152), and resistance to lytic viral infection (79).

It should be noted that turbidity measurements (unless explicity shown otherwise) are not an adequate viability assay since absorbance has been shown to be a nonlinear function of the number of viable cells (86, 164). "Good turbidity" generally corresponds to stationary-phase cultures (69, 70, 86). In the discussion below, emphasis will be given to those studies that clearly involve exponentially growing cells.

Cell Size

The volume of individual mycoplasma cells is equivalent to spheres in the size range of 0.33 to 1.0 μm diameter. The sizing involves a simplification because, for example, the 0.9-μm spherical *Acholeplasma laidlawii* cell is seen only in dying cultures; in growing cultures the cell is usually a 0.5-μm by 2-μm filament, which has the same volume as the 0.9-μm sphere (87). Some degenerating cells have been reported to contain or release small bodies which have been called "elementary bodies," but no evidence exists that these are viable particles (2, 87).

The 0.33-μm cell (85) is about three times larger than the early reports of 0.1-μm clone-forming units, and the reasons for the erroneous size estimates must be considered. One of the techniques used to estimate cell size was passage through known pore-size filters (74, 109). Razin (126) has reviewed studies indicating that the plastic mycoplasmas can be squeezed through filter pores smaller than the actual cell size, and Lemcke (77) has recently examined the filtration conditions that allow mycoplasmas to pass through filter pores smaller than the cell diameter. It has also been reported that staphylococcal L-forms with diameters greater than 0.6 μm, when grown on filters, are able to pass through filter-pore sizes as small as 0.05 μm (99). In experiments on *A. laidlawii* cells, which are 0.5 μm by 2 μm, the authors have found (*unpublished data*) that, with positive pressure (using a Swinney hypodermic adapter), 10% of the titer can be recovered in a 0.45-μm pore-size filtrate. However, no titer was recovered when the filtration was done with negative pressure (using a water aspirator). Hence, filtration efficiency of the mycoplasmas depends on the conditions and cannot be used as a size measure, as can be done with rigid virus particles.

The small size of the mycoplasmas puts them close to the 0.25-μm limit of resolution of optical phase-contrast microscopy (e.g., 87). In addition, the optical measurements are complicated by: (i) the "halo" artifact produced by the phase-contrast optics (9) and (ii) the nature of the phase-contrast image formation in which the size of a circular object appears smaller than the true object (21, 145). Hence, it must be concluded that measurements in

this size range, based on optical microscopy, are inaccurate and, in a comparison with light and electron micrographs, it was not always possible to decide whether a light microscopy image represented a single cell or a clump of cells (87). Bredt (15–17) has been able to solve this problem by using time-lapse photography to follow the growth of the individual viable units in his samples.

Sizing by electron microscopy, because of the difficulties described above, lacks the important correlative data that can be obtained in other systems by light microscopy observations of living cells. Razin (126) has discussed the problems inherent in electron microscopic size measurements. In general these are: (i) for thin sections, dependence of the size on the plane of section; (2) for negative stain preparations, morphological distortions caused by the heavy metal stains (to be discussed further below); and (3) for electron microscopy in general, the difficulty in knowing whether the particle observed represents a viable cell. The last problem can be approached by studying cells growing on electron microscope grids (85, 91, 105), in which case each microcolony must represent a viable clone.

Cell Morphology

The limitations of light and electron microscopy observations discussed above must be kept in mind in evaluating reports on cell morphology. In addition, it has been shown that mycoplasma shape can be vastly altered and deformed by the physical and chemical agents used in the preparations. For example, the pear-shaped *Mycoplasma gallisepticum* forms: (i) spheres, if centrifuged before fixation (93), (ii) filaments, if exposed to hypotonic solutions (11), (iii) aberrant cells having many blebs, if grown on a surface (91), and (iv) a variety of distorted shapes, if unfixed cells are negatively stained with phosphotungstic acid (10, 93). Phosphotungstic acid has also been reported to alter the spherical shape of unfixed cells of other mycoplasmas (141, 142), producing bizarre forms with many protrusions and filaments, and causing the detachment of small protrusions giving rise to what could be falsely interpreted as small cellular particles. Presumed enzymatic deoxyribonucleic acid (DNA) degradation during centrifugation of unfixed cells has also been reported in *M. mycoides* (63) and *M. gallisepticum* (143), reflecting certain cytological alterations during cell harvesting. Filament production in hypotonic solutions has also been reported for *M. pulmonis* (141). One should distinguish between filaments as a morphological form resulting from growth which has been clearly demonstrated in phase microscopy, and filamentous protrusions resulting from effects of physical and chemical agents, both of which may appear in a given species.

A detailed review of all of the published papers on morphological forms seen in mycoplasma cultures would indeed be an extensive task. From our survey of those reports showing the best cytological preservation, the morphotypes may tentatively be grouped into the following categories: (i) *coccoid cells*, e.g., *M. pulmonis* (64, 141) and Caprine PPLO strain 14 (J. Maniloff, 1968. Bacteriol. Proc., p. 78), the latter forms streptococcal-like chains (Fig. 1); (ii) *coccoid cells with membrane tubules*, which frequently appear as tubule-connected diplococci (Fig. 2), e.g., *M. arthritidis* H39 (85), originally erroneously classified as a *M. hominis* and possibly *M. felis* (13); (iii) *filamentous cells*, sometimes showing branching (Fig. 3), e.g., *M. orale* (3), *M. hominis* (98), *A. laidlawii* (87, 129), and *M. mycoides* (41, 49); (iv) *filamentous cells with terminal structures*, the only known example of which is *M. pneumoniae* (12, 68); and (v) *pear-shaped cells with terminal structures*, the only known examples of which are the blebs of the strains of *M. gallisepticum* (Fig. 4; references 1a, 10, 91, 93, 105, 182). The morphology of the T strains has still not been adequately described. This classification does not imply that a species has a unique shape under all growth conditions, but rather asserts that under given growth conditions the morphological character is distinct enough to be classified into one of a small number of forms.

These morphologies characterize the exponentially growing mycoplasmas. There is no evidence that mycoplasmas can retain their viability if taken up in an intracellular form; they can be phagocytized by animal cells (64, 182, 183). On the other hand, the studies of plant and insect parasites presumed to be mycoplasmas have demonstrated intracellular particles, and it is not possible at the present time to generalize about their possible morphological forms (e.g., 22, 51–53, 83, 84, 94, 112, 118, 119, 157).

Cell Growth

When colony-forming units are assayed, mycoplasma cultures follow typical bacterial growth curves with exponential, stationary, and death phases (43, 45, 69, 70, 80, 86, 129, 180). Mycoplasma colonies have been shown to form from single cells for *M. gallisepticum*

(105) and *M. arthritidis* H39 (unpublished results of the present authors, using same experimental protocol as reference 105). In the exponential phase at 37 C, most cultures have a doubling time in the range of 1 to 6 hr (86, 166). The length of the stationary phase varies among the species (86, 129) but is frequently shorter than 24 hr. The subsequent death rates are quite rapid; the titer drops by a factor of 10^2 to 10^4 per hr (80, 86).

The few studies of macromolecular nucleic acid synthesis during culture development show growth patterns similar to those found in bacterial cultures (58). When stationary-phase *M. gallisepticum* cells are transferred to fresh medium, there is an exponential increase in cell titer but a lag in DNA synthesis, resulting in a decrease in DNA per cell. Exponential DNA synthesis begins and continues while the cell number begins to level off as the culture reaches stationary phase, resulting in an increase in the DNA per cell (122). These data, together with the multi-hit ultraviolet inactivation studies (*discussed below*), indicate that stationary-phase cells may be multinucleate. The ribonucleic acid (RNA)/DNA ratio in exponential-phase *M. gallisepticum* is reported to be 4.5 (73) and for stationary-phase cells to be 2.0 (110).

The problem of replication in the mycoplasmas can now be restated in somewhat different terms. The fundamental act of replication in these cells and all other procaryotes is the replication of the DNA genome, a single macromolecule which usually occurs in a loop configuration. The two DNA structures must then be packaged into separate nuclear bodies. If the cell then divides into two nearly equal halves, each containing one nuclear body, the process is designated binary fission. An unequal cytoplasmic division at this stage would appear as a budding process. Alternatively, several nuclear divisions and cellular elongation followed by cell division would constitute replication by filament formation. In any case, there is a primary process of nuclear division followed by secondary cell division. The details of the cell division have not been clarified in all mycoplasma strains.

The growth of exponential-phase cells has been followed by the beautiful cinematographic studies of Bredt (15–17) and is in agreement with the interpretations of well-preserved cells from growing cultures examined by electron microscopy. Binary fission is the characteristic mode of replication and has been observed for *M. gallisepticum* (91, 105), *M. hominis* (17), *M. pulmonis* (W. Bredt, 1972, Med. Mikrobiol. Immunol.; **157**:169), *M. pneumoniae* (15, 16), *M. arthritidis* (85), and Caprine PPLO strain 14 (J. Maniloff, 1967, J. Cell Biol. **35**:87A). Bredt (1972, Med. Mikrobiol. Immunol. **157**:169) has also found that some of the filamentous cells (e.g., *M. hominis*), in addition to binary divisions, can elongate and constrict at a couple of points giving rise to several viable filamentous cells.

There are reports of cell numbers doubling in cultures which are supposed to be synchronized, of *M. orale* (43), *M. pneumoniae* (45), and two avian PPLO strains (71). These are expected for binary division as the mode of cell replication, but this agreement may be fortuitous in view of the difficulties in obtaining high degrees of synchrony, the problem of reproducibly counting mycoplasma colonies, and, in the case of the *M. pneumoniae* study, the long time interval used makes it difficult to decide whether there are indeed plateaus in the growth curve.

As was mentioned above, there are no currently acceptable data supporting the existence of "elementary bodies" or an endomycelial-endofilamentous theory of mycoplasma growth. The experiments that were interpreted to suggest such forms appear, in hindsight, to have suffered from the various artifacts discussed above relating to sizing, preparation for microscopy (including fragmentation of cells during preparation), and morphological changes in drying cultures. Three further points should be noted here. (i) If "elementary bodies" exist, it should be possible because of the nature of phase-contrast light microscopy to observe colonies growing from no preexisting image, since the hypothetical "elementary body" would be below the limit of resolution. No such observations have been reported. (ii) The report of "elementary body" formation in stationary-phase cultures (129) cannot reflect a growth phenomenon since the viability of such cultures is constant or slightly decreasing. (iii) One further argument may be offered against the logical possibility of a stage of the mycoplasma life cycle of a size of 0.15 μm or less. We know that the smallest mycoplasma genomes are of the order of 5×10^8 daltons (102). Assuming minimal hydration (1 g of water/g of DNA), such a molecule cannot be packed into a membrane-bounded sphere of less than 0.15 μm external diameter. Any other necessary cellular apparatus would raise this minimum. Conservation of information demands that the cell retain its genome over all stages of the life cycle. Therefore, reported viable mycoplasma units of less than around

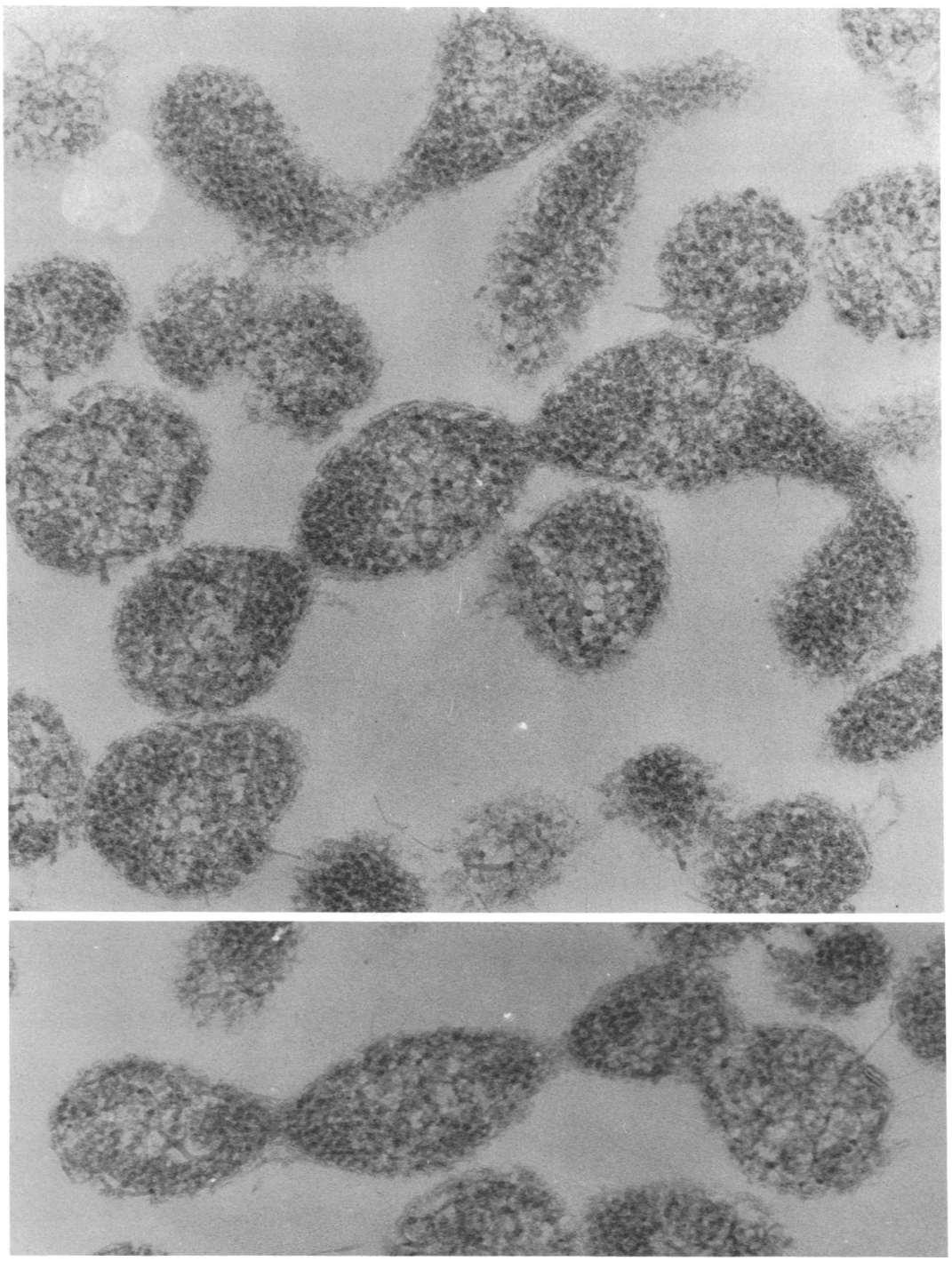

FIG. 1. *Micrographs of sections of Caprine PPLO strain 14 logarithmic phase cells; glutaraldehyde fixed, Epon embedded, and uranyl stained (87). The cells form streptococcal-like chains. The cytological elements seen are the cell membrane, fibrous nuclear material, and ribosome granules.* ×81,000.

0.2 μm diameter are not possible on the basis of current knowledge.

SUBCELLULAR ORGANIZATION

The view of mycoplasma cytology has changed greatly since Morowitz and Tourtellotte (107) showed a schematic representation of a typical PPLO cell as a round body bounded by a lipoprotein membrane and containing disorganized DNA, ribosomes, and soluble RNA. The known subcellular structures now consist of the cell membrane, possible extracellular structures, the chromosome, ribosomes and macromolecular RNA, plasmids, and other cytoplasmic organelles.

Some mycoplasmas have been found to have special cytological features that relate to their interaction with animal cells: the capsular substance around *M. mycoides* (49), the terminal bleb on *M. gallisepticum* (182, 183), and the terminal knoblike structures of *M. pneumoniae* (28). These mycoplasmas, perhaps coincidentally, are among the more pathogenic species. The *M. neurolyticum* exotoxin should probably be included as a special case; the cell must certainly have an interesting method of exporting a toxin with a molecular weight over 200,000 (172).

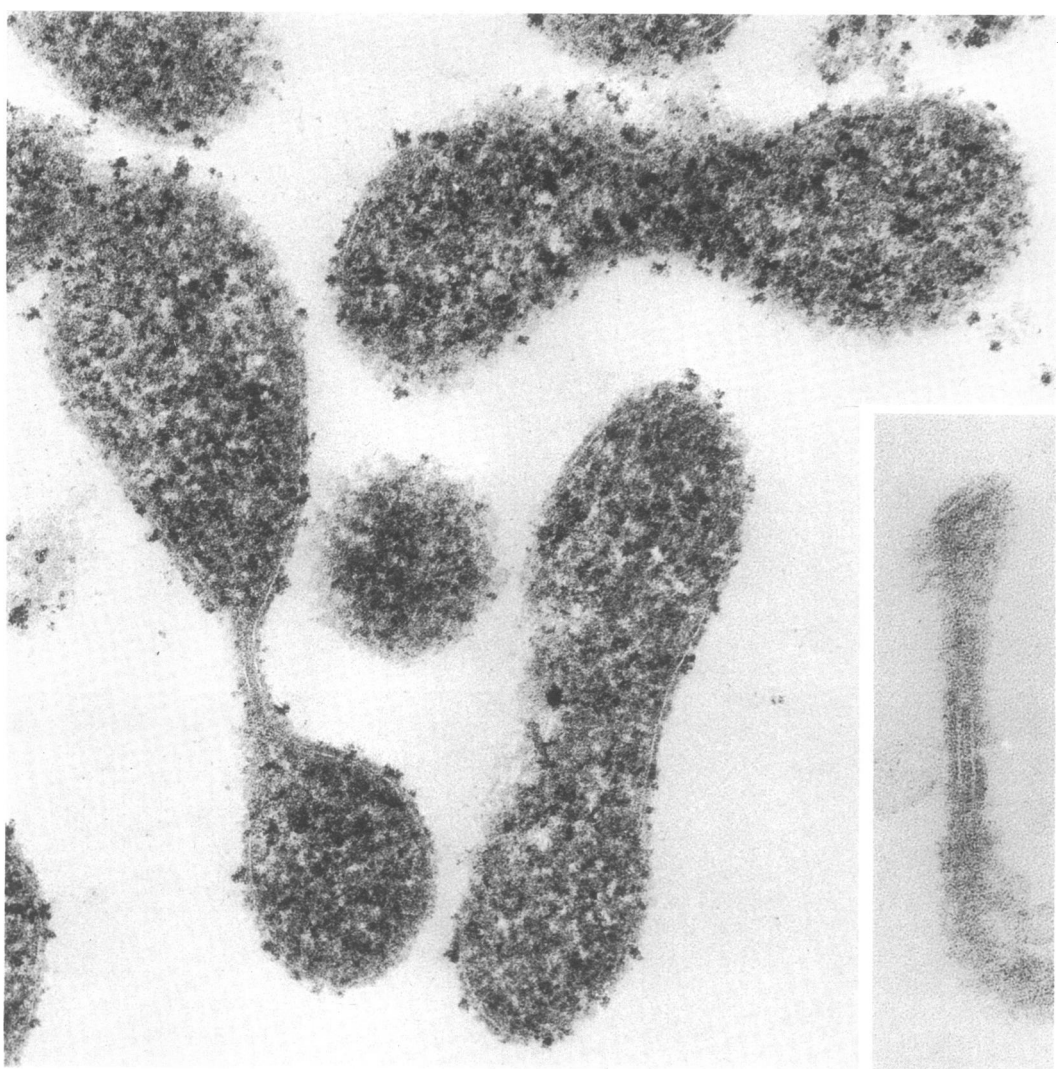

FIG. 2. *Section of M. arthritidis H39, formerly named M. hominis H39 (85). Elongated coccoid cells and a dividing cell, with the coccoid cells connected by a membrane tubule, are seen. ×81,000. Inset: Detail of part of a membrane tubule between two cells. ×162,000.*

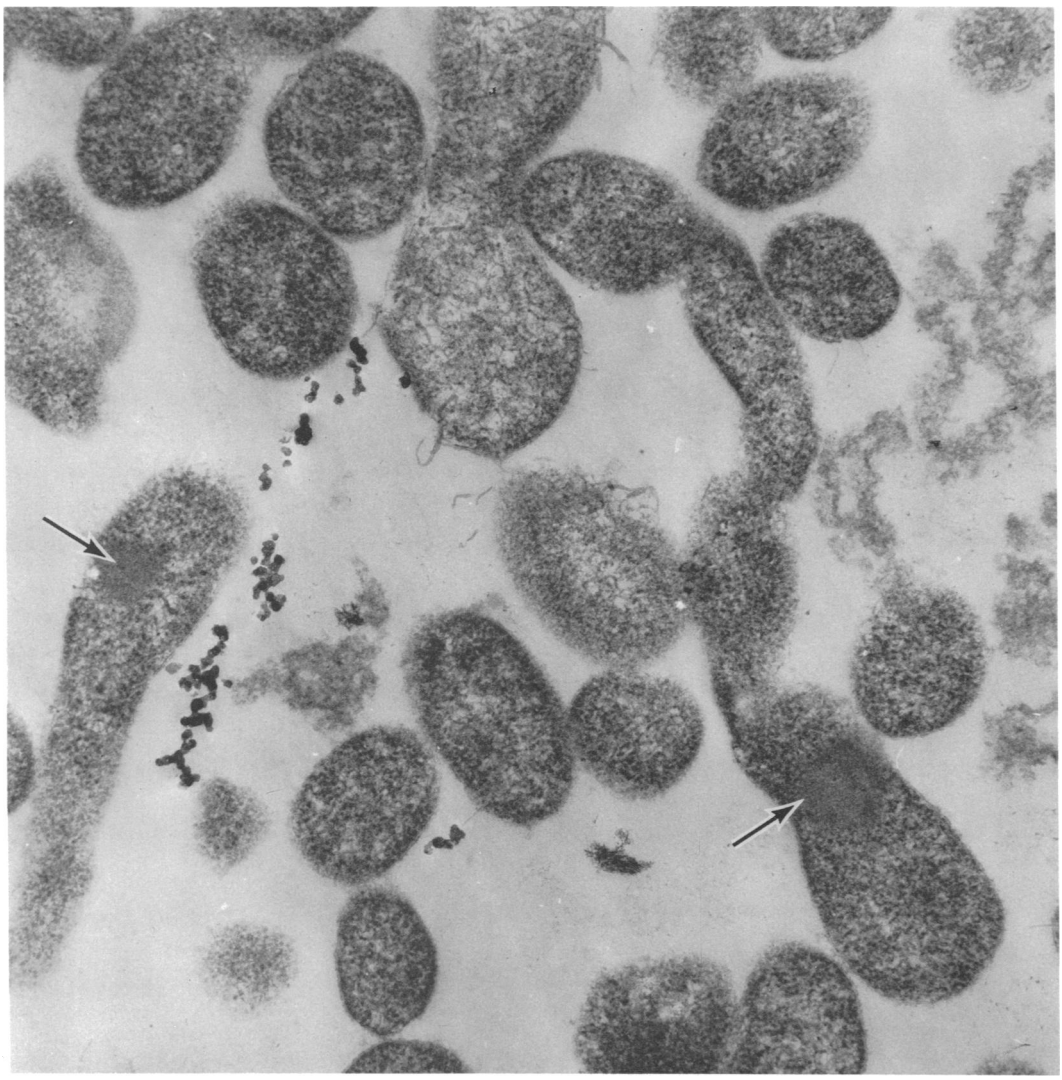

FIG. 3. *Section of A. laidlawii B cells (87). Large cytoplasmic granular particles can be seen in the filamentous cells (arrows).* ×*40,500.*

Cell Membrane

All electron microscopy studies that have been done confirm the absence of any membraneous organelle, except for the bounding cell membrane (see references above and 124). This, plus the absence of other observable surface envelope layers, has allowed the preparation of membranes for further study by gentle procedures (e.g., osmotic shock), and these fractions are relatively uncontaminated, i.e., they are of a single membrane type and have no adhering extracellular components.

Biochemical properties. The chemical composition of mycoplasma membranes is reported as 50 to 59% protein, 32 to 40% lipid, 0.5 to 2% carbohydrate, 2 to 5% RNA, and about 1% DNA (124, 127). The RNA and DNA are regarded as cytoplasmic contaminants and can be removed by washing and nuclease treatment (124, 126, 127). The carbohydrate component may be significantly underestimated, since Engelman and Morowitz (36) have shown that the analytical method used does not detect glucosamine and galactosamine, which are present in rather large amounts (about 5%) in *A. laidlawii* membranes. Morowitz and Terry (106) indicated that the hexosamine is in long-chain polysaccharides. The glycolipid and protein mem-

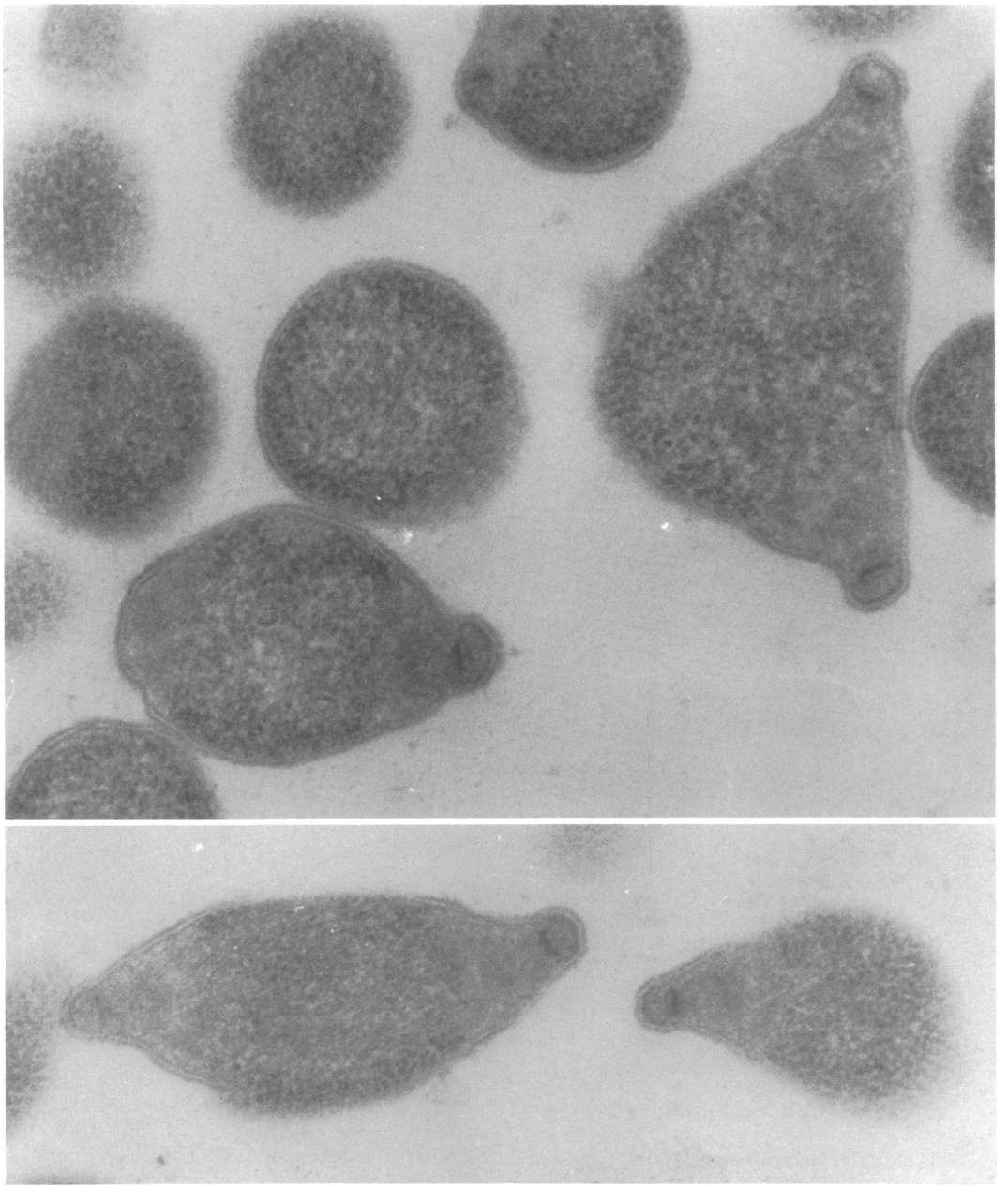

FIG. 4. *Sections of exponentially growing M. gallisepticum A5969 (105). Both one-bleb daughter cells and two-bleb predivision cells are seen. The subcellular organelles include the cell membrane, nuclear material, ribosomes, bleb, and infra-bleb region.* ×*81,000.*

brane components specify the mycoplasma antigenic properties (5, 65, 138, 139).

Polyacrylamide gel electrophoresis of the urea-phenol-acetic acid-solubilized membrane proteins reveals 20 to 30 bands (125, 131, 148, 171). Since not all of the proteins enter the gels, the actual number of membrane proteins may be larger. The band patterns tend to be similar for strains within a species and the species differences in gel patterns correspond to known serological and nucleic acid homology differences. The membrane amino acid compositions of the mycoplasmas that have been studied are summarized in Table 1. Cor-

TABLE 1. *Amino acid composition of membranes*

Amino acid	*M. gallisepticum*[a] (mole %)	*A. laidlawii* A[b] (mole %)	*A. laidlawii* B[c] (mole %)	*A. laidlawii* B[d] (mole %)	Erythrocytes[e] (mole %)	*B. megaterium*[f] (mole %)
Lysine	9.38	7.1	6.37	5.77	5.17	6.7
Histidine	1.41	1.4	1.46	1.32	2.31	1.6
Arginine	3.41	3.0	2.95	2.76	4.74	3.3
Aspartic acid	12.32	12.5	11.43	12.30	8.94	8.8
Threonine	6.21	7.5	6.77	7.90	5.75	5.7
Serine	6.70	6.4	6.40	7.44	7.20	5.5
Glutamic acid	10.34	9.4	8.23	9.59	13.34	7.2
Proline	4.42	3.9	3.60	4.29	4.84	5.0
Glycine	6.60	8.1	6.95	8.31	6.68	7.8
Alanine	7.48	8.1	8.23	9.62	7.71	7.0
Cysteine	0.30	trace	0.19	0.12	1.15	0.1
Valine	7.31	7.0	7.55	5.18	6.57	7.0
Methionine	1.83	2.3	2.33	2.15	1.86	2.3
Isoleucine	6.11	7.0	7.39	5.27	4.71	6.6
Leucine	8.60	9.5	9.79	8.96	11.50	8.6
Tyrosine	3.93	4.1	4.81	4.36	2.36	2.1
Phenylalanine	4.54	4.8	5.40	4.70	3.95	4.6
Tryptophan	—	2.3	—	—	1.43	—
Amide	—	6.4	—	—	—	3.3

[a] Cells grown on Tryptose medium (110).
[b] Cells grown on peptone-yeast extract medium (24).
[c] Cells grown on Tryptose medium (36).
[d] Cells grown on beef-heart infusion medium (106).
[e] Average of four preparations cited by Rosenberg and Guidotti (144).
[f] Data for *Bacillus megaterium* protoplast membranes (181).

responding data for erythrocyte and *Bacillus megaterium* (as a representative bacterium) membranes are included for comparison. The mycoplasma values are not appreciably different from the erythrocyte and bacterial data, but the latter do show some variation in acidic, basic, and uncharged residues, and the erythrocytes have a 4- to 10-fold higher cysteine content.

The biochemistry of the membrane lipids has been recently reviewed by Smith (165, 166) and Razin (126, 127) and hence only certain topics will be briefly summarized here. Most of the mycoplasmas seem to require fatty acids for growth (166); in addition, the species of the genus *Mycoplasma* require cholesterol, and several *Mycoplasma* strains also require glycerol (126, 127). The major membrane lipid constituents are phospholipids and glycolipids. In the *Mycoplasma* species, cholesterol makes up 12 to 30% of the total lipid (4). Although the *Acholeplasma* do not require cholesterol, if grown in a cholesterol-containing medium they incorporate it as 3 to 4% of their total lipid (4). *A. laidlawii* also contains four major carotenoid pigments (166) which comprise at most 1% of the lipid (126, 127).

In a study of nongrowing *A. laidlawii*, Kahane and Razin (66) showed that, at least in these cells, membrane lipid and protein syntheses are not synchronized. Unfortunately, because of the state of their cells, the data on turnover rates of proteins and lipids cannot be applied to actively growing cells.

When *A. laidlawii* is grown in media supplemented with various fatty acids, including non-biologically occurring compounds, the exogenous fatty acid is incorporated into the membrane and can comprise 60 to 80 mole per cent of the total fatty acids (81, 114). This allows the lipids to be varied, to examine the relationship of the lipid composition to membrane properties. These data indicate the range of fatty acids that can be incorporated and constitute a functional cell membrane, and also show the relative lack of specificity required for the membrane lipid components.

Smith (166) has reviewed the reports of the various biochemical activities which have been shown to be associated with the membrane: redox enzymes (including quinones and cytochromes in some species), adenosine triphosphatase, nucleases, cholesterol esterase, and several lipid synthetases. In addition, a membrane lysophospholipase (177) and peptidase (25) have been found. Rottem and Razin (147) found that the *A. laidlawii* adenosine triphosphatase hydrolyzes other nucleotide triphos-

phates and hence is probably a nonspecific membrane nucleotide triphosphatase (nucleotide monophosphates were not hydrolyzed). In addition, the triphosphatase was not K^+- or Na^+-activated and was not affected by ouabain. The pattern of membrane-bound versus soluble enzymes varies among the mycoplasmas (120, 121); e.g., while adenosine triphosphatase activity is localized in membrane subcellular fractions, $NADH_2$ oxidase activity has been found in the soluble fractions of the *Mycoplasma* examined but in the membrane fraction of *A. laidlawii*.

Several membrane transport processes have been observed in the mycoplasmas. Rottem and Razin (147) showed that K^+ and Na^+ transport in *A. laidlawii* are not coupled and are independent of the adenosine triphosphatase activity in the cell. Using the same species, Cho and Morowitz (23) found that K^+ transport is energy-dependent and, since it is inhibited by sulfhydryl blocking agents, is probably mediated by cysteine-containing proteins. Membrane amino acid transport systems, resembling microbial permease systems, have been demonstrated for methionine uptake by *M. hominis* and histidine uptake by *M. fermentans* (133). The methionine system was highly specific, but the histidine system was less specific and could be competitively inhibited by lysine and arginine. Two energy-dependent noninducible sugar transport systems have been found in *M. gallisepticum* (149), one for D-glucose and α-methyl-D-glucoside and one for D-mannose and D-fructose. An inducible α-glucosidase has also been demonstrated in *A. laidlawii* (158), but whether this activity or a coordinate permease are membrane-associated has not been shown yet.

A number of recent studies indicate that the mycoplasma cell membrane is also involved in determining the cells' antibiotic susceptibility. In a study of one *Acholeplasma* and six *Mycoplasma* species (39, 155), it has been shown that resistance to chloramphenicol, dihydrostreptomycin, and tetracycline appears to involve a reduced permeability to the antibiotics, rather than an alteration in a ribosomal protein. Also, in a study of *A. laidlawii* sensitivity to viral infections, Liss and Maniloff (79) found that cells selected for streptomycin or novobiocin resistance were also virus-resistant. These examples indicate the significance of the cell membrane in considerations of antibiotic susceptibility, but there is no reason to believe that there is a common mechanism for these diverse phenomena.

Biophysical properties. Electron microscopy studies show the mycoplasma to be bounded by a membraneous structure having a "unit" membrane appearance (20, 31, 127). The generally reported thickness of the membrane is 7.5 to 1.0 nm (126), but in fact the membranes studied seem to fall into two size ranges, 7 to 8 nm and 1.0 to 1.2 nm (20, 31). More accurate measurements are needed before it can be decided whether this represents a significant difference among the mycoplasmas. In addition, consideration must be given to the ionic conditions, in view of Bernstein-Ziv's observation (11) that the *M. gallisepticum* membrane thickness is increased in hypotonic solutions.

An interesting membrane arrangement has been described in *M. salivarium* (75) where many cells on the surface of colonies were surrounded by membrane lamellae, giving a myelin-like appearance. Similar observations have recently been made in virus-infected *A. laidlawii* cultures (J. Maniloff and A. Liss, *unpublished data*).

In freeze-etching studies of *A. laidlawii* cells (Fig. 5) and membranes (173, 175), the membrane is split along an internal plane exposing 7.5- to 12.5-nm particles. Since these are not removed by lipid solvents (J. Das and J. Maniloff, 1971, Biophys. Soc. Abstr., p. 291a), they probably represent proteins within the membrane. The outer and inner (cytoplasmic) membrane surfaces had a rough-textured appearance (173). Similar results have been observed for *M. gallisepticum* (Fig. 6), Caprine PPLO strain 14, and *M. arthritidis* (J. Maniloff and R. Steere, *unpublished data*).

It is extremely difficult to evaluate the observations of surface spikes in negatively stained preparations (26, 59, 60, 142). Most of these experiments involve the negative staining of unfixed (sometimes disrupted) cells and, hence, there is concern about the production of staining artifacts. On the other hand, there must be specific types of surface sites in the mycoplasmas in order to explain their interaction with mammalian cells (167). *M. gallisepticum*, for example, binds to mammalian cells by the bleb end of the mycoplasma cell (182, 183). Therefore the ends of this mycoplasma must have the binding site constituents. There have been, as yet, no data localizing the surface spikes on the bleb structures. Therefore there are two unknowns: the status of the spikes and the structure of the surface-binding sites.

The electrical properties of *M. gallisepticum* and *A. laidlawii* membranes have been measured (20, 154) and found to be similar to those

FIG. 5. *Micrograph of freeze-etched A. laidlawii B cells (90). The arrow shows the shadowing direction. The cells are filamentous, as seen in sections (Fig. 3). The fracture plane through the filamentous cell has (starting from the part of the cell at the lower left) travelled along the inside face of the membrane, through the cytoplasm, and through an internal membrane plane, exposing particles on a convex surface. ×81,000.*

FIG. 6. *Micrograph of freeze-etched M. gallisepticum A5969 (90). The arrow shows the shadowing direction. The cell morphology is the same as seen in sections (Fig. 4). The dividing two-bleb cell has been fractured along an internal membrane plane, showing particles on a convex surface.* ×81,000.

of other cell membranes. The cells have a negative surface charge of about -0.01 coul/m^2, which gives rise to a very small surface conductivity (about 0.003 mho/m), and have a membrane capacitance of about 0.9 μf/cm^2.

Mycoplasmas are much more osmotically stable than bacterial protoplasts (127). It is interesting to note that, using cells grown so that their membranes are enriched with regard to specific fatty acids (*discussed above*), both the rate of glycerol permeability (82) and the osmotic fragility (137, 146) follow the order linoleic (18:2) > oleic (18:1 cis) > eladic (18:1 trans). This would argue against leakiness stabilizing the cell, since then high leakiness should correspond to low osmotic fragility. All of these studies have involved *A. laidlawii* which is much more osmotically fragile than other *Acholeplasma* species (J. Maniloff and J. Robbins, *unpublished data*) and the *Mycoplasma* (123).

Lenard and Singer (78) reported that the optical rotatory dispersion spectrum of *A. laidlawii* membranes showed a minimum at 235

nm which indicated the presence of some α-helical structure in the membrane proteins. Similar data were obtained by Choules and Bjorklund (24) who also observed spectral patterns, characteristic of α-structure, in circular dichroism and infrared studies. Choules and Bjorklund have summarized the *A. laidlawii* membrane protein conformation, based on the three physical chemical studies, as 23 to 31% α(helix), 30 to 57% β(pleated sheet), and 13 to 45% random coil. The α content is in agreement with other membranes (78), but no significant amount of β structure has been reported in other membranes (179). Since all the different membranes have the same spectral patterns, the structure differences arise from the interpretations of the small band shifts that occur between the membranes and the polypeptides of known conformation that are used as standards. The various problems that might give rise to band shifts unrelated to the protein structure (such as turbidity artifacts and spectra perturbation by other chromophores) have been discussed by Choules and Bjorklund (24) and Wallach and Gordon (179). Except for the quarter to third of the protein that is α-helix, there is no agreement on the configuration of the rest of the membrane protein structure.

Lipids in the mycoplasma membrane undergo a melting type of thermal phase transition from highly ordered fatty acid chains to a liquid paraffin-line configuration. The existence of this thermal phase transition in *A. laidlawii* cells, membranes, and lipids was first demonstrated by Steim et al. (168; see also 95, 140) using differential thermal calorimetry. The phase transition has also been measured by electron paramagnetic resonance (175) and X-ray diffraction studies (34, 35). From these studies, it has been concluded that most of the membrane lipid is in a bilayer arrangement. Since the transition temperature is a function of the lipid composition, both can be varied by supplementing the medium with a specific lipid (168) or by changing the growth temperature (95). Melchior et al. (95) concluded that the cells try to regulate their lipid composition so that the transition temperature is slightly below the growth temperature—i.e., the cells attempt to grow on the disorder side of the order-disorder phase transition. Rottem et al. (152), using electron paramagnetic resonance studies, also noted an increase in membrane "fluidity" when the cells were grown at lower temperatures—i.e., when "fluidity" is measured at 37 C, cells grown at 37 C will be just past their lipid transition temperature, but cells grown at 15 C will be well beyond their transition temperature and hence will show more "fluidity."

The membrane electron paramagnetic resonance studies also show an increase in the freedom of motion of the probe molecule as it is moved away from the polar end of the fatty acids (152), which would be expected for a lipid bilayer structure. Tourtellotte, Branton, and Keith (175) found a decrease in the probe mobility in membranes as compared with lipid extracts, an indication that membrane lipid mobility may be influenced by proteins. The protein-lipid interaction is also shown by the inaccessibility of some of the membrane protein to proteolytic attack (106), to binding a nuclear magnetic resonance probe (96), and to binding a fluorescent probe (97).

Reaggregation studies. Razin, Morowitz, and Terry (134) showed that *A. laidlawii* membrane could be solubilized by detergents and that, if the detergent was removed, membrane reaggregates were obtained which had most of the original reduced nicotinamide adenine dinucleotide oxidase activity, a "unit" membrane appearance, and consisted of lipid and protein. Similar results have been found for membranes of *M. gallisepticum* (135, 151) and *M. mycoides* (135). An extensive review of the solubilization and reconstitution studies is in press (128).

Most data have been accumulated for the interaction of *A. laidlawii* membrane and sodium dodecyl sulfate (SDS), and these have been reviewed by Engelman (33). The SDS solubilizes the native membrane to form separate protein-SDS and lipid-SDS complexes, and not lipoprotein membrane subunits, as had been thought earlier. Auborn, Eyring, and Choules (6) have recently shown that the solubilization kinetics are complex, half-order with respect to both membranes and SDS micells and second-order with respect to SDS monomers. The soluble components have molecular weights in the range of 20,000 to 49,000. Kahane and Razin (67) have shown that, if too low SDS concentrations (10 mM) are used, the native membrane is not completely solubilized and this partially disaggregated membrane can be subsequently recovered and erroneously thought to be reaggregated membrane. When the SDS is removed by dialysis, a membrane reaggregate is formed, showing that no preexisting structure is needed for this assembly process. The lipid-protein ratio in the reaggregate is a function of the Mg^{2+} concentration during dialysis (33). Kahane and Razin (67) have shown that, at 20 mM Mg^{2+}, the reaggre-

gate lipid-protein ratio is the same as in the native membrane.

A variety of techniques have been used to examine the reaggregated membrane structure for comparison with the native membrane. Electron paramagnetic resonance studies have shown that the "fluidity" of the reaggregate was similar to that of the native membrane (152). X-ray diffraction patterns of the reaggregate resembled that of the native membrane, and a thermal phase transition was observed (96). These data indicate that a lipid bilayer structure has been formed in the reaggregated membrane similar to that in the native membrane. However, since the 0.415-nm lipid X-ray reflection is about twice as broad for the reaggregate as compared with the native membrane, the ordered region giving rise to the reflection must be smaller in the reaggregate than in the native membrane. Freeze-etching examination of the reaggregated membrane has revealed the absence of particles on the fracture face (173). These particles appear to be proteins (*discussed above*) and indicate a basic structural difference between the reaggregated and native membranes. Confirming this, the nuclear magnetic resonance (96) and fluorescence probes (97) show many protein-binding sites exposed in the reaggregate that were not available in the native membrane. Hence, in the reaggregate the membrane proteins are incorrectly reassembled into the structure; either they have not been able to reassume the correct conformation or they have not been able to interact with the lipids correctly so as to make their binding sites inaccessible to the probes.

The lack of specificity in the reassembly process has been demonstrated by: the production of hybrid membranes, reaggregates containing lipids and proteins from both *A. laidlawii* and *M. gallisepticum* (130), reaggregates containing *M. pneumoniae* glycolipids and *A. laidlawii* proteins (27, 136, 139), and reaggregates of *A. laidlawii* membranes which have incorporated extraneous proteins such as penicillinase and bovine serum albumin (150).

The lack of lipid specificity (*discussed above*) as far as membrane structure is concerned should be noted and may reflect the significance of lipid as a common denominator in establishing the membrane structure. The lipid composition sets the membrane "fluidity" and the lipid bilayer structure seems to reassemble spontaneously into a thermodynamically stable structure. By contrast, membrane protein specificity is higher (e.g., without the appropriate permease, substrates cannot be utilized), and protein reassembly into membranes seems to be a more complicated architectural problem.

Extracellular Aspects

Extracellular structures. In considering extracellular structures, it seems reasonable to question the possible existence of surface envelopes external to the cell membrane and of surface appendages. The problem of the latter structure arises because of the motility of some of the mycoplasmas (15, 18; W. Bredt, 1972, Med. Mikrobiol. Immunol. **157**:169). However, since no surface appendages have been observed by electron microscopy the motility is referred to as gliding.

Since the mycoplasmas are defined as cells bounded only by a cell membrane, the question of other surface envelopes is a serious one. With one possible exception (*to be discussed*), electron microscopy shows no external surface envelopes, and electrical measurements of the surface conductivity of unfixed cells confirms the absence of surface structures other than the membrane (20). The possible exception is the mycoplasma prototype strain *Mycoplasma mycoides*. In their biochemical studies of the galactan produced by *M. mycoides*, Plackett, Buttery, and Cottew (117) suggested that the galactan might form a capsular or slime-layer substance, although they could not see any such structure by electron microscopy. However, Gourlay and Thrower (49) showed that growing *M. mycoides* cells were within a mucinous homogenous matrix, which if precipitated by specific antibody was frequently seen around the cells as a capsular envelope. They also mentioned the possibility that the virulence of particular *M. mycoides* strains may be related to the amount of capsule. In a study of Caprine PPLO strain 14 (a *M. mycoides*), Maniloff (Bacteriol. Proc., p. 78, 1968) showed that, in older, dying cultures, cells were frequently surrounded by an amorphous surface envelope—compare Fig. 1 of exponentially growing cells with Fig. 7 of the same culture in early death phase. Future considerations of mycoplasma taxonomy should include the significance of *M. mycoides* capsular material.

The osmotic stability of the mycoplasmas suggests that consideration should be given to possible special properties or components of the surface envelope of these organisms. The absence of a bacterial type cell wall has been inferred from penicillin resistance and the lack of wall-specific biochemical components (166). A possible exception is the presence of poly-

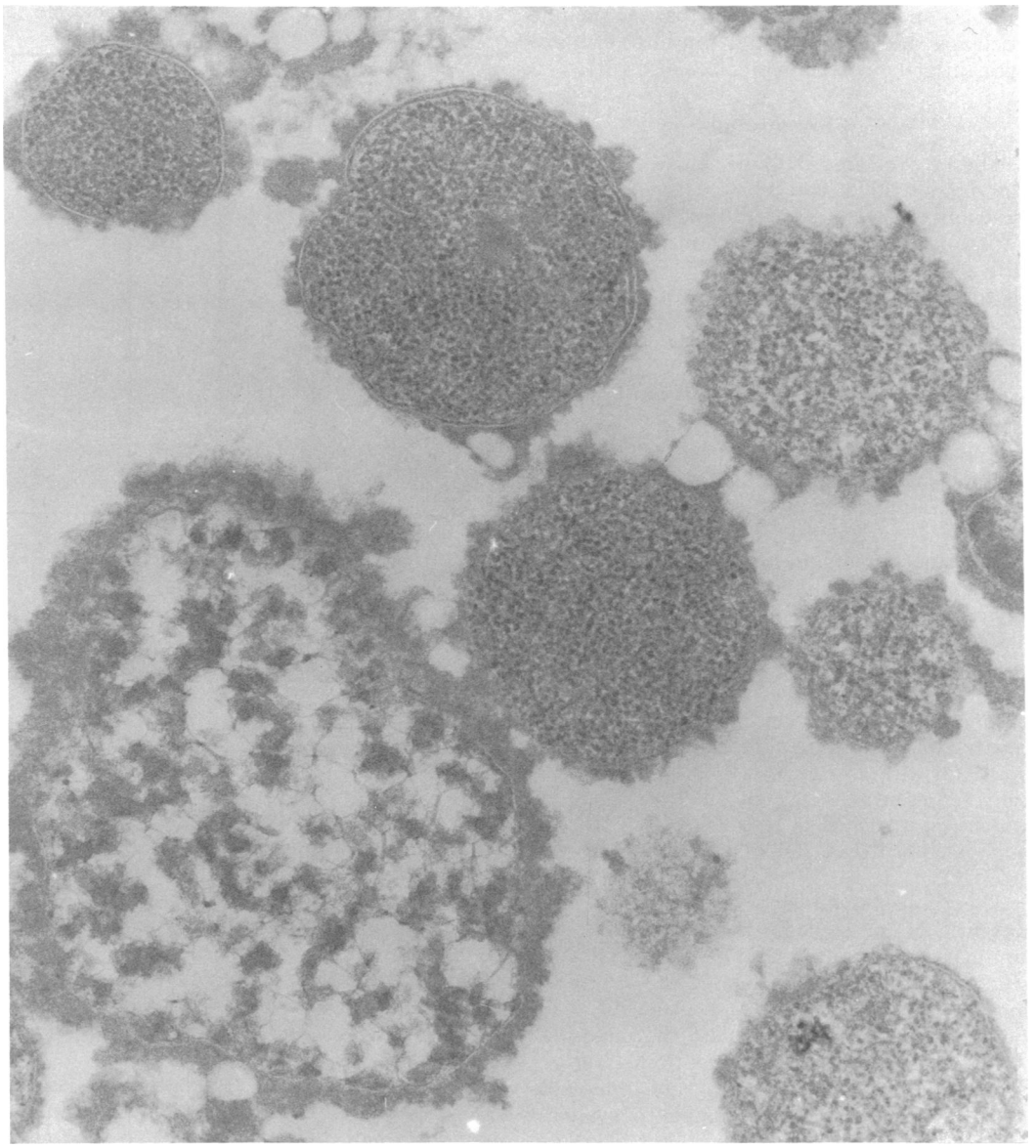

FIG. 7. *Section of late-stationary-phase Caprine PPLO strain 14 cells. Cells are seen either swollen or densely packed with particles. An extracellular material surrounds these cells. Compare with Fig. 1, which is of the same culture sampled in log phase.* ×*60,750.*

hexosamine, which is about 5% of the dry weight of the *A. laidlawii* membrane (46).

Extracellular products. Smith (166) recently reviewed the mycoplasma extracellular products: neurotoxins, hemolysins, and exoenzymes. However, the cytological mechanism for the elaboration of these products is not known. Thomas (172) has reviewed the data on the two known mycoplasma neurotoxins, those of *M. neurolyticum* (an exotoxin) and *M. gallisepticum*. The *M. neurolyticum* toxin is a thermolabile protein. Thomas (172) postulated that the pathogenicity of *M. arthritidis* and *M. pulmonis* may also be due to toxin production. Some mycoplasmas have alpha or beta hemolytic activity, depending on the culture conditions and the erythrocyte species being used. Hemolysis seems to be due to peroxide, but it has not been possible to demonstrate a relationship between peroxides and pathogenicity. The exoenzymes have been found to be responsible for some of the effects of myco-

plasmas on animal cells and appear to include nucleases and enzymes that can alter erythrocyte antigenic determinants (see also 167).

Chromosome

The mycoplasma DNA comprises 4 to 7% of the dry weight of the cell (132); the variation probably reflects species differences and also variations in the DNA per cell at different culture phases, as discussed above. The adenine-thymine and guanine-cytosine (GC) ratios are about 1.0, in agreement with the Watson-Crick DNA double-helix structure (166). The mycoplasma genetic information is in a single chromosome, which is a circular DNA molecule (102).

Genome structure. The base ratios of the mycoplasmas span the range of 23 to 41% GC (113). Within this range the *Acholeplasma* species are 30 to 36% GC, the *Mycoplasma* species (except for *M. pneumoniae*) are 23 to 36% GC, and *M. pneumoniae* is 39 to 41% GC. The seven T strains that have been studied are all about 28% GC (7). For comparison, the GC content of bacteria ranges from 25 to 75%.

The variation in base composition within a chromosome was shown by Ryan and Morowitz (153) who were able, using *Mycoplasma* sp. (Kid) with 25% GC, to isolate a DNA fraction representing 1.4% of the total genome which was enriched in ribosomal RNA (rRNA) and transfer RNA (tRNA) cistrons and was 46% GC.

The published values of the DNA molecular weights of mycoplasma chromosomes are summarized in Table 2. The most extensive studies were done by Bak et al. (8), using measurements of the DNA renaturation reaction rate constants to calculate the molecular weights. The other values come from a variety of techniques and, unfortunately, adequate data are not generally available to evaluate some of the reported numbers. For example, the molecular weight of *M. gallisepticum* measured by Riggs (143) is frequently quoted to be 10×10^8 daltons. However, the 10×10^8 daltons measured (by radioautography, the Kleinschmidt technique and chemical analysis) is the DNA per growing cell and, to correct for exponential growth, it must be multiplied by 0.69 to obtain the DNA per newly formed cell. Therefore, 6.9×10^8 daltons is the DNA content of a daughter *M. gallisepticum* cell, and the true chromosome size (depending on ploidy and the temporal relationship between cell division and DNA replication) will be less than or equal to this value. The sizes of the *Mycoplasma* chromosomes are in the range of 4.2 to 6.9×10^8 daltons, whereas those of the *Acholeplasma* chromosomes are 7.6 to 11.0×10^8 daltons. As noted by Bak et al. (8), the *Acholeplasma* genomes are about twice as large as those of the *Mycoplasma*. The smallest reported bacterial genome is 8×10^8 daltons and values range as high as several billion daltons (100).

TABLE 2. *Molecular weights of Mycoplasma chromosomes* ($\times 10^8$)

Organism	By renaturation studies[a]	By other methods
M. agalactiae bovis		5.9[b], 6.9[c]
M. arthritidis	4.4	4.4[c], 5.1[d]
M. fermentans	4.8	
M. gallisepticum	4.9	6.9[e]
M. hominis	4.5	
M. meleagridis	4.2[f]	
M. orale	4.7	
M. pneumoniae	4.8	
M. salivarium	4.7	
Mycoplasma sp. (Kid)		6.8[g]
T strain 27	4.7	
T strain 58	4.4	
Acholeplasma laidlawii A	11.0	7.8[h]
A. laidlawii B	10.0	7.6[i], 9.9[j]
A. granularum	9.5	

[a] Bak et al. (8).
[b] By Kleinschmidt technique (108).
[c] By Kleinschmidt technique (102).
[d] By Kleinschmidt technique (14).
[e] Calculated from data of Riggs (143).
[f] *M. meleagridis* data from Allen (1).
[g] By Kleinschmidt technique (153).
[h] By radioautography (143).
[i] By radioautography (102).
[j] By sucrose gradient centrifugation (32).

The early reports of 10^8 daltons or less of DNA per cell (103, 110), although now considered too small, did stimulate interest in cells with only a limited amount of genetic information. The mycoplasma genomes, while shown to be larger, still only code for about 600 to 1,000 cistrons per cell (102, 108), and this limits the ultimate complexity. This was demonstrated by the experiments of Ryan and Morowitz (153), using *Mycoplasma* sp. (Kid), which showed the genome of this cell to have only one cistron each for the 16*S* and 23*S* rRNA and only about 44 cistrons for tRNA molecules.

Those chromosomes which have been spread out and examined by radioautography or the

Kleinschmidt technique have shown a circular topology; they are *M. agalactiae bovis* (108), *M. arthritidis* H39 (14), *M. gallisepticum* (143), and *Mycoplasma* sp. (Kid) (153). Double Y's or replication forks with two arms of equal length were observed in the chromosomes of *M. agalactiae bovis* (102), *M. arthritidis* H39 (14), *M. gallisepticum* (143), and *A. laidlawii* (102). With respect to topology, chromosone replication in the mycoplasmas involves DNA circles similar to those shown for *Escherichia coli* (19).

Replication. Smith (160) has shown that DNA replication in *A. laidlawii* is semiconservative and proceeds unidirectionally from at most a few growing point regions. The analysis of this datum and of the experiments on thymineless death in these organisms (162) suggests that DNA replication continues throughout the cells' generation time. A small account of nonconservative repair replication has been found in normally growing *A. laidlawii* (162, 163) and is believed to occur in *A. laidlawii* after thymine starvation (162); a similar type of replication has been demonstrated in irradiated cells.

The DNA growing point region is believed to be a membrane-associated complex in *A. laidlawii* and *M. gallisepticum*. Using osmotically shocked *A. laidlawii* lysates on sucrose gradients (159, 161), a subcellular fraction was isolated which contained a twofold enrichment of pulse-labeled (i.e., newly synthesized) DNA. Since the DNA could be released by Pronase or detergents, it was suggested that the growing point is localized at the cell membrane. In *M. gallisepticum* preparations (122), lysed by freeze-thaw cycles, a low-speed subcellular fraction has been isolated which has a four- to fivefold enrichment and contains 15% of the total cellular DNA and 80% of the pulse-labeled DNA. These experiments have shown that this is the DNA growing region and that the subcellular fraction is enriched in the *M. gallisepticum* terminal structures (see Fig. 4), suggesting that this structure is the DNA replication complex site.

Ultraviolet and X-ray irradiation experiments of mycoplasma cultures show multihit or multitarget kinetics; i.e., the survival curves show an initial shoulder, followed by exponential inactivation (30, 38, 43, 44, 104). The multiple-hit character of the inactivation curves may be due to cell clumping or polyploidy. In *A. laidlawii* it has been shown that both the ultraviolet sensitivity and the number of lethal events to kill a cell vary with the cell's growth phase (30). The multiorder survival kinetics can be altered by the experimental conditions, giving rise to first-order kinetics. Furness et al. (44) noted the inconsistency in titer depending on whether their irradiated cells were grown in broth or on plates; hence the counting method can affect the type of survival curve obtained.

The ultraviolet repair replication studies reported thus far have all examined *A. laidlawii*. Folsome (38), Smith and Hanawalt (163), and Das et al. (30) have all shown that the cells are capable of photoreversal of some of the DNA damage. Das et al. (30) found that this is maximal in middle to late exponential-phase cells. Smith and Hanawalt (163) and Das et al. (30) also demonstrated that the cells have a dark-repair capability by the excision-repair mechanism. Smith and Hanawalt (163) estimated that the size of the region excised, the region around the radiation-induced pyrimidine dimer, is about 150 to 600 nucleotides. Das et al. (30) found that the single-strand DNA regions, between the breaks, were about $\frac{1}{23}$ of the total strand length, and 60% of the breaks were repaired by 2 hr after irradiation. Hence, at least this one mycoplasma has the genetic information and enzymatic capabilities for both dark and light repair of its DNA.

The multiorder ultraviolet survival curves of *A. laidlawii* have suggested that these cells might be multinucleate (30). A similar conclusion was reached for *M. gallisepticum*, based on the difference in rates of DNA synthesis in different phase cultures (*discussed above*). However, in *M. arthritidis* H39, Bode and Morowitz (14) found the DNA per cell to be 1.2 to 1.5 times the genome size, in agreement with the expectation that an exponentially growing cell with only one copy of its chromosome should contain about 1.4 times its genome. From so few cases, no generalization can be made, but it may be expected that small coccoid mycoplasmas, like *M. arthritidis* H39, will have only one genome per cell, and the larger mycoplasmas, especially the filamentous ones, will be multinucleate.

Genetics. The stability of mycoplasma mutants has not been carefully examined, but Steinberg et al. (169) noted leakiness in their temperature-sensitive mutants, and Liss and Maniloff (*unpublished data*) have found that naladixic acid-resistant *A. laidlawii* reverts to sensitivity about a month after selection; the selection may, in fact, be for a slower-growth-rate cell, which will be naturally selected against by a back mutation. It must be concluded that there are few stable mycoplasma

mutants for which the mechanism of the mutational alteration is known.

Mycoplasma mutation frequencies for antibiotic resistance and for carbohydrate utilization have been measured (86). Mutants (stable variants) have been selected for antibiotic resistance (76), temperature sensitivity using the mutagens nitrosoguanidine (169, 170), aminopurine or bromodeoxyuridine (C. E. Folsome and J. Folsome, Bacteriol. Proc., p. 30, 1966), and constitutivity for the inductible glucosidase enzyme using the mutagen nitrosoguanidine (158). The antibiotic-resistant mutants that have been studied, which involved antibiotics known to interfere with protein synthesis, turned out to be resistant due to a reduced antibiotic uptake rather than a specific ribosomal protein alteration, as had been expected (39, 155).

Unsuccessful attempts at DNA transformation of *A. laidlawii* have been reported by Folsome (38) and Smith (166). Folsome (38) reported that the cells were able to bind DNA so that it was not accessible to deoxyribonuclease attack, but no recombinants could be found. The data are completely negative for both DNA-mediated (transformation) and cell-mediated (conjugation) transfer of genetic information.

The discovery of mycoplasma viruses (47) opens the possiblity of other mechanisms of gene transfer, in particular transduction and transfection. Most of the known mycoplasma viruses are bullet-shaped particles (47, 50, 79) and are believed to contain single-stranded DNA (A. Liss and J. Maniloff, *unpublished data*); hence, transduction by these viruses may be quite rare. A better possibility for transduction may be the few enveloped spherical mycoplasma viruses (48). Experiments have shown that transfection can occur, i.e., *A. laidlawii* can be infected with mycoplasma virus DNA, undergo a lytic infection, and produce mature viruses (A. Liss and J. Maniloff, *unpublished data*).

Ribosomes and RNA

RNA represents 8 to 17% of the mycoplasma cell mass (132). For *M. gallisepticum*, the RNA-DNA ratio is 4.5 for exponentially growing cells (73) and 2.0 for cells in early stationary phase (110). The RNA species are 22*S*, 16*S*, and 5*S* rRNA, 4*S* tRNA, and unstable messenger RNA (mRNA). The RNA is involved with protein synthesis, which Tourtellotte (174) has shown follows transcription and translation mechanisms similar to those of other procaryotes. Kirk and Morowitz (73) found that about 86% of the RNA is present as rRNA and the rest as soluble RNA. Kirk (72) estimated that mRNA represents about 4.5% of the total RNA.

Ribosomes and rRNA. Ribosomes are seen as cytoplasmic granules in electron micrographs of the mycoplasmas. The 70*S* particle size is 1.7 nm, determined from the optical analysis of *M. gallisepticum* micrographs (89). The ribosome composition in *M. hominis* is 61% RNA and 39% protein (61), and in *M. gallisepticum* is 55% RNA and 45% protein (73).

The so-called "70*S* particle" in *M. hominis* has a sedimentation coefficient (corrected to standard conditions) of 71*S* and dissociates into 53*S* and 33*S* subunits (61), and in *M. gallisepticum* has a corrected value of 74*S* and dissociates into 56*S* and 36*S* subunits (73). Johnson and Horowitz (61) have reported that *M. hominis* 70*S* ribosomes require higher Mg^{2+} concentrations for stability than those of *E. coli*. Polysomes with corrected sedimentation coefficients of 110*S* and 135*S* have been reported in *M. gallisepticum* (73). Figure 8 shows a polysome distribution from *M. gallisepticum* with polysomes of uncorrected sedimentation coefficients at 98*S* (corresponding to the corrected 110*S* particle), 122*S* (the 135*S* particle), 143*S*, 163*S*, and 182*S* (J. Maniloff, *unpublished data*).

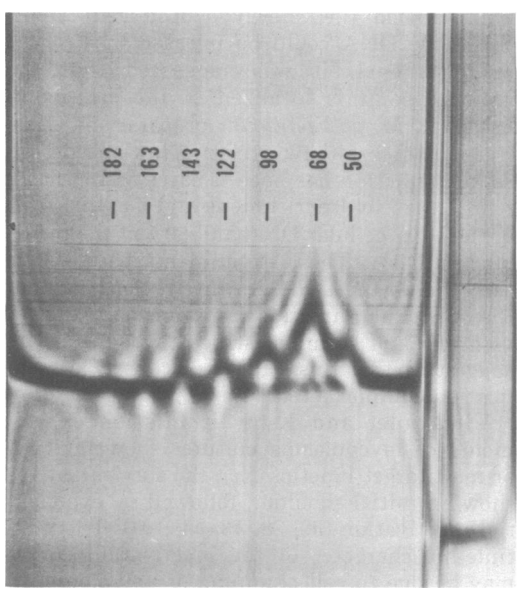

FIG. 8. *Schlieren pattern of polyribosome distribution of M. gallisepticum A5969. The numbers are the uncorrected sedimentation coefficients of each peak. The cells were broken in a French pressure cell (J. Maniloff, unpublished data).*

Johnson and Horowitz (61) examined *M. hominis* ribosomal proteins by polyacrylamide disc-gel electrophoresis. They observed 18 protein bands for the 30*S* subunit, as compared to 15 bands for *E. coli* 30*S* particles, and 21 bands for the 50*S* subunit as compared to 20 bands for *E. coli* 50*S* particles. Hence, mycoplasma ribosomes are as complex as eubacterial ribosomes both with regard to the numbers of proteins and RNA species. Johnson and Horowitz (61) also noted that about half the proteins from both *M. hominis* and *E. coli* showed similar electrophoresis mobilities and half were different.

Kirk and Morowitz (73) found two rRNA species in *M. gallisepticum* ribosomes, at 16*S* and 22*S*. The first corresponds to *E. coli* 16*S* rRNA (from the 30*S* subunit), but the second definitely sediments slower than the *E. coli* 23*S* rRNA (from the 50*S* subunit). Similar results are cited by Tourtellotte (174) for *M. pneumoniae* which has rRNA at 16*S* and slightly lower than 23*S*. In *M. hominis*, Johnson and Horowitz (61) reported the 16*S* and 22*S* rRNA, but also were able to isolate the 5*S* rRNA in the ratio of one 5*S* molecule for each 16*S* and 22*S* pair, so mycoplasma ribosomes have the same three rRNA species as the eubacteria. The difference between the mycoplasma 22*S* rRNA and the bacterial 23*S* rRNA is not due to nuclease action during preparation (61) so must reflect either slight size or conformational differences in the two RNA species.

Kirk and Morowitz (73) reported that 63% of the *M. gallisepticum* rRNA was 22*S* RNA. This is in agreement with the observation that sedimentation patterns of *M. gallisepticum* show more 50*S* than 30*S* particles (73; see Fig. 8). The sedimentation pattern of *M. hominis* rRNA also shows more 22*S* RNA than 16*S* RNA (61). The reason why these amounts are unequal is not known.

Table 3 summarizes the reported nucleoside compositions of mycoplasma ribosomes. Data on *E. coli* ribosomes and on the DNA compositions have been included for comparison. The mycoplasma rRNA compositions are all similar and not too different from that of *E. coli;* the difference between the mycoplasma and *E. coli* data is seen to be the small decrease in guanosine and small increases in adenosine and uridine, making the mycoplasma GC lower than that of *E. coli*. The *M. hominis* and *E. coli* 16*S* and 22 to 23*S* RNA species are also similar to each other and to the bulk rRNA composition. Kirk and Morowitz (73) cite similar results for *M. gallisepticum* 16*S* and 22*S* rRNA. However, large differences in both aspects are seen for the 5*S* RNA species. Although earlier workers were unable to find minor bases (54), Johnson and Horowitz (61) also found that *M. hominis* 16*S* and 22*S* rRNA contained the modified nucleosides pseudouridine, dihydrouridine, ribosylthymidine, 3-methyluridine, 5-methylcytidine, inosine, 1-methyladenosine, 6-*N*-methyladenosine, 6,6-*N*-dimethyladenosine, 2-*N*-methylguanosine, and 7-methylguanosine. Hence, at least *M. hominis* rRNA does contain a number of modified nucleosides and, although they are not quantitatively as large as in *E. coli*, they are significant. In 16*S* rRNA, 1.02 mole% of the *M. hominis* bases are modified and 1.85 mole% of those in *E. coli;* in 22 to 23*S* rRNA, 0.58 mole% are modified in *M. hominis* and 1.12 mole% in *E. coli*.

Thre is a significant difference between the percent GC of the mycoplasma DNA and the percent GC of the rRNA (Table 3). This indicates the heterogeneity in percent GC of different cistrons and, since the mycoplasma rRNA GC is close to that of most procaryotes, probably reflects the evolutionary conservation of the rRNA base sequences. Ryan and Morowitz (153) showed that, although *Mycoplasma* sp. (Kid) DNA is 25% GC, a DNA fraction containing the rRNA cistrons was 46% GC, close to the rRNA 48% GC, and they were able to use the per cent GC heterogeneity in *Mycoplasma* sp. (Kid) to partially purify the rRNA cistrons. They were able to show that the *Mycoplasma* sp. (Kid) genome contains only one cistron each for its 16*S* and 22*S* rRNA. This compares with *E. coli* which has five cistrons each for 16*S* and 23*S* rRNA.

Ribosome superstructures have been reported in the form of helices in *M. gallisepticum* (92) and as tetrads in T strains (F. T. Black, 1972, Med. Mikrobiol. Immunol. **157:** 170). The helices have been shown to arise during preparation (93) and are not polysome condensations (88). They have been shown to be self-assembly structures of 70*S* ribosomes, with 10 particles per 3 turns as the repeat, and there are indications that the helices are stabilized by interactions between 50*S* particles (88, 89).

Mycoplasma protein synthesis, both in vivo and in vitro, is sensitive to those antibiotics known to be inhibitory through their action on 70*S* ribosomes: chloramphenicol, puromycin, erythromycin, and tetracycline (39, 174, 176). Fraterrigo and Perlman (39) have shown that ribosomes from a tetracycline-resistant *A. laidlawii* were antibiotic-sensitive, indicating that

TABLE 3. *Nucleoside composition of Mycoplasma rRNA*

RNA source	Nucleosides (mole %)					DNA (%GC)[a]
	G	C	A	U	G + C	
M. hominis ribosomes[b]	27	20	29	25	46	27
M. mycoides ribosomes[c]	26	20	30	25	45	25
Mycoplasma sp. (Kid) ribosomes[d]	27	21	28	24	48	25
M. gallisepticum ribosomes[e]	25	18	29	26	43	35
Escherichia coli ribosomes[b]	32	22	26	21	54	50
M. hominis 5*S* rRNA[b]	22	21	29	28	43	—
M. hominis 16*S* rRNA[b]	25	19	30	25	45	—
M. hominis 22*S* rRNA[b]	26	20	29	24	45	—
E. coli 5*S* rRNA[b]	34	30	19	17	64	—
E. coli 16*S* rRNA[b]	29	22	27	21	51	—
E coli 22*S* rRNA[b]	29	24	26	21	52	—

[a] Neimark (113).
[b] Johnson and Horowitz (61).
[c] Walker (178).
[d] Ryan and Morowitz (153).
[e] Kirk and Morowitz (73).

the mutation has not been in a ribosome protein gene. Maniloff (88) was able to show that chloramphenicol and lincomycin, which bind to the 50*S* part of the bacterial ribosomes, do not allow ribosome helices to form in *M. gallisepticum*, indicating that these antibiotics block the interaction sites that stabilize the helix. Conversely, cycloheximide, which blocks eucaryotic protein synthesis involving 80*S* ribosomes, has no effect on mycoplasmas (174, 176).

tRNA. Mycoplasma tRNA has been shown to sediment at 4*S*, along with *E. coli* tRNA (55, 61), and to have a thermal denaturation curve similar to *E. coli* tRNA, indicating similar secondary structures (55).

Some *M. hominis* tRNA species (61) and *Mycoplasma* sp. (Kid) tRNA species (55) can be charged by some *E. coli* aminoacyl-tRNA synthetases, and *Mycoplasma* sp. (Kid) tRNA can also substitute for *E. coli* tRNA in in vitro cell-free protein synthesis. Redundancy has been observed in the tRNA isoaccepting species: *A. laidlawii* was found to have only a single tRNA each for tyrosine, phenylalanine, alanine, glycine, and cysteine; two tRNA species for arginine, methionine, and isoleucine; and three tRNA species for valine, serine, and leucine (37, 55). *Mycoplasma* sp. (Kid) has three isoleucine tRNA species (62). The *Mycoplasma* sp. (Kid) tRNA redundancy is interesting in view of the data of Ryan and Morowitz (153) showing that these cells only have about 44 tRNA cistrons, three of which are for Ile-tRNA species and two for Met-tRNA species.

One of the two Met-tRNA species has been found to fMet-tRNA in *M. gallisepticum* (55), *Mycoplasma* sp. (Kid) (55), *A. laidlawii* A (37), and *A. laidlawii* B (55). This suggests that, like other procaryotes, the mycoplasmas initiate their peptide chains with formylmethionine.

Studies have been carried out on the base compositions of bulk tRNA of several mycoplasmas and isolated *Mycoplasma* sp. (Kid) Ile-tRNA (Table 4). In addition to these, *M. gallisepticum* tRNA has been found to contain isopentenyladenosine (55), and *M. mycoides* tRNA contains pseudouridine, 4-thiouridine, ribothymidine, and dihydrouridine (178). Although these studies are still in a relatively early stage, it is clear that mycoplasma tRNA species contain a variety of minor bases. The absence of ribothymidine in two *Mycoplasma* species (Table 4) indicates that these tRNA species may differ significantly from eubacterial tRNA. The mycoplasma tRNA percent GC is (Table 4): 54% for *A. laidlawii*, 56% for *M. hominis*, 54% for *Mycoplasma* sp. (Kid), 50% for *Mycoplasma* sp. (Kid) Ile-tRNA, 55% for *M. gallisepticum* (73), and 61% for *E. coli*. These values are quite high compared with the DNA percent GC of these mycoplasmas (Table 3: 25 to 35%). The tRNA base ratio, as well as the rRNA base ratio, seems to have been conserved during the evolution of these organisms.

mRNA. Using actinomycin D, which inhibits RNA synthesis, Tourtelotte (174) was able to follow mRNA decay in seven mycoplasmas by the decay in protein synthesis. There was a variation in the rate of cessation of protein

TABLE 4. *Nucleoside composition of Mycoplasma tRNA*

Nucleoside	Nucleosides per tRNA molecule				
	Acholeplasma laidlawii[a]	*M. hominis*[b]	*Mycoplasma* sp. (Kid)[c]	*Mycoplasma* sp. (Kid)[d] Ile tRNA	*Escherichia coli*[b]
Adenosine	17	16	18	17	18
1-Methyladenosine	0.4	0.22	Trace	—	0.06
6-*N*-Methyladenosine		0.35	Trace	—	<0.01
Isopentenyl adenosine	<0.3	—	—	—	—
6,6-*N*-Dimethyladenosine	—	0.04	—	—	<0.01
Guanosine	21	23	23	17	25
1-Methylguanosine	0.8	—	—	—	—
2-*N*-Methylguanosine		0.02	Trace	—	0.03
7-Methylguanosine	1.0	0.64	Trace	0.5	0.32
2'-*O*-Methylguanosine	0.4	—	—	—	—
Inosine	—	0.12	—	—	0.14
Cytidine	19	22	20	17	21
5-Methylcytidine	0.6	0.02	—	—	<0.01
2'-*O*-Methylcytidine	0.4	—	—	—	—
Uridine	14	16	19	13	13
Pseudouridine	2.4	1.02		1.0	1.78
Ribothymidine	0.9	0.01		—	0.99
Dihydrouridine	0.8	0.94	—	3.0	1.55
4-Thiouridine	0.2	0.32	Trace	0.2	0.64
2'-*O*-methyluridine	<0.2	—	—	—	—
2'-*O*-Methyluridine	—	0.05	—	—	<0.01

[a] Feldman and Falter (37).
[b] Calculated from data of Johnson and Horowitz (61).
[c] Calculated from data of Hayashi, Fisher, and Söll (55), assuming 80 nucleotides/tRNA.
[d] Johnson, Hayashi, and Söll (62).

synthesis, but in all cases it was complete in 15 min. From these data, the mRNA half-life was calculated to be less than 4 min, which is close to the 2-min mRNA half-life which Kirk (72) reported for *M. gallisepticum*.

Plasmids

Plasmids are extranuclear genetic structures which can reproduce autonomously (56). The finding that many mycoplasmas carry viruses (48, 79) makes it fairly certain that there are mycoplasma plasmids.

In electron microscopy studies of *M. arthritidis* DNA, Morowitz (102) reported, in addition to the large pieces of DNA, small circles of less than 20×10^6 molecular weight. Also in *M. arthritidis*, Haller and Lynn (Bacteriol. Proc., p. 68, 1968) found a satellite DNA band in cesium chloride gradients. It is not known whether this is a plasmid; the one reported effort to isolate a virus from *M. arthritidis* was unsuccessful (79), but one has been isolated in more recent experiments (A. Liss and J. Maniloff, *unpublished data*). There is also the possibility of nonviral plasmids.

A virus was isolated from *A. laidlawii* B (79) and, in other studies of these cells, in addition to the cells' chromosomal DNA, a low-molecular-weight DNA peak was identified in neutral and sucrose gradients (30, 32). This peak, which is 35% of the total DNA in the cell, is believed to be viral plasmids (90).

Other Cytoplasmic Organelles

Cytoplasmic granules or structures have been described in *M. gallisepticum*, *A. laidlawii*, and *M. pneumoniae*. The structures appear morphologically different in each case.

The pear-shaped *M. gallisepticum* cells (Fig. 4, 6) have a terminal bleb structure, connected to the cell by an infra-bleb region (91, 93, 105). The bleb is hemispherical, about 80 by 125 mm, and the base, its attachment to the infra-bleb, is a flat, circular plate. The infra-bleb region is about 200 nm in diameter and appears to be two concentric spherical regions. *M. gallisepticum* binds to mammalian cells by the bleb end of the cell (182, 183); hence, the terminal structures must have some sort of binding sites. During the life cycle of the cell, these structures are replicated and form opposite poles of the cell at division (91, 105). These structures are found in the subcellular fraction containing the DNA growing region (122) and appear to be involved in DNA replication. Munkres and Wachtel (111) showed

that the cells' adenosine triphosphatase activity is localized along the inside of the membrane bounding the bleb and infra-bleb region, and acid phosphatase is localized only in the infra-bleb region. There is a suggestion that acid phosphatase activity may be different in the one-bleb daughter cell versus the two-bleb predivision cell. Maniloff (90) has shown that the central core of the infra-bleb region and the periphery of the bleb are rich in basic protein and, with tellurite used as an electron acceptor, redox activity was localized in the infra-bleb region. In summary, the *M. gallisepticum* terminal structures seem to be the sites of a variety of enzymatic processes and to be involved, at least as the probable site of DNA synthesis, in cellular regulation.

Each filamentous *A. laidlawii* cell seems to contain a granular region (Fig. 3) which partitions the filament (87). It is spherical and as wide as the cell, about 0.2 to 0.3 μm. There are no data on the possible function of this material.

Biberfeld and Biberfeld (12) described the knoblike structures on the ends of *M. pneumoniae* filaments; these consist of a densely stained rod surrounded by cytoplasm and ending with a platelike structure. The only data on the functional aspect of these terminal structures is the recent observation of Collier and Clyde (28) that they are the regions of interaction between *M. pneumoniae* cells and human fetal trachea organ culture cells.

SUMMARY

The basic points of this review of mycoplasma cell biology were presented at the beginning as a set of generalizations: (i) difference of morphology among species, with species characteristic cell structure; (ii) 0.33 μm (instead of 0.1 μm) as the smallest viable mycoplasmas; (iii) cell replication primarily by binary division; and (iv) a biochemistry similar to that of other procaryotic cells. The "elementary bodies" with their promise of novel modes of cell growth and division have proven to be nonexistent.

Regarding the last generalization, mycoplasma biology is seen to be qualitatively the same as that of other procaryotes; the only differences are small quantitative ones arising because of the limited cell size and genome. All the complexity of molecular biology is seen in the mycoplasmas, and this must mean that the subtle sophistications seen in the biochemical structures and mechanisms are not evolutionary additions but constitute the fundamental requirements of all biological systems.

The linear dimensions of the smallest mycoplasmas are about two times that of the theoretical minimal 0.15-μm cell; in other words, there are living cells only 10 times more massive than the most restrictive cell that can be postulated, assuming conventional terrestrial biology. Mycoplasma are indeed near the limits imposed by the required apparatus of molecular biology. If appreciably smaller cells are ever found to exist, the finding will challenge our ideas of what constitutes the necessary biochemical processes for life.

ACKNOWLEDGMENTS

We thank the many members of our research groups who have contributed to our information and ideas concerning the mycoplasmas. Support for this work has come from the Public Health Service National Institutes of Health, the National Aeronautics and Space Administration, the U.S. Atomic Energy Commission, and the University of Rochester Atomic Energy Project (Report No. UR-3490-54). J.M. is the recipient of Public Health Service Research Career Development Award AI17480.

We thank our friend and colleague, Mark Tourtellotte, for many valuable discussions, guidance in experimental problems, and his advice in planning this review.

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