Ultrastructure and Ribosomes of *Mycoplasma gallisepticum*

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**Abstract**

Maniloff, Jack (Yale University, New Haven, Conn.), Harold J. Morowitz, and Russell J. Barnett. Ultrastructure and ribosomes of *Mycoplasma gallisepticum*, J. Bacteriol. 90:193-204. 1965.—The ultrastructure of *Mycoplasma gallisepticum* A5069 has been studied by electron microscopy (thin-section and negative staining), ultracentrifugation, and chemical analysis. The list of ultrastructure is: membrane, nuclear material, ribosomes, ribosomal structures, infra-bleb region, and blebs. The nuclear material, containing the cell’s deoxyribonucleic acid, appears as an unbounded region containing 30-A fibrils. The ribosomes have a diameter of about 140 A, a ribonucleic acid-protein ratio of 0.68, and an uncorrected sedimentation coefficient of 70.2S. The 70.2S particle can be broken into 49.3S and 32.4S particles. Ribosomal arrays were found filling the intracytoplasmic space between the nuclear material and the membrane. Under certain conditions, these arrays formed cylindrical arrangements of ribosomes. The infra-bleb region is composed of a granular material, although little internal structure could be found. The bleb was highly structured.

The ultrastructure of the Avian pneumonia-like organism (PPLO) A5069, *Mycoplasma gallisepticum*, has been shown to consist of a “unit” membrane, nuclear area, ribosomes, cylindrical arrays of particles, and surface blebs (Maniloff, Morowitz, and Barnett, 1965). The isolation of a subcellular fraction that contained all the morphologically identifiable nuclear material and that was found by chemical analysis to be the only fraction containing deoxyribonucleic acid (DNA) indicated that the DNA was localized in the nuclear area. Due to their staining properties, the particles forming the cylindrical arrays were identified as ribosomes.

In the studies reported here, the nuclear material was shown, by the use of the indium staining procedure (Watson and Aldridge, 1961; Aldridge and Watson, 1963), to contain all of the cell’s DNA. Also, the particles making up the cylindrical arrays seen in the cell have been identified as ribosomes, by combined microscopic, chemical, and sedimentation studies.

The above studies were done by first harvesting the cells, and then fixing the pellets. Other studies, in which the cells were first fixed and then harvested, gave a differently shaped cell. The reasons why this second form of the cell (elongated rather than spherical) is believed to be representative of the living organism are discussed. The elongated cell is shown to have a fibrillar nuclear material, but not to contain cylindrical ribosomal arrays, indicating that these latter structures, when seen, arise during preparation.

The studies reported here involved the use of glutaraldehyde (as a bacterial fixative) and negative staining (to study bacterial morphology). The former technique showed nuclear ultrastructure similar to that of the standard fixation procedure of Ryter et al. (1958). Under the conditions reported, negative staining enabled the morphology of whole cells to be studied.

**Materials and Methods**

Preparation for ultrastructure studies. The A5069 strain of *Mycoplasma gallisepticum* was grown and harvested as previously described. The procedure of preparing the pellets for electron microscopy (6.25% glutaraldehyde fixation, Epon embedding, thin-sectioning, and uranyl acetate staining) has also been reported (Maniloff et al., 1965). In some studies, cells were first fixed and then harvested. For these experiments, broth culture samples were taken from the 37 C incubator and poured into an equal volume of cold (4 to 6 C) fixative (12.5% glutaraldehyde in 0.2 M sodium cacodylate, final pH about 7.2), to give a final

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was at harvest of the cells. For some studies, it was desired to quickly cool the cells. In this case, a sample from the 37°C incubator was poured into an equal volume of cold (4 to 6°C) medium, harvested at 4 to 6°C, and fixed and prepared for microscopy as described above.

Preparation of ribosome fractions. A ribosomal pellet was prepared as follows: cells from a 500-ml culture were resuspended in 15 ml of 0.01 M Tris-0.004 M succinate-0.01 M magnesium buffer (TSM), pH 7.4, sonic treated (with a Mullard Ultrasonic 100-w generator, type E-7580A) for 30 min, the debris was removed by centrifugation for 15 min at 14,250 × g at 4 to 6°C, and the ribosomes were pelleted by centrifugation for 2 hr at 93,000 × g. The pellet was divided three ways: one part for ultracentrifugal analysis, one part for chemical analysis, and one part for thin-section electron microscopy. This procedure yielded about 0.6 mg of ribosomes.

Electron microscopy. The grids, containing thin sections or a dried suspension, were examined with either an Akasaki Tronscope-50 or an RCA EMU-3B electron microscope. Micrographs for ultrastructure measurements were taken immediately after the magnification of the RCA microscope had been calibrated with a carbon-shadowed diffraction grating replica (E. F. Fullam, Schenectady, N.Y.).

For negative staining, 200 mesh stainless-steel grids, coated with both Formvar and carbon, were placed on a clean slide. A drop of fixed suspension was put on each grid, and, after about a minute, most of the drop was drained off with filter paper. A drop of negative staining solution [2% phosphotungstic acid (PTA), adjusted to pH 7.0 with 2 N potassium hydroxide] was put on the moist grid. After about 2 min, the PTA was drained off; the grids were placed on filter paper to dry, and were then examined.

For positive staining, the grids containing unstained samples were stained with uranyl acetate, as described previously (Manioff et al., 1965).

For nucleic acid staining, the indium procedure (Watson and Aldridge, 1961; Aldridge and Watson, 1963) was used, with certain modifications suggested by M. L. Watson (personal communication). The following protocol was used to stain for DNA:

(i) Put glutaraldehyde-fixed blocks into weighing bottle. Wash with solution of 6.25% glutaraldehyde-3.3% perehloric acid (PCA) in 0.01 M cacodylate for 10 min at 4 to 6°C.

(ii) Wash with solution of 6.25% glutaraldehyde-6.7% of PCA in 0.001 M cacodylate for 10 min at 4 to 6°C.

(iii) Incubate overnight (about 18 hr) in 6.25% glutaraldehyde-10% PCA, at 4 to 6°C.

(iv) Wash by diluting 1:1 with 6.25% glutaraldehyde in water for 10 min at 4 to 6°C.

(v) Wash six times, each for 10 min, with 6.25% glutaraldehyde in water; the first three at 4 to 6°C, the last three at room temperature.

(vi) Dehydrate, at room temperature, in increasing concentrations of ethyl alcohol.

(vii) Wash with 100% ethyl alcohol for 10 min at 4 to 6°C.

(viii) Wash at 4 to 6°C as follows: 5 min with solution of 1:3 pyridine-ethyl alcohol; 5 min with 1:1 pyridine-ethyl alcohol; 5 min with 3:1 pyridine-ethyl alcohol.

(ix) Follow the indium procedure of Watson and Aldridge (1961), beginning with their Step 4. Embed in Vestopal, not methacrylate.

When total nucleic acid staining was desired, the steps involved in the PCA extraction were omitted. Controls, to determine the effect of the procedure on the ultrastructure, were done with the same procedure and solvents, but without the reagents. Some sections were subsequently given a 5-min uranyl acetate stain, to check the general morphology.

To check the nucleic acid extraction during the indium procedure, the DNA was labeled by growing the cells on medium containing only 3.4 g of tryptose per liter, to reduce competition to the labeled compound, and supplemented with thymidine-3-C14 to a final activity of 2 mc per ml of medium. The cells were harvested and carried through step iii (the end of the PCA extraction) of the above procedure. Samples from the medium, fixative, Wash I (6.25% glutaraldehyde-3.3% PCA), Wash II (6.25% glutaraldehyde-6.7% PCA), PCA (6.25% glutaraldehyde-10% PCA), and pellets were analyzed for radioactivity and optical density (OD) at 260 μm. The nucleic acid of the pellet was hydrolyzed for this procedure by putting it, in 5% trichloroacetic acid, into a boiling-water bath for 10 min. Ribonucleic acid (RNA) assays, as described be-

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<th>Table 1. Results of nucleic acid extraction*</th>
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* The counts per minute per milliliter are the actual experimental values (from a 10-min count); the total counts per minute have been corrected for dilution. Symbol: I = value indistinguishable from background.
FIG. 1. Control to determine the effect of the indium staining procedure on the ultrastructure of PPLO A5969. The cellular fine structure is seen to be unaltered by the preparation and includes: surface "unit" membranes, nuclear areas, and cylindrical ribosomal arrays. 70,000X. Abbreviations: b = bleb; c = cylindrical ribosome arrays; i = infra-bleb region; m = surface membrane; n = nuclear area; r = ribosomes; and ra = ribosomal arrangements.

FIG. 2. Cells glutaraldehyde-fixed and stained for DNA by the indium staining procedure (with use of a PCA extraction of RNA). Only the nuclear material has taken up the stain. 70,000X. For abbreviations, see Fig. 1.
FIG. 3. Cells glutaraldehyde-fixed and stained for total nucleic acid by the indium staining procedure. The stain was taken up by the nuclear material and the ribosomes. Some of the latter can be seen to be in cylindrical arrangements. Cylinders, apparently cut perpendicular to the long axis can be seen (arrow), having hollow cores and four of five ribosomes in the plane of section. 70,000X. For abbreviations, see Fig. 1.
Fig. 4. Glutaraldehyde-fixed ribosomal fractions showing particles about 140 A in size. (a) Thin-section of ribosomal fraction found to contain 72.2S particles and to have an RNA/protein ratio of 0.68. (b) Positively (uranyl acetate) stained ribosomal suspension. (c) Negatively (PTA) stained ribosomal suspension. 72,800X. 

Fig. 5. Cells harvested, glutaraldehyde-fixed, and prepared for microscopy as described in text. The spherical cells show a loss of cytoplasmic ground substance, but the other fine structural features can be seen: "unit" membrane, granular nuclear material, infra-bleb regions, cylindrical ribosomal arrays, randomly arranged ribosomes and blebs. 72,800X. For abbreviations, see Fig. 1.
low, could not be done, since the samples precipitated the orcinol reagent.

Analytical ultracentrifugation. For ultracentrifuge studies, ribosomal solutions were diluted with TSM so as to have an OD of about 0.45 at 260 m. Analytical ultracentrifugation was carried out in a Spinco Model E ultracentrifuge at 20 to 21 C, with ultraviolet absorption optics and a 30-mm centerpiece. Centrifugation was done at 20,410 rpm, and pictures were taken every 5 min. Densitometer traces were made with a Joyce double-beam recording microdensitometer. Sedimentation coefficients and their 95% confidence limits were calculated by the method of least squares (Wilks, 1948).

Chemical analysis. The RNA of the ribosome pellet was hydrolyzed by putting the pellet, in 5% trichloroacetic acid, into a boiling-water bath for 10 min. The precipitate was removed by centrifugation at 14,350 X g for 20 min, dissolved in 0.1 N sodium hydroxide, and analyzed for protein. The decanted trichloroacetic acid supernatant fluid was analyzed for RNA.

Protein determinations were done with the Folin-Ciocalteu reagent (Lowry et al., 1951). Bovine albumin, fraction V (Armour Pharmaceutical Co., Kankakee, Ill.), was used to calibrate the assay.

RNA was determined by the modified orcinol procedure of Ceriotti (1955). Yeast nucleic acid (Calbiochem) was used to calibrate the assay.

When the cells were grown on media containing

![Cell images](https://example.com/cell_images)

**Fig. 6.** Cells cooled, harvested, and glutaraldehyde-fixed. In the ellipsoidal-shaped cells can be seen "unit" membranes, ribosomal arrays, thick fibrous nuclear material, infra-bleb regions, and blebs. 72,800X. For abbreviations, see Fig. 1.

**Fig. 7.** Elongated cells glutaraldehyde-fixed before harvesting. The ultrastructure can be seen to include: "unit" membrane, fibrillar nuclear material, random ribosomal arrangements, infra-bleb regions, and blebs. 72,800X. For abbreviations, see Fig. 1.
thymidine-$2\text{-C}^{14}$, the label was followed by drying 1-ml samples on planchets and counting in a gas-flow counter.

**RESULTS**

Nucleic acid staining. To definitely identify the nucleic acid-containing structures, the indium staining procedure (Watson and Aldridge, 1961) was used. Specificity for DNA was accomplished by a PCA extraction of the RNA (Aldridge and Watson, 1963). For these studies, it was important to be certain that no DNA was extracted. Hence, the DNA was labeled with thymidine-$2\text{-C}^{14}$, enabling accurate monitoring of DNA extraction. The results of the study of nucleic acid extraction during the indium staining procedure are given in Table 1. The values for the media and fixative are only of qualitative significance, since the counter was not corrected for coincidence. The background for the experiment was 47 count/min (during a 30-min period) and, hence, had a standard deviation of ±2.2 count/min for a 10-min count. Therefore, at the 99% confidence limit, 6 count/min above background would have been significant, and, if 0.2% of the labeled DNA had been extracted, it would have been detected. During the experiment, most of the cell's 260 m$\mu$-absorbing material was extracted. In summary, during the PCA extraction of the indium procedure, at least 99.8% of the cell's DNA was retained, whereas most of the cell's RNA was extracted.

Controls of the indium staining procedure (Fig. 1) indicated an ultrastructure similar to that previously reported (Maniloff et al., 1965). Samples stained for DNA (Fig. 2) showed the stain taken up only by the nuclear material, i.e., by a centrally located region, unbounded by membranes and containing granules smaller than ribosomes and amorphous and fibrillar material. The small amount of peripheral density seen in these cells could be either partially extracted ribosomes or dispersed DNA particles. Since, as will be discussed, these cells have undergone a

**Fig. 8.** Elongated cells glutaraldehyde-fixed. (a and c) Sections showing the fine structure. 72,800X. (b and d) Enlargements of the blebs in the cells of (a) and (c), respectively, showing the bleb ultrastructure: dense elliptical outer plate (op), flat plate (fp), threads (t) connecting the flat and elliptical plates, and the unstained regions (ur) between the threads. It should be noted that the threads and unstained regions may form microtubules. 168,800X. For abbreviations, see Fig. 1.
shape change, little significance can be placed in the threads radiating from the nuclear material, although DNA involvement in the cylindrical ribosomal arrays remains a possibility. In the cells stained for total nucleic acid (Fig. 3), both the nuclear material and the 140-A particles took up the stain, indicating the presence of RNA in the particles. Subsequent uranyl staining of sections, stained for either total nucleic acid or DNA, again showed the preservation of ultrastructure during the procedure.

Ribosomes. Thin-section electron micrographs of the ribosomal fraction (93,000 × g for 2 hr) showed approximately 140-A uranyl-stained particles (Fig. 4a). Chemical analysis of the fraction gave an RNA-protein ratio of 0.68. A sedimentation study of the fraction showed a single component with an uncorrected sedimentation coeffi-

**Fig. 9.** Cells having elongated shape stained for DNA by the indium procedure (with a PCA extraction of RNA) and showing the stain only taken up by the nuclear material. 66,000X. For abbreviations, see Fig. 1.

**Fig. 10.** Cells stained for total nucleic acid by the indium procedure, showing the stain taken up only by nuclear material and the ribosomes. 66,000X. For abbreviations, see Fig. 1.
cient (± the 95% confidence limits) of 72.2 ± 6.5S. Upon 1:6 dilution with water (to adjust the OD of the solution to 0.45 at 260 μm), peaks were found at 70.2 ± 7.0S, 49.6 ± 3.4S, and 33.3 ± 3.4S. Part of the sample was kept at 4 to 6°C for 24 hr and then diluted 1:6 with water. Peaks were found at 68.3 ± 5.0S, 48.9 ± 5.1S, 43.6 ± 4.2S, 31.5 ± 4.3S, and 20.1 ± 3.2S.

A ribosomal fraction was examined by both positive (Fig. 4b) and negative staining (Fig. 4c).

Both preparations showed particles measuring about 140 Å.

Cell morphology. To study changes in cell morphology during preparation, an exponential phase culture was divided into three parts. One part was prepared for thin-section electron microscopy by the procedure used in the previously reported ultrastructure studies (harvesting followed by fixation in cold glutaraldehyde) and one part by an alternate procedure (diluting the culture with water).

![Fig. 11. (a) and (b) Cells unfixed and unstained. (c) and (d) Cells unfixed and negatively (PTA) stained. (e) and (f) Cells glutaraldehyde-fixed and unstained. (g) and (h) Cells glutaraldehyde-fixed and negatively (PTA) stained. 36,400X.](http://jb.asm.org/)

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an equal volume of cold glutaraldehyde followed by harvesting. As an additional control (to study the temperature effect), part of the culture was diluted with an equal volume of cold medium, harvested in the cold, and fixed.

The cells prepared by the ultrastructure procedure (harvesting followed by fixation) appeared similar to those reported previously (Maniloff et al., 1965). They were spherical (Fig. 5), about 0.50 to 0.55 \( \mu \) in diameter, and, while much fine structure could be seen, the cytoplasmic ground substance was lost. The degree of loss varied from preparation to preparation, the other features remaining fairly uniform. A bounding “unit” membrane, granular nuclear material, infra-bleb regions (the region between the bleb and the rest of the cell), abundant cylindrical ribosomal arrays, and surface blebs could be seen (Fig. 8). The 110-A membrane consisted of two dense 30-A lines separated by a less dense 50-A area.

Cells which were cooled, harvested, and fixed were shaped approximately like prolate ellipsoids of revolution (Fig. 6), 0.55 to 0.70 \( \mu \) by 0.25 to 0.40 \( \mu \). Some vacular area could be seen in these cells, but not nearly as much as in cells examined by the ultrastructure procedure. The cells had a “unit” membrane, ribosomal arrays, thick fibrous nuclear material, infra-bleb regions, and blebs.

When cells were first fixed and then harvested, each cell had an elongated appearance of about 0.15 to 0.30 \( \mu \) in diameter and about 1.0 to 1.25 \( \mu \) long (Fig. 7). The nuclear material was in the center part of the posterior section and had a fibrillar appearance (most threads were about 30 A thick). Ribosomal arrays, showing irregular packing, filled the intracytoplasmic space between the nuclear area and the “unit” membrane. The infra-bleb region of the cell was approximately spherical, with a diameter of about 0.25 \( \mu \). Although some cells seemed to have a circular pattern of material in this region, a more definitive description of the infra-bleb region fine structure could not be obtained from these studies.

A structured bleb made up the anterior of the cell (Fig. 7 and 8). The bleb, shaped approximately like an oblate ellipsoid, measured about 800 A by 1,300 A, excluding the bounding membrane. The outer elliptical surface was formed by a plate densely stained with uranyl (Fig. 8b and d), about 60 A thick. About 300 A below this curved plate was a flat plate, about 160 A thick, with dense margins and a less dense center. The flat plate was connected to the elliptical surface by a series of densely stained threads, about 25 to 50 A thick. These threads were separated by unstained regions, about 60 by 300 A. The row of alternating threads and unstained regions appeared to form a hemisphere. It should be noted (Fig. 8b and d) that the geometric arrangement of the threads may be in the form of microtubules connecting the dense elliptical surface to the flat plate. Below the flat plate, the cytoplasmic ground substance was lightly granular.

Cells were prepared by this latter method and put through the indium staining procedure. Samples stained for DNA showed the stain taken up only in the nuclear area (Fig. 9). In samples stained for total nucleic acid, the stain was taken up by both the nuclear material and the ribosomes (Fig. 10). In neither case did the bleb or infra-bleb region take up the stain, indicating and absence of nucleic acids in these structures.

**Negative staining studies.** Unfixed and fixed cells were put on grids, some of each were negatively stained, and the cells were examined. The unfixcd unstained cells (Fig. 11a and b) were approximately tear-drop shaped, with the mass of the posterior section concentrated in a ring at the periphery (probably a drying artifact). Unfixed negatively stained cells (Fig. 11c and d) were grossly distorted and bore little resemblance to the cells prepared by the other procedures. The fixed cells, both unstained (Fig. 11e and f) and stained (Fig. 11g and h), appeared similar, and both were roughly tear-drop shaped. The differences in the latter two procedures were a lack of contrast and resolution in the unstained cells.

**Discussion**

Three shapes of cells were seen in these experiments: elongated, tear-drop, and spherical. The spherical-shaped cell is considered an artifact for several reasons. First, it seems most probable that artifacts of preparation would cause the formation of a spherical cell. [The transition to a spherical cell has been demonstrated in amebae, where mechanical injury, electrical shock, or ultraviolet radiation causes a retraction of the pseudopodia and the change to a spherical shape (Heilbrunn, 1952).] Second, the procedure used to obtain the elongated and tear-drop shapes seems better designed to preserve cellular morphology than the one used to get the spherical shape. This is reflected in the structure of the nuclear material: an oriented fibrillar network in the elongated shape, as opposed to a homogeneous area in the sphere. It should be noted here that the cylindrical ribosomal structures become more apparent as the cell shape changes from elongated to ellipsoidal to sphere. This may be due
to the following: since a condensation of nuclear material can be seen to occur during the shape transition, there may arise, during the preparation, intracellular forces (osmotic, for example) that enhance the formation of a tightly packed conformation of ribosomes from irregularly arranged ribosomes. Third, the elongated and tear-drop shapes have been seen in cells prepared by many methods: unfixed cells on grids (this obviates the possibility of the shape being a fixation artifact), fixed cells on grids, fixed cells negatively stained, and fixed cells prepared by thin-sectioning.

The finding that the cells began to become spherical when they were cooled suggested that the maintenance of shape in Avian PPLO A5969 is dependent upon metabolic activity. Thompson (1962) has noted that nonspherical form, in cells lacking rigid walls, indicates a state of unstable equilibrium (of chemical and physical change). The elongated and tear-drop shapes are so close to each other that it is impossible to consider one representative of the free living cell and exclude the other. The cell may also be a variety of shapes between these two extremes, shape transitions being normal reflections of fluctuations in metabolic activity.

In view of the present studies, it is necessary to revise the list of ultrastructure of M. gallisepticum, strain A5969 (Maniloff et al., 1965). The catalog of ultrastructure is:

Membrane. The 110-A “unit” membrane thickness obtained here appears to be a more realistic value than that previously published because of the calibration in the present studies.

Nuclear material. The DNA appears as an unbounded fibrillar and granular region, similar to that observed in bacteria (Brieger, 1963). Hence, glutaraldehyde fixation would seem to provide an alternative method to the standard fixation procedure of Ryter et al. (1958) of preserving nuclear fine structure, but without introducing a heavy metal into the sample. The 30-A fibril thickness reported here is in good agreement with the 20 to 50 A fibrils found in bacteria (Brieger, 1963), the 20-A T2 phage extracted DNA (Beer and Zobel, 1961), and the theoretical DNA double helix value of 20 A (Watson and Crick, 1953).

Ribosomes. The properties of Avian PPLO A5969 ribosomes are in agreement with those of other organisms. The RNA-protein ratio of 0.68 is within the 0.6 to 1.7 range found for isolated ribosomes and detached microsomal RNP particles (Hultin, 1964). The 140-A diameter is close to the 150 to 210-A values from other organisms (Hultin, 1964). The uncorrected 70.2S, 49.3S, and 32.4S sedimentation coefficients are in rough agreement with the 77S, 56S, and 38S values (at standard conditions of 20 C, water solvent, and infinite dilution) reported for the bacteria (Deley, 1964). By analogy with pea seedling (Bayley, 1964) and rat liver ribosomes (Dass and Bayley, 1964), it can be suggested that the 43.6 and 20.1 particles arise from the breakdown of the 49.3S particle. A theoretical calculation (with use of the ultracentrifuge equation and the conservation of mass equation, and assuming that all components have the same density and are spherical) leads to the supposition that the 20.1S and 43.6S particles came from a 52S particle, consistent with the above suggestion. Ribosomal subunits, with sedimentation coefficients similar to those reported here, have also been found, under a variety of different experimental conditions, to arise from yeast (Morgan, 1962) and Escherichia coli ribosomes (Meselson et al., 1964).

Ribosomal structures. The cylindrical ribosomal structures were apparent in the spherical cells, but not in the elongated ones. This suggests that they may exist in vivo as loose arrangements, and the tight packing seen in spherical cells may be an artifact. The ribosomal structures are important whether or not the ribosomes exist in vivo in this conformation, for they are a reflection of ribosomal arrangements in the cell and also can yield information regarding the symmetry of the ribosome (Kilkson, 1964).

Infra-bleb region. The lack of nucleic acid in the infra-bleb region, as shown in the indium staining studies, implies that this material is mainly protein. There is an indication that the material in this region may be in a circular arrangement.

Blebs. The blebs lack nucleic acid and must be largely composed of protein. The presence of components that did not stain with uranyl suggests lipids might also be present, by analogy to the staining of the plasma membrane. In view of the absence of nucleic acids, the lack of any indication of infectious behavior, and differences in morphology, the blebs are not viruses. In addition, there is no indication of any similarity (in size, organization, or morphology) between the blebs and any components of the mitotic apparatus.

The difference in the micrographs of the indium staining procedure in spherical and elongated cells was unexpected. It can only be concluded that the difference in degree of staining reflects the change in availability of binding sites when the DNA changes from a fibrous to an aggregated material. Watson and Aldridge (1961) discussed
the difficulties in estimating the amount of complex that will be formed.

A reasonably complete description of the ultrastructure of Avian PPLO A5969 has been obtained. These studies have also shown that negative staining, preceded by glutaraldehyde fixation, is a good technique for the examination of whole cells. Studies of the integration of these components into the replicative process will be discussed in a future communication.

Acknowledgments

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