THE INTERACTION OF MYCOPLASMAS WITH MAMMALIAN CELLS

I. HeLa Cells, Neutrophils, and Eosinophils*

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Plates 39 to 43

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The pathogenicity of mycoplasmas for members of the animal kingdom has been recognized for many years (1–3), and isolated reports (4–7) have indicated that the pleuropneumonialike organisms (PPLO's) may also be pathogenic for man. Recent knowledge that the Eaton agent, a PPLO, is a cause of atypical pneumonia (8) and that myringitis can be caused by mycoplasma (9) has greatly stimulated the interest of medical investigators in these infectious agents. It is now considered likely that this order of microorganisms may also play a role in other human diseases of as yet unknown etiology, particularly where clinical manifestations resemble known PPLO infections in animals. In addition, mycoplasmas have been found associated with two human tumors (10–16) though the connection of this microbe with neoplasia is still entirely in the realm of speculation.

Studies have been hampered by the fastidious growth requirements of the microorganism in vitro, and the difficulty of its detection in the infected host. Though the initial isolation of many PPLO's has been achieved in mammalian cell cultures, the organism is able to proliferate in a variety of cell-free media, and the relationship to the host cell has remained obscure. It is well known that the presence of mycoplasmas is detrimental to tissue cultures, but it is still controversial whether actual cell penetration is responsible for this cytopathogenic effect (13, 14) or whether depletion of essential nutrients alone, or in combination with a cytotoxin (15–17), leads to the destruction of infected cultures. Similarly, the underlying histopathogenicity of the lesions in experimentally infected animals is not yet understood, though it has become possible to delineate mycoplasma colonies with the fluorescence antibody technique (18).

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In an attempt to elucidate some of these questions, a series of electron microscope studies have been initiated. This report presents ultrastructural analyses of spontaneously infected HeLa cells as well as of tissue cultures which had been inoculated with a variety of mycoplasma species. In addition, human peripheral blood leukocytes and human thoracic duct lymph were incubated with PPLO's for varying periods of time. It was hoped that the result of these studies would aid the recognition of PPLO's in spontaneously infected tissues and elucidate the interaction of mycoplasmas with mammalian cells to some extent.

Materials and Methods

Mycoplasma pneumoniae, *Mycoplasma neurolyticum*, and *Mycoplasma gallisepticum* were used for these studies. *M. pneumoniae* strain FH was obtained from Dr. R. M. Chanock as the 206th agar passage (19). This strain was isolated originally by Dr. C. Liu (20) from a patient with atypical pneumonia. *M. pneumoniae* strain Bru[1] was isolated originally from the sputum of a young man with atypical pneumonia by Dr. W. A. Clyde (21).

M. neurolyticum strains KSA and type A were received from Dr. J. G. Tully (22). Despite multiple broth passages since their isolation, these strains had retained the ability to cause the "rolling disease" syndrome in mice. The KSA strain had been isolated by Dr. R. M. Lemcke from a normal mouse brain (23). The type A strain had been recovered from a 20-yr-old lyophilized culture of mouse mycoplasma in Dr. A. B. Sabin's laboratory (22). *M. gallisepticum* strain A5969 was kindly made available to us by Dr. H. J. Morowitz (24). It had been isolated from a chicken with respiratory disease by van Roekel (24).

All mycoplasmas were grown in a medium consisting of seven parts Difco PPLO broth or agar base, two parts unheated horse serum, and one part 25% yeast extract supplemented with 0.5 g % glucose and 10^3 units penicillin G per ml (8). 1 to 5% of overnight cultures of the mycoplasmas were inoculated into 200 to 1000 ml of this medium. The microorganisms were harvested during logarithmic growth phase by centrifugation at 13,800 *g* for 10 min. In some instances, the mycoplasmas were washed in Hank's saline containing 20% human or rat serum.

Tissue Culture Cells.—HeLa cell cultures strain S_3 were grown at 37°C in monolayers in milk dilution bottles and made available to us by Dr. R. P. Cox (25). The medium consisted of Waymouth formula (26) containing 10% calf serum. They were passed weekly and refed fresh medium on the 4th day of each pass. Sterility of the cultures was established by periodic plating of the cells on PPLO agar. The cultures were infected with 10^4 to 10^6 c.f.u/ml of either *M. pneumoniae* or *M. gallisepticum*. *M. neurolyticum* was not studied in tissue culture. In addition, several electron microscopic studies were carried out on the Ch strain of HeLa cells (25), which were found to be spontaneously contaminated with a nontypable aerobic strain of mycoplasma.

Preparation for electron microscopy was carried out following two to ten passages of the PPLO's in the cell cultures during the logarythmic growth phase of the microorganism. At this time, plating of the infected HeLa cells onto PPLO agar yielded titers ranging from 10^2 to 10^4 c.f.u/ml.

Human Blood Cells.—Leukocytes were separated from 100 to 200 ml of fresh blood collected either in Fenwall ACD bags or test tubes which contained 10 units of Heparin per ml. Buffy coats were removed with a Pasteur pipette following sedimentation of the erythrocytes

[1] This strain was kindly supplied to us by Dr. Z. McGee in Dr. Ruth Wittler's laboratory.

in a clinical centrifuge at 1200 RPM for 8 min. Human thoracic duct lymph was collected as described before (27). The white blood cells were resuspended in a medium consisting of 80% Hank's saline and 20% of autologous plasma to a final concentration ranging from one to three 10^8 cells per ml.

Incubation Mixture.—From 10^8 to 10^{10} mycoplasmas, resuspended in 1 to 2 ml of broth base (i.e. without serum or yeast), were added to 1 to 2 ml of the leukocyte suspension. The cells were gently agitated by hand in a water bath at 37°C for 3, 5, and 15 min, and on a rotating tumbler in a 37°C incubator for 30 min, 1, 2, 3, and 4 hr.

Fixation.—In the case of tissue cultures, the medium was decanted and immediately replaced by either cold 1% osmium tetroxide (28) or 3% glutaraldehyde (29) sufficient to cover the monolayer of cells attached to the glass bottle. The fixatives did not cause the cells to detach from the glass and, after 15 min, they were scraped off the glass with a wooden applicator. The suspended cells were kept in the fixative for an additional 15 min. In most instances, the glutaraldehyde-fixed cells were postfixed with 2% osmium tetroxide for 1 hr.

Leukocytes which had been incubated with PPLO's were fixed by addition of an equal volume of cold 2% osmium tetroxide or 10 ml 3% glutaraldehyde at the end of the incubation period. Usually the glutaraldehyde-fixed preparations were also postfixed with 2% osmium tetroxide for 1 to 2 hr. In general, both types of fixation seemed satisfactory except that the fine structure of *M. gallisepticum* fixed in glutaraldehyde differed somewhat from the ultrastructure of the organism fixed in osmic acid alone. However, for the purpose of the experiments reported here, the differences appeared immaterial.

Electron Microscopy.—The suspended cells were sedimented at 400 RPM for 5 min. Dehydration in alcohol and propylene oxide and embedding in Epon 812 (30) were carried out as described in detail elsewhere (27). Thin sections were obtained with a LKB ultrotome and stained with lead hydroxide (31), uranyl acetate (32), or both. The sections were examined and photographed with a Siemens Elmiskop I electron microscope, using an accelerating voltage of 60 kv at instrument magnifications ranging from 6000 to 38,000.

RESULTS

Mycoplasmas.—The ultrastructure of *M. gallisepticum* (Figs. 1 *a* to 1 *c*) and *M. pneumoniae* (Fig. 2) in thin sections has been described by others (33–36); and in general, our findings concur with theirs. For orientation, representative samples are shown in Figs. 1 *a* to 1 *c*, and Fig. 2 respectively. *M. gallisepticum* is an ovoid cell which measures about 500 to 700 mμ in length and 300 to 450 mμ in width. It is limited by a trilaminated membrane, the so called "unit membrane" (37), which is about 110 A thick. Ribosomes and a nuclear area are easily discerned. The organism may be distinguished from other PPLO's studied to date by virtue of a "bleb" at one or both ends of the cell. At higher magnifications, this protrusion reveals a complicated structure and since, as can be seen below, this may play a role in intercellular reactions, it will be described in greater detail. The membrane surrounding the protrusion is a continuation of the unit membrane though it seems somewhat thicker and stains more intensely, particularly when uranyl and lead stains are used in combination (Figs. 1 *a* and 1 *b*). About 300 to 400 A below the outer membrane is a structure which varies in appearance with the plane of section and perhaps also with the growth phase of the organism. In some section, the structure consists of two plates, the outer one convex, the inner one flat. The area within this

lenslike structure is less electron opaque and measures about 400 by 1200 A. In fortuitous sections, threads may be resolved between this structure and the inner aspect of the surface membrane (Figs. 1 *b* and 1 *c*). The threads are 30 to 50 A thick. Cross-sections through one of the plates show the central structure to be very dense and osmiophilic while the threads surrounding it, like the spokes of a wheel, can be easily identified (Fig. 1 *c*).

M. pneumoniae (Fig. 2) is very pleomorphic during its exponential growth phase. Round, oval, and tear-drop shapes were encountered. The largest organisms measured about 1000 mμ in longest diameter, the smallest were less than 100 mμ. Their unit membrane seemed somewhat thinner than the membrane surrounding *M. gallisepticum* and measured about 90 A. Densitometric analysis is necessary to confirm these measurements. Frequently, one or more small forms were attached to the larger cells by a bridge of cytoplasm while the membrane surrounded both forms in continuity. This suggests that *M. pneumoniae* reproduces by single or multiple budding. The organism contains ribosomes, varying from 100 to 140 A in size, and thick strands of osmiophilic material as well as thinner filaments, which on the basis of chemical analysis and staining characteristics are likely to consist of DNA (35).

The ultrastructure of *M. neurolyticum* has not been described before and will be presented in more detail elsewhere (38). In thin sections, one-third to one-half of the organisms appeared oval shaped with a hole somewhat off center. The hole was limited by a unit membrane of the same dimensions as the membrane which surrounded the cell. A similar number of organisms appeared as longitudinal or cross-sections of this configuration (Fig. 3). At the present time it is not possible to conclude whether *M. neurolyticum* is shaped like an egg with a deep invagination or whether the cell has a real hole in the center like a doughnut. Smaller forms may represent tangiential or peripheral sections of either shape.

The species of mycoplasma which was found in the spontaneously contaminated HeLa cells was not studied in isolated form. The organism seemed even more pleomorphic than the Eaton agent and its fine structure closely resembled that of *M. hominis* type I (39).

PPLO's in Tissue Cultures.—Preservation of the microorganism in infected HeLa cells proved satisfactory with all methods used. Cell cultures inoculated with *M. gallisepticum* or *M. pneumoniae* showed no gross cytopathologic effect despite many passages. However, even at very low magnifications it became apparent that innumerable thin cytoplasmic processes had formed along the entire surface membrane of the cells. The processes were of such length that they were located partially above or below the plane of section, so that cross-sections of these pseudovilli were found at some distance from the main body of the cell. Frequently sections of the processes were similar in shape and size to the PPLO's, but they could be distinguished without difficulty on the basis

of their internal fine structure (Fig. 6). Countless mycoplasmas were seen in spaces between the HeLa cells, mostly in close apposition to the membranes of the cytoplasmic processes. Complete obliteration of the space between the PPLO and the surface of the HeLa cell was often noted. At the point of such contact it never seemed possible to resolve either the unit membrane of the PPLO or that of the tissue culture cell. Not infrequently it appeared as if the microorganism was continuous with the tip of a HeLa cell process. As a rule, mycoplasmas were not seen intracellularly though much time was spent looking for this occurrence. Occasionally, a few PPLO's were found in vacuoles just under the surface membrane of a HeLa cell (Fig. 4). However, since the border of the HeLa cell is undulating, it is conceivable that what appear to be vacuoles are sometimes actually cellular invaginations. Phagocytosis or evidence indicative of active invasion of the tissue culture cells by the PPLO's was not apparent. On the other hand, necrotic and partially disintegrated cells showed an abundance of microorganisms within their cytoplasm. Frequently, the surface membrane of such cells had almost completely disappeared in spite of which the cellular components had remained coherent. However, in most instances, the cellular anatomy had become so distorted that the relationship of the microorganism to cellular organelles or membranes became difficult to define. This was also true of the tissue cultures which had become contaminated spontaneously. Only a few cells seemed to have an increased glycogen content, more vacuoles and rough endoplasmic reticulum (ER), and bizarre shaped mitochondria. However, the PPLO's were seen only within HeLa cells which were markedly disrupted in spite of the fact that the microorganism covered the surface membrane of almost every cell in the culture.

PPLO's Incubated with Leukocytes.—Incubation with blood cells at 37°C for up to 15 min had little detrimental effect on the ultrastructure of the microorganisms provided that the cells were fixed in suspension prior to centrifugation. Incubation for more than 15 min usually affected the morphology of *M. pneumoniae* and *M. neurolyticum* adversely whereas *M. gallisepticum* appeared somewhat more resistant to experimental procedures. The affinity of mycoplasmas to cell membranes again became strikingly evident when the microorganisms were incubated with human blood cells for as short a time as 2 min. All types of leukocytes as well as the platelets were almost completely covered with the PPLO's. There was no appreciable difference in this reaction whether human or animal mycoplasma species were employed. Eaton agent and *M. neurolyticum* seemed to attach to white cells anywhere along their plasma membrane whereas *M. gallisepticum* appeared to display some polarity. The majority of electron micrographs suggested that this organism attached preferentially with its longitudinal axis at right angles to the mammalian cell surface, and that the "bleb" constituted the site of contact more often than could be attributed to chance alone. An example of this phenomenon is seen in

Fig. 7. The illustration also shows the attachment of isolated "blebs" to the leukocyte membrane. However, since the organism may adhere to the leukocyte with its long axis perpendicular to the plane of section, and the size of the "bleb" may coincide with the thickness of the section, it is possible that the "body" of the organism was excluded from view. Moreover, the "bleb" may have become severed from the "body" during the experimental procedures. It is also noteworthy that *M. gallisepticum* adherent to leukocytes appeared more elongated than the unattached microorganisms or those examined in the absence of white blood cells. All three mycoplasmas were avidly phagocytosed by neutrophils and eosinophils within a few minutes of incubation (Figs. 7 to 10). Neutrophils underwent degranulation as is the case when these cells ingest bacteria (40). In Fig. 8, several *M. pneumoniae* can be seen in vacuoles while other organisms are still adherent to the surface membrane of the cell. Fig. 9 shows *M. neurolyticum* within a phagocytic vacuole of a polymorphonuclear leukocyte while one of the cell's lysosomes has been fixed in the process of discharge into the vacuole. Interestingly, PPLO's are as avidly phagocytozed by eosinophils as by neutrophils which is not the case for most bacteria and zymosan particles (40). The eosinophils showed large vacuoles which contained one or more mycoplasmas (Fig. 10). The osmiophilic central bands which are characteristic of eosinophilic granules could always be identified within these phagosomes even after 4 hr. of incubation when the microorganism was no longer recognizable. This is in contrast to what was observed with rabbit eosinophils ingesting staphylococci or zymosan in which case the entire granule content, including the osmiophilic band, surrounded the bacterium (40, 41).

The effect of PPLO's on human and rat mononuclear cells resembled the effect observed in tissue culture cells to some extent. On the other hand, the microorganisms were seen in the cytoplasm of some mononuclear cells which, as a rule, had not been considered phagocytic (42). Since the interaction of mycoplasmas with mononuclear cells appears to be a complicated one, it will be dealt with in greater detail in an accompanying communication.

DISCUSSION

The most striking observation to emerge from these studies was the intimate relationship which mycoplasmas have to the plasma membrane of mammalian cells (Figs. 4 to 6). A similar electron micrograph finding has been pictured by Barile (43) but the significance of the observation was not dealt with by this author.

It has been suggested that PPLO's, which unlike bacteria, lack a rigid cell wall, use the mammalian cell membrane mainly as support for growth. However, if the microorganisms were merely resting on the surface of the HeLa cells, it should have been possible to delineate the juxtaposed unit membranes with the electron micrographs. This could not be achieved unless a resolvable space

had remained between the surface membrane of the PPLO and that of the HeLa cell. Though the possibility of a tangential section can always be invoked when the limiting membrane of any structure cannot be resolved at every point, the fact that neither the plasma membrane of the cell nor that of the microorganism could ever be resolved at the point of their contact suggests that more than simple adherence of one cell to the other is involved. The development of numerous cytoplasmic processes, which differ from the pseudopods of other cells in that they are longer and thinner and do not serve phagocytosis or locomotion, may be an attempt on the part of the infected cells to compensate for the loss of surface area occupied by the PPLO's. Frequently, it appeared as if the microorganism arose from the tip of a cellular process, an impression which had also been gained by others (35). However, without the use of special markers such impressions could be erroneous. The studies were unable to answer whether mycoplasmas actually utilize structural components of the membrane or whether this intimate association permits the diffusion of cellular nutrients. It is known that the PPLO's require certain proteins, sterols, and phospholipids to maintain cellular integrity (44). The mammalian cell membrane may make such a lipoprotein available in "presynthesized" form. Surprisingly, the tissue culture cells were able to survive heavy PPLO infections. Only the cultures which had become contaminated spontaneously with *M. hominis* type I showed some cells which seemed to have an increased glycogen content, a larger number of vacuoles and bizarre mitochondria. However, since HeLa cells are very pleomorphic, and electron microscope techniques limit the extent of sampling possible, it cannot be decided with certainty whether the morphology of the infected cells differed much from the controls except for the described membrane changes. Therefore, it is likely that the gross cytopathologic effect observed in contaminated tissue cultures, such as the detachment of cells from glass surfaces, the penetration of vital dyes and the release of cellular products into the medium, may all be manifestations of the membrane alterations just described.

It seems probable that some PPLO's seen within the cells of tissue cultures are not situated in vacuoles, but lie at the bottom of crypts which open into the surrounding medium. This would explain the report that some contaminated tissue cultures can be "cured" with specific antisera (45). Since there is no evidence that antisera can penetrate living cells, it may well be that the effect of the antiserum on the microorganism takes place extracellularly.

The attraction of mycoplasmas to the surface membranes of peripheral blood leukocytes and platelets was much more pronounced than the reaction which takes place between leukocytes and bacteria under identical conditions (40). When leukocytes were incubated with staphylococci or streptococci for 15 min, a number of bacteria were seen adherent to the phagocytic cells while most remained free in the medium. The PPLO's, on the other hand, surrounded the

white cells like iron filings surrounding a magnetic pole. In the case of *M. gallisepticum* the marked elongation of the microorganism, when about to become attached to the plasma membrane of leukocytes, indicated that stretching had taken place as a result of a positive taxis exerted by the mammalian cell on the mycoplasma. The relationship between the two seems to deserve the term, reciprocal chemotaxis. Very few organisms remained free in the medium unless clumping of the microbes had occurred. Clumping was a recurring problem only with the Bru strain of *M. pneumoniae.*

Phagocytosis was rapid and in specimens which had been incubated for more than 1 hr, polymorphonuclear leukocytes and eosinophils showed large vacuoles in which the organism could only rarely be recognized. Presumably, degradation of the PPLO's by the lysosomal enzymes had already taken place since in earlier specimens, coalescence of lysosomes with phagosomes was readily observed (Fig. 9).

The phagocytic vacuoles in eosinophils presented a peculiar appearance in that the central crystal of the eosinophilic granule had remained intact (Fig. 10). The fact that the microorganisms were degraded in eosinophils equally well as in neutrophils may indicate that the osmiophilic substance may not partake in this reaction.

The studies presented here have shown that mycoplasmas are as subject to phagocytosis as bacteria and that their digestion by lysosomal enzymes is as effective. Because of the greater surface affinity to the cells and the greater pliability of the microorganism, phagocytosis may even be more efficient. This point would have to be established by kinetic studies. Thus, it is likely that the phagocytic cells constitute the first line of defense against invading mycoplasmas, and that the local inflammatory reaction induced by PPLO's does not differ in essence from that caused by bacteria. On the other hand, PPLO's seem to affect the surface membranes of cells in a manner which has not been observed for other microbes and which tempts one to speculate on cellular and systemic consequences beyond the data presented here.

SUMMARY

The ultrastructure of three mycoplasma species, *Mycoplasma pneumoniae*, *Mycoplasma gallisepticum*, and *Mycoplasma neurolyticum*, has been studied in isolated form as well as in HeLa cell cultures and following incubation with human peripheral blood leukocytes. During log growth phase, the organisms could be distinguished from each other on the basis of their fine structure. In mammalian cell cultures, PPLO's appeared to proliferate on the plasma membranes which had markedly increased their surface area by means of long cytoplasmic processes which extended toward and surrounded them. Some of the microorganisms affected in this way may well have lain, not in vacuoles, but at the bottom of crypts.

It is suggested that the cytopathogenic effect exerted by PPLO's on some tissue cultures may be attributable to membrane damage. Mycoplasmas adhered to leukocyte plasma membranes in a similar manner. They were avidly phagocytosed by neutrophils and eosinophils with accompanying degranulation of the white cells. It is thus likely that the local inflammatory reaction induced by PPLO's does not differ in essence from that caused by bacteria.

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EXPLANATION OF PLATES

PLATE 39

FIGS. 1 *a* to 1 *c*. *Mycoplasma gallisepticum* obtained from specimen incubated with leukocytes for 15 min. Fixed with glutal and osmic acid.

FIG. 1 *a*. Thickness of "unit membrane" surrounding the cell is noteworthy. Plane of section shows platelike layers of bleb (*B*). Small dots represent ribosomes. Nuclear area (*N*). × 70,000.

FIG. 1 *b*. Organism taken from same preparation shows only part of unit membrane probably due to mechanical damage. Dense strands of material in center presumed to represent DNA. Selected because plane of section appears to traverse one of the plates in bleb (*B*). × 69,000.

FIG. 1 *c*. Detail of *M. gallisepticum* flattened against leukocyte. Plane of section demonstrates threads which extend from central structure to periphery of bleb (*B*). × 75,000.

FIG. 2. *Mycoplasma pneumoniae* strain FH grown in HeLa cell culture. Fixed in 2% osmic acid. Pleomorphism and bud formation is apparent. Osmiophilic strands of material in center of organism presumed to represent DNA again noted. × 38,000.

FIG. 3. Electron micrograph illustrating various planes of section through *M. neurolyticum*. Fixed in glutal followed by 2% osmic acid. Arrows point to unit membrane. At asterisk, unit membrane surrounding the central hole cannot be seen, probably because section passed tangentially. × 70,000.

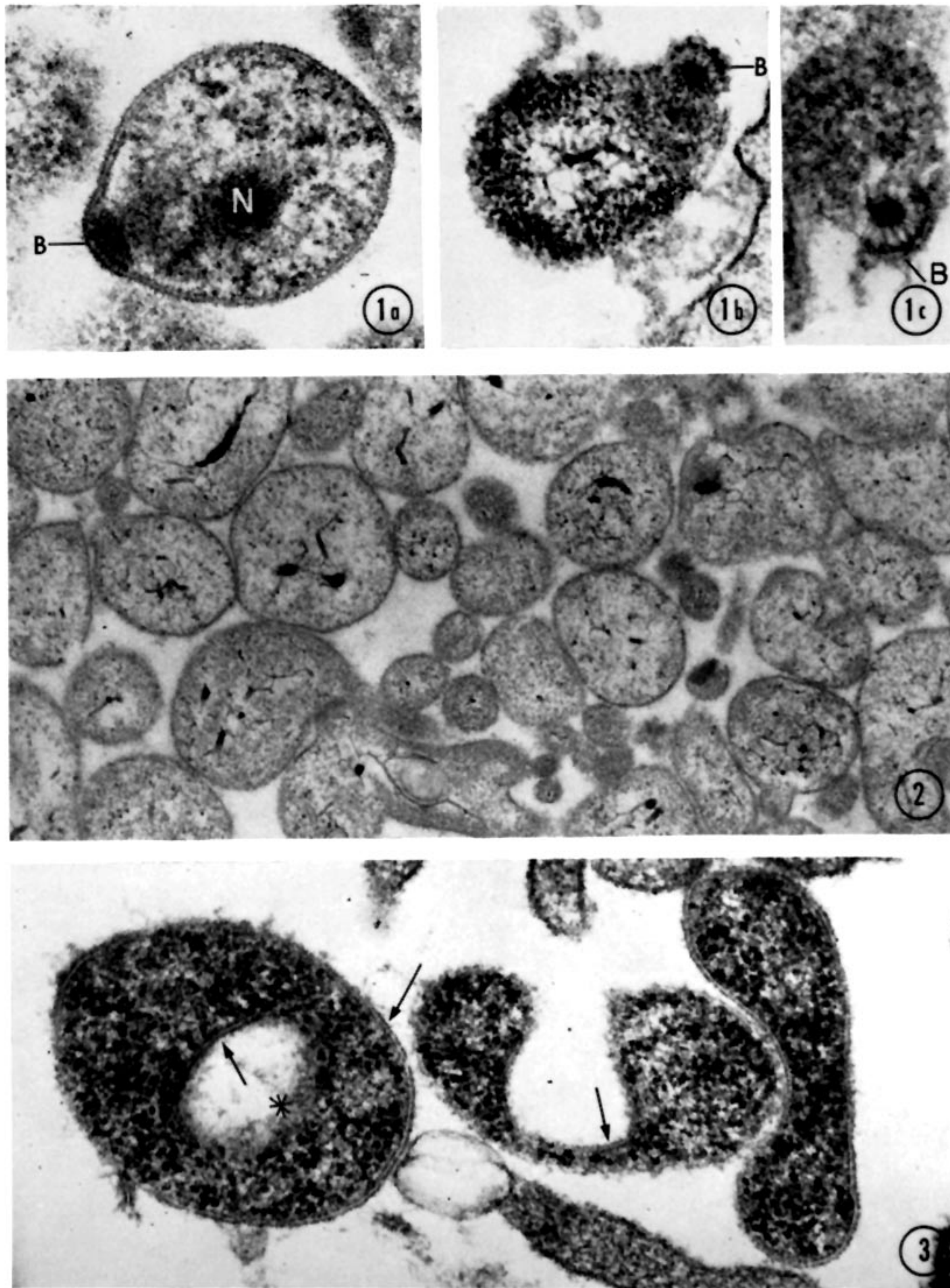

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PLATE 40

FIGS. 4 and 5. HeLa cell cultures inoculated with *M. pneumoniae* strain FH, 7th passage. Fixed in 2% osmic acid. Fig. 4 illustrates the extremely rare finding of microorganisms (*P*) in apparent vacuoles (*V*). In thin sections, the possibility exists that some of these actually represent cytoplasmic invaginations (Compare Fig. 4 with Fig. 5.). The intimate relationship of the PPLO's with the cytoplasmic processes (*C*) can be appreciated. Fig. 5 shows the periphery of two adjacent HeLa cells with intervening space which is occupied by PPLO's and cellular processes. An apparent vacuole (arrow) is present in the cell on the left of Fig. 5. It does not contain an organism. Glycogen (Gl). × 25,000.

FIG. 6. HeLa cell culture found to be spontaneously contaminated with PPLO's. Fixed with glutal followed by 2% osmic acid. The periphery of three cells appear in this field. The intercellular space shows numerous thin cytoplasmic processes (*C*) which can be distinguished easily from the densely osmiophilic microorganisms (*P*). Mitochondria (*M*), nucleus (*N*). × 20,000.

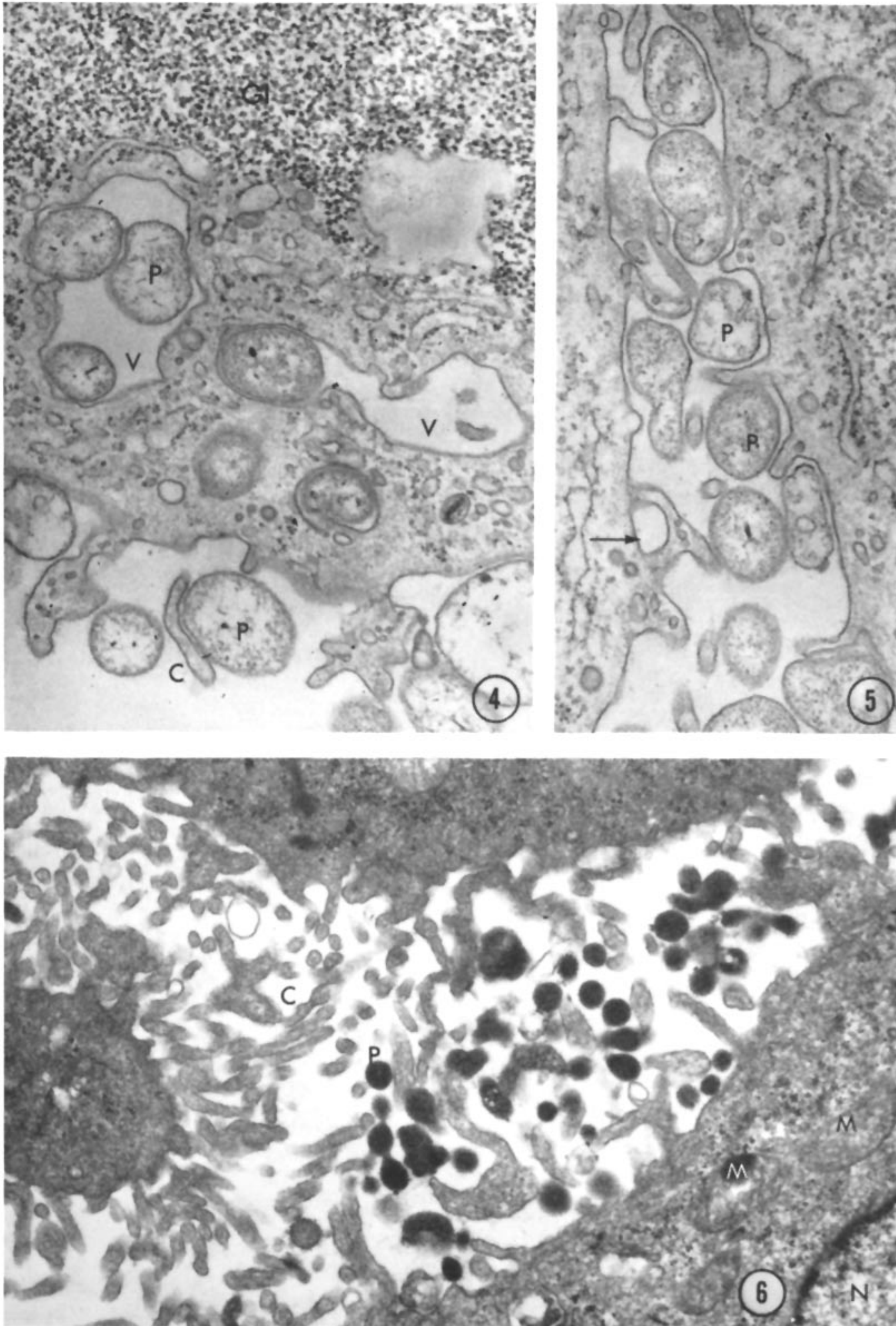

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PLATE 41

FIG. 7. Section through pole of a neutrophil which had been incubated with *M. gallisepticum* for 15 min. Fixed in glutal followed by 2% osmic acid. The polarity of these particular microorganisms in respect to the surface of the cell is noteworthy. Many "blebs" can be seen which have either been severed from the remainder of the organism or where the remainder of the microorganism is beyond the plane of section (arrows). Many organisms appear within phagocytic vacuoles at this time of incubation. Granules (*G*). × 22,000.

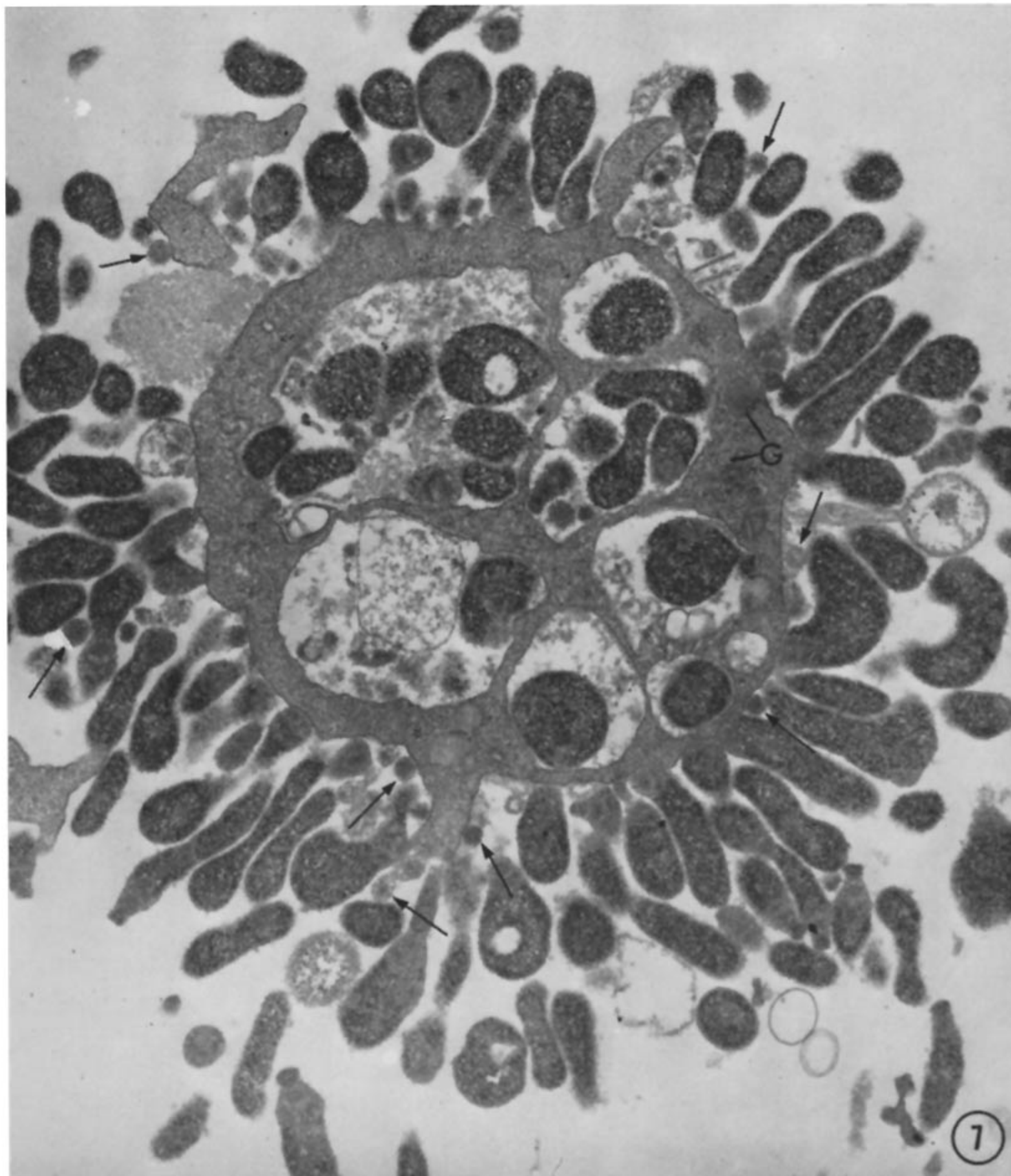

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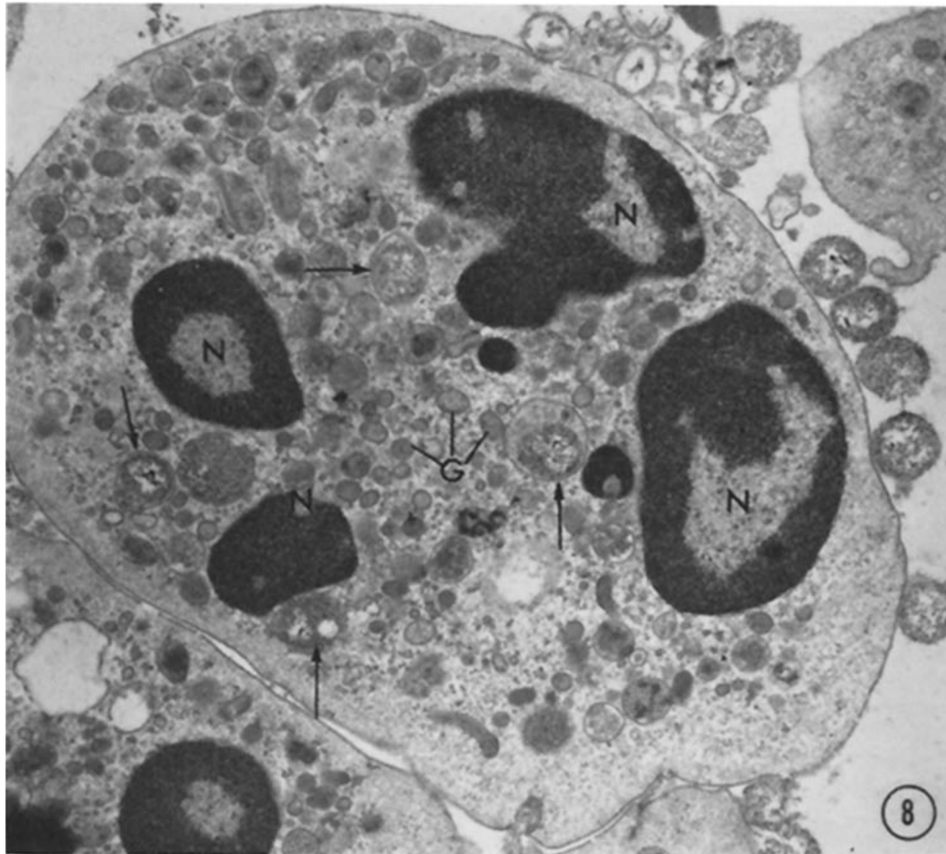
PLATE 42

FIG. 8. Human peripheral blood neutrophil taken from specimen incubated with *M. gallisepticum* for 5 min. Fixed in suspension with 2% osmic acid. Several vacuoles containing PPLO's are seen (arrows). Nucleus (*N*), specific granules or lysosomes (*G*). × 15,000.

FIG. 9. Detail of human peripheral blood polymorphonuclear leukocyte incubated with *M. neurolyticum* for 5 min. Fixed in 2% osmic acid. Phagocytic vacuole shows microorganism (*P*) and one of the cell's lysosomes fixed in the process of discharge (arrow). Nucleus (*N*), granules (*G*). × 26,000.



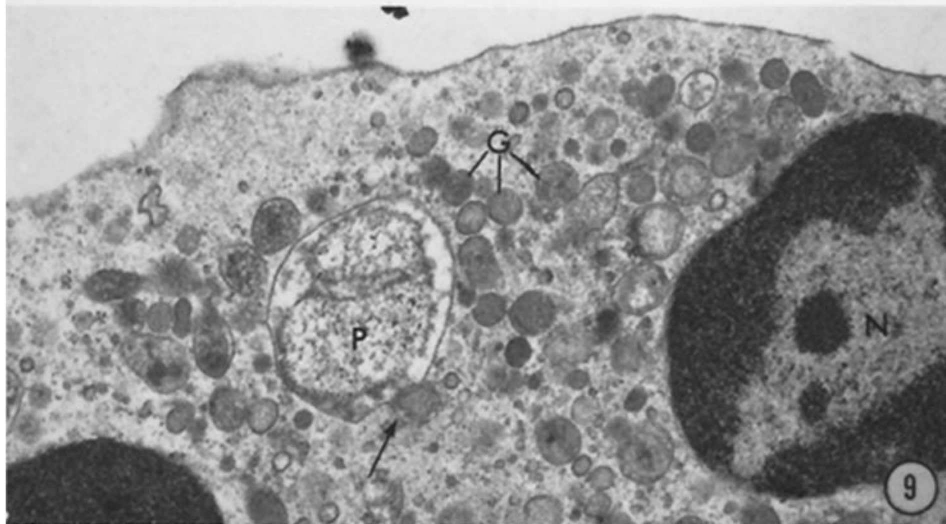


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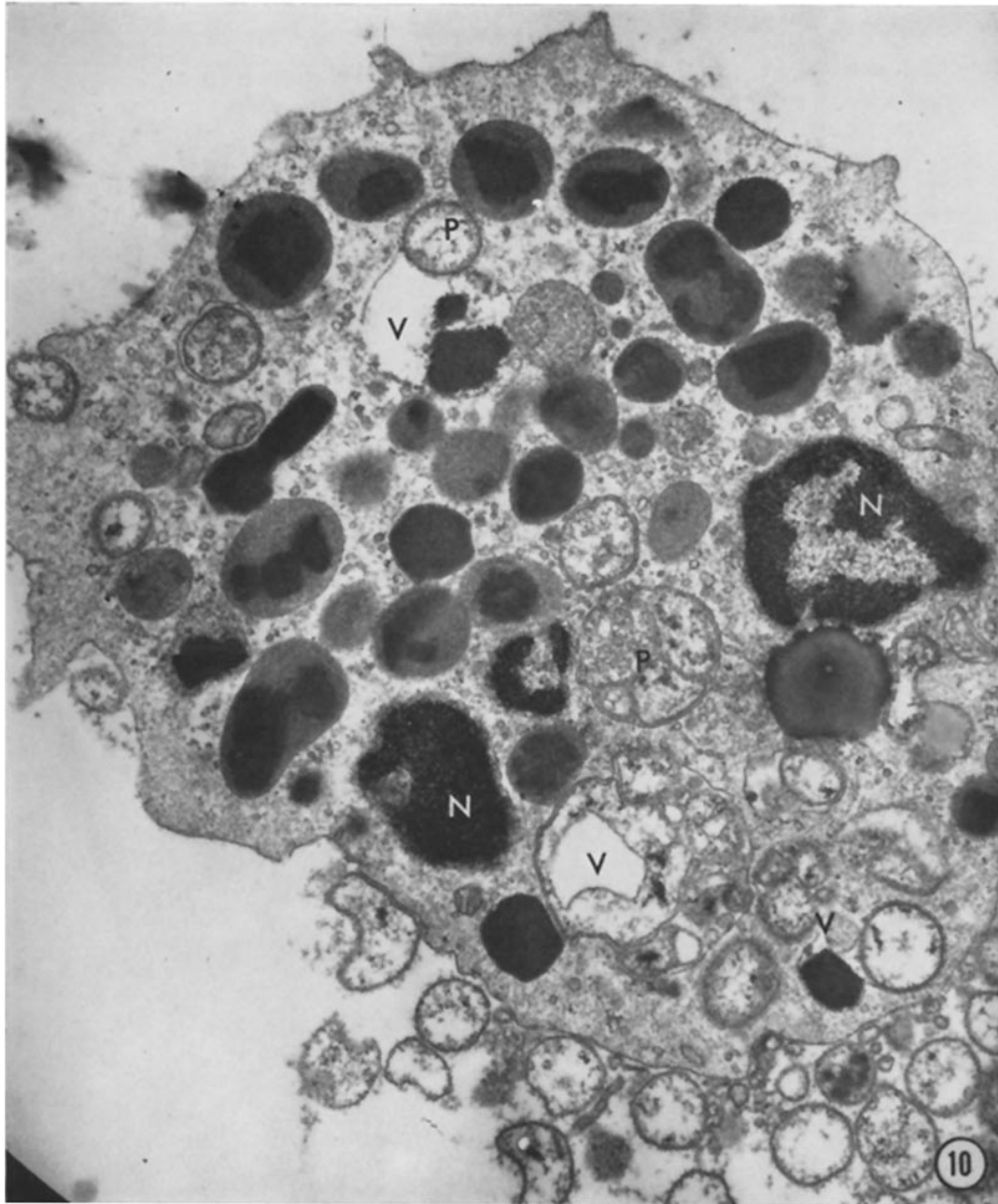
PLATE 43

FIG. 10. Human peripheral blood eosinophil taken from specimen agitated with *M. neurolyticum* at 37C°. for 1 hr. Fixed in osmic acid. Though the fine structure of the microorganism is markedly damaged, it can be recognized in several phagocytic vacuoles (*V*). Note that the black osmiophilic crystals characteristic of eosinophilic granules have remained intact within the phagosomes. At upper left, an organism can be seen in the process of being ingested by the cell. At the bottom, several organisms can be seen adhering to the cell's surface membrane. × 18,000.



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