THE INTERACTION OF MYCOPLASMAS WITH MAMMALIAN CELLS

II. Monocytes and Lymphocytes*

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Plates 44 to 51

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The accompanying report described the phagocytosis of mycoplasmas by neutrophils and eosinophils and alluded to the interaction of mononuclear leukocytes with these microorganisms (1). The present communication will deal with the uptake of pleuropneumonialike organisms (PPLO's) by monocytes and lymphocytes contained in the peripheral blood buffy coats used in these studies. As mentioned in a preliminary report (2), the fraction of mononuclear leukocytes which took up PPLO's was much in excess of the 3 to 7% accepted to represent monocytes in normal human blood (3). In addition, mycoplasmas were seen in a small percentage of cells which could be classified as lymphocytes on the basis of their ultrastructure (2, 4, 5). A more detailed electron microscopic analysis of these observations will be presented here. Furthermore, an attempt was made to determine whether the property displayed by this type of mononuclear cell is specific for mycoplasmas, or whether bacteria, viruses or inert particles can also be engulfed by lymphocytes. The experiments were extended to include human and rat thoracic duct lymph since there is evidence indicating that a proportion of peripheral blood lymphocytes differ from the majority of the thoracic duct cells in their origin and function (6, 7).

Materials and Methods

The source and preparation of *Mycoplasma pneumoniae, Mycoplasma neurolyticum,* and *Mycoplasma gallisepticum* used in these studies has been described in detail (1). In brief, these organisms were harvested during their logarithmic growth phase by centrifugation at 13,000 *g* for 10 min. They were resuspended in PPLO broth (8) to final concentrations ranging from 10^8 to 5×10^{10} organisms per ml depending on the concentration of leukocytes available for the experiment. Streptococcus Group A Type I was suspended in the same broth at similar concentrations. Thorotrast was obtained from Testagar and Co., Detroit. It contained 25%

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thorium dioxide by volume and was used by adding 0.1 ml of the undiluted preparation to 1 ml of the cell suspension. Polystyrene particles, obtained from Dow Chemical Corp., Midland, Michigan, had an average diameter of 0.8 μ. They were dialized against 0.85% saline for 24 hr following which 0.1 ml of a 30% suspension was added to the cells.

Coliphage strain T_2 was used in concentrations of 4×10^{11} organisms per ml.

Cells.—Buffy coats were taken from heparinized blood of normal subjects and prepared as described before (1). Human thoracic duct lymph was obtained on only two occasions. The specimens were divided in half; one aliquot was used in combination with *M. neurolyticum*, the other with *M. gallisepticum*. Since human thoracic duct lymph was not readily available, the remainder of the studies was carried out with rat thoracic duct lymph.[1] For this purpose, the thoracic ducts of 250 to 300 g male Sprague-Dawley rats were cannulated by the method of Bollman (9), as modified by Gowans (10). Several hours after cannulation, the cannula was cleared of clots and free flow was established. All lymphocytes were obtained during 8 to 12 hr of drainage within the first 24 hr after cannulation. The lymph was collected at room temperature in saline containing 20 units of heparin per ml. Before use, the cells were centrifuged at 150 *g* for 5 min and washed twice in Hank's saline. On conventional microscopy, the cell population had the usual characteristics of cells collected in this manner (10).

As a rule, all leukocytes were resuspended in 80% Hank's saline and 20% fresh autologous plasma to a final concentration of 10^8 cells per ml.

Incubation.—An attempt was made to adjust the concentration of microorganisms to leukocytes to a ratio of 50:1. However, it was difficult to control a certain amount of clumping of the mycoplasmas, especially when *M. pneumoniae* were used. Therefore, in some of the experiments the concentration of PPLO's was doubled or tripled. Incubation was carried out with gentle agitation at 37°C for 3, 5, 15, and 30 min, 1, 2, and 3 hr. Altogether, ten experiments were conducted with human buffy coats, ten with rat thoracic duct lymph and four with human lymph. Human blood leukocytes and rat thoracic duct lymphocytes were used with all three mycoplasma species, whereas human lymph was studied only with *M. gallisepticum* and *M. neurolyticum*. Following incubation, some specimens were sedimented and fixed as a pellet with 1% osmium tetroxide (11). In later experiments the specimens were fixed in suspension with 3% glutaraldehyde (12), following which they were sedimented and postfixed with 2% osmium tetroxide. As can be seen from the electron micrographs, the second method yielded better preservation though it did not otherwise affect the experimental results. Embedding was carried out in Epon 812 (13) essentially as described before (1). Thin sections were stained with lead hydroxide (14) and uranyl acetate (15) and viewed with a Siemens Elmiskop I electron microscope. For the purpose of counting mononuclear cells in the peripheral blood specimens, survey electron micrographs were taken at initial magnifications of 750. Such plates were photographically enlarged to make identification of intracytoplasmic microorganisms or particles possible.

RESULTS

Adherence to Cells.—Mycoplasmas adhered to all leukocytes regardless of whether the cells originated from peripheral blood or from lymph. No PPLO's were seen attached to erythrocytes.[2] The large number of microorganisms

[1] The thoracic duct lymph was kindly prepared for us by Dr. Bertran Gesner, whose collaboration in these studies is gratefully acknowledged.

[2] *M. gallisepticum* has been observed to adhere to turkey erythrocytes in the absence of serum (Gesner, B. and Thomas, L., Sialic acid binding sites; role in hemagglutination by M. gallisepticum, *Science*, 1966, **151**, 590). The present studies were carried out in the presence of serum which may account for the failure of the microorganisms to adhere to red blood cells.

surrounding each lymphocyte (Fig. 1 *a*) suggested a strong affinity of mycoplasmas for the white cells since centrifugation at 500 RPM would not have sedimented unattached PPLO's unless clumping had occurred. In contrast, when streptococci were incubated with rat leukocytes, attachment of bacteria to mononuclear cells was rarely seen (Fig. 1 *b*). Though streptococci and mycoplasmas were used in similar concentrations, fewer bacteria were present among the sedimented cells on the electron micrographs. This was attributed to loss of bacteria in the supernates which were not processed for electron microscopy. T_2 phage adhered to peripheral blood mononuclear cells as well as to human and rat lymphocytes. Since only a small number of experiments were carried out with phage, it is difficult to assess at the present time to what extent the viruses were attracted to the leukocytes. However, no phage particles were seen at a distance of more than 1 μ from the plasma membrane of the cells, which again suggested that particles which did not adhere to the cells had been removed during the dehydration or embedding procedures. Thorotrast, a heavy metal which sedimented together with the leukocytes, was not only seen randomly dispersed among the cells, but also adhered to their plasma membranes to a considerable extent. This was not the case for latex particles which neither adhered to nor sedimented with the cells at 150 *g* and which were thus only rarely found in the embedded specimens.

Phagocytosis.—The phagocytosis of mycoplasmas by neutrophils and eosinophils has been described in detail (1). Unexpectedly, PPLO's were seen within more than 50% of the mononuclear cells derived from human peripheral blood buffy coats. On the basis of their morphology, approximately 10% of these cells could be considered monocytes, another 10% had the appearance of lymphocytes, and the remaining 30% were difficult to classify. Since a comparative description of the ultrastructure of human mononuclear leukocytes is not readily available, a morphological analysis of these two cell types seems necessary.

Typical monocytes show a peripheral distribution of nuclear chromatin and a relatively small nucleolus or none at all (Figs. 2, 3 *a* and 3 *b*). Their cytoplasm contains many vesicles, a varying number of osmiophilic granules, and slender mitochondria. Though monocytes have a well developed Golgi zone, centrioles and spindle fibers are less commonly encountered than in lymphocytes. 15 min of incubation with PPLO's resulted in the appearance of many phagocytic vacuoles which contained more than one and often as many as ten mycoplasmas in a single section (Fig. 3 *b*). Lymphocytes (Fig. 4 *a*) are somewhat smaller in diameter and have a larger nucleo-cytoplasmic ratio than monocytes. Their nuclear chromatin is not as peripherally located and is usually clumped throughout the nucleus. The cytoplasm of lymphocytes shows fewer vesicles except in the Golgi region and is almost completely devoid of lysosomes. Their mitochondria appear wider than those of monocytes, and centrioles are almost always seen when the plane of section traverses the Golgi zone (Figs. 4 *a*, 7 *a*

and 8 *a*). After 15 min of incubation with PPLO's, lymphocytes showed few phagocytic vacuoles which rarely contained more than three organisms. As has already been mentioned, the remaining 30% of blood mononuclear cells which had ingested PPLO's could not be classified with such certainty. However, in the absence of phagocytosis, most of these cells would probably have been considered lymphocytes on the basis of their morphology. Thus, the cell depicted in Fig. 4 *b* has the "hand mirror" (3) configuration and the chromatin distribution believed to be characteristic of lymphocytes. The cytoplasm shows only a few small profiles of rough endoplasmic reticulum (ER) and a paucity or perhaps even complete absence of lysosomes. The perinuclear fibrils seen in this cell are more often found in macrophages (16), but they have also been encountered in lymphocytes (4). In spite of the fact that this specimen had been incubated with *M. gallisepticum* for 1 hr only two vacuoles with three organisms are observed. Nevertheless, the vesicular appearance of the cytoplasm makes it impossible to exclude the possibility that this particular electron micrograph represents a thin section of a monocyte rather than a lymphocyte. When incubation was prolonged beyond 15 min, another feature appeared useful in distinguishing monocytes from lymphocytes. Within monocytes, the phagocytized microorganisms became altered or unrecognizable within a short time. Examples are shown in Figs. 5 *a* and 5 *b*. Fig. 5 *a* depicts a section of a monocyte which had been incubated with *M. gallisepticum* for only 15 min. The organisms surrounding the cell show a well preserved ultrastructure, but within the phagocytic vacuoles, their morphology has been altered. The osmiophilic particles, believed to be ribosomes (17), are now lined up in a spiral array giving the organism the so called corncob appearance. This phenomenon has been considered evidence of denaturation by Manilof et al. (17). Peripheral blood monocytes which had been incubated with the Bru strain of *M. pneumoniae* showed large osmiophilic inclusions within 15 min (Fig. 5 *b*). Since mycoplasmas could often be identified within these inclusions, it is likely that the structures represented phagolysosomes; i.e., that they were the result of coalescence of PPLO-containing phagocytic vacuoles with the cells' lysosomes. In typical lymphocytes such bodies were not seen up to 3 hr of incubation with the microorganisms. The mycoplasmas remained identifiable over this period of time even though prolonged incubation in this medium damaged the ultrastructure of the lymphocytes as well as that of the microorganisms.

Because of the difficulty in classifying mononuclear cells, and because the concept that lymphocytes may phagocytose microorganisms is a controversial one, the experiments were repeated with rat and human thoracic duct lymph. As can be seen in Figs. 1 *a*, 6, 7 *a*, 7 *b*, 8 *a* and 8 *b*, adherence of PPLO's to thoracic duct lymphocytes was as striking as the affinity of the microorganisms for peripheral blood mononuclear leukocytes. Some cells (Fig. 6) showed the development of long cytoplasmic processes which were reminiscent of those

described in PPLO-infected tissue cultures (1). Mycoplasmas were seen along and in between these processes rather than on their tips. In the absence of PPLO's, the cells had only short pseudopods which rarely exceeded 0.5 μ in length. Thoracic duct lymphocytes also took up mycoplasmas (Figs. 7, 8 *a*, and 8 *b*), but the number of cells which showed intravacuolar PPLO's averaged only 6% and never exceeded 8% of the total cell count. In two specimens, one originating from human lymph incubated with *M. neurolyticum* and one rat lymph incubated with *M. gallisepticum*, the incidence of cells with PPLO's was as low as 4%. In all instances the intracellular microorganisms were located within vacuoles, allowing for clear delineation of the vacuolar unit membrane, the plasma membrane of the PPLO, and the intervening space. Since prolonged incubation was detrimental to the ultrastructure of the lymphocytes as well as the mycoplasmas, many inclusions which could not be identified with certainty had to be disregarded. In general, the morphology of the mycoplasmas within lymphocyte vacuoles resembled that of the PPLO's surrounding the cells. At times, specific structures, such as strands of "nuclear" material (1, 18) (see inset, Fig. 7 *a*), helped to identify the microorganism.

Though streptococci and latex particles were readily ingested by monocytes present in these preparations, these agents could never be demonstrated within lymphocytes. On the other hand, T_2 phage and thorotrast were always seen in a small percentage of thoracic duct lymphocytes (Figs. 8 *c* and 8 *d*). Precise counts have not been carried out, but it is estimated that these particles were also taken up by about 4 to 6% of the thoracic duct cells. As was the case with PPLO's, both T_2 phage and thorotrast were found in membrane-bound vesicles and neither one of these agents was ever seen free in the cytoplasm.

DISCUSSION

Phagocytosis of microorganisms or foreign particles by peripheral blood "lymphocytes" has been reported by a number of investigators (19–22). Others have claimed that "lymphocytes" first undergo transformation into larger cells before they become phagocytic (23, 24). Most of these studies were carried out with the limitations of conventional microscopy, and in none of them were the cell type or its source clearly defined. Suggestions have appeared in the literature indicating that "blood lymphocytes" comprise a mixture of cells of varying origin and function and that only a certain proportion of these mononuclear cells have the properties attributed to the majority of thoracic duct lymphocytes (6, 7, 25). It has been reported that rabbit thoracic duct lymphocytes cannot phagocytose microorganisms or starch grains (26), and that rat thoracic duct lymphocytes do not appear as macrophages in artificially induced inflammatory sites (7). Moreover, while phytohemagglutinin-stimulated lymphocytes assume the morphology of macrophages, it has been impossible to show that such cells become phagocytic (27, 28). On the other hand, tagged rat thoracic

duct lymphocytes infused into syngeneic recipients could be traced to the liver sinusoids where they were observed to phagocytose intravenously injected bacteria (29). In view of these controversial findings, data concerned with the phagocytosis of microorganisms or foreign particles by lymphocytes must be interpreted with caution even when the cells are defined on the basis of their ultrastructure. In the studies under discussion, more than 50% of peripheral blood mononuclear cells showed intravacuolar PPLO's. In some instances, it could be argued that the vacuoles merely represented cross-sections of cytoplasmic invaginations occupied by mycoplasmas as had been shown in tissue culture cells (1). However, in most cases, the indentations of the remaining plasma membrane were not deep enough to make this possibility likely (Figs. 4 *a*, 7 *a*, 7 *b*, 8 *a* and 8 *b*), and the cells which had developed the longest processes (see Fig. 6) did not necessarily show any cytoplasmic vacuoles. Moreover, unrelated studies in this laboratory have shown that a similar percentage of blood mononuclear cells can ingest rheumatoid factor complex precipitates (30) which do not induce the development of long cytoplasmic processes. The buffy coats used in these experiments were obtained from normal subjects who did not have a monocytosis. Thus, it may be deduced that the PPLO's were taken up by cells which looked like lymphocytes on routine blood smears. Perhaps the term "lymphocyte" when applied to the peripheral blood mononuclear cell will have to be redefined.

In thoracic duct lymph, the number of mononuclear cells which had taken up mycoplasmas amounted to only 6%. Here the classification of individual cells as lymphocytes was not as difficult since typical monocytes are rarely present in lymph. It is noteworthy that a similar percentage of thoracic duct lymphocytes was able to engulf T_2 phage and thorotrast, but that streptococci or latex particles were never found in lymphocytes under identical experimental conditions. In this regard, it may be relevant to consider that lymphocytes do not exhibit chemotaxis towards most test objects as do granulocytes and monocytes (26). For phagocytosis by lymphocytes to occur, it may be necessary for the test substance first to be attracted to the cell. Adherence to the plasma membrane may then initiate the complex series of reactions which lead to its invagination. Mycoplasmas, T_2 phage, and thorotrast appeared to fulfill such a requirement, whereas streptococci and latex particles displayed no affinity for the plasma membrane of lymphocytes. The observation that mycoplasmas were rarely seen on the tips of pseudopods but more commonly along and in between the cytoplasmic processes of the cells is consistent with this hypothesis. Moreover, agitation of the cell suspension increases the chances of collision and may enhance a process which may have remained inapparent if the suspension had been kept stationary during the incubation period. The fact remains that, under defined experimental conditions, a certain percentage of "lymphocytes" can be shown to take up mycoplasmas. The implications of this

observation are manifold. Firstly, the uptake of a potential antigen by cells of the lymphoid series is of interest since such a process could bypass the presumed role of the macrophage in the immune response. In this connection, it should be mentioned, however, that within the 3 hr period of observation no changes suggestive of antibody synthesis, such as an increase in ribosomes or development of rough endoplasmic reticulum, were noted in lymphocytes which had taken up phage particles or PPLO's. Secondly, lymphocytes which had engulfed mycoplasmas did not develop osmiophilic bodies which monocytes displayed within a short time following phagocytosis (Figs. 5 *a* and 5 *b*). In monocytes, PPLO's had become unrecognizable when coalescence of the cells' lysosomes with the phagocytic vacuoles had occurred. In lymphocyte vacuoles the morphology of the microorganism remained essentially unaltered as phagolysosomes did not seem to develop. Thus, the paucity of lysosomes in lymphocytes may protect mycoplasmas from early intracellular digestion. Indeed, if it could be demonstrated that PPLO's were taken up by the class of lymphocytes which are known to be long-lived (31–33), it would not be inconceivable that viable components of the microorganisms could reside in lymphocytes for prolonged periods of time. Lastly, the studies seem to support the concept that blood lymphocytes consist of a different mixture of cells than thoracic duct lymphocytes. In human as well as rat thoracic duct lymph, only a very small percentage of cells took up PPLO's even though the microorganism adhered to all cell types. Correlation of cell size to phagocytosis was not impressive in thoracic duct lymph, though in blood, PPLO's were never seen in cells which had only a small rim of cytoplasm. The unexpectedly high incidence of peripheral blood lymphocytes which had taken up PPLO's suggests that phagocytosis is more frequently a function of the type of lymphocyte that circulates primarily in peripheral blood than the type that is primarily associated with lymph. Since the two circulations are in continuity, a certain amount of admixture is to be expected. The observation is consistent with the existence of two populations of lymphocytes (6, 7, 33) and can be subjected to further experimental verification.

SUMMARY

The incubation of mycoplasmas with human peripheral blood buffy coats resulted in the uptake of these microorganisms by more than 50% of the mononuclear cells. Mycoplasmas adhered to the plasma membranes of all leukocytes, most of which developed long cytoplasmic processes not seen in the controls. In human and rat thoracic duct lymph, about 6% of the cells ingested the microorganisms. T_2 phage and thorotrast were taken up by a similar percentage of lymphocytes. On morphological grounds, the cells which were able to take up PPLO's or particles could not be distinguished from the cells which were incapable of this function. Following phagocytosis, neither the cell nor the micro-

organism showed any morphological alterations over a 3 hr period of observation. The demonstration that a small percentage of "lymphocytes" are able to phagocytose may have pathological and immunological implications.

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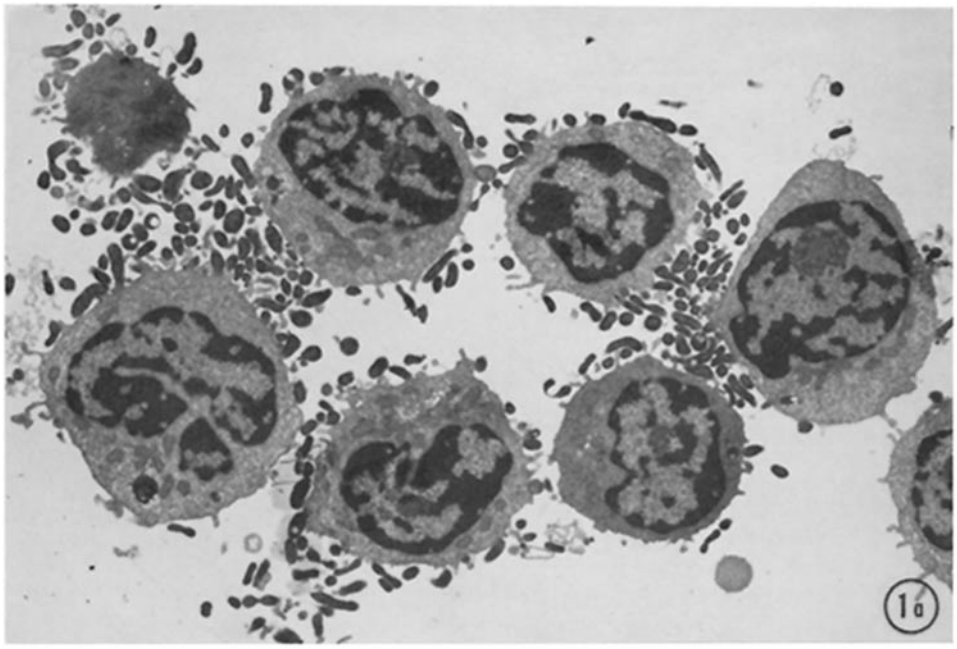
EXPLANATION OF PLATES

PLATE 44

FIG. 1 *a*. Human thoracic duct lymphocytes incubated with *M. gallisepticum* for 15 min. Affinity of the microorganisms to the plasma membrane of the mammalian cells is readily apparent. Fixed in 3% glutaraldehyde, 2% osmium tetroxide. × 5500.

FIG. 1 *b*. Rat thoracic duct lymphocytes incubated with streptococci for 15 min. Bacteria appear randomly distributed. No affinity to the plasma membrane of the lymphocytes is apparent. Fixed in 3% glutaraldehyde, 2% osmium tetroxide. Erythrocytes (*E*), streptococci (*S*). × 5000.



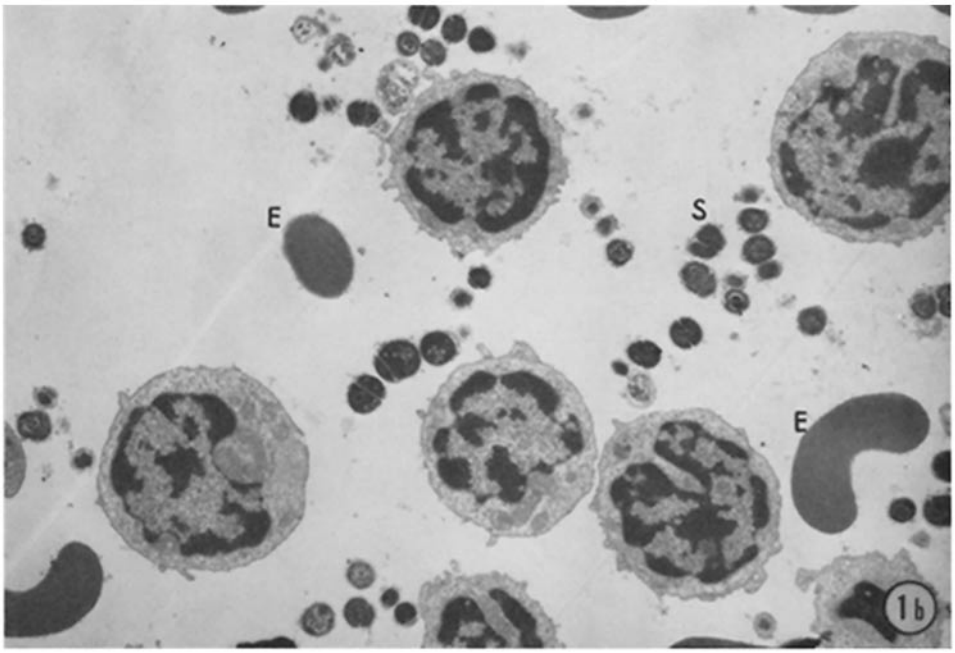


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PLATE 45

FIG. 2. Typical peripheral blood monocyte (see text) from specimen which had been incubated with *M. gallisepticum* for 3 min. Arrow points to vacuole containing microorganism. The characteristic "bleb" (1) is still recognizable. Note peripheral distribution of nuclear chromatin. Lysosomes (*L*); mitochondrion (*M*); golgi zone (*G*); nucleus (*N*). Fixed in suspension with equal amount of 2% osmium tetroxide. × 22,000.

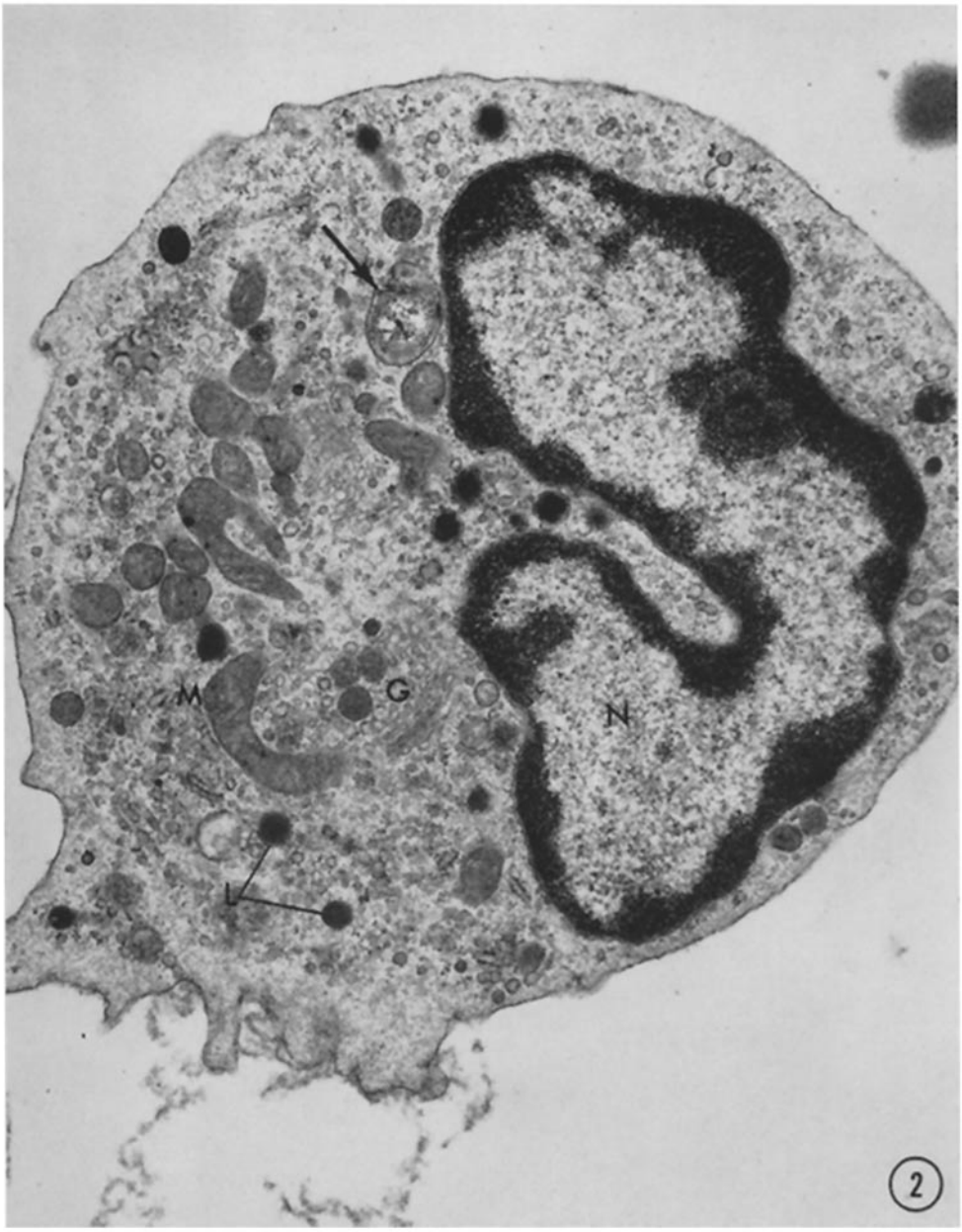

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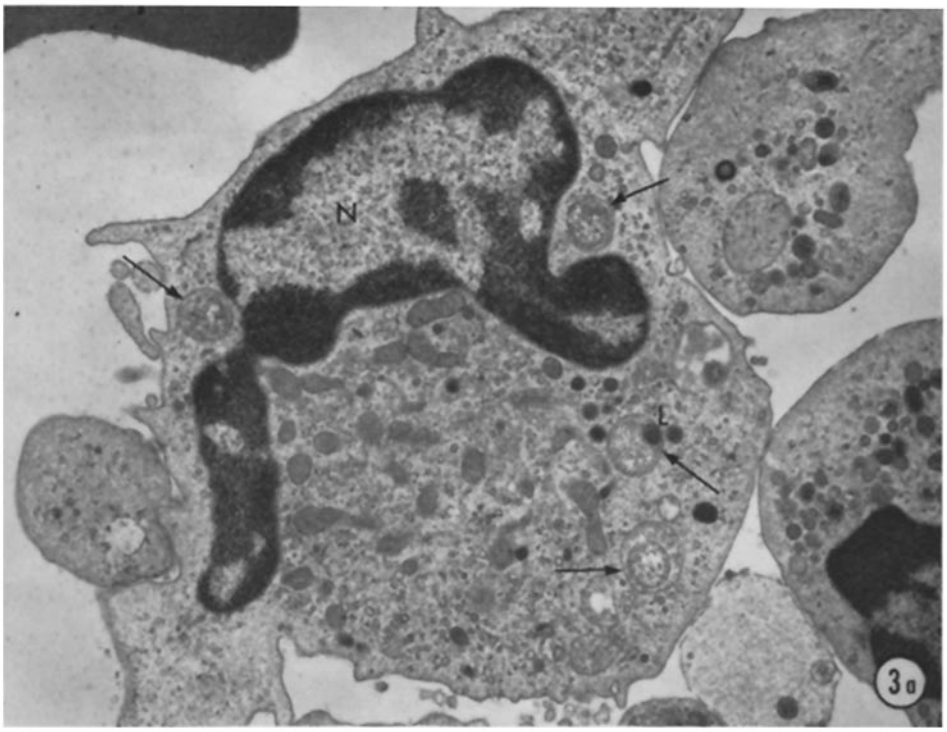
PLATE 46

FIG. 3 *a*. Peripheral blood monocyte taken from same specimen as cell depicted in Fig. 2. Several phagocytic vacuoles containing *M. gallisepticum* are seen (arrows). Vacuole on the right contains the microorganism as well as one of the cell's lysosomes (*L*). Nucleus (*N*). × 12,000.

FIG. 3 *b*. Human peripheral blood monocyte which had been incubated with *M. neurolyticum* for 15 min. Many phagocytic vacuoles can be seen. The largest shows about fourteen mycoplasmas, one of which has maintained the characteristic ultrastructure of *M. neurolyticum* (arrow) in the log growth phase (1). Several poorly preserved organisms are seen outside the cell. Fixed in suspension with equal amounts of 2% osmium tetroxide. Mitochondrion (*M*); nucleus (*N*); mycoplasmas (*P*). × 13,000.



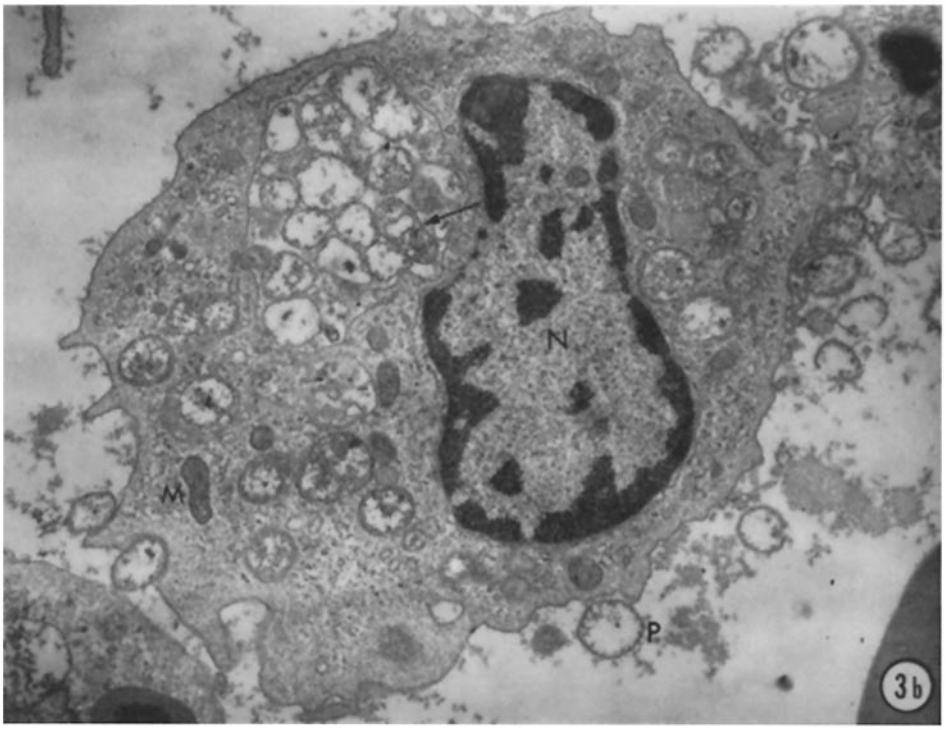


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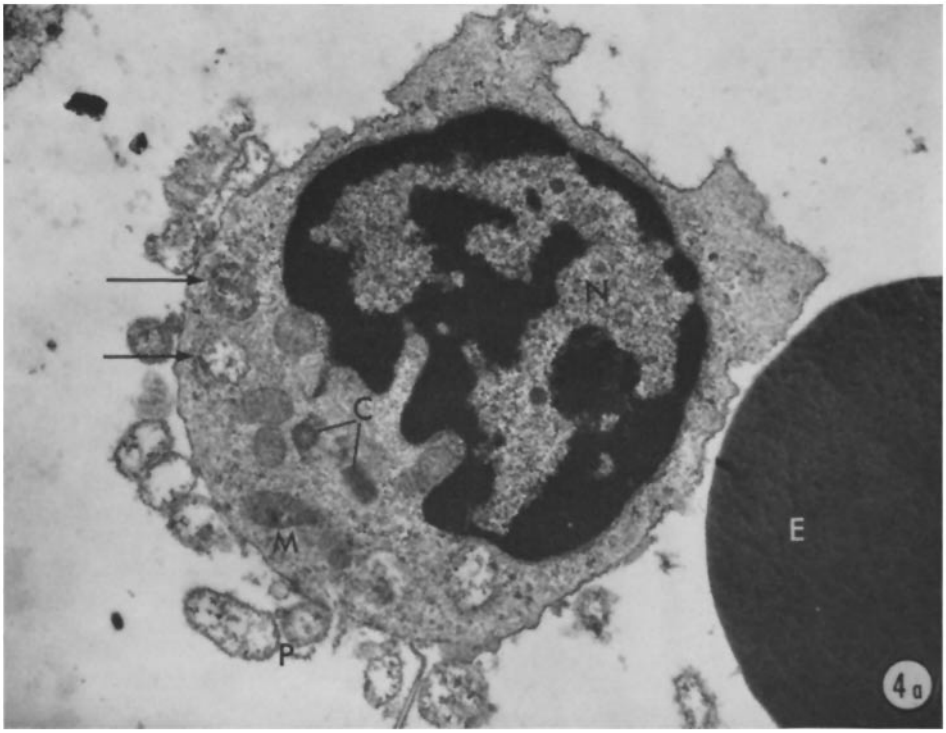
PLATE 47

FIG. 4 *a*. Small lymphocyte taken from specimen of human peripheral blood buffy coat incubated with *M. neurolyticum* for 15 min. Organisms on plasma membrane as well as those in phagocytic vacuoles (arrows) are poorly preserved. Note centrioles (*C*) which are rarely seen in monocytes. Mitochondrion (*M*); nucleus (*N*); erythrocyte (*E*); mycoplasmas (*P*). Fixation with 1% osmium tetroxide following sedimentation of pellet. × 17,000.

FIG. 4 *b*. Peripheral blood monocyte with "hand mirror" configuration. In thin section this cell is difficult to distinguish from a lymphocyte (see text). Specimen was incubated with *M. gallisepticum* for 15 min. One of the phagocytized PPLO's still shows characteristic "bleb" (arrow). Nucleus (*N*); mitochondrion (*M*); perinuclear fibrils (*F*). Fixed in 2% osmium tetroxide. × 16,000.



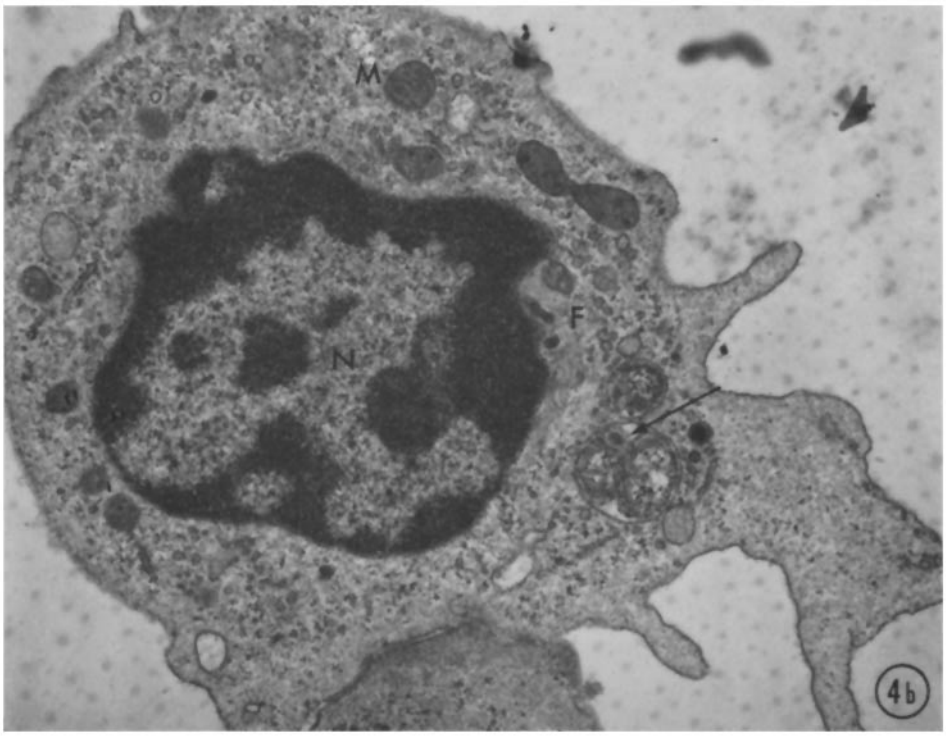


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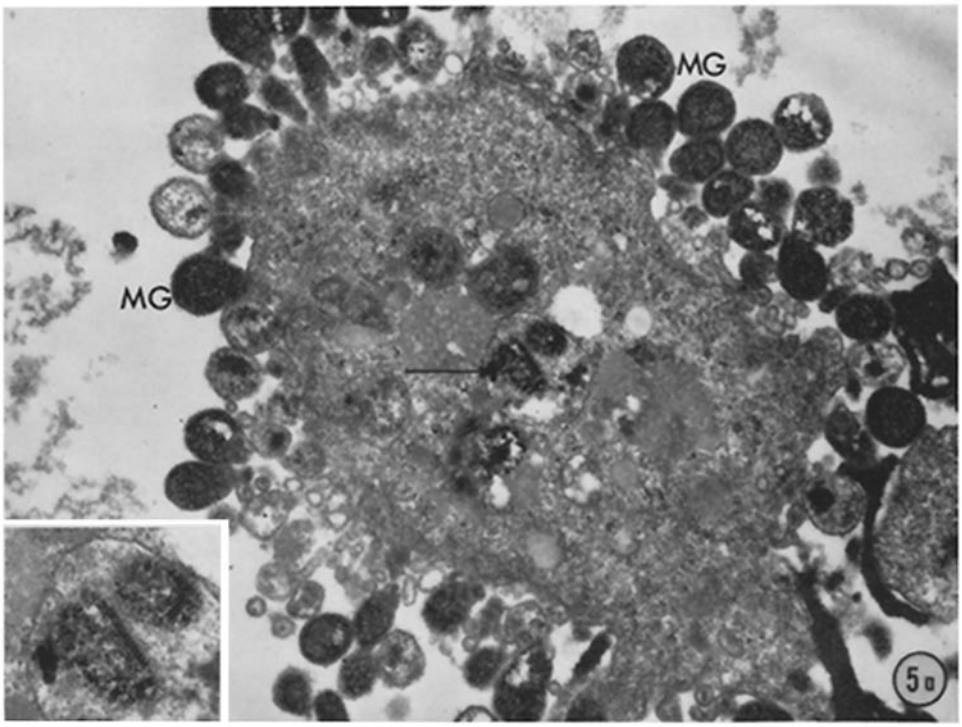
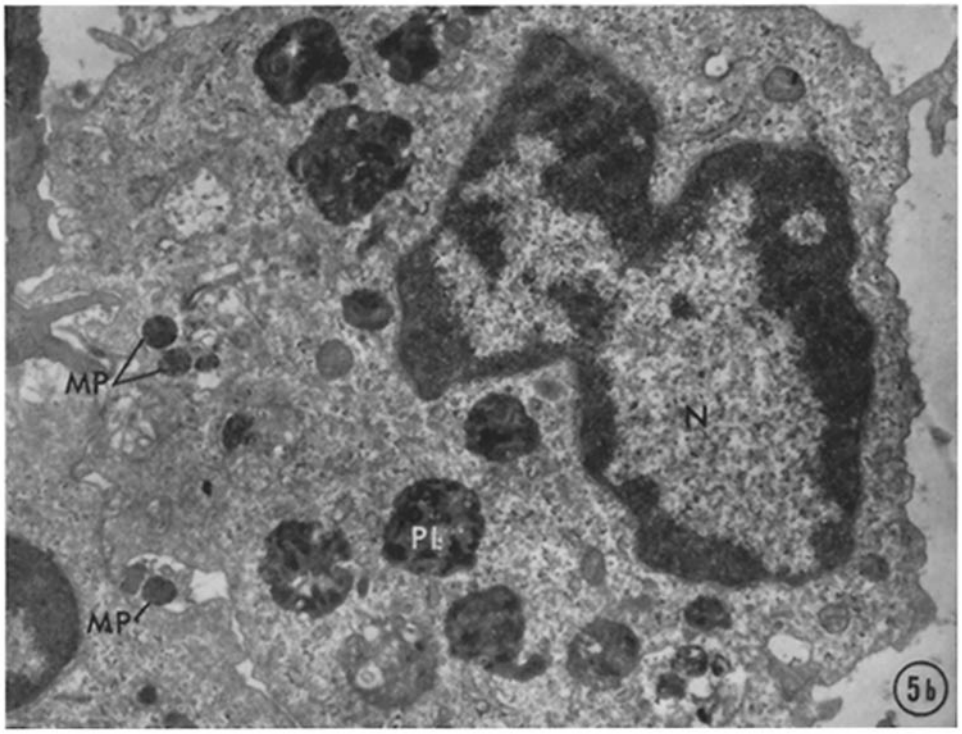
PLATE 48

FIG. 5 *a*. Peripheral blood monocyte incubated with *M. gallisepticum* (*MG*) for 15 min. Note polarity of the microorganisms in relation to the plasma membrane of the cell. The ribosomes of mycoplasmas in phagocytic vacuoles have assumed a spiral arrangement. Arrow points to organism which is seen to better advantage at higher magnification in inset. None of the PPLO's surrounding the cell show such arrays. Fixed in suspension with 3% glutaraldehyde followed by 2% osmium tetroxide. × 19,000 (inset, × 40,000).

FIG. 5 *b*. Peripheral blood monocyte incubated with *M. pneumoniae* for 15 min. Large osmiophilic bodies are presumed to be phagolysosomes (*PL*). In this particular cell, mycoplasmas can no longer be identified except extracellularly (*MP*). Nucleus (*N*); phagolysosomes (*PL*). Fixed in glutaraldehyde and osmic acid. × 15,000.





(Zucker-Franklin et al.: Interaction of mycoplasmas with mammalian cells. II)

PLATE 49

FIG. 6. Human thoracic duct lymphocyte incubated with *M. gallisepticum* (*MG*) for 15 min. Cytoplasmic processes (*CP*) extend over considerable distance from the cell surface sometimes giving the appearance of being detached from the cell since portions are located beyond the plane of section. Mycoplasmas are attached alongside and in between the processes rather than to their tips. No phagocytosis is evident in this section. Mitochondrion (*M*); nucleus (*N*). × 21,000.

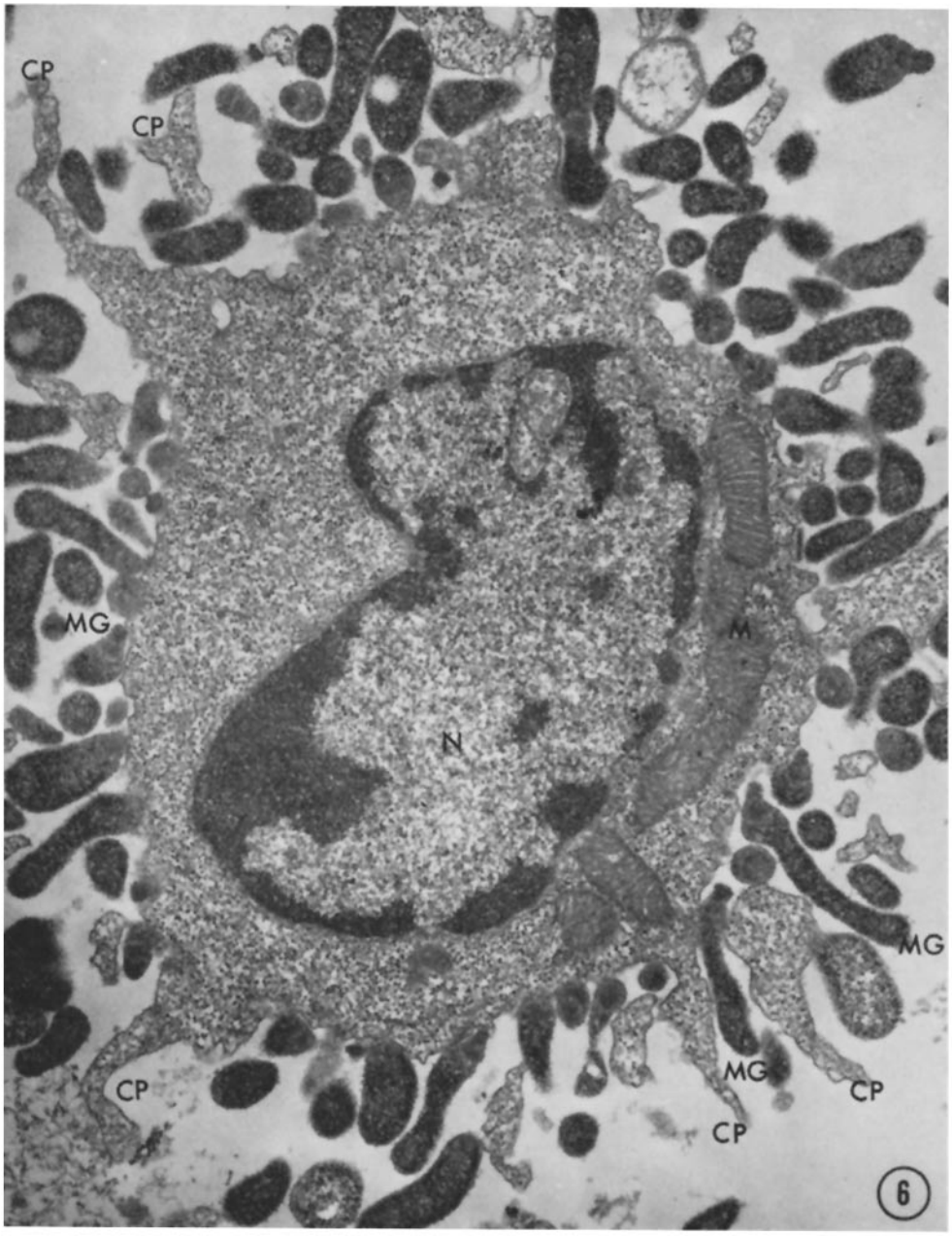


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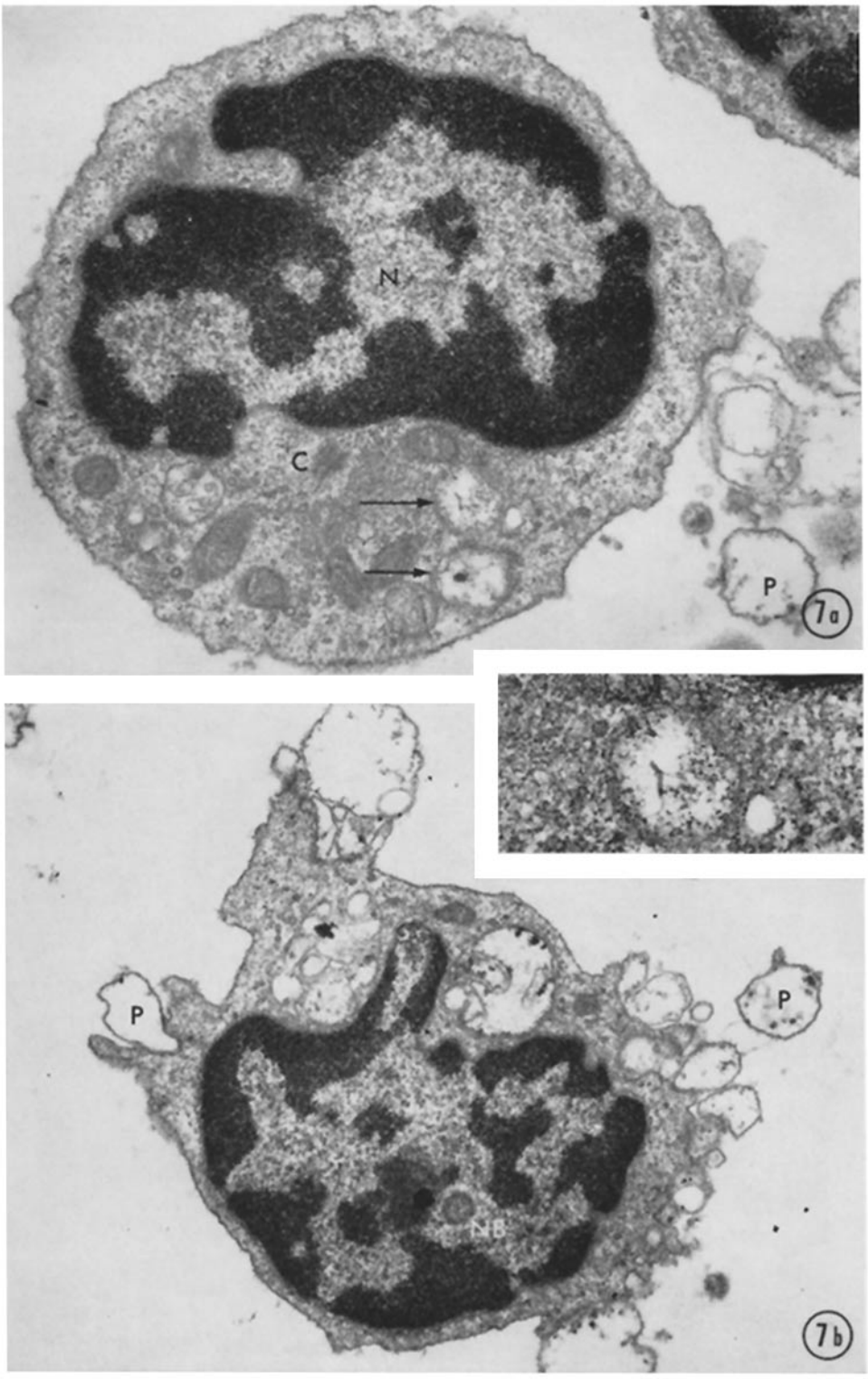
PLATE 50

FIGS. 7 *a* and 7 *b*. Rat thoracic duct lymphocytes incubated with *M. neurolyticum* for 15 min. In this specimen, the microorganisms were poorly preserved but in both electron micrographs, the structures within the vacuoles clearly resemble the mycoplasmas (*P*) in the surrounding medium.

FIG. 7 *a*. The organism in the upper vacuole shows the strand of fibrillar material which is presumed to be DNA and has been described in the intact organism (1, 18). The inset shows this at higher magnification. Centriole (*C*); nucleus (*N*). × 23,000 (inset, × 44,000).

FIG. 7 *b*. Depicts several partially lysed microorganisms in the process of being engulfed as well as two phagocytic vacuoles containing the structures. Myco-plasmas (*P*); nuclear body (*NB*) often seen in the lymphocytes of rats. Fixed in 1% osmium tetroxide. × 14,000.



(Zucker-Franklin et al.: Interaction of mycoplasmas with mammalian cells. II)

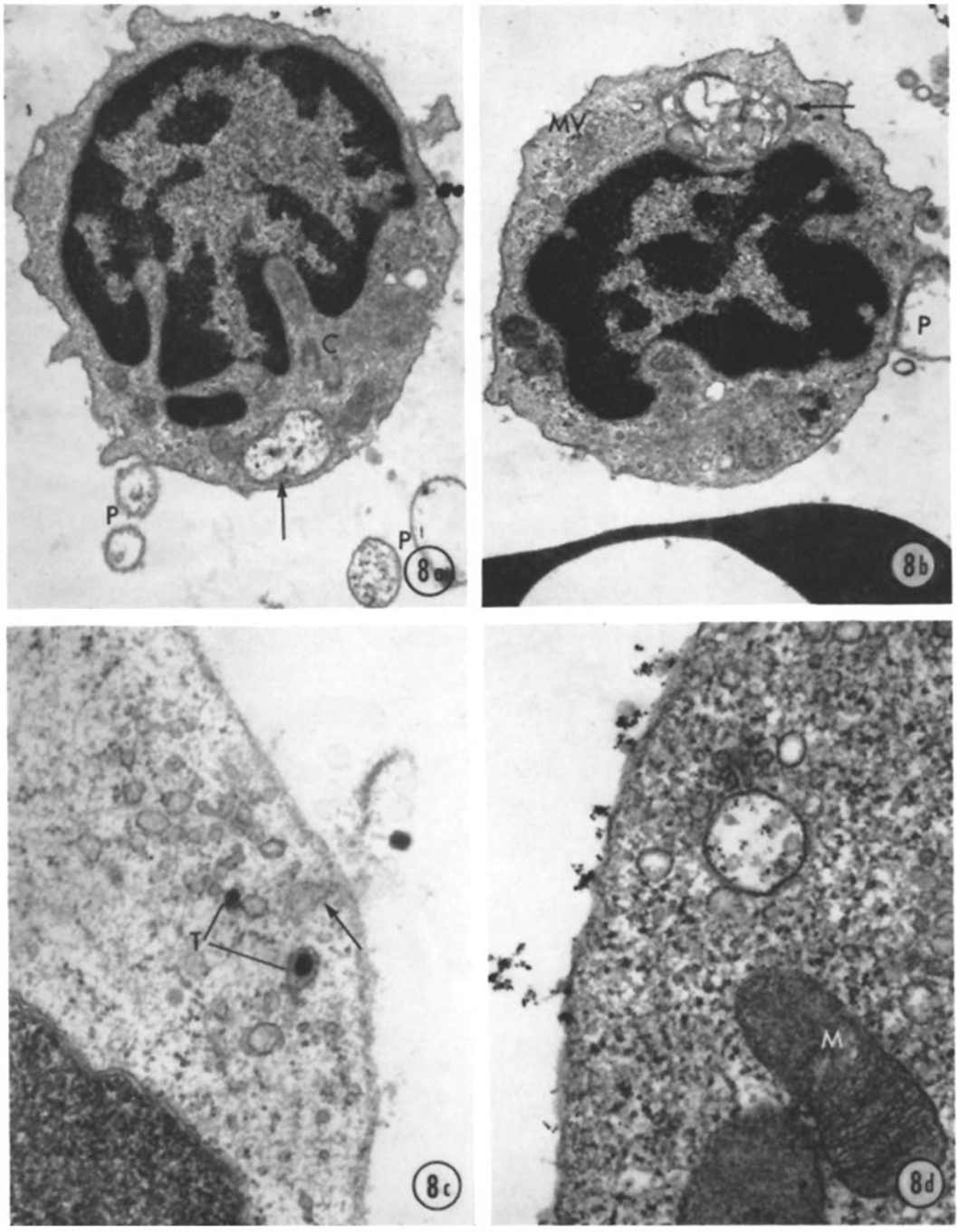
PLATE 51

FIG. 8 *a*. Rat thoracic duct lymphocyte incubated with *M. neurolyticum* for 2 hr. Appearance of mycoplasma within the vacuole (arrow) does not differ much from the organisms outside the cell (*P*). Note centriole (*C*), and absence of lysosomes. × 14,000.

FIG. 8 *b*. Rat thoracic duct lymphocyte incubated with *M. neurolyticum* for 3 hr. Organisms surrounding cell appear as badly lysed as those within phagocytic vacuole (arrow). There are no typical lysosomes though two multivesicular bodies (*MV*) can be seen within the vicinity of the vacuole. Mycoplasma (*P*). × 15,000.

FIG. 8 *c*. Detail of rat thoracic duct lymphocyte incubated with T_2 phage for 30 min. Two phage particles can be seen within cytoplasmic vesicles (*T*). One particle devoid of a nucleoid appears to be in the process of being engulfed (arrow). Several particles are seen outside the cell attached to some membranous material adherent to the cell. Fixed in osmium tetroxide. × 43,000.

FIG. 8 *d*. Detail of rat thoracic duct lymphocyte incubated with thorotrast for 30 min. Particles adhere to the plasma membrane and can also be seen in a cytoplasmic vacuole. Fixed in osmium tetroxide. Mitochondrion (*M*). × 51,000.



(Zucker-Franklin et al.: Interaction of mycoplasmas with mammalian cells. II)