CHAPTER 8

The Mycoplasmataceae—"Pleuropneumonia-like Organisms" (PPLO)

BY RUTH M. LEMCKE

It may be questioned by purists whether this group of micro-organisms, the *Mycoplasmataceae*, should be included in a book entitled "Focus on Bacteria". However, on aesthetic considerations alone, the bizarre and often beautiful forms exhibited by organisms of this group warrant their inclusion in such a microbial picture gallery. Moreover, the group is interesting biologically just because it does not quite fit into any of the three man-made groups of micro-organisms, that is to say, bacteria, fungi or protozoa. Of course, this only goes to show the limitations of any system of biological classification, however useful and necessary such systems may be for practical purposes.

Unlike true bacteria, mycoplasmas do not have a rigid limiting cell wall, only a thin cytoplasmic membrane. This means that the cells are more plastic than those of bacteria and that the mycoplasmas are able to assume quite a variety of different shapes. In cultures of mycoplasmas, also, some very minute cells are formed which can pass through fine filters capable of holding back the very smallest bacteria. The smallest cells appear to be of the same order of size as the larger viruses such as *vaccinia*, that is to say 100–200 mμ in diameter. However, mycoplasmas can be grown on artificial media in the laboratory, whereas viruses need living cells, either in animals, chick embryos or tissue cultures in which to grow. In this respect mycoplasmas resemble bacteria, although they are fastidious organisms and most species require the presence of complex substances such as serum and yeast extract in the artificial culture media. Mycoplasma cells differ from protozoa in being much less highly organized; there are no special organelles (for example, vacuoles through which food is ingested) and no well defined nuclear apparatus.

Studies of the chemical structure of the *Mycoplasmataceae* suggest that, apart from the absence of a cell wall, they resemble bacteria and it is probably to the latter that they are most closely related. However, it is perhaps not surprising that they have been regarded rather as oddities by microbiologists.

What, then, of the appearance of these curious micro-organisms? When grown in broth the cells vary in shape from spherical or disc-like forms to more elongated rods and filaments. Some of the forms seen in a broth culture of the mycoplasma causing *agalactia* of sheep and goats are shown in Fig. 74. The predominant form here seems to be ring-like

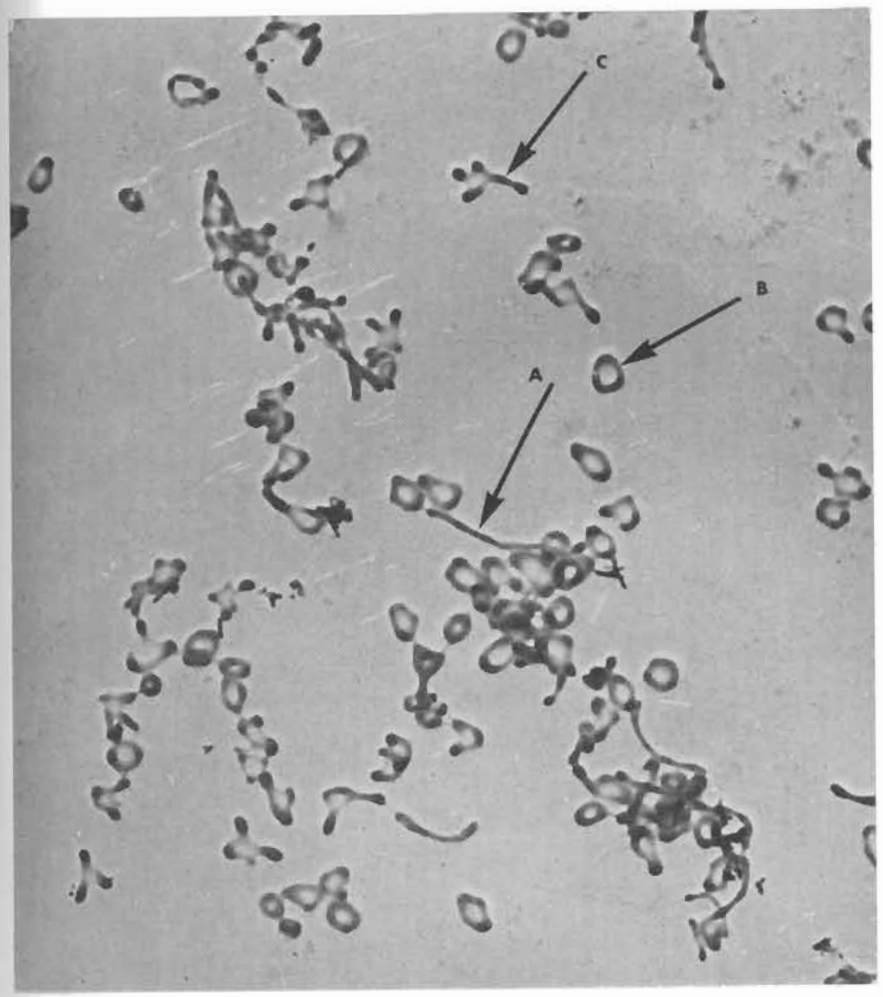

FIG. 74. *Mycoplasma* causing agalactia of sheep and goats. Electron micrograph by E. Klieneberger-Nobel and R. C. Valentine. *See* E. Klieneberger-Nobel (1963) "Recent progress in Microbiology", Vol. VIII, plate 3, p. 504, University of Toronto Press. This photograph shows the various forms which the organism of agalactia of sheep and goats can develop in a broth culture. There are filamentous forms (A) and many ring-like forms with a thickened periphery and thin centre (B). Some organisms have produced sprouts from which new outgrowth seems to develop (C). Magnification: × 8500.

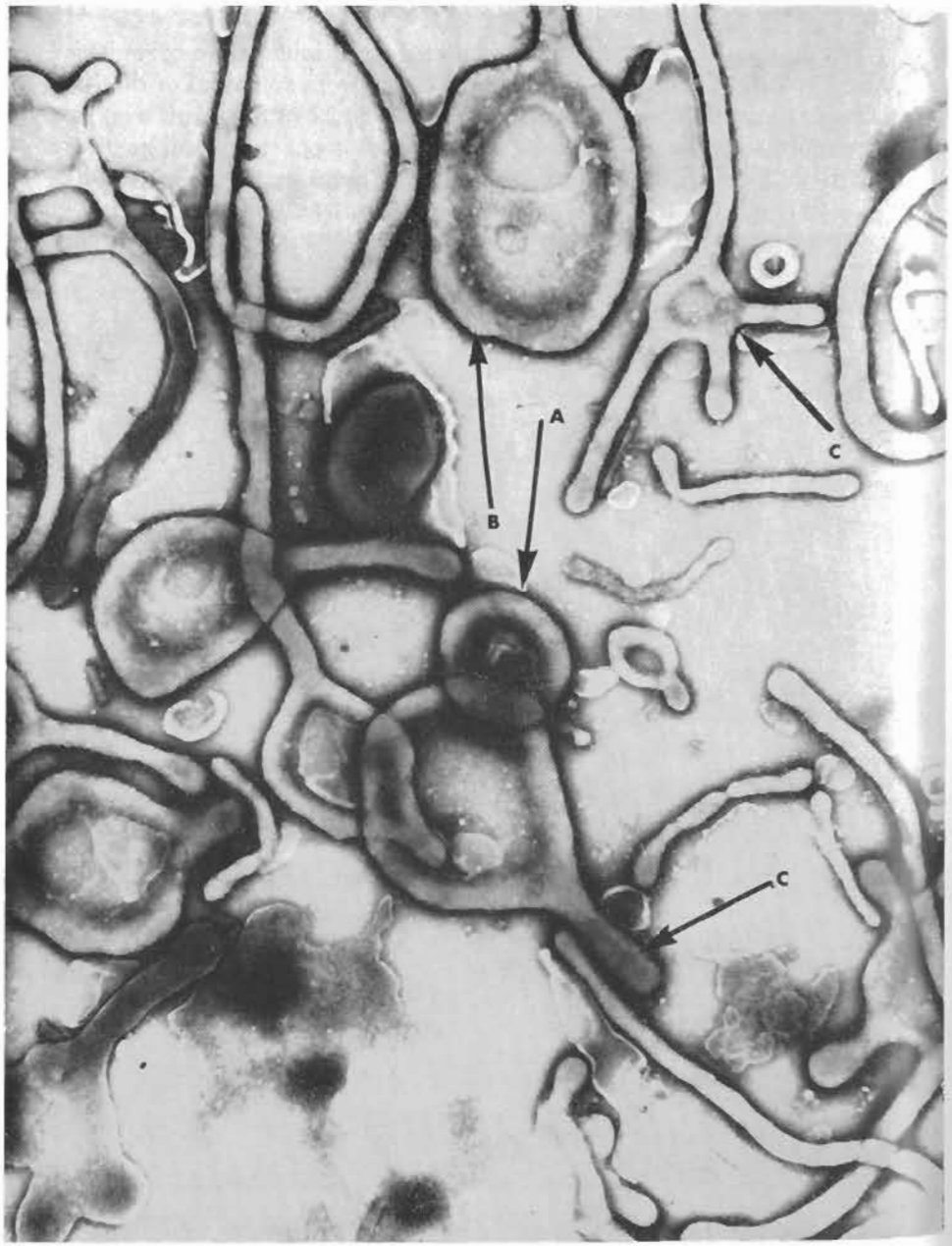

FIG. 75. *Mycoplasma* causing contagious bovine pleuropneumonia. Electron micrograph (by courtesy of H. P. Chu). Negative staining with phosphotungstic acid. The organism is shown here at a very high magnification; the negative stain, condensed round the edges of the organisms, shows them up in clear relief. There are round (A) and oval (B) forms with thickened edges appearing white. Many organisms have one or several filamentous sprouts (C) Magnification: × 40,000.

with a thickened periphery and thin centre. In actual fact, these forms may be more nearly spherical in life, but the procedures involved in preparing specimens for the electron microscope may cause some collapse of the cells, especially if the material at the centre of the cells is different from that nearer the outer membrane. Some of these ring-like forms appear at their thickened edges to be giving rise to fresh outgrowths or "sprouts" so that they have a medusa-like appearance; some of these sprouts have already elongated into distinctly rod-like forms.

The appearance of rods or filaments growing out from the rounded forms is shown still more clearly in Fig. 75. Here the mycoplasma causing *contagious pleuropneumonia of cattle* has been negatively stained for the electron microscope with an electron-dense substance, phosphotungstic acid. This substance, deposited round the limiting membrane of the cells, shows them up in clear relief. Magnified 40,000 times, the appearance is indeed bizarre, and it is not difficult to see the resemblance of these forms to the germinating spores of larger micro-organisms such as streptomycetes and fungi. It is not surprising that some of the early microbiologists described this species—the first mycoplasma to be discovered—as a miniature fungus.

Other species, such as the mycoplasma isolated from a tissue culture (Fig. 76), grow predominantly in long threads or filaments. Sometimes, as in Fig. 77, which is a picture of a mycoplasma causing *bronchopneumonia* in rats, the long threads consist of chains of very minute round forms, like a string of beads. As this micrograph is at a magnification of 7500, the extreme fineness and delicacy of the smallest round forms can be appreciated; only the magnifications possible with the electron microscope can reveal them properly.

On solid media, where the cells are supported by the underlying agar, they are very thin but grow to a larger size than in liquid medium. These thin cells vary quite considerably in size and often in shape. The micro-colony of the agalactia organism shown in Fig. 78 illustrates this. The individual cells are best seen at the very edge, where the colony is only one cell thick. They are thin and delicate with the most densely-staining material at the periphery of the cell next to the limiting membrane, giving the whole colony a lace-like appearance. In a bacterial colony the individual cells are more sharply delineated due to the presence of the cell wall (see for example Fig. 21), but in this photograph, where the mycoplasma cells have only a delicate cell membrane, the cell contours are more difficult to pick out. The colony shown in Fig. 78 was grown on a thin plastic film overlying an agar medium. If the mycoplasma is not separated from the agar in this way, the centre of the colony embeds itself in the agar, forming a thick core, whereas peripherally the cells spread out in a thin layer on the surface.

This gives a characteristic "fried egg" appearance to the colony (Fig. 3 (g)). Recent work suggests that the minute mycoplasma cells inoculated on to the surface of the agar are first drawn down by *capillarity* into the narrow spaces which separate the *fibrils* of agar. Inside the agar they

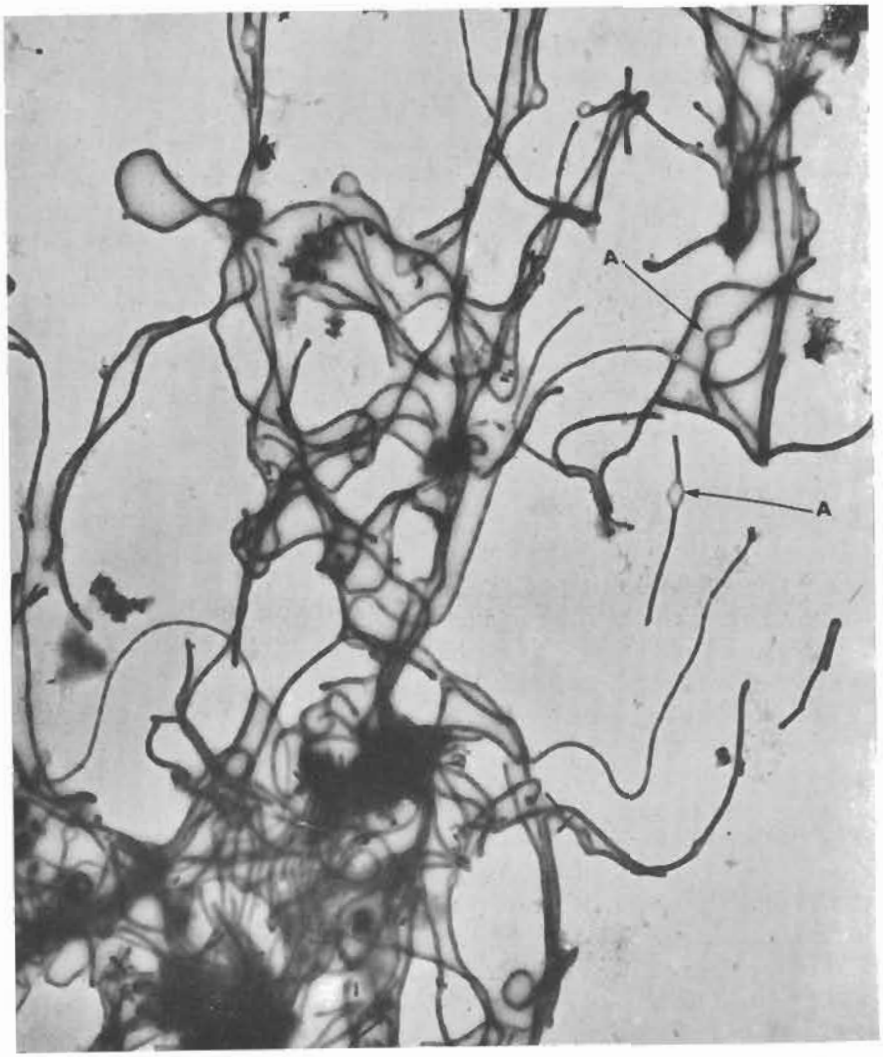

FIG. 76. *Mycoplasma* isolated from tissue culture, but originating from the human throat. Electron micrograph by E. Klieneberger-Nobel and R. C. Valentine. *See* E. Klieneberger-Nobel (1963). "Recent progress in Microbiology", Vol. VIII, plate 3, p. 304, University of Toronto Press. The broth culture of this organism showed mainly filamentous growth; however, oval bodies were also present (A). Magnification: × 8000.

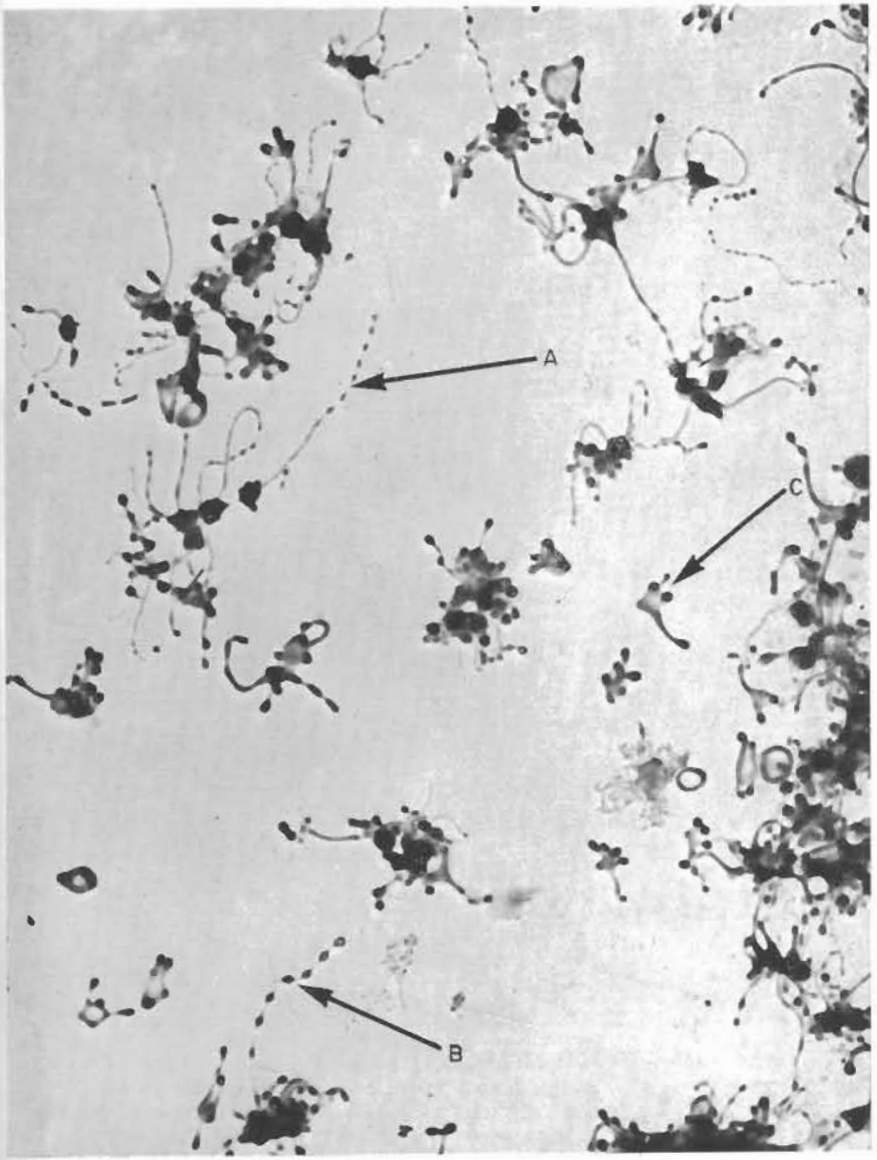

FIG. 77. *Mycoplasma* causing bronchopneumonia in rats. Electron micrograph by E. Klieneberger-Nobel and R. C. Valentine. *See* E. Klieneberger-Nobel (1963). "Recent progress in Microbiology", Vol. VIII, plate 3, p. 304, University of Toronto Press. This photograph shows filamentous forms with minute granules (A); these increase and grow into bigger elements (B). Forms which have produced sprouts are seen at (C). Magnification: 7500.

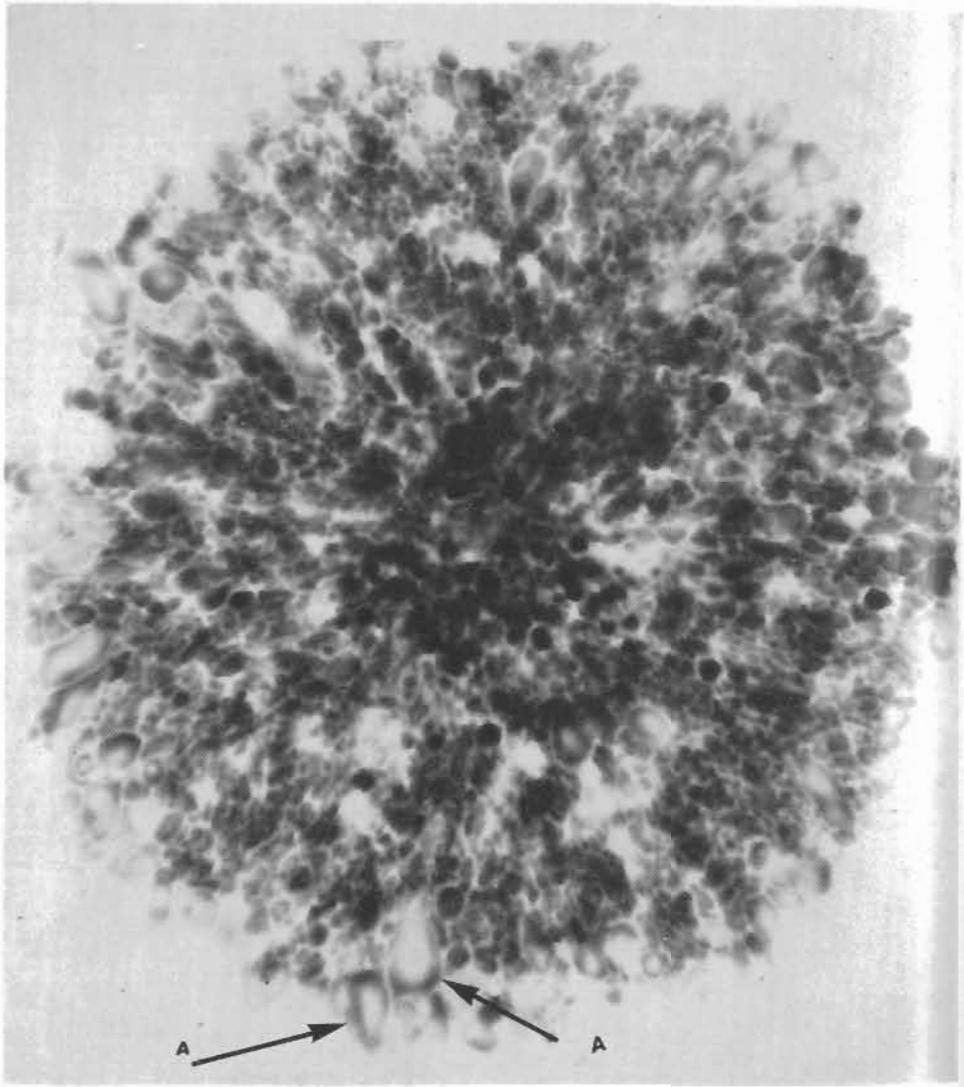

FIG. 78. Microcolony of mycoplasma causing agalactia of sheep and goats. Overnight growth on thin plastic film overlying agar. Fixed formalin, stained Giemsa solution. E. Klieneberger-Nobel, photographed by Mr J. Smiles. This photograph shows some of the very large but thin cells which develop on solid medium (A); as in the liquid material, the densely stained matter is found at the very edge. In contrast to bacteria the cells have no definite outline. This is due to their delicate outer membrane. Magnification: × 2500.

grow and multiply, forming the thick colony centre. As growth continues, the cells spread out over the surface to form the thinner periphery. When bacteria have lost their cell-walls and are growing in the L-form (Chapter 4), they form a similar type of colony (see Fig. 3(h)).

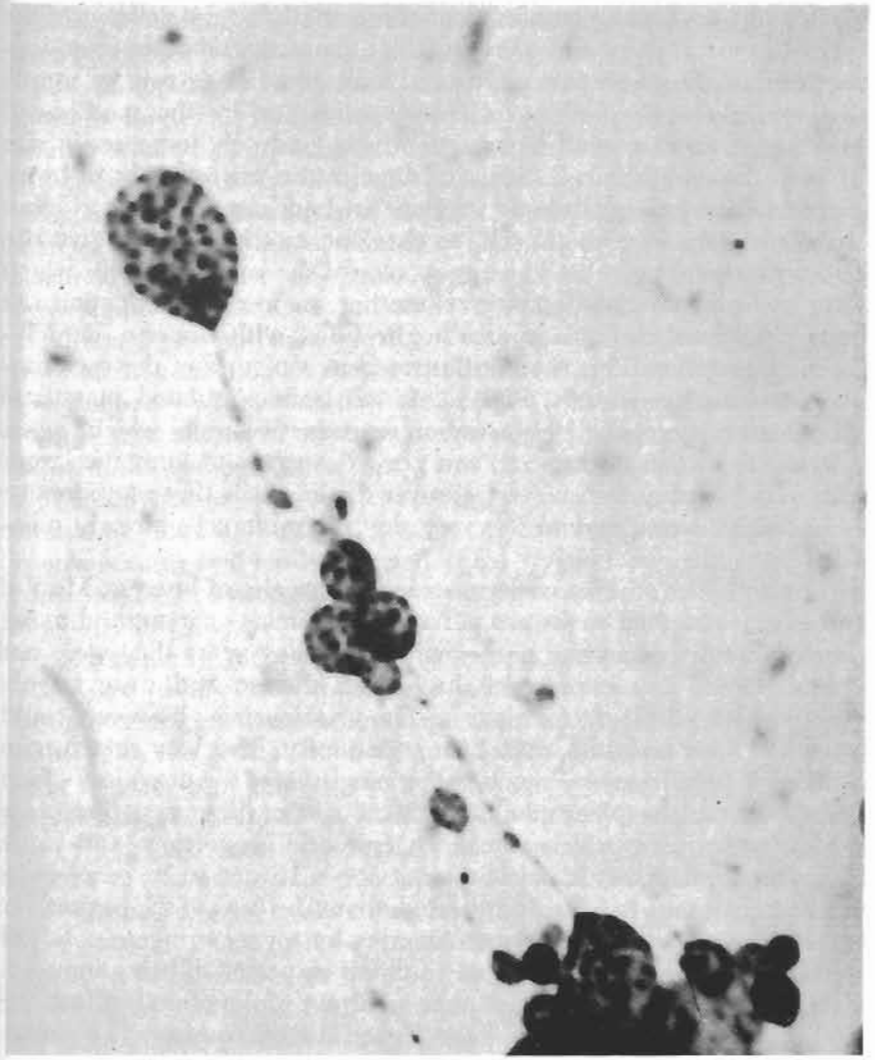

FIG. 79. *Mycoplasma* causing bronchopneumonia in rats. Three-day growth on solid medium, Bouin's fixative, stained Giemsa solution. E. Klieneberger-Nobel (1962). Fig. 16b, p. 40, "Pleuropneumonia-like Organisms (PPLO), Mycoplasmataceae", Academic Press, London. This photograph shows several of the thin but large elements developing on solid medium; they are packed with small granular, reproductive units.

It is possible that in both cases the lack of a rigid cell wall facilitates the entry of the cells into the narrow interfibrillar spaces of the agar.

The best way of finding out how any micro-organism grows and reproduces itself is to follow the development of individual cells by continuous observation under the microscope. It has been very difficult to do this with mycoplasma on account of the small size and delicacy of the cells. As a result there are several theories about the mode of reproduction of the *Mycoplasmataceae*. One cell may give rise to two by simple division into two equal parts (*binary fission*) or by the mother-cell budding to form a small outgrowth which gradually increases in size to form the daughter-cell. Some of the electron micrographs of broth cultures (Figs. 74–77) strongly suggest that budding can occur at more than one place in a single cell, so that one mother-cell can give rise to several daughter-cells. The appearance of the large, thin cells which form on solid media suggests yet another mode of reproduction. At certain stages these cells appear to be filled with discrete, densely-staining granules. This is well illustrated in a picture of the rat bronchopneumonia organisms, Fig. 79. It has been postulated that these granules are very small elements or reproductive units which, when released from the mother-cell, can grow out again to form the larger cells. Then the cycle can start all over again. Such tiny reproductive units might correspond to the very tiny filterable cells already mentioned.

The activities of these curious organisms are also of interest. Most of the species observed so far are parasitic in animals or man, and many are frankly or potentially pathogenic. For many years they were not considered of any importance in human disease and were largely ignored by medical bacteriologists. The veterinarians, however, could not view their activities with such equanimity. The very first species isolated in 1898 by two French bacteriologists was the organism which causes contagious pleurophneumonia of cattle (Fig. 75). This is a devastating disease which can reach epizoötic proportions and cause grave economic losses in cattle-rearing areas. It used to be enzoötic in Europe but seems to have disappeared about the turn of the century. It was only eradicated from North America by a very stringent policy of testing cattle and slaughtering any animals suspected of being infected. Even now, no one is allowed to take a culture of the organism into the U.S.A., for fear of reintroducing the disease into the country. The disease still occurs in Africa and Australia, although much research has been done to find more efficient methods of detecting the disease in cattle with sub-clinical infections and of protecting cattle by vaccination. Other species of mycoplasma cause genital tract infections and mastitis in cattle as well as arthritis in calves. Other animals of economic

importance can also be infected by different mycoplasma species. The organism causing agalactia of sheep and goats has already been shown (Figs. 74 and 78). As its name suggests, this is a disease primarily of the mammary glands in lactating ewes, but the joints and eyes of both sexes can be infected, with resultant lameness and blindness. There is also a pleuropneumonia of goats similar to the disease in cattle, but not of such great economic importance.

It will probably have been noted that diseases caused by mycoplasmas frequently affect the respiratory tract. This is also true in poultry where they have been implicated in chronic respiratory disease —a complex syndrome in which certain viruses are also involved. With the more intensive rearing of poultry in recent years, it has become increasingly important to control and eradicate this disease. Recent research also suggests that enzoötic pneumonia in pigs is caused by a mycoplasma, but the agent has not yet been isolated.

Although rats and mice are not of economic importance like the livestock already mentioned, they are widely used as experimental animals. The frequency with which they are infected with mycoplasmas can be a complicating factor in experimental work. For example, it is difficult to assess the pathogenic effect of a new virus by inoculating it into mice if the inoculation causes an existing mycoplasma infection in the animal to "flare up". Again, it is the respiratory tract of these animals which is most frequently infected (Figs. 77 and 79), although other species of *Mycoplasma* can cause polyarthritis in rats and a brain infection—"rolling disease"—in mice.

As previously mentioned, mycoplasmas were for a long time not considered to be of any importance in human disease. The first one to be isolated from a human being was from an abscess in a gland associated with the genital tract. Since then mycoplasmas have frequently been isolated from genital tract infections of various types. Sometimes they are the only organisms found; at others they are present together with recognized pathogens such as the gonococcus, the spirochaete of syphilis or the protozoön *Trichomonas vaginalis*. Like these pathogens mycoplasmas are probably transmitted by venereal contact. Infections are mostly localized in the genital tract but sometimes, after trauma caused by an operation or after childbirth, the organisms get into the blood stream and a more generalized illness with fever develops. Thus, the genital mycoplasmas are at least potentially pathogenic. Others found in the human mouth seem to be present as harmless *commensals*.

Although mycoplasmas have been so frequently implicated in respiratory diseases of animals, it is only fairly recently (1962) that a mycoplasma has been incriminated as the cause of one of the atypical pneumonias in man. The agent responsible for this atypical pneumonia

was called Eaton agent after its discoverer and was for many years thought to be a virus. The discovery that it could be grown on artificial media and that it resembled a mycoplasma caused a great deal of excitement among doctors and microbiologists interested in respiratory disease. Thus, the *Mycoplasmataceae*, hitherto rather neglected by medical bacteriologists have become a focal point for new researches. As a result of this new interest in these fascinating organisms, our knowledge of their activities and significance in both animal and human disease should expand considerably in the next few years.