JOURNAL OF BACTERIOLOGY, Jan. 1969, p. 441-444

Vol. 97, No. 1
Printed in U.S.A.

NOTES

Association of a Mycoplasma-like Agent with Chronic Pneumonia and Bronchiectasis in the Rat

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Received for publication 5 September 1968

In a previous communication (2), the fine structure and location of a mycoplasma-like agent in experimentally infected mouse lung was described. The agent which caused a chronic pneumonia in mice was originally derived from laboratory rats with naturally acquired chronic pneumonia, as defined by J. R. M. Innes et al. (3), and it was found to be indistinguishable from the grey lung agent (1). All attempts to activate a latent infection in the conventional mice used to study the mycoplasma-like pneumonia agent were unsuccessful, and 10th passage mouse lung homogenate produced the chronic pneumonia in specific-pathogen-free (SPF) rats. Nevertheless, it is believed that the grey lung agent was originally activated in laboratory mice (J. S. F. Niven, *personal communication*), and it remained possible that the mycoplasma-like pneumonia organism was activated in the same way. Thus, its relationship with the natural rat disease has remained in question.

This note reports the direct demonstration of the pneumonia agent in the lungs of conventional rats with naturally acquired chronic pneumonia and bronchiectasis. The techniques used were designed to overcome the difficulty of finding organisms in samples of such relatively small size as are used in the electron microscopy of large organs, such as rat lung.

Whole lung was thoroughly homogenized to give a 10% (w/v) suspension in 3% (v/v) phosphate-buffered glutaraldehyde (*p*H 7.4) at 4 C, and then centrifuged at 3,000 rev/min for 5 min. The supernatant fluid was removed and centrifuged at 30,000 rev/min for 30 min. After 2 hr in the fixative, the pellet was washed with phosphate buffer. It was then postfixed in 1% (w/v) phosphate-buffered osmium tetroxide (*p*H 7.4) at 4 C for 2 hr, washed in phosphate buffer, dehydrated in graded alcohols, and embedded in Maraglas by standard procedures. Ultrathin cross sections of the pellet were stained with ethanolic uranyl acetate followed by lead citrate, and were examined by using an A.E.I. EM6B microscope.

Rat pneumonia organisms were seen in very large numbers in lung preparations from all the conventional diseased rats examined, that is, 10 12-month-old rats with chronic pneumonia and 5 15-month-old rats with bronchiectasis. The organisms were not seen in lung preparations from 5 10- to 15-month-old SPF rats examined as controls. Conventional mice inoculated intranasally with 10% (w/v) normal mouse lung homogenate on each of 5 successive days were also examined by the same techniques, and no organisms were seen. The organisms in rat lung preparations were identical in size and fine structure with the pneumonia agent examined in 15th passage mouse lung (Fig. 1-5). An interpretation of the three-dimensional structure of the pneumonia agent is shown in Fig. 6.

The results strongly reinforce the belief that the mycoplasma-like organism which has been studied in conventional mice was not activated in these animals, but was derived from the naturally acquired chronic pneumonia in the rat. The association of this agent with the rat disease is probably causal, because the organism readily produced the chronic pneumonia in SPF rats known to be free from lung disease. It is probable that the chronic inflammation produced by this agent in the rat is the primary factor predisposing the lungs to secondary invasion by upper-respiratory-tract organisms, including *Mycoplasma pulmonis* and *Streptobacillus moniliformis*. This is supported by the observation that both these pathogenic organisms can be established in the upper respiratory tract of SPF rats, but they fail to invade the lungs in the absence of the pneumonia agent (F. W. Gay, Ph.D. Thesis, The Queen's University, Belfast, 1967).

The relationship of the mycoplasma-like agent to the enzootic bronchiectasis virus of Nelson (4) remains to be determined. As the latter has never been isolated or characterized (J. B. Nelson,

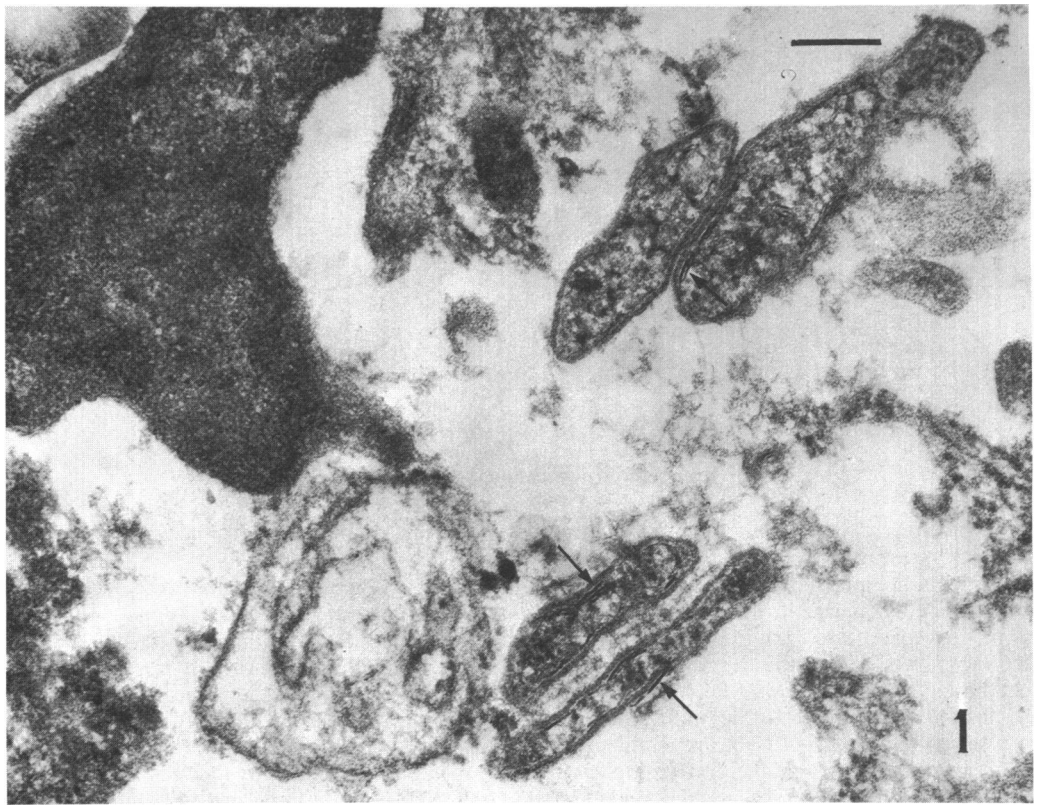

FIG. 1–5. *The mycoplasma-like pneumonia agent in naturally infected rat and experimentally infected mouse lung preparations. The bar represents 0.2 μm.*

FIG. 1. *Rat lung preparation. Elongated forms predominated (95%) in all sections, showing characteristic 5- to 7-nm peripheral fibrils (arrows).*

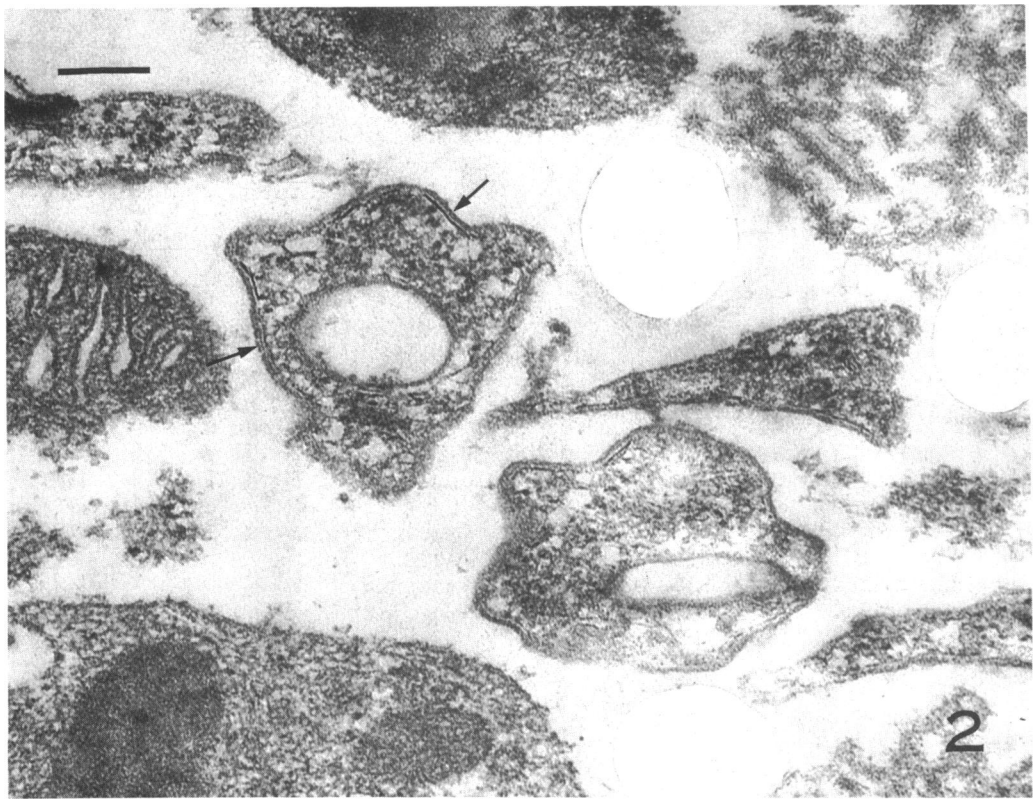

FIG. 2. *Rat lung preparation. Forms seen much less frequently (5%) than those in Fig. 1; interpreted as horizontal sections through thick plates. Note the 11-nm unit membrane (arrows) and adjacent peripheral fibril. Ribosomes can be seen in the internal fibrillar network which is interspersed with electron-lucent spaces.*

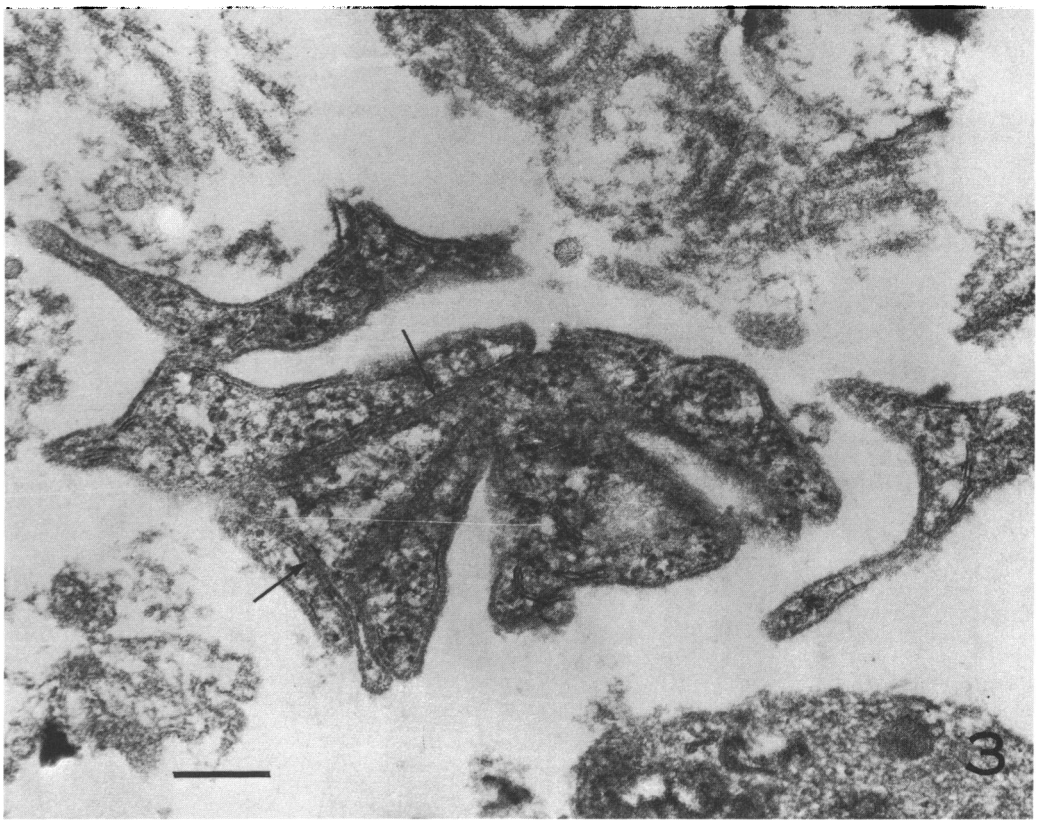

FIG. 3. *Rat lung preparation. A microcolony of organisms in which cell division appears to be taking place by the formation of cross-walls (arrows).*

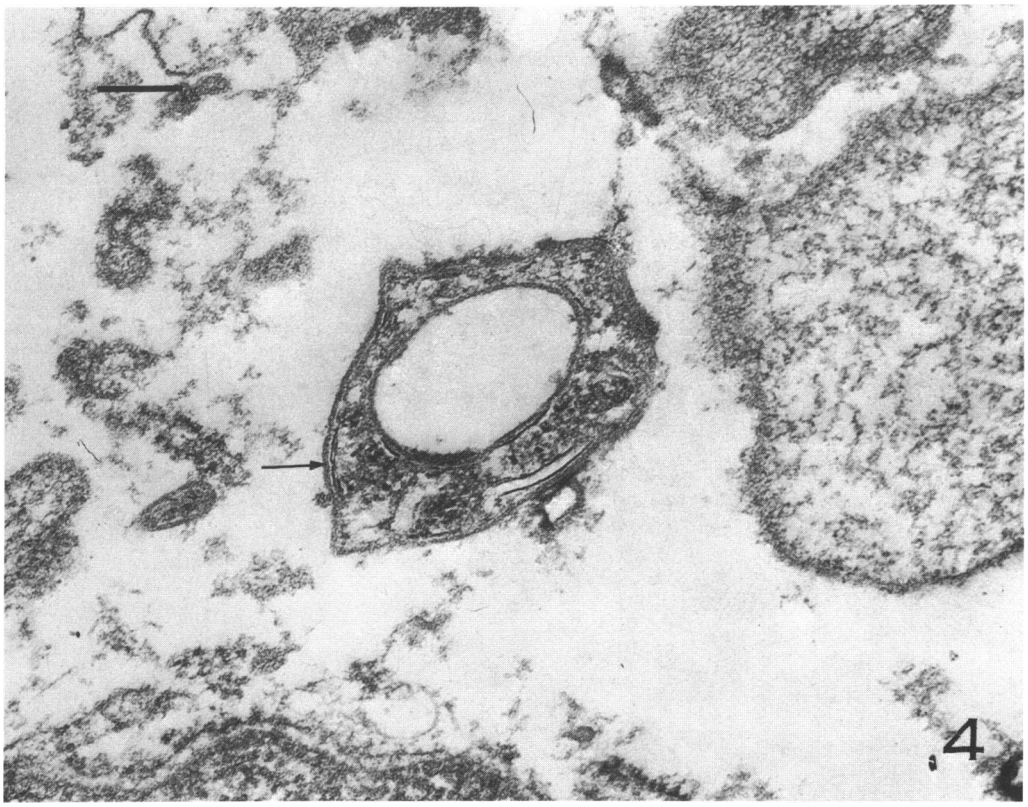

FIG. 4. *Mouse lung preparation. Organism showing the characteristic peripheral fibril and adjacent unit membrane (arrow).*

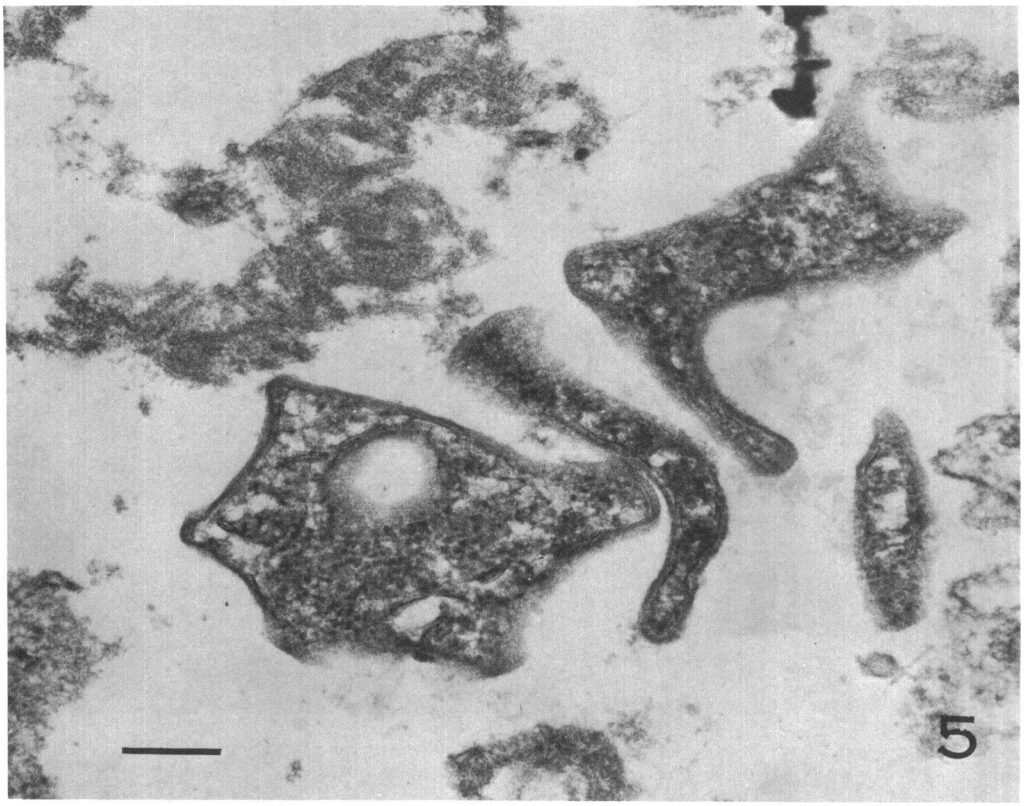

FIG. 5. *Mouse lung preparation. A group of organisms showing the range of shapes seen in sections. Characteristic peripheral and internal fibrils and ribosomes interspersed with electron-lucent spaces are clearly seen.*

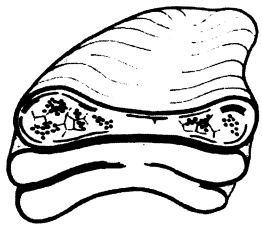

FIG. 6. *Three-dimensional interpretation of a microcolony composed of three cells, sectioned vertically. Each cell is shown as a thick plate of irregular outline.*

personal communication), and all attempts to culture a virus from rat pneumonia and bronchiectasis in this laboratory have failed, identity is probable.

I am indebted to D. P. Bell and P. C. Elmes for providing conventional and specific-pathogen-free rats.

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