

# ELECTRON MICROGRAPHS OF PLEUROPNEUMONIA-LIKE ORGANISMS

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There has been considerable and increasing interest in the field of pleuropneumonia-like organisms in the past eight to ten years, and a number of new morphologic and chemotherapeutic observations have been recorded, particularly through the work of Klieneberger (1935, 1936, 1938, 1940a, 1940b, 1940; also Klieneberger and Smiles, 1942; and Klieneberger and Steabben, 1940), Dienes (1938, 1939a, 1939b, 1940; also Dienes and Smith, 1943), and Sabin (1938a, 1938b, 1939a, 1939b; also Sabin and Warren, 1940). Recently there has been isolated in these laboratories a new organism taken from the swollen arthritic joints of the rat and resembling in many clinical effects and morphological appearances some of the previously recorded pleuropneumonia-like organisms. All of the experimental work with this new organism has been reported (Powell and Rice, 1944), and this paper presents a series of electron micrographs taken from representative cultures of the organism.

## METHODS

All the organisms were grown in a beef-infusion broth with 30 per cent ascitic fluid added. For observation under the electron microscope, they were centrifuged, the broth supernatant was removed, and the organisms were resuspended in a small volume of physiological saline solution. A drop of the suspension was placed on the stainless steel screen with the usual thin collodion film used in electron microscopy and washed with distilled water to remove any salt crystals or extraneous debris. All micrographs were made at the magnification of 6000  $\times$  and photographically enlarged to approximately 15,000  $\times$ .

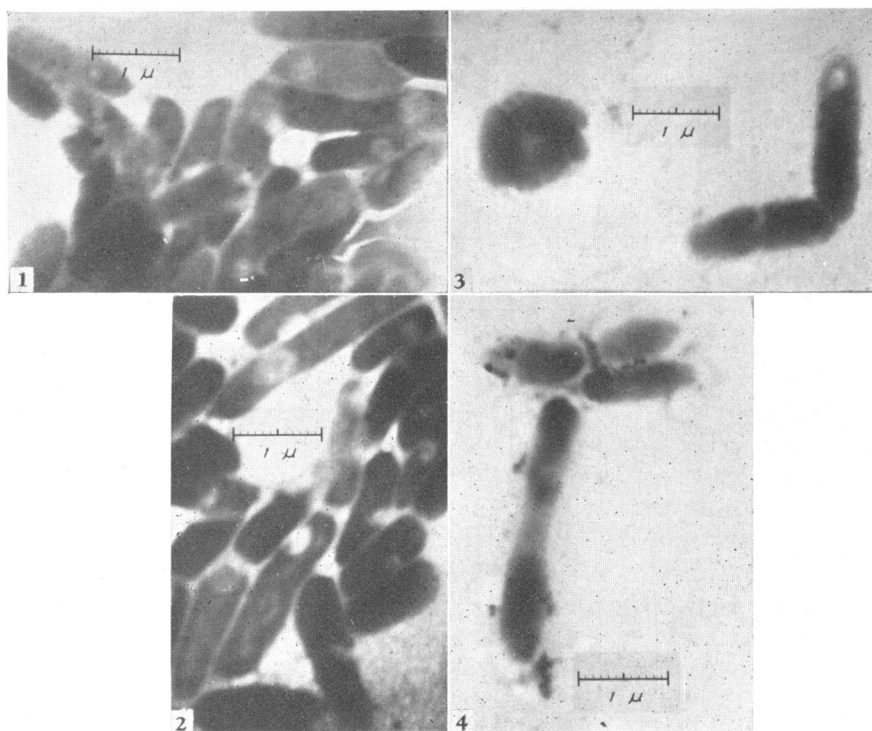
## MORPHOLOGY

The cultures usually comprise gram-negative bacilli which seem chained or clumped when stained and observed with the ordinary laboratory microscope. Figures 1 to 4 give representative electron micrographic views of this bacillus; figures 1, 2, and 3 were taken after four hours' growth in broth which was warmed to incubation temperature at the time of inoculation; figure 4 was taken after six hours' growth in broth chilled at the time of inoculation. These organisms were subcultured only once in broth after their isolation from the fluid of the swollen joint of the rat. In the four-hour growth it is interesting to note the close arrangement of the cells as they lie clumped together and the very apparent light spot or vacuole appearing in almost every cell. In one or two cells, elongate light areas are visible.

In the cultures from time to time there have appeared other forms; and in

one culture particularly (7-19-43) the bacillary forms seemed to be completely absent, with only bizarre forms, as micrographs 5 to 8 show, to account for the turbidity in the broth. The dense, opaque granules in these micrographs, as in figures 9 and 11, are very possibly sodium chloride crystals which were not completely removed in the washing process. Figures 9 to 12 are representative of a culture containing both bacillary and pleuropneumonia-like forms.

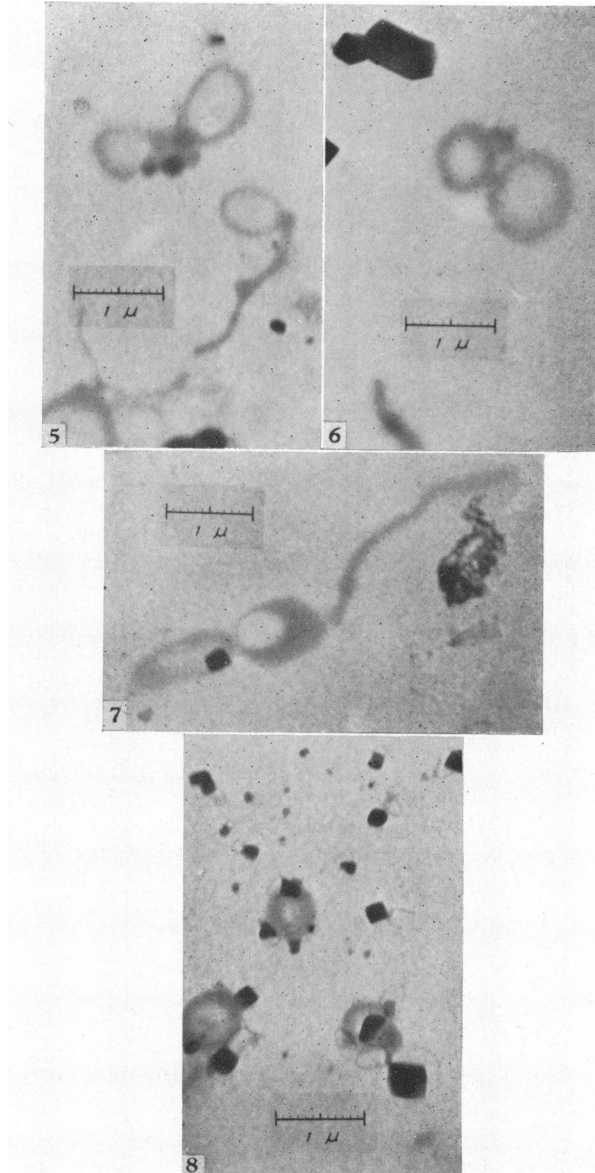
In all the literature concerning pleuropneumonia-like organisms they are reported as exceedingly fragile, and the usual method of handling, for observation under a regular microscope, is agar-block-fixation (Klieneberger and Smiles,



FIGS. 1, 2, 3, AND 4. Gram negative bacilli from pleuropneumonia-like organism cultures, 4 hours' incubation—Figure 4, 6 hours' incubation. 15,000X.

1942). Obviously this is impracticable with the electron microscope. It has been stated that in liquid media external forces or surface tension play an important part in determining the morphology of the organism, even of the largest pleuropneumonia-like elements. The treatment necessary in preparation for electron micrographic observation has been violent, and the specimen has been subjected to repeated changes in surface tension and a final entry into high vacuum ( $0.4 \mu$ ) in the tube of the electron microscope. However, it seems illogical that such consistency as was found in these pictures could be attributed to artefacts. Pictures 5 to 8 particularly are only representatives from a considerable number of similar micrographs. Admittedly, the bizarre rings are

broken in figures 5 and 7 where there is the impression of streaming protoplasmic material still attached to one or a pair of rings. However, there are several pairs of rings still intact.

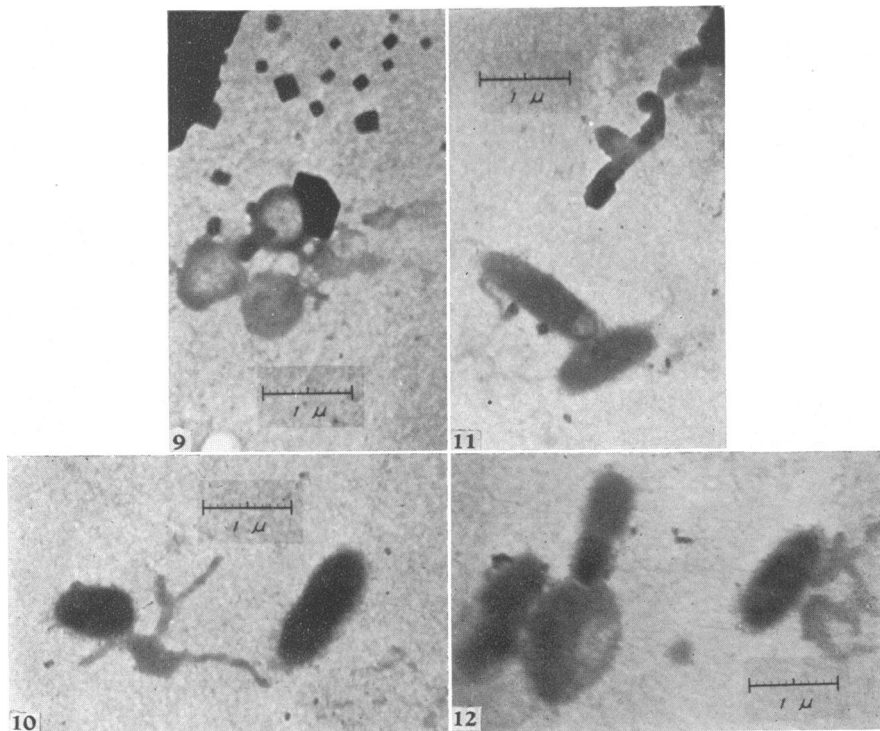


FIGS. 5, 6, 7, AND 8. Pleuropneumonia-like organisms, 24 hours' incubation, 15,000X.

#### SUMMARY

Micrographs are presented of a bacillary and pleuropneumonia-like stages of an organism recently isolated from the rat. Despite extreme cellular fragility,

it is believed that at least two forms in the supposed life cycle of the pleuropneumonia-like organism are represented in these micrographs.



FIGS. 9, 10, 11, AND 12. Gram negative bacilli and pleuropneumonia-like organisms within the same culture, 24 hours' incubation, 15,000X.

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