JOURNAL OF BACTERIOLOGY, Jan., 1967, p. 490-492

Vol. 93, No. 1
Printed in U.S.A.

NOTES

Influence of Fixation on Gross Morphology of *Mycoplasma*

KARL REUSS

Hygiene-Institut der Stadt und Universität, Frankfurt/Main, West Germany

Received for publication 12 September 1966

In recent publications on the morphology of *Mycoplasma*, these organisms were shown to be highly pleomorphic, varying in shape from roughly spherical to bizarre forms with long branched filaments (D. R. Anderson and M. F. Barile, J. Bacteriol. **90**:180, 1965; J. B. Nelson and M. J. Lyons, J. Bacteriol. **90**:1750, 1965). Some strains seemed to consist almost entirely of filamentous forms (D. R. Anderson and M. F. Barile, J. Natl. Cancer Inst. **36**:161, 1966). The question has been discussed whether some of these filaments in negatively stained preparations might be artifacts produced by the drying of mycoplasmas in phosphotungstate acid. This possiblity has not been precluded by any detailed experiments which have been published. We therefore started an investigation of this problem by comparing negatively stained preparations of fixed and unfixed specimens.

The *Mycoplasma* strain used in this study was the agent first described as a virus (Negroni, Brit. Med. J. **1**:927, 1964), and subsequently discovered to be a mycoplasma (Girardi et al., Nature **205**:188, 1965; Grist and Fallon, Brit. Med. J. **2**:1263, 1964). It was recently identified as *M. pulmonis* (Fallon et al., Brit. Med. J. **2**:388, 1965). It was grown in Brain Heart Infusion (Difco), supplemented with 1% yeast extract (Difco), 0.5% glucose, 0.2% Gelysate (BBL), and 20% horse serum. The strain was adapted to this medium by several passages at 48-hr intervals. For electron microscopy, broth cultures were centrifuged for 30 min at 6,500 × *g*. One part of the pellet was removed and suspended in several drops of 3% potassium phosphotungstic acid (PTA), *p*H 6.1 to 6.3. The remaining part of the pellet was suspended in 2 to 3 drops of 10% Formalin (in phosphate-buffered saline, *p*H 7.2). After fixation for 15 min at 22 C, PTA was added. Drops of the fixed or unfixed mixtures were placed on Formvar-coated grids. Excess fluid was removed after a few seconds by touching the side of the grid with filter paper. The air-dried grids were examined with a Siemens-Elmiskop I electron microscope.

In the unfixed preparation, the mycoplasmas had bizarre shapes with irregular outlines and long filaments (Fig. 1), whereas in the fixed part of the same pellet they were roughly spherical without any protrusion or filament (Fig. 2). A more detailed examination of the unfixed specimen revealed great variations in shape occurring on the same grid. In some areas a thick layer of PTA, containing highly distorted filamentous organisms, had dried onto the Formvar film. In other areas, where more PTA had heen removed, the mycoplasmas were roughly spherical, with only an occasional short filament. These observations clearly demonstrated that the filaments in unfixed preparations were artifacts caused by distortion during the drying process on the grid. Comparable results were obtained with viruses (Bonar et al., J. Natl. Cancer Inst. **30**:949, 1963; Reuss et al., Zentr. Bakteriol. Parasitenk. Abt. I Orig., *in press*).

Long filaments, closely resembling those in mycoplasma preparations, were shown emerging from stromalytic erythrocytes (Baker, J. Ultrastruct. Res. **11**:494, 1964). The distorting effect of hypotonic solutions on mycoplasmas could be demonstrated in the following experiment. Sedimented mycoplasmas were suspended in distilled water and centrifuged for 30 min at 6,500 × *g*. The pellet was suspended in a drop of distilled water and fixed for 15 min with 10% Formalin. After negative staining, filaments were observed emerging from irregularly shaped organisms (Fig. 3). This result reveals that mycoplasma filaments can be produced by hypotonic effects and that fixed filaments withstand the negative staining procedure without alteration. Therefore, the conclusion seems to be justified that the spherical forms without filaments in fixed preparations give a better impression of the real morphology of the mycoplasmas than the pleomorphic forms observed in unfixed preparations.

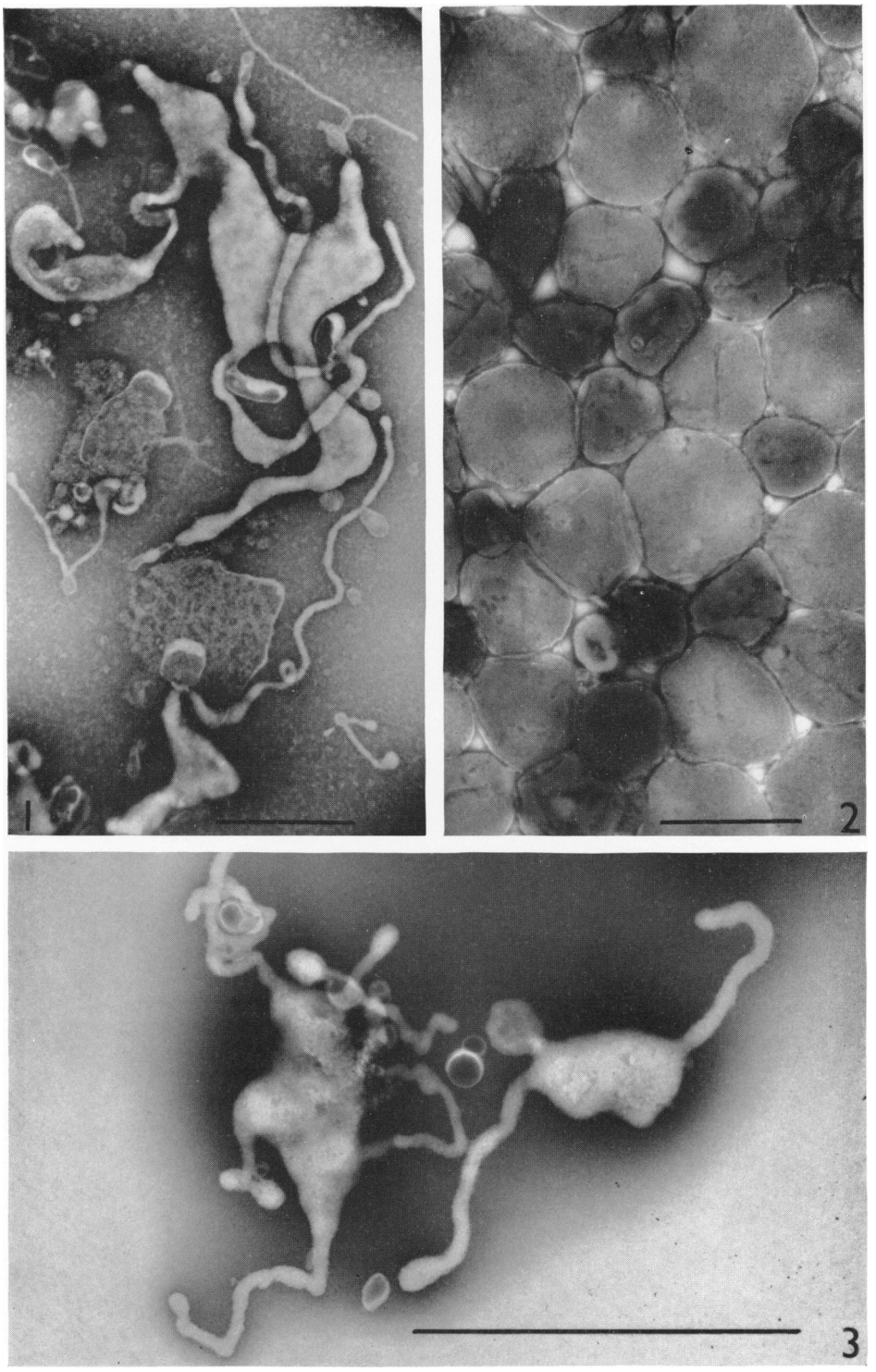

FIG. 1. *Negroni strain of Mycoplasma, stained with PTA. Unfixed preparation showing pleomorphic forms with long filaments.* × *20,000.*

FIG. 2. *Same material as Fig. 1, but fixed with 10% Formalin before PTA staining. All cells are roughly spherical and without filaments.* × *20,000.*

FIG. 3. *Mycoplasmas treated with distilled water for 30 min, then fixed with 10% Formalin and stained with PTA. Pleomorphic forms with filaments as in unfixed preparations.* × *55,000. (Bars equal 1* μ)

Similar results with other *Mycoplasma* strains will be described in detail elsewhere (Reuss et al., Zentr. Bakteriol. Parasitenk. Abt. I Orig., *in press*). To date, we have not been able to demonstrate filaments in Formalin-fixed preparations of untreated mycoplasmas. This does not preclude, however, the possibility that under different cultural conditions filamentous forms may occur.

I am indebted to L. Hayflick, Philadelphia, Pa., for the Negroni strain of *Mycoplasma*.

This investigation was supported by the Deutsche Forschungsgemeinschaft.