inflammation exudate more rapidly, to a greater concentration, and persisted longer than did the other antibiotics studied.

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Growth Morphology of *Mycoplasma pneumoniae* Strain FH on Glass Surface (33009)

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The variable morphology of mycoplasma still evokes different views about growth and multiplication of these microorganisms (1). The results of experiments to determine morphology are further complicated by the possibility of artifacts due to the preparation. The ability of *Mycoplasma pneumoniae* to adhere on a glass surface (2), provides a chance of observing living organisms without further manipulation. The following experiments were undertaken to study the growth morphology of *M. pneumoniae* on a glass surface in a fluid environment.

Material and Methods. Mycoplasma. Mycoplasma pneumoniae strain FH used in the study was kindly supplied by Dr. Del Giudice, Baltimore Biological Laboratories (BBL).

Media. The growth medium consisted of PPLO broth or PPLO agar (Difco Lab.), both supplemented with 20% horse serum and 10% yeast extract (Microbiological Assoc.) (1). For biochemical tests 1% glucose was added together with final concentrations of 0.005% phenol red, 0.025% 2,3,-5-triphenyltetrazolium chloride (Tetrazolium) or 0.002% methylene blue. To demonstrate the lysis of red blood cells (RBC), 3% sheep RBC were added to PPLO agar or the enriched PPLO agar.

Growth chamber. A coverslip chamber described for the demonstration of cytophilic antibody (3) was used. Glass rings (9 mm i.d., 2–3 mm high) were mounted on 22-mm square coverslips by silicone grease. The chambers were autoclaved, inoculated with about 0.2 ml of 1:10 dilution of a 24-hour culture, sealed by a second sterile coverslip and incubated at 37°C. Growth was observed by phase contrast microscopy.

Identification tests. After opening and removal of broth, the chambers showing growth were either filled with the test medium, or, after removing the rings, the coverslips with the adherent organisms were placed upside down on a test agar plate. Serological identification by growth inhibi-

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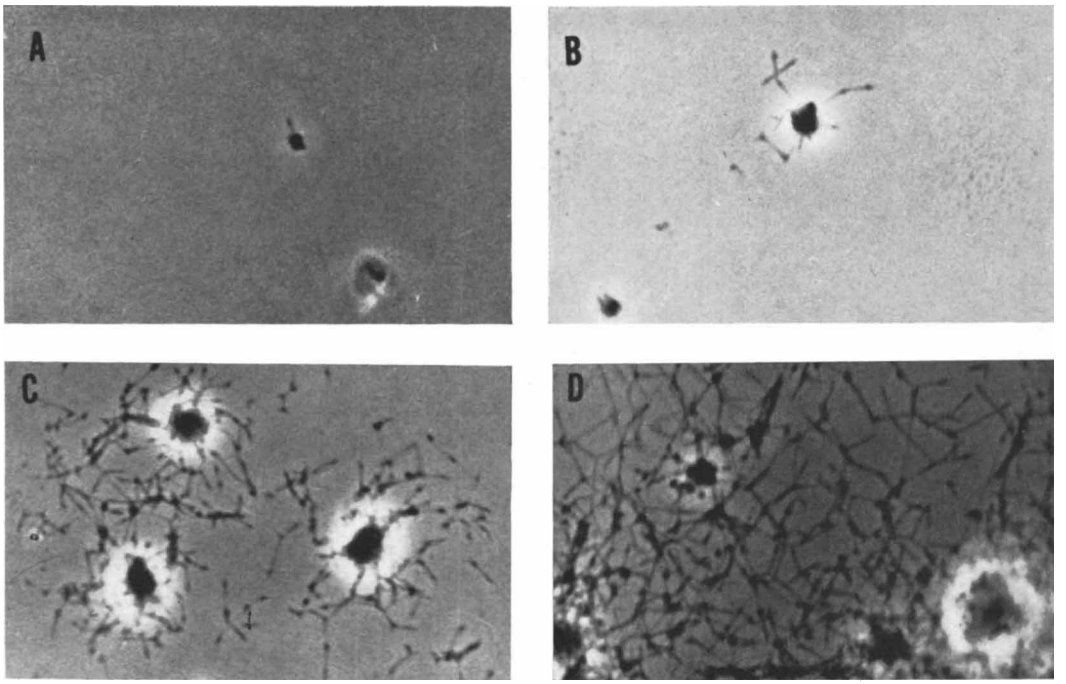

FIG. 1. Growth structures of *M. pneumoniae* at different times after inoculation. A. After 3.5 hours. B after 7 hours. C. After 22 hours. D. After 5 days (total magnification × 1600).

tion was performed by the method of Clyde (4).

Fluorescent antibody test (FA). Chambers with growth were opened, washed twice and incubated with *M. pneumoniae* horse antiserum (BBL) for 45 min at room temperature. They were then washed twice for 5 min, the glass rings were removed and the coverslips were placed upside down on microslides. Incubation with fluorescein-labeled antisera against *M. hominis* (horse) and *M. orale* I (mule) and inhibition with unlabeled *M. pneumoniae* antiserum were used as controls. A Zeiss photomicroscope with fluorescence equipment was used during the investigation.

Results. Morphology. The development of structures on glass started with tiny dark spots sticking on the surface of the coverslips. At 2–4 hours little filaments were present (Fig. 1A). Within the next few hours the colonies grew larger and the filaments branched off to a network-like structure around the growing colonies (Fig. 1 B and C). Later on filaments not connected with the colonies were also found (Fig. 1C). The filaments were straight, very often showing knob-like ends, from which other filaments originated, sometimes in branching forms. Filaments, which partially lost their contact to the glass, bent and waved with the free end in the broth, thus demonstrating their soft consistency. After about 24 hours "grape-like" structures began to grow out from the colonies into the broth. After 4–5 days a network of filaments covered the glass, the colonies had grown large and granular, and within the network new small colonies developed everywhere (Fig. 1D). The "grape-like" structures had grown out of nearly every colony (Fig. 2).

Identification tests. Broth from the chambers subcultured on enriched PPLO agar showed aerobic growth of colonies typical for *M. pneumoniae*, whereas no other mycoplasma or bacteria could be detected aerobically or anaerobically. The subcultured microorganisms were able to ferment glucose within 48 hours, to grow on and to reduce methylene blue agar, and to reduce Tetrazo-

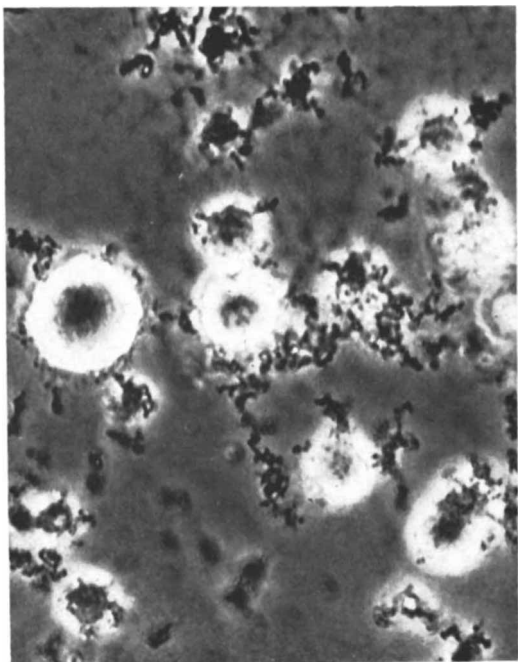

FIG. 2. Growth after 5 days, focused into broth. Note numerous "grape-like" structures (total magnification × 640).

lium beginning within 24 hours. They also lysed sheep RBC. The growth of the microorganisms was specifically inhibited by *M. pneumoniae* antiserum. The structures adhering to the coverslip gave similar reactions in the foregoing tests, thereby conforming to the behavior of *M. pneumoniae* as described by Hayflick and Chanock (1).

Fluorescent antibody test. Colonies and filaments were brightly stained by fluorescein-labeled *M. pneumoniae* antiserum. The "grape-like" structures could not be detected and might have been washed off during the staining procedure. Heterologous sera stained colonies and filaments very poorly. Inhibition by unlabeled specific antiserum showed marked decrease of the fluorescence.

Discussion. The demonstration of *M. pneumoniae* antigen, as well as the results of the biochemical tests, suggest that structures adherent on the bottom of glass chambers inoculated with *M. pneumoniae* are indeed living mycoplasma. The growth occurs in different forms. There are large round colonies, which are probably related to the "spherules" of Kenny and Grayston (5). There are short straight filaments, which resemble the description given by Freundt (6) for other mycoplasma, and there are also "grape-like" structures which grow out of the colonies into the broth. The antigenic nature of the latter, which could not be detected by fluorescent antibody, has yet to be proven to be *M. pneumoniae.*

Filamentous growth of different species of mycoplasma has often been described, recently again by Razin *et al.* (7). These authors showed a support of filamentous growth by unsaturated fatty acids. Whether this factor is responsible for the development of filaments by *M. pneumoniae* is not yet known. On the other hand there might be some influence of other environmetnal factors such as a fluid medium and an available surface. Our experiments do not show whether the single filaments really possess all qualities of a colony-forming unit of *M. pneumoniae.* They do seem to have surface antigens specific for this mycoplasma species and they could be observed to grow.

Summary. Mycoplasma pneumoniae (grown in broth on coverslips showed large round colonies and a network of short straight filaments adherent to the glass surface. From the colonies, "grape-like" structures grew into the broth. Antigens of *M. pneumoniae* could be demonstrted on colonies and filaments by fluorescent antibody technique. Biochemical tests showed the presence of living Mycoplasma. The results give evidence for an ability of *M. pneumoniae* to grow in different morphological forms.

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