

Spheroplast-like Cells of Pneumococci

Although theoretically there is a great possibility for the detection of bacterial spheroplasts directly in nature, only occasional findings have been described¹. However, in many cases of routine bacteriological examinations we might encounter phenomena which could be interpreted as transitional stages to the L form of bacteria. Our aim is to describe and to demonstrate spheroplast-like cells of *Diplococcus pneumoniae* found in the smears prepared from the cerebrospinal fluid taken from a case of pneumococcal meningitis.

Cerebrospinal fluid was centrifuged and the smear dried, fixed in the flame, and stained with the Gram method. Microscopically we found in the smear (Figure 1) a few normal and many unusual forms of pneumococci. Most of these forms were approximately three times larger in size than the normal ones. Some were still in the

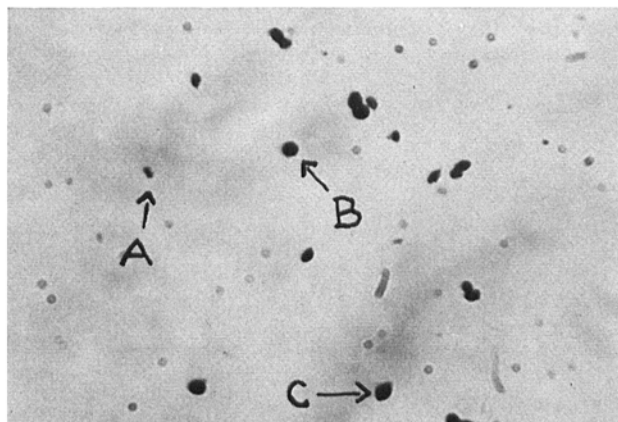


Fig. 1. A, a normal diploform of *Diplococcus pneumoniae*. B, a larger rounded single cell of *D. pneumoniae*. C, a larger pointed single cell of *D. pneumoniae*. Gram stain. Magnification ca. 900 ×.

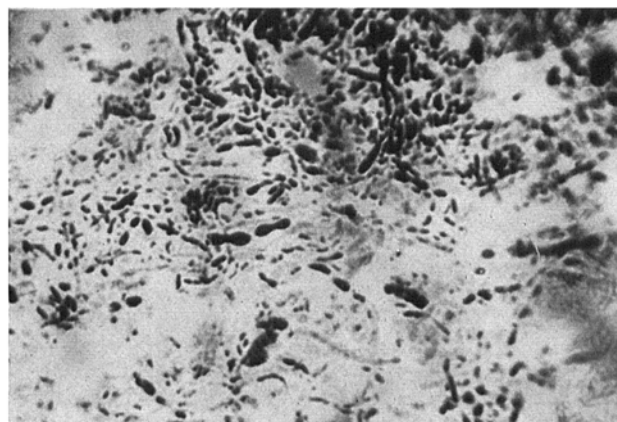


Fig. 2. Bigger, rounded or elongated cells of pneumococci in the bacteriostatic zone of penicillin. Gram stain. Magnification ca. 900 ×.

diplo-forms, the others were singular. Most of them had one or both ends still pointed, but many completely rounded forms were observed. It seemed that the capsular material in small and in larger forms was still preserved.

The growth of the microorganism on the bacteriological media was not visibly inhibited. The colonies grew to normal dimensions in 48 h of incubation at 37°C. The smear prepared from these colonies showed cells which were still enlarged. Most of them were pointed at the poles of diplo-formations. In contrast to the picture seen in the smears prepared from the purulent cerebrospinal fluid, long chains of large pneumococci were found in the smears prepared from first cultures of the organisms described. The size and form of the microorganisms returned to normal in the course of subsequent subcultures.

We consider the globular organisms described in this communication to be spheroplasts of pneumococci, which have caused purulent meningitis. We believe they were produced by the spheroplasting effect of penicillin² used as therapeutic agent in this case.

We tried to find *in vitro* evidence of the action of penicillin on the form of pneumococci. We did not succeed well with the incorporation of penicillin into the fluid or solid media. Probably the right concentration of penicillin was missed. At the border of the inhibitory zone around the disc soaked with penicillin (50–500 U of penicillin/1 ml), many larger rounded cells of pneumococci were found (Figure 2). Some of the cells were not rounded but elongated, and some clostridium or plectridium-like forms were observed. On this occasion it should be mentioned that also in the direct preparations of purulent liquor taken in cases of pneumococcal meningitis, long 'bacillary' forms of pneumococci were occasionally found. They were especially pronounced around the border of the inhibitory zone which was seen in the primary culture of the purulent liquor in the spot where the liquor was inoculated more massively. These unusual forms of pneumococci persisted also in the first few subcultures of pneumococci.

The concentrations of antibiotic required for the conversion of normal to spheroplast-like cells of microorganisms are subbactericidal, i.e. those generally readily attained in the tissue, blood and body fluids already during the first hours of antibiotic therapy. TUCKER and EAGLE³ found a concentration of 35 units/ml $\frac{1}{2}$ h after intramuscular injection of 1,2 million units of G-penicillin⁴.

Zusammenfassung. Spheroplasten-ähnliche Formen von Pneumokokken wurden in den Präparaten aus Liquor cerebrospinalis bei purulenter Meningitis gefunden, die vermutlich durch Penicillintherapie verursacht waren.

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¹ A. G. MELONI and C. MONTI-BRAGADIN, Ann. Sclavo 4, n. 2 (1962).

² K. MCQUILLEN, Fourth International Congress of Biochemistry, vol. XIII, Colloquia (Pergamon Press).

³ H. A. TUCKER and H. EAGLE, Amer. J. Med. 4, 343 (1948).

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