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Preparation of Cell Walls and Protoplasm of *Neisseria* with the Ribi Cell Fractionator

JOHN E. MARTIN, JR., WILLIAM L. PEACOCK, JR., GILBERT REISING, DOUGLAS S. KELLOGG, JR., EDGAR RIBI,[1] AND JAMES D. THAYER[2]

Venereal Disease Research Laboratory, Venereal Disease Program, National Communicable Disease Center, Atlanta, Georgia 30333

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The varied pressures required for disruption of *Neisseria gonorrhoeae* and other species of *Neisseria* when the Sorvall-Ribi refrigerated cell fractionator is used in the preparation of cell walls and cellular protoplasm are reported. Optimal disruption pressure for the gonococcus was considerably less than that required for other members of the genus *Neisseria*. Pressures varied from 8,000 psi for *N. gonorrhoeae* F62, colony type 4, to 22,000 psi for the nonpathogenic *Neisseria*—*N. sicca*, *N. flava*, and *N. catarrhalis*. Representative electron photomicrographs are shown.

Disruption of bacteria to obtain their main morphological elements—cell walls and protoplasm—for the purpose of chemical, immunological, and serological examination has been accomplished by various procedures, including ultrasonic disintegration, grinding with abrasives, freeze-thawing, shaking with glass beads, and high-pressure extrusion (1, 2, 4, 6-9). For the investigations reported here, the last method has been employed, and the Sorvall-Ribi refrigerated cell fractionator (3) was the apparatus of choice. With the aid of this device, pressurized bacterial suspensions were released under carefully controlled pressure and temperature at the orifice of a pressure cell whereby the bacteria were disrupted by the sudden reduction in pressure together with shear forces upon extrusion from the needle valve. Pressures were selected which permitted the cell walls to be merely cracked without excessive fragmentation. In addition, the temperature at the orifice was maintained between 4 and 10 C to prevent denaturation of the important antigenic fractions by heat (3). The shell-like cell walls were separated from the soluble cell components by differential centrifugation.

Because of the rapid rise in the incidence of gonococcal infections and in an effort to develop a serological test based on a more precise knowledge of gonococcal antigens, we have undertaken the systematic study of the antigens of *Neisseria gonorrhoeae*. To this end, we have used the cell fractionator for cellular disruption as the preliminary step in the separation of cell walls from protoplasm.

This paper describes the application of the disruption procedure to *N. gonorrhoeae*, *N. meningitidis*, and nonpathogenic *Neisseria* species. The optimal disruption pressures are reported, and representative electron photomicrographs are shown.

MATERIALS AND METHODS

The following cultures of *Neisseria* were examined: *N. gonorrhoeae* F62, colony types 1, 2, 3, and 4 (5); *N. meningitidis* group B; *Neisseria sicca*; *Neisseria flava*; and *Neisseria catarrhalis*. Each strain was cultured on GC Medium Base (Difco) enriched with a defined supplement (10). After 18 hr of incubation under increased carbon dioxide tension (candle extinction) at 35 C, the bacteria were prepared for disruption.

The bacterial growth was scraped from the plates with a glass "L" rod, suspended in distilled water, and dispersed by pipetting. All further processing was carried out at 4 C. The bacterial suspension was centrifuged for 30 min at 5,000 × *g*, and the resulting sediment was suspended in distilled water (200 mg of wet weight of cells per ml).

The pressure in the cell was increased from 5,000 psi in 1,000-psi increments. During the entire period of this study, the needle valve of the pressure cell was cooled with nitrogen gas chilled to −50 C to maintain the temperature of the effluent at the outlet between 4 and 10 C. The effluent from the cell was examined electron microscopically after each increment in pressure until optimal disruption was obtained. Optimal disruption was defined as disruption of approximately 95% of the cells with a minimal amount of cell wall fragmentation.

[1] Permanent address: National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Mont. 59840.

[2] Deceased 18 July 1968.

Bacterial material taken from the effluent of the pressure cell at optimal disruptive pressure was centrifuged at 36,000 × *g* for 30 min. The supernatant material was decanted, and the sedimenting insoluble cell wall material was washed once with distilled water.

RESULTS

The optimal pressures for disruption of *Neisseria* cell walls are shown in Table 1. Although all organisms examined were of the same genus, optimal disruptive pressure varied from 8,000 psi for *N. gonorrhoeae* type 4 to 22,000 psi for the nonpathogenic *Neisseria*—*N. sicca*, *N. flava*, and *N. catarrhalis*. *N. gonorrhoeae* F62 types 1 and 2, were disrupted at 12,000 psi and type 3 was disrupted at 10,000 psi, whereas *N. meningitidis* group B was optimally disrupted at 18,000 psi.

The electron photomicrographs (Fig. 1 and 2) are representative of the *Neisseria* both before and after disruption. Uniform shading density and an intact cell wall are shown before disruption. After extrusion from the high-pressure cell, the density of the cell changes and what appears to be a hole is seen in the cell wall (note arrow, Fig. 2). The change in electron opacity presumably arises from removal of protoplasm from the cell.

DISCUSSION

Pressure disruption of bacteria allows the rapid preparation of undenatured soluble and insoluble bacterial fractions (3). Although the terms "cell wall" and "protoplasm" have been used interchangeably in this paper with the terms "insoluble" and "soluble," this is a dubious distinction. Precursors of the cell wall, capsular material, and finely particulate cell wall fragments undoubtedly appear in the so-called protoplasm fraction. In this sense, the terms "cell wall" and "protoplasm" refer only to physical characteristics of the bacterial material, the protoplasm being that material remaining in the supernatant fluid after centrifugation at 36,000 × *g* for 30 min, and the cell walls being the sediment of that centrifugation.

TABLE 1. *Optimal disruption pressures for various Neisseria species*

Organism	Optimal disruption pressure
	psi
N. gonorrhoeae F62, type 4	8,000
N. gonorrhoeae F62, type 3	10,000
N. gonorrhoeae F62, type 2	12,000
N. gonorrhoeae F62, type 1	12,000
N. meningitidis group B	18,000
N. sicca	22,000
N. flava	22,000
N. catarrhalis	22,000

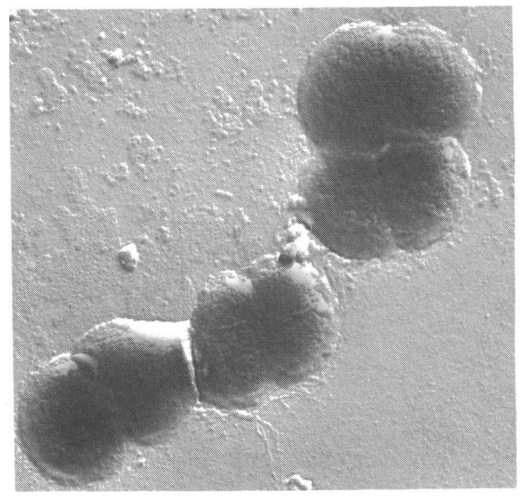

FIG. 1. *Typical electron photomicrograph of N. gonorrhoeae diplococci before high-pressure extrusion. Note the rounded appearance of the cells with a clear, continuous cell-wall outline.* × *18,000.*

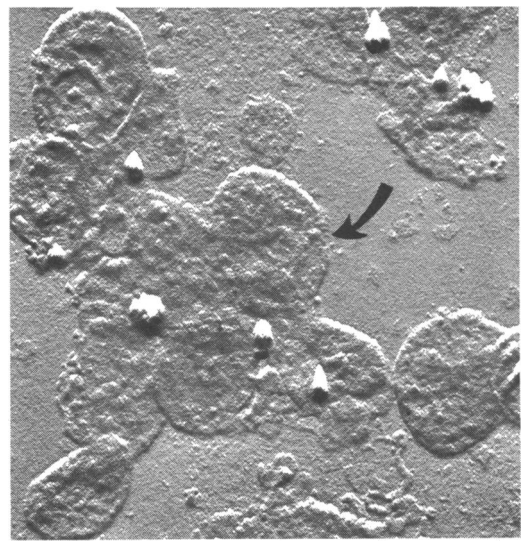

FIG. 2. *N. gonorrhoeae cells after high-pressure extrusion. The cell wall has a wrinkled, grannlar appearance and no longer gives a continuous outline. The break in continuity is probably caused by rupture of the wall by the extrusion process.* × *18,000.*

The variation in the pressures required for disruption of various members of the genus *Neisseria* speaks strongly for differences in structural composition of the cell wall. The sig-

nificant difference between the pressures necessary to disrupt type 1 and type 4 cells of *N. gonorrhoeae* F62, suggests that these two colonial types might also be distinguished on a chemical or immunological basis. If so, then the difference would be expected to be found in the cell wall fraction.

Additional work is in progress to identify and characterize antigenic components of the cell wall and the protoplasm. By such detailed analysis of the pathogenic and nonpathogenic *Neisseria*, it is hoped that specific antigens can be found which are important in the human immune response to gonococcal infection and which will aid in the development of a serological detection system for *N. gonorrhoeae* infection.

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