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Fine Structure of *Haemophilus vaginalis*

B. SUE CRISWELL, WAYNE A. STENBACK, S. H. BLACK, AND HERMAN L. GARDNER

Department of Microbiology, Division of Experimental Biology, and Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, Texas 77025

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Haemophilus vaginalis 594 has a trilaminar cell wall, ribosomes, a fibrillar nucleoid, and both convoluted and simple mesosome-like bodies. Polar bulbous enlargements of the cell and multiple cross wall formations may explain its pleomorphism.

Haemophilus vaginalis 594 is a small gram-negative organism that displays moderate degrees of pleomorphism and may appear in end-to-end pairs or in small, palisading clumps. Poorly defined granules develop in older cultures, but no endospores or capsules have been found (2). Volutin granules (3) and fat granules (4) have been reported in the organism. The present study employed the electron microscope to distinguish additional structural features of *H. vaginalis* unresolvable by light microscopy. Furthermore, this study is consistent with prior findings that *H. vaginalis* 594 is structurally a gram-negative organism (1).

H. vaginalis 594 was grown in a biphasic culture system (6) consisting of Casman broth (Difco) plus 5% rabbit serum, with agar as the solid phase and water as the liquid phase. After equilibration for 12 to 24 hr on a rotary shaking incubator set at 35 C and aerated with 5% CO_2, in air, cells were harvested by centrifugation and placed in Millonig's phosphate buffer (7), *p*H 7.2. The cell harvest was then fixed in glutaraldehyde and osmic acid, stained in 5% uranyl acetate in 10% Formalin, and embedded in araldite. Sections of 60 to 90 nm thickness were cut with a glass knife on a Porter-Blum MT 1 ultramicrotome and picked up on copper grids containing carbon-coated parlodion membranes. Sections were stained with uranyl acetate and lead citrate (5) and examined in a Siemens Elmiskop 1A electron microscope set at 80 kv with an objective aperature of 50 nm.

H. vaginalis 594 appears to have a fibrillar nucleoid and ribosomes dispersed throughout the cytoplasm (Fig. 1 and 2). The cell wall, 12 nm thick, is a trilaminar structure consisting of two electron-dense layers separated by an electron-translucent layer. Biochemically, the wall resembles that of the gram-negative group of organisms (1). The fibrillar material adhering to the cell walls in Fig. 1 and 4 may represent the presence of a microcapsule, fimbriae, or adherent components of the media in which the organism was grown. Some of the pleomorphism of the organism observed by light microscopists (2) may be due to forms showing a polar bulbous development of the cell as in Fig. 3. The nucleoid region appears fibrillar, and a mesosome-like structure is present in the rod portion of the cell. The hole may indicate inadequate fixation and subsequent removal of material such as volutin or fat, as others have described (3, 4). A similar hole appears in Fig. 4. Cell division occurs through distinct cross wall formation (Fig. 4), and, in a number of sections, cells were seen in which new cross walls apparently were forming before complete separation of the initial cross wall. This observation could explain the large variation in length among cells of *H. vaginalis* seen with the light microscope.

Various intracytoplasmic membranous structures may be seen in *H. vaginalis* 594. A complex structure was observed in the polar region (Fig. 2), and simpler structures were seen in the cytoplasm and in the nuclear regions. The laminated structure in the pleomorphic cell in Fig. 3 may represent another type of mesosome-like structure.

Interpretation of structural detail from the electron micrographs of Reyn et al. (4) is difficult because of inadequate definition of cellular components other than intracytoplasmic mesosomes. The use of glutaraldehyde and osmic acid fixation has greatly enhanced interpretation of the fine structure of *H. vaginalis* 594. Furthermore, these findings are consistent with prior observations that *H. vaginalis* 594 has a cell wall complex which resembles that of the gram-negative group of organisms both in size and fine-structural appearance.

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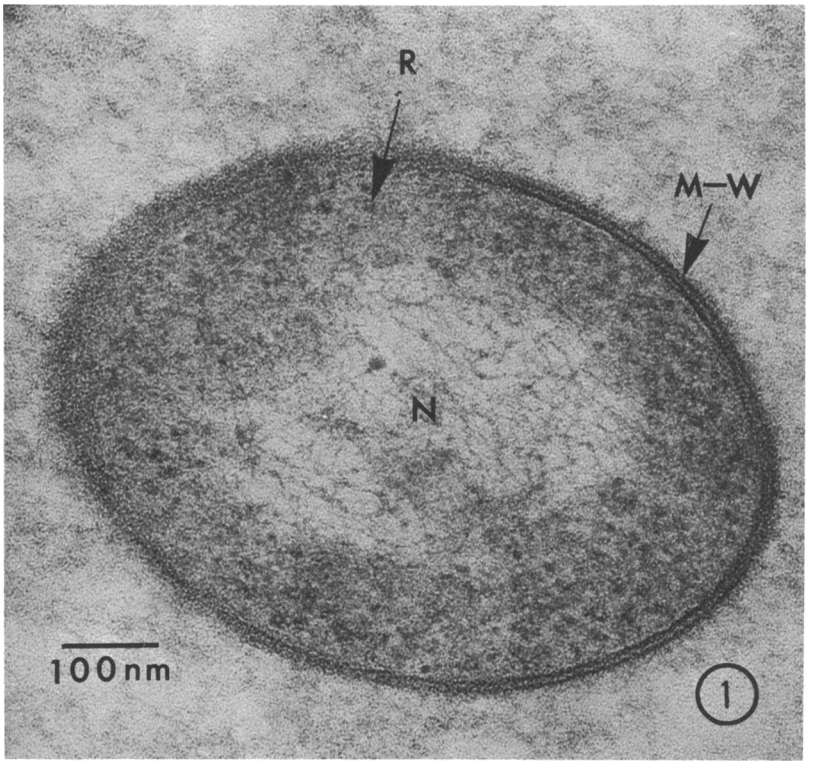

FIG. 1. *A section through Haemophilus vaginalis 594 showing a fibrillar nucleoid (N), ribosomes (R), and a multilayered cell wall-membrane complex (W-M).*

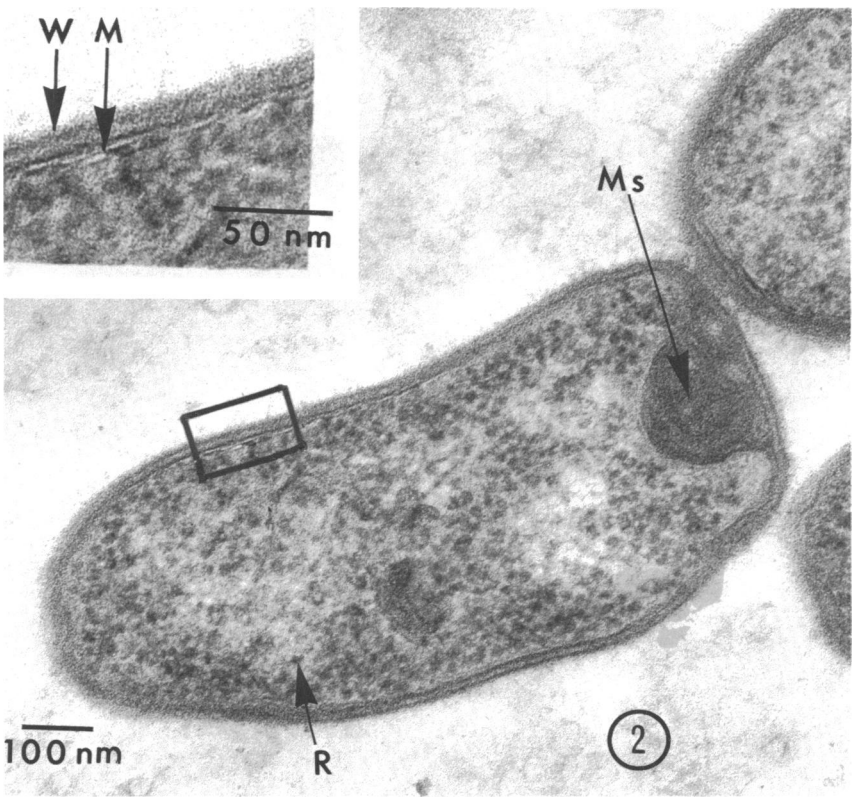

FIG. 2. *A longitudinal view of Haemophilus vaginalis 594 showing ribosomes (R) and an intracytoplasmic membranous structure resembling a mesosome (Ms). The wall-membrane complex is enlarged in the insert to show the structure more clearly. W, wall; M, membrane.*

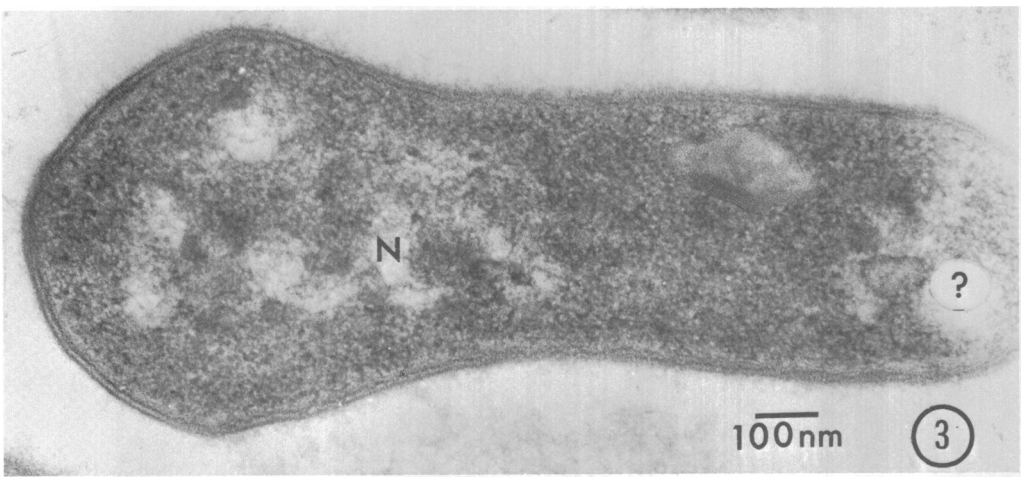

FIG. 3. *Longitudinal view of Haemophilus vaginalis 594 showing the pleomorphic bulbous enlargment of one end of the cell. Much of the nuclear material (N) appears to be contained within the bulbous area of the cell.*

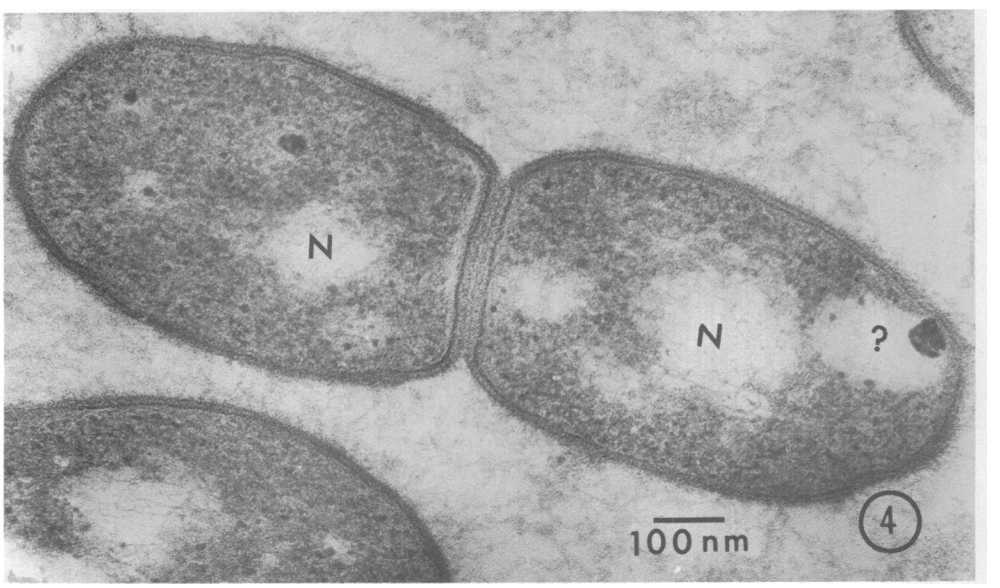

FIG. 4. *Sections of Haemophilus vaginalis 594 showing distinct cross-wall formation during division. Nuclear regions (N) appear in both daughter cells.*

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