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Arrangement of Pili in Colonies of *Neisseria gonorrhoeae*

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The morphology and arrangement of pili in the P^{++} colony phenotype of *Neisseria gonorrhoeae* were examined by a variety of electron microscopic techniques. The apparent structure and organization of gonococcal pili varied depending upon the method of specimen preparation. Pili as thin, individual, unbranched structures were demonstrated by negative staining and in sections of epoxy-embedded specimens. Pili forming thick structures which branch, subdivide, and rejoin to form an irregular lattice were demonstrated in specimens processed by the critical-point drying method and by rapid freezing and low temperature sublimation. We propose that in gonococcal colonies of the P^{++} phenotype, pili exist as individual threadlike structures only on the bacterial surfaces; as the pili leave the bacterial surfaces, they form thick bundles which branch, subdivide, and rejoin to form a supporting framework interconnecting the colony members. This arrangement of pili is usually disrupted by the commonly used method of negative staining and cannot be clearly detected within epoxy-embedded specimens. These data are summarized in a model depicting the organization of pili in the P^{++} colony phenotype of *N. gonorrhoeae*.

Pili are nonflagellar bacterial surface appendages. They are constructed from identical protein subunits (pilin) linked in tandem to form long thin polymers which radiate from the bacterial surface. These structures were first described by electron microscopy 30 years ago as surface features of *Escherichia coli* (1, 13) and were subsequently shown to be important for the pathogenicity of *E. coli* and other species of gram-negative bacteria (7, 10, 11, 33, 34).

In 1971, pili were reported on some colony types of *Neisseria gonorrhoeae*, the etiological agent of gonorrhea (17, 41). In published electron micrographs of negatively stained preparations, gonococcal (GC) pili appear as threadlike structures, and occasionally, lateral aggregates are observed (17, 35, 41). GC pili are also thought to be composed of linear polymers of pilin, a protein which ranges between 17,000 and 22,000 apparent molecular weight. The molecular weight, isoelectric point, antigenicity, and other biological properties of pili vary depending upon the strain of origin (3, 20–22, 30, 31).

The functions attributed to pili are wide ranging. Many studies indicate that pili are important to the pathogenicity of *N. gonorrhoeae*. In primary cultures isolated from gonorrhea patients, piliated colonies predominate (15, 18, 29). Experimentally, piliated gonococci (GC) are more virulent for humans (18), chimpanzees (2, 23), and chicken embryos (4, 6) and attach more readily to various eucaryotic cells including human vaginal epithelial cells (24), human fallopian tubes (44), human sperm (16), and human amnion cells (34). In different studies piliated GC were reported to be both more and less resistant to phagocytosis by human neutrophils than nonpiliated GC (27, 31–34, 37, 40, 42, 43) (for excellent reviews on the role of pili as mediators of interactions between GC and eucaryotic cells, see references 37 and 45). Piliated GC exhibit movement described as twitching motility whereas nonpiliated GC do not (12), and piliated GC are more readily transformed than nonpiliated GC (32). Piliated colony phenotypes are easily grown and maintained on clear typing medium (15). On this medium, the GC lack carbohydrate coats such as a glycocalyx but may express other polymers such as polyphosphates (26).

The structure and arrangement of GC pili may be important in understanding some of these proposed pathogenic functions; we have, therefore, by a variety of electron microscopic procedures, examined the ultrastructural features of these bacterial appendages.

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MATERIALS AND METHODS

GC. Strain JS1 was obtained from John Swanson, Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories. Isolation, identification, and propagation have been previously described (39). GC were grown for 18 to 20 h on GC clear typing medium as described by James and Swanson (15) and modified by Swanson (39). Piliated transparent colonies (P^{++} O^{-}) were used in these experiments (38). Nonpiliated transparent colonies were processed in parallel as controls (P^{-} O^{-}).

Negative staining. GC were removed from colonies by touching the colony surface with Formvar-coated grids and negatively staining the grids with 1.5% phosphotungstic acid, pH 7.0.

Embedded specimens. Colonies of GC were fixed in situ for 45 min at room temperature in a glutaraldehyde-based fixative described by Ito and Rikihisa (14). Fixed colonies were washed from the agar plates and then postfixed for 30 min in 1.5% osmium tetroxide in 0.05 M cacodylate buffer, pH 7.2. The glutaraldehyde- and osmium-fixed colonies were stained en bloc for 45 min with 0.5 mg of ruthenium red per ml, 0.5% uranyl acetate, or 0.5% tannic acid. The specimens were dehydrated in a graded series of ethanol and embedded in Spurr embedding medium (43).

For embedding in polyethylene glycol, the colonies were fixed as described above, stained en bloc in uranyl acetate, and processed as described by Wolosewick (46). Carbon and platinum replicas of sections dried by the critical-point drying (CPD) method were studied.

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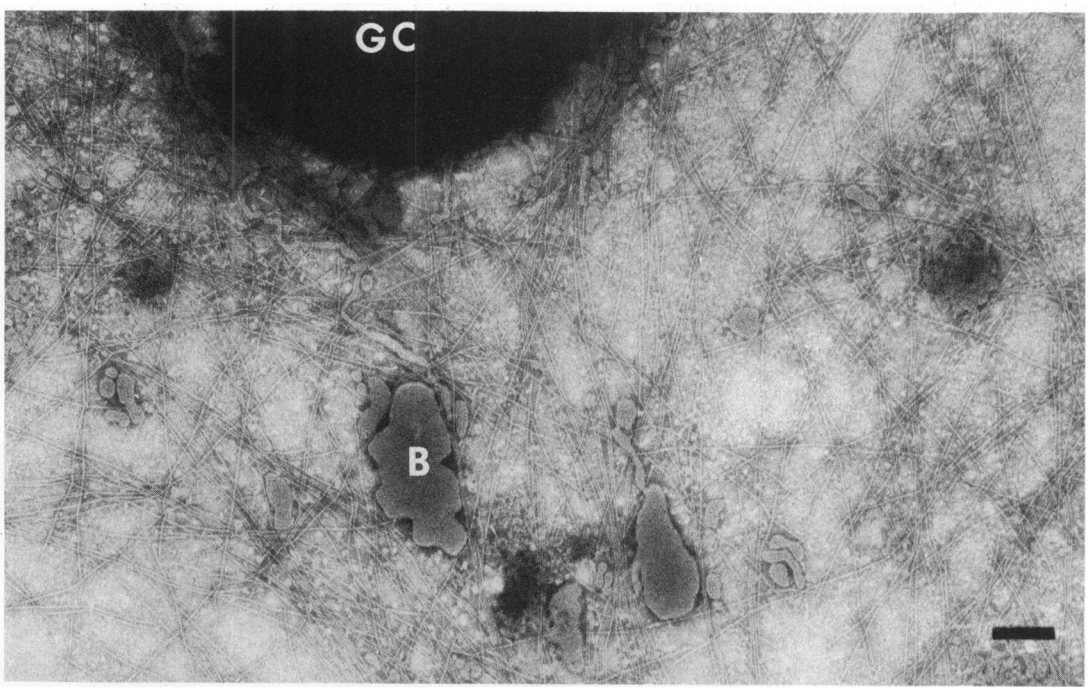

FIG. 1. Negatively stained preparations of piliated GC reveal the numerous linear, unbranched pili. Membrane blebs (B) are commonly detected. 75 kV. Bar, 0.1 μm. ×78,000.

CPD processing. GC were removed from the surface of colonies as described for negative staining. The GC were fixed for 45 min at room temperature as described by Ito and Rikihisa (14) followed by 1.5% osmium tetroxide for 10 min. The specimens were stained en bloc with 1% uranyl acetate and then dehydrated in a graded series of ethanol and dried by the CPD method (CPD) in CO_2. Carbon-coated and uncoated specimens were examined at 75 kV (transmission electron microscope) and 1,000 kV (high-voltage electron microscope [HVEM]) accelerating voltage.

Freeze-drying. GC were fixed on Formvar-coated grids as described for CPD processing. The fixed specimens were rinsed in distilled water and rapidly frozen in propane cooled with liquid nitrogen. The specimens were dried by sublimation for 24 h at −100°C and 7.6×10^{-8} mHg in a Denton vacuum chamber modified for low-temperature freeze-drying by the Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder. Carbon-coated and uncoated specimens were examined by HVEM at 1,000 kV.

Freeze-fracture. Unfixed colonies were washed from agar plates with Dulbecco buffer (8) and rapidly frozen in Freon cooled with liquid nitrogen. The frozen colonies were fractured and etched in a Balzers apparatus. Carbon- and platinum-shadowed replicas were cleaned in 6% sodium hypochlorite and collected on grids after washing with distilled water. Glutaraldehyde-fixed colonies rinsed in water were also prepared by this same method.

RESULTS

Piliated GC negatively stained with phosphotungstic acid (Fig. 1) show numerous long threadlike structures (pili) dispersed across the grid surface. The pili, ca. 7 nm in diameter, are unbranched and sometimes are found in aggregates or parallel arrays. Nonpiliated colonies (P^- O^-) processed in parallel lack these structures (data not shown).

In thin sections of epoxy-embedded colonies (Fig. 2), the pili also appear as individual threadlike structures, occasionally in bundles or parallel arrays. The pili are difficult to detect in sections unless the colonies are first stained en bloc with reagents such as tannic acid or ruthenium red. Similar features of the piliated colonies, the presence of numerous thin threads and scattered bundles, are observed when 0.5-μm-thick sections were studied with HVEM (data not shown).

When GC are removed from the surface of piliated colonies (P^{++} O^-) with Formvar-coated grids and the specimens are processed by the CPD method, stabilized with carbon, and studied by HVEM, strikingly different images of pili are obtained (Fig. 3). The GC are seen to be joined together by thick structures which branch and fuse together to form an irregular three-dimensional lattice. At higher magnification and in stereo, these structures can be seen both to connect the GC and occasionally appear to contain electron-dense material (Fig. 4). The electron-dense material associated with the thick structures was not demonstrated with other preparative techniques.

The diameters of the thick structures vary but are usually 30 nm or larger. To test whether the increased thickness is due to the carbon coating, we examined similarly prepared specimens directly without carbon coating at both 1,000 and 75 kV (Fig. 5). The images obtained are similar, independent of the presence of a carbon coating.

In an attempt to view these thick structures within the interior of the piliated colonies, rather than just at the surface, fixed colonies embedded in polyethylene glycol were sectioned. The polyethylene glycol was removed, and the sections were processed by the CPD method. Platinum-carbon replicas of the sections were prepared and examined. Although the fine structural features are inapparent, the irregular lattice remains intact within the piliated colonies (Fig. 6). The individual threadlike structures readily detected in negatively stained and in epoxy-embedded specimens are

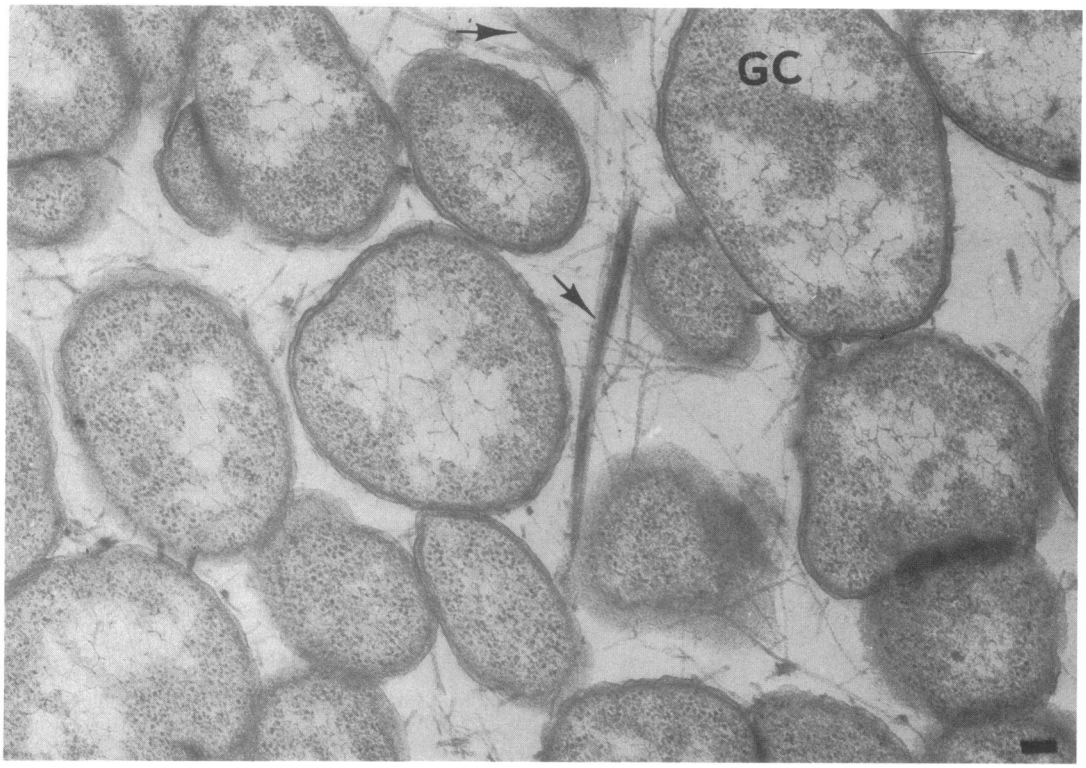

FIG. 2. Pili, both as individual strands and as aggregates (arrows), are shown in sections of epoxy-embedded colonies of *N. gonorrhoeae*. 75 kV. Bar, 0.1 μm. ×41,000.

not detected in similar specimens processed by the CPD method, and conversly, the large lattice-like network shown in CPD specimens is absent in the negatively stained and epoxy-embedded specimens. In nonpiliated colonies of GC processed in parallel as controls, neither thick nor thin structures are detected. This lattice-like arrangement of pili is also preserved when GC are removed from the surface of piliated colonies (P^{++} O^{-}) with Formvar-coated grids, fixed,

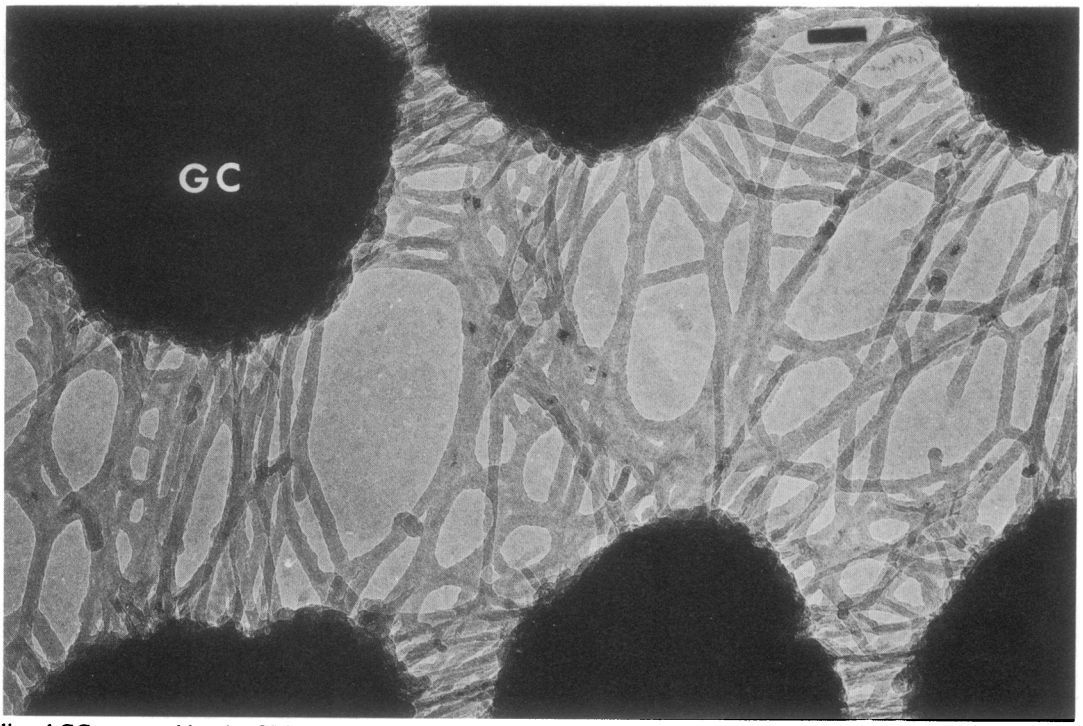

FIG. 3. Piliated GC prepared by the CPD method reveal a network of thick structures which branch and rejoin. The individual thin linear pili were not revealed by this method. 1,000 kV. Bar, 0.1 μm. ×72,000.

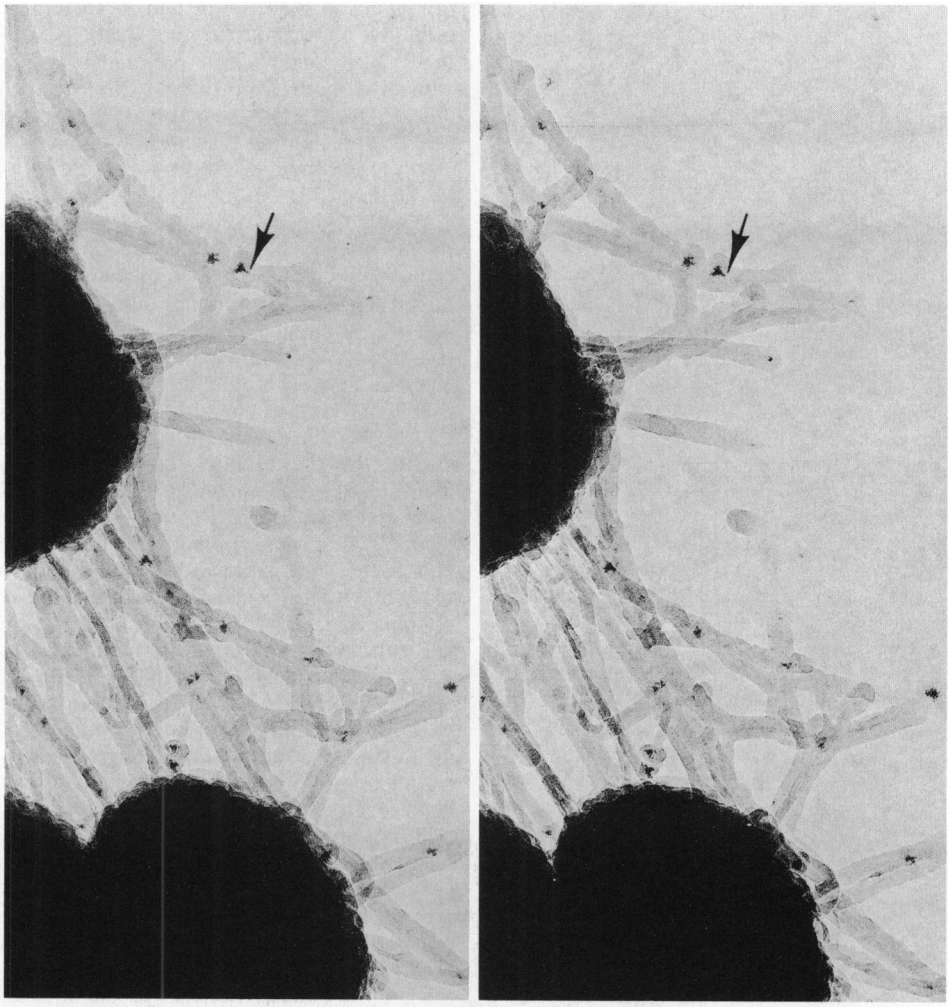

FIG. 4. When studied with a stereo viewer, the thick structures of piliated GC, preserved by CPD, often appear to contain electron-dense material (arrows). 1,000 kV. ×68,000.

rapidly frozen, and dried at −100°C. The HVEM images of specimens prepared by this freeze-drying method (Fig. 7) are similar to the HVEM images of specimens prepared by CPD.

In an attempt to determine how the different images of pili are related and to identify artifacts present in the different methods of specimen preparation used, unfixed piliated colonies were rapidly frozen in Freon cooled with liquid nitrogen, fractured, and etched. Carbon-platinum replicas were made and examined (Fig. 8). Replicas of fractured and etched colonies clearly reveal individual pili closely associated with the surface of the GC outer membrane (Fig. 8). However, as the pili leave the GC surface, they invariably form bundles. The bundles branch and, where they can be traced, spread out again as individual threads upon contact with another GC. Thin individual pili are not clearly seen in the medium between the GC. Similar images are obtained whether or not GC were fixed in glutaraldehyde and rinsed in water before freezing.

DISCUSSION

As demonstrated in this study, the apparent size, morphology, and arrangement of GC pili vary depending upon the method of specimen preparation employed. The long thin strands of unbranched pili were readily detected in negatively stained preparations. This observation was confirmed in the sections of epoxy-embedded piliated colonies. The methods of negative staining and embedding in epoxy are by far the most common electron microscopic procedures used to study GC pili (5, 17, 28, 30, 35, 36, 41, 42). In this respect, our results are similar to reports of others, independent of the strain of piliated GC used.

In contrast to the images of pili obtained by using these traditional techniques, when piliated GC were removed from colonies (in the same manner as for negative staining) and processed by the CPD method, strikingly different images of pili were obtained. The pili now appeared as much larger structures which branched to form a three-dimensional irregular lattice. This lattice-like arrangement was also detected within the colonies when polyethylene glycol sections were dried by the CPD method and examined either directly or from replicas. Nonpiliated colonies processed in parallel lacked these structures.

Although the appearance of pili is markedly affected by the preparative techniques commonly used in electron microscopy, in numerous studies with negatively stained or

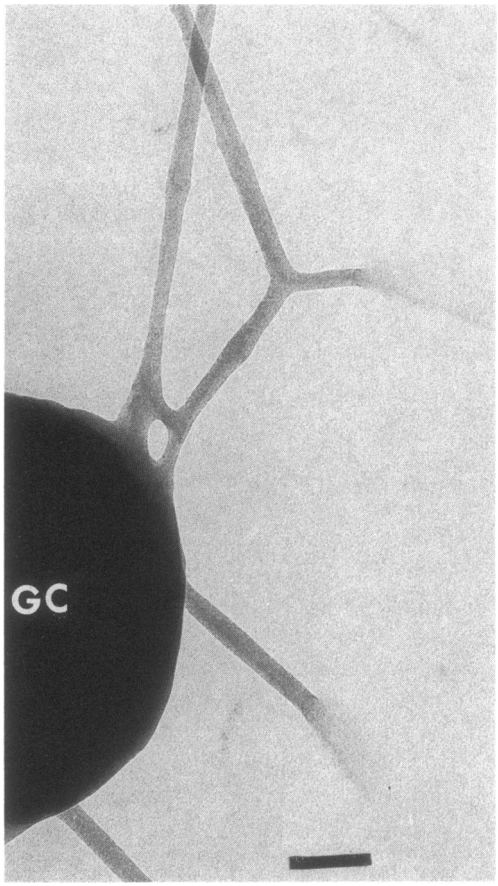

FIG. 5. Thick structures of piliated *N. gonorrhoeae* are also detected without the presence of carbon coating usually used to stabilize specimens prepared by CPD. 75 kV. Bar, 0.1 μm. ×105,000.

epoxy-embedded preparations, bundles or aggregates of pili have been demonstrated. Robertson and co-workers published an electron micrograph of purified pili negatively stained with uranyl acetate (30). The pili were arranged largely in bundles formed by parallel aggregates of individual pili. Penn and co-workers (28) included micrographs of GC which bore pili radiating separately from the cell and others which aggregated laterally to form bundles. Swanson and co-workers (5, 41) published numerous electron micrographs of pili negatively stained with phosphotungstic acid or uranyl acetate, in which aggregates were more frequently seen in those preparations stained with uranyl acetate. Bundles or aggregates of pili were also present in electron micrographs published by Jephcott et al. (17). Using scanning electron microscopy, Elmros et al. (9) showed that within piliated colonies (which were prepared by CPD) individual GC were interconnected by thick structures which they termed "intracellular strands"; these strands were absent in nonpiliated colonies. Kraus and Glassman also published scanning electron microscopic photomicrographs of colonies with thick intracellular strands (19). The possible organization of pili is, however, not addressed in these papers. Pili which appear to radiate from the bacterial surface as individual threads and pili which appear as thick structures or bundles are both commonly reported depending primarily on the method of specimen preparation employed. How the pili might be organized in the colony before specimen preparation is of potential importance to understand how pili function within the colony and in the pathogenesis of gonorrhea. Most clinical isolates from human urogenital tracts are found in piliated form. The structural arrangement of pili may be effective in holding the colony members together in this in vivo environment which is subjected to extensive mechanical flushing.

Whether the native forms of pili are similar to the thin-strand images commonly produced by negative staining and epoxy embedding or to thick interconnecting strands produced by CPD techniques and occasionally produced by negative staining and epoxy embedding is an engaging question of potential importance to the analysis of the methods of electron microscopy as well as to understanding the pathogenesis of gonorrhea. The carbon coat used to stabilize the CPD specimens adds little to the image and cannot alone account for the thickness of the pili (Fig. 5). Does the CPD itself induce artificial aggregations and distortions which form images as seen in Fig. 3 through 6? Or do negative staining or epoxy embedding or both usually disassociate and destroy the native structural arrangements of pili, perhaps due to the surface tension of air drying in negative staining and by heating at 65°C in the presence of organic solvents during the epoxy-embedding process?

To understand how the different images are related, we studied piliated colonies by methods of rapid freezing. In specimens prepared by freeze-drying (Fig. 7), the lattice-like arrangement of pili was apparent. We were unable to detect significant differences in the images of piliated GC prepared by freeze-drying in comparison to CPD. In freeze-fracture micrographs individual pili were found on the etched surfaces of GC similar to those in images published by Swanson (35); however, as determined in this study, the intercellular spaces contained only thick structures, pili arranged in bundles (Fig. 8). (Although Buchanan et al. published micrographs of freeze-fracture- and freeze-etch-prepared GC with thick intracellular strands, the significance of this observation was not addressed by the authors [5].) The bundles present within the inter-GC spaces appeared to branch and rejoin to form an irregular lattice consistent with those images seen with CPD techniques. Individual pili were not detected in the inter-GC spaces. It seems likely that in situ pili are arranged in this manner before specimen preparation. It is difficult to explain the bundles of pili as aggregation artifacts induced by the freeze-fracture method because: (i) it is doubtful that ice crystal formation could force scattered pili together to form the thick bundles which interconnect the GC; (ii) possible aggregation of pili during etching (at −100°C) is ruled out because the pili, if naturally dispersed when frozen, would remain so as they continued into the unetched frozen matrix, and this is clearly not the case (Fig. 8B); and (iii) similar freeze-fractured, freeze-etched images were obtained whether the colonies were unfixed and frozen in buffer or fixed in glutaraldehyde, rinsed, and frozen in distilled water. Therefore, fixation-induced aggregation or images of putative salt artifacts (25) are not of importance here.

In summary, the appearance of pili varies depending upon the techniques used to prepare the specimens. Our hypothesis of how pili are arranged in the P^{++} colony phenotype of *N. gonorrhoeae* before specimen preparation is shown in Fig. 9. This model and the body of data presented suggest that in situ pili exist as individual threads only on the bacterial surfaces. As the threadlike pili leave the GC surfaces, they do not radiate as individual threads but are

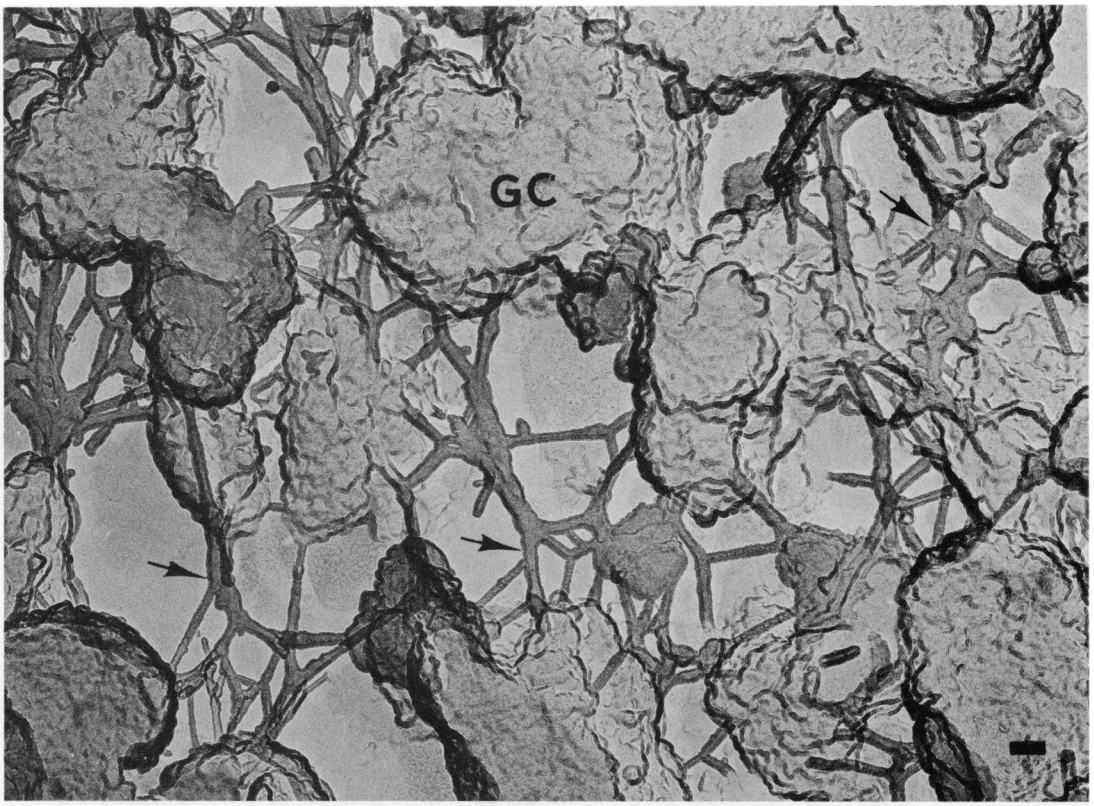

FIG. 6. Thick structures (arrows) of piliated *N. gonorrhoeae* which branch and rejoin to form a lattice-like arrangement are detected throughout the colony by examination of sections and replicas of polyethylene glycol-embedded specimens. 75 kV. Bar, 0.1 μm. ×45,000.

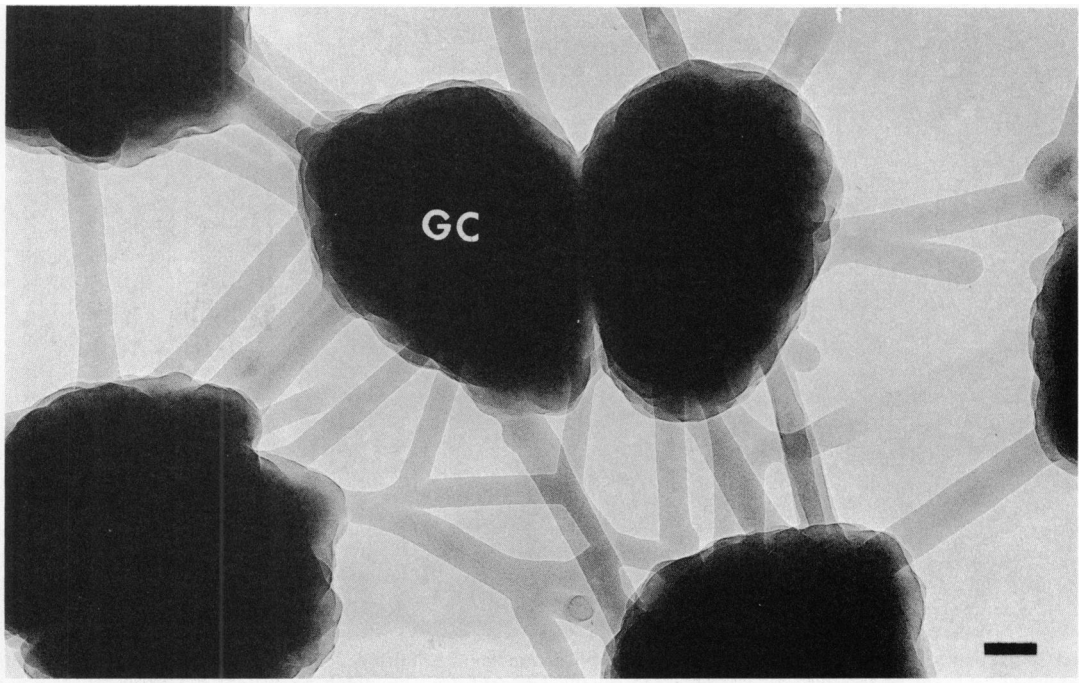

FIG. 7. Piliated GC prepared by rapid freezing following by low-temperature sublimation reveal a network of thick structures which branch and rejoin. 1,000 kV. Bar, 0.1 μm. ×68,000.

arranged in thick bundles which branch, subdivide, and rejoin to form a supporting network interconnecting the colony members. The presence of electron-dense material detected by HVEM within these aggregates of pili (Fig. 4) suggests the possibility that constituents other than pilin may be present.

This lattice-like arrangement of pili is preserved by the techniques of CPD and rapid freezing, but is inapparent in epoxy-embedded specimens and is largely destroyed when specimens are air dried as in negative staining.

From a biological perspective, the existence of a lattice-like network composed primarily of pili is consistent with the functions attributed to piliated GC. The formation in vivo of microcolonies held together by this lattice would accomplish the following: (i) provide multiple sites for adherence of the colony to host cells; (ii) minimize the mechanical flushing away of GC not directly attached to host cells; (iii) provide for optimal spacing of GC within the colony to maximize sharing of nutrients and exchange of genetic information; (iv) present a large surface area to the phagocytic cells of the host and minimize phagocytosis of individual GC; and (v) provide a large focus of GC for successful transmission to another host.

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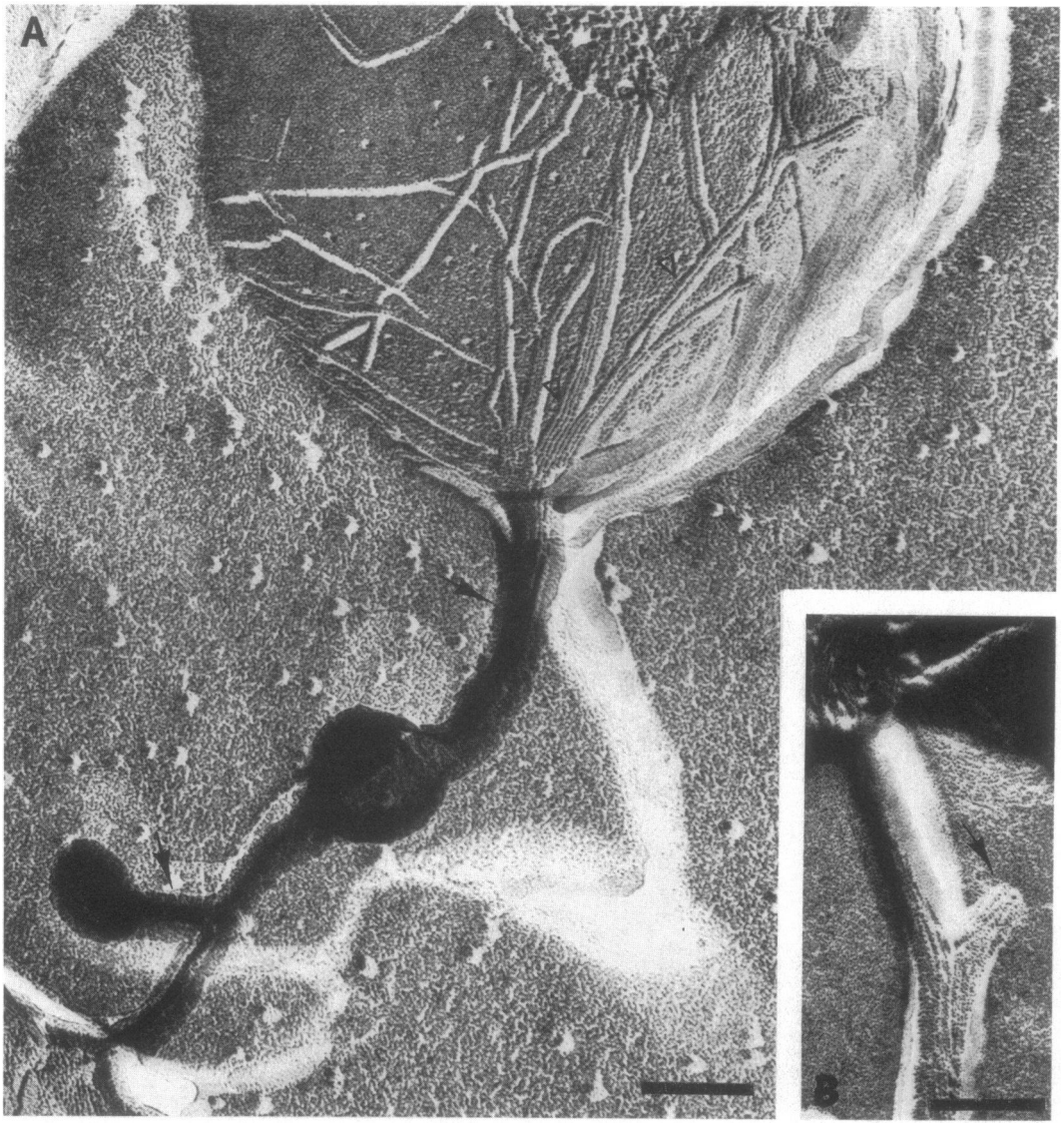

FIG. 8. Thick branched structures (arrows) and thin linear pili (triangles) are both revealed by freeze-fracture and etching of unfixed specimens of piliated GC. (A) Individual pili are detected on the GC surfaces; the thick branched structures are found between GC, continuous with individual threads. 75 kV. Bar, 0.1 μm. ×145,000. (B) Away from the GC surfaces the thread-like pili remain in bundles as they continue into the unetched matrix (arrow). 75 kV. Bar, 0.1 μm. ×150,000.

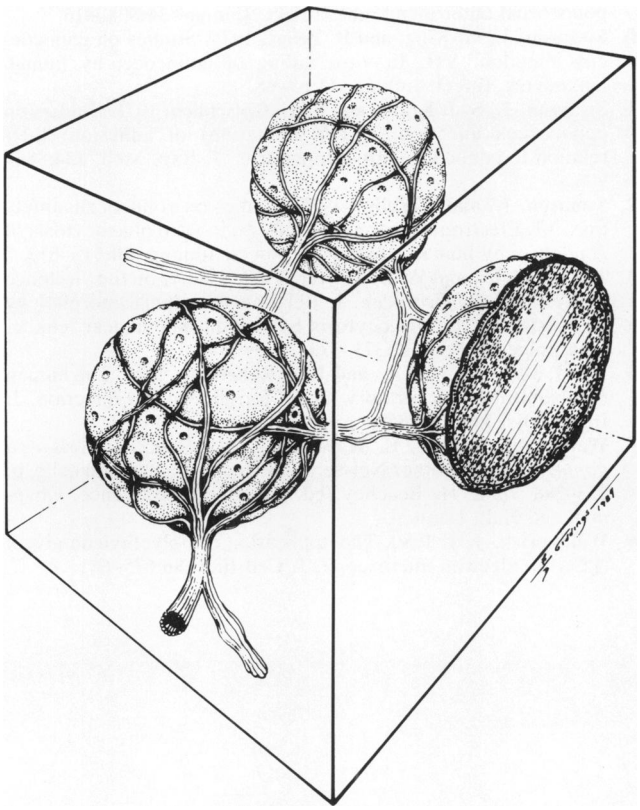

FIG. 9. Model depicting the arrangement of pili in the P^{++} colony phenotype *N. gonorrhoeae*. A portion of a colony is shown. The long thin pili appear as individual threads on the GC surfaces. The pili leave the GC surfaces in the form of bundles which branch and rejoin to form an irregular network interconnecting the colony members.

LITERATURE CITED

1. **Brinton, C. C., Jr., A. Buzzell, and M. A. Lauffer.** 1954. Electrophoresis and phage susceptibility studies on a filament-producing variant of the *E. coli* B bacterium. Biochim. Biophys. Acta **15**:533–542.
2. **Brown, W. J., C. T. Lucas, and U. S. G. Kuhn.** 1972. Gonorrhea in the chimpanzee. Infection with laboratory passed gonococci and by natural transmission. Br. J. Vener. Dis. **48**:177–178.
3. **Buchanan, T. M.** 1975. Antigenic heterogeneity of gonococcal pili. J. Exp. Med. **141**:1470–1475.
4. **Buchanan, T. M., and E. C. Gotschlich.** 1973. Studies on gonococcus infection. III. Correlation of gonococcal colony morphology with infectivity for the chick embryo. J. Exp. Med. **137**:196–200.
5. **Buchanan, T. M., J. Swanson, K. K. Holmes, S. J. Kraus, and E. C. Gotschlich.** 1973. Quantitative determination of antibody to gonococcal pili. J. Clin. Invest. **52**:2896–2909.
6. **Bumgarner, L. R., and R. A. Finkelstein.** 1973. Pathogenesis and immunology of experimental gonococcal infection: virulence of colony types of *Neisseria gonorrhoeae* for chicken embryos. Infect. Immun. **8**:919–924.
7. **Burrows, M. R., R. Sellwood, and R. A. Gibbons.** 1976. Haemagglutinating and adhesive properties associated with the K99 antigen of bovine strains of *Escherichia coli*. J. Gen. Microbiol. **96**:269–275.
8. **Dulbecco, R., and M. Vogt.** 1954. Plaque formation and isolation of pure lines with poliomyelitis virus. J. Exp. Med. **99**:167–182.
9. **Elmros, T., P. Hörstedt, and B. Winblad.** 1975. Scanning electron microscopic study of virulent and avirulent colonies of *Neisseria gonorrhoeae*. Infect. Immun. **12**:630–637.
10. **Evans, D. G., and D. J. Evans, Jr.** 1978. New surface-associated heat-labile colonization factor antigen (CFA/II) produced by enterotoxigenic *Escherichia coli* of serogroups O6 and O8. Infect. Immun. **21**:638–647.
11. **Evans, D. G., D. J. Evans, Jr., W. S. Tjoa, and H. L. DuPont.** 1978. Detection and characterization of colonization factor of enterotoxigenic *Escherichia coli* isolated from adults with diarrhea. Infect. Immun. **19**:727–736.
12. **Henrichsen, J.** 1972. Bacterial surface translocation: a survey and a classification. Bacteriol. Rev. **36**:478–503.
13. **Houwink, A. L., and W. van Iterson.** 1950. Electron microscopical observations on bacterial cytology. II. A study on flagellation. Biochim. Biophys. Acta **5**:10–44.
14. **Ito, S., and Y. Rikihisa.** 1981. Techniques for electron microscopy of rickettsiae, p. 213–240. *In* W. Burgdorfer and R. L. Anacker (ed.), Rickettsiae and rickettsial diseases. Academic Press, Inc., New York.
15. **James, J. F., and J. Swanson.** 1978. Studies on gonococcus infection. XIII. Occurrence of color/opacity colonial variants in clinical cultures. Infect. Immun. **19**:332–340.
16. **James-Holmquest, A. N., J. Swanson, T. M. Buchanan, R. D. Wende, and R. P. Williams.** 1974. Differential attachment by piliated and nonpiliated *Neisseria gonorrhoeae* to human sperm. Infect. Immun. **9**:897–902.
17. **Jephcott, A. E., A. Reyn, and A. Birch-Andersen.** 1971. *Neisseria gonorrhoeae*. III. Demonstration of presumed appendages to cells from different colony types. Acta Pathol. Microbiol. Scand. Sect. B **79**:437–439.
18. **Kellogg, D. S., Jr., W. L. Peacock, Jr., W. E. Deacon, L. Brown, and C. I. Pirkle.** 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. J. Bacteriol. **85**:1274–1279.
19. **Kraus, S. J., and L. H. Glassman.** 1974. Scanning electron microscope study of *Neisseria gonorrhoeae*. Appl. Microbiol. **27**:584–592.
20. **Lambden, P. R.** 1982. Biochemical comparison of pili from variants of *Neisseria gonorrhoeae* P9. J. Gen. Microbiol. **128**:2105–2111.
21. **Lambden, P. R., J. E. Heckels, H. McBride, and P. J. Watt.** 1981. The identification and isolation of novel pilus types produced by variants of *N. gonorrhoeae* P9 following selection in vivo. FEMS Microbiol. Lett. **10**:339–341.
22. **Lambden, P. R., J. N. Robertson, and P. J. Watt.** 1980. Biological properties of two distinct pilus types produced by isogenic variants of *Neisseria gonorrhoeae* P9. J. Bacteriol. **141**:393–396.
23. **Lucas, C. T., F. Chandler, Jr., J. E. Martin, Jr., and J. D. Schmale.** 1971. Transfer of gonococcal urethritis from man to chimpanzee. An animal model for gonorrhea. J. Am. Med. Assoc. **216**:1612–1614.
24. **Mårdh, P.-A., and L. Weström.** 1976. Adherence of bacteria to vaginal epithelial cells. Infect. Immun. **13**:661–666.
25. **Miller, K. R., C. S. Prescott, T. L. Jacobs, and N. L. Lassignal.** 1983. Artifacts associated with quick-freezing and freeze-drying. J. Ultrastruct. Res. **82**:123–133.
26. **Noegel, A., and E. C. Gotschlich.** 1983. Isolation of a high molecular weight polyphosphate from *Neisseria gonorrhoeae*. J. Exp. Med. **157**:2049–2060.
27. **Ofek, I., E. H. Beachey, and A. L. Bisno.** 1974. Resistance of *Neisseria gonorrhoeae* to phagocytosis: relationship to colonial morphology and surface pili. J. Infect. Dis. **129**:310–316.
28. **Penn, C. W., N. J. Parsons, D. R. Veale, and H. Smith.** 1980. Antigenic heterogeneity associated with pilus aggregation and autoagglutinability in *Neisseria gonorrhoeae*. J. Gen. Microbiol. **121**:195–202.
29. **Punsalang, A. P., Jr., and W. D. Sawyer.** 1973. Role of pili in the virulence of *Neisseria gonorrhoeae*. Infect. Immun. **8**:255–263.
30. **Robertson, J. N., P. Vincent, and M. E. Ward.** 1977. The preparation and properties of gonococcal pili. J. Gen. Microbiol. **102**:169–177.
31. **Salit, I. E., M. Blake, and E. C. Gotschlich.** 1980. Intra-strain heterogeneity of gonococcal pili is related to opacity colony

variance. J. Exp. Med. **151**:716–725.
32. **Sparling, P. F.** 1966. Genetic transformation of *Neisseria gonorrhoeae* to streptomycin resistance. J. Bacteriol. **92**:1364–1371.
33. **Stirm, S., F. Ørskov, I. Ørskov, and B. Mansa.** 1967. Episome-carried surface antigen K88 of *Escherichia coli*. II. Isolation and chemical analysis. J. Bacteriol. **93**:731–739.
34. **Svanborg Edén, C., and H. A. Hansson.** 1978. *Escherichia coli* pili as possible mediators of attachment to human urinary tract epithelial cells. Infect. Immun. **21**:229–237.
35. **Swanson, J.** 1972. Studies on gonococcus infection. II. Freeze-fracture, freeze-etch studies on gonococci. J. Exp. Med. **136**:1258–1271.
36. **Swanson, J.** 1973. Studies on gonococcus infection. IV. Pili: their role in attachment of gonococci to tissue culture cells. J. Exp. Med. **137**:571–589.
37. **Swanson, J.** 1977. Surface components affecting interactions between *Neisseria gonorrhoeae* and eucaryotic cells. J. Infect. Dis. **136**:S138–S143.
38. **Swanson, J.** 1977. Surface components associated with gonococcal-cell interactions in the gonococcus, p. 369–401. *In* R. B. Roberts (ed.), The gonococcus. John Wiley & Sons, Inc., New York.
39. **Swanson, J.** 1981. Surface-exposed protein antigens of the gonococcal outer membrane. Infect. Immun. **34**:804–816.
40. **Swanson, J., G. King, and B. Zeligs.** 1975. Studies on gonococcus infection. VII. In vitro killing of gonococci by human leukocytes. Infect. Immun. **11**:65–68.
41. **Swanson, J., S. J. Kraus, and E. C. Gotschlich.** 1971. Studies on gonococcus infection. I. Pili and zones of adhesion: their relation to gonococcal growth patterns. J. Exp. Med. **134**:886–906.
42. **Swanson, J., and B. Zeligs.** 1974. Studies on gonococcus infection. VI. Electron microscopic study on in vitro phagocytosis of gonococci by human leukocytes. Infect. Immun. **10**:645–656.
43. **Thongthai, C., and W. D. Sawyer.** 1973. Studies on the virulence of *Neisseria gonorrhoeae*. I. Relation of colonial morphology and resistance to phagocytosis by polymorphonuclear leukoctyes. Infect. Immun. **7**:373–379.
44. **Ward, M. E., P. J. Watt, and J. N. Robertson.** 1974. The human fallopian tube: a laboratory model for gonococcal infection. J. Infect. Dis. **129**:650–659.
45. **Watt, P. J., and M. E. Ward.** 1980. Adherence of *Neisseria gonorrhoeae* and other Neisseria species to mammalian cells, p. 253–288. *In* E. H. Beachey (ed.), Bacterial adherence. Chapman and Hall, London.
46. **Wolosewick, J. J.** 1980. The application of polyethylene glycol (PEG) to electron microscopy. J. Cell Biol. **86**:675–681.