

PILI ON MENINGOCOCCI FROM PRIMARY CULTURES OF NASOPHARYNGEAL CARRIERS AND CEREBROSPINAL FLUID OF PATIENTS WITH ACUTE DISEASE*

BY I. W. DEVOE AND J. E. GILCHRIST

(From the Department of Microbiology, Macdonald Campus of McGill University, Macdonald College H9X 3M1, Quebec, Canada)

Although pili have been observed on the surfaces of several strains of *Neisseria meningitidis* (2, 4), the proportion of cells in a population that possess these structures is generally less than 5%. Furthermore, these few piliated cells seldom exhibit more than one or two pili per cell (2). One exceptional strain of meningococcus (ATCC 13090), however, routinely produces abundant pili on nearly all the cells of the population (>80%) under a variety of laboratory growth conditions (2).

In the close relative, *Neisseria gonorrhoeae*, Kellogg et al. (5, 6) found a correlation between colony type and virulence. Swanson et al. (10) and Jephcott et al. (4) reported that only cells from the virulent colony types were piliated suggesting that pili may be linked to the property of virulence in the gonococcus. Furthermore, the piliated gonococci were found to have a greater ability to adhere to both tissue culture cells (9) and sperm cell surfaces (3), both of which could give these cells a selective advantage in the urinary tract. Other data suggest that pili may also aid this bacterium in resisting phagocytosis (7). The work presented here is the result of a field survey of meningococci from known carriers at the Canadian Forces Base, Cornwallis, Nova Scotia and also from patients with acute meningococcal disease in São Paulo, Brazil.

Materials and Methods

Organisms. Meningococci for this study were obtained from the nasopharynx of known carriers (Canadian Forces Base, Cornwallis, Nova Scotia) without symptoms of acute disease and the cerebrospinal fluid (CSF) of patients suffering from acute meningococcal disease (Emilio Ribas Hospital, São Paulo, Brazil). Specimens from throat swabs of CSF were cultured on Thayer-Martin agar plates [containing both Supplement B (Difco Laboratories, Detroit, Mich.) and antibiotic inhibitors vancomycin (3 µg/ml), colistin (7.5 µg/ml), and nystatin (12.5 U/ml)]. The plates were incubated at 37°C (16 h) in candle jars with 100% humidity. All strains were identified as

* This work was supported in part by the National Research Council of Canada.

meningococci and serogrouped by procedures described by Vedros (11). Serogroups were determined by means of antisera supplied by the Neisseria Repository, Naval Medical Research Unit No. 1, Berkeley, Calif. All subcultures of the various isolates were grown on the medium described above.

Electron Microscopy. Suspensions of cells from colonies were prepared in either physiological saline or Gey's balanced salts. Either of these solutions was found to be equally satisfactory for the purpose of this study. Formvar-coated, carbon-coated grids were floated upon drops of various cell suspensions. Negative staining was carried out as previously described (1). Electron micrographs were taken with an AEI electron microscope EM6B (AEI Scientific Apparatus, Inc., Elmsford, N.Y.).

Results

The significance of surface pili in the meningococcal disease process is not understood. These fibrillar structures have been detected on a number of meningococcal laboratory strains (2, 4); however, with the exception of one strain (ATCC 13090) (2) the number of cells, if any, in a population which exhibit pili is very low. In order to further define this situation freshly isolated strains of meningococci taken from both known carriers and individuals with meningococcal disease were analyzed to establish whether cells in primary cultures were distinctly different from those of laboratory strains.

Cells from approximately 30 primary cultures of carrier strains and a similar number of strains from CSF were analyzed after suspending cells from single colonies in an appropriate salt solution. From these suspensions negative-stain preparations were prepared for electron microscopy. Although our main concern involved the possible pili on these cells, we were also interested in cell wall surface characteristics especially with regard to the blebs previously reported (1) on cells of three prototype meningococcal laboratory strains.

The initial findings from all primary cultures were especially interesting in that pili were observed in over 80% of cells from every colony analyzed, irrespective of the individual from which they were isolated or the serogroup of the bacterium. The piliation was relatively heavy on some cells while others exhibited only a moderate number of these structures (Fig. 1 *a* and *b*). The parallel aggregate masses of pili similar to that reported for the gonococcus (4, 8) were not observed in any of these preparations. However, clumps of pili not associated with the cell surfaces (Fig. 2) were occasionally observed, but such random aggregation may be an artifact introduced by the shearing and clumping of pili during the suspension of cells from various colonies.

The finding that over 80% of the cells from all of the colonies examined were piliated prompted us to investigate the effects of subculturing on the presence of pili on these same cells. Sequential subcultures were carried out on primary cultures from 10 individual carriers and 15 CSF samples (Table I). The results were similar in each instance. On the first subculture (Fig. 3 *a* and *b*) approximately 50% of the cells from single colonies possessed pili; however, the number of pili on individual cells was reduced significantly from that observed on cells in primary cultures. A second subculture of these cells resulted in either the complete loss of detectable pili or in a very low frequency of piliation (Fig. 4 *a* and *b*) similar to that previously observed in laboratory strains (2). Therefore, it seems reasonable to conclude that meningococci are piliated in the carrier state and in the disease state and that either partial or complete loss of these

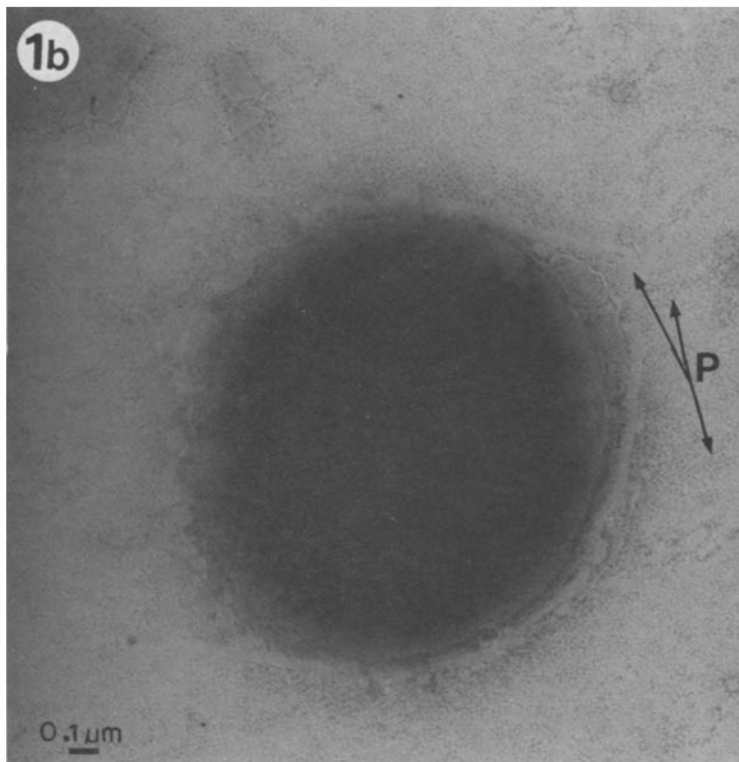
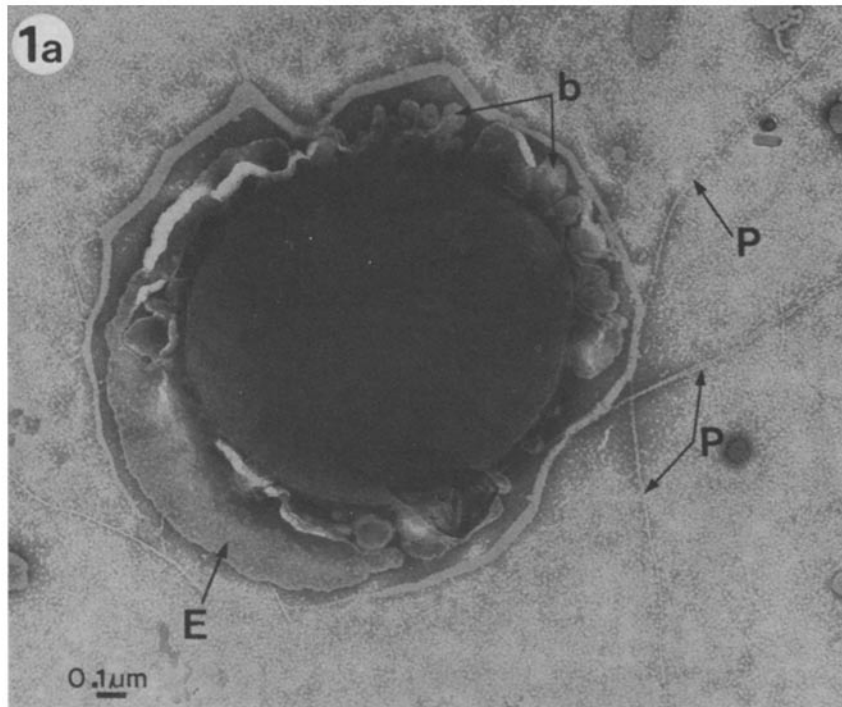


FIG. 1. (a) Meningococcus from primary culture of nasopharynx of carrier. (P), pilus; (b), cell wall bleb; (E), extracellular material of unknown origin. $\times 40,000$. (b) Meningococcus from primary culture of cerebrospinal fluid of patient with acute meningococcal disease. $\times 45,000$.

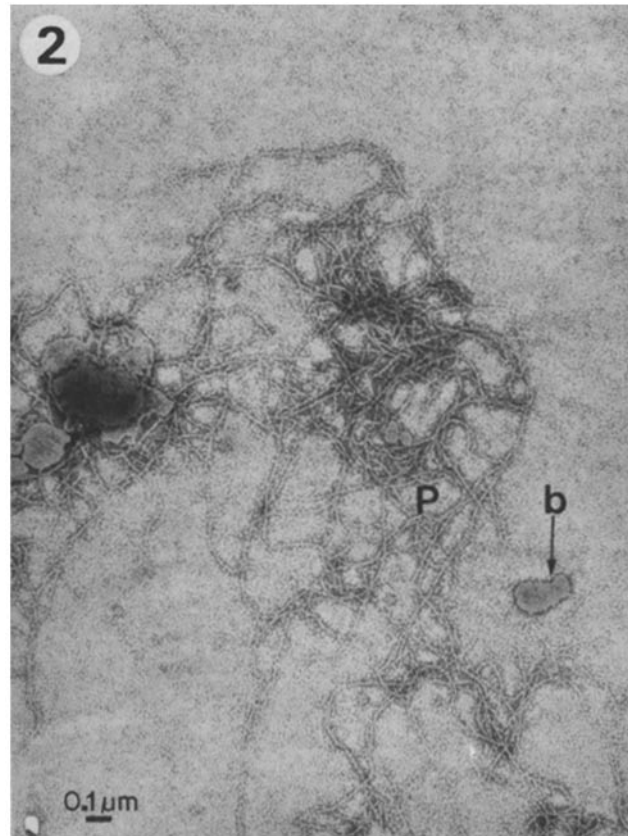


FIG. 2. Aggregate of cell-free pili from primary culture. $\times 30,000$.

structures from the population occurs as a result of serial subculture under laboratory conditions.

The extracellular membranous material surrounding each of the cells (Fig. 1-4) from either primary or serial subcultures was a prominent feature of all cells in these preparations and, therefore, deserves mention here. We have previously reported (1) that in actively growing cultures of meningococci, endotoxin-containing blebs of the outer-trilaminar cell wall layer were produced and released into the surrounding medium under normal growth conditions. In the study presented here, the extracellular wall material surrounding cells from primary cultures (Fig. 1) was frequently more amorphous than that observed on cells after subculture (Figs. 3 and 4); however, generally the morphology of blebs was qualitatively similar in cells from either primary cultures or subcultures. Although conversion of cells to laboratory strains involves the loss of pili, the characteristic release of cell wall material during growth is retained after serial subculture.

There were no detectable morphological differences between cells of strains taken from carriers or those taken from patients with acute disease. Furthermore, all serogroups were indistinguishable on the basis of gross morphological differences.

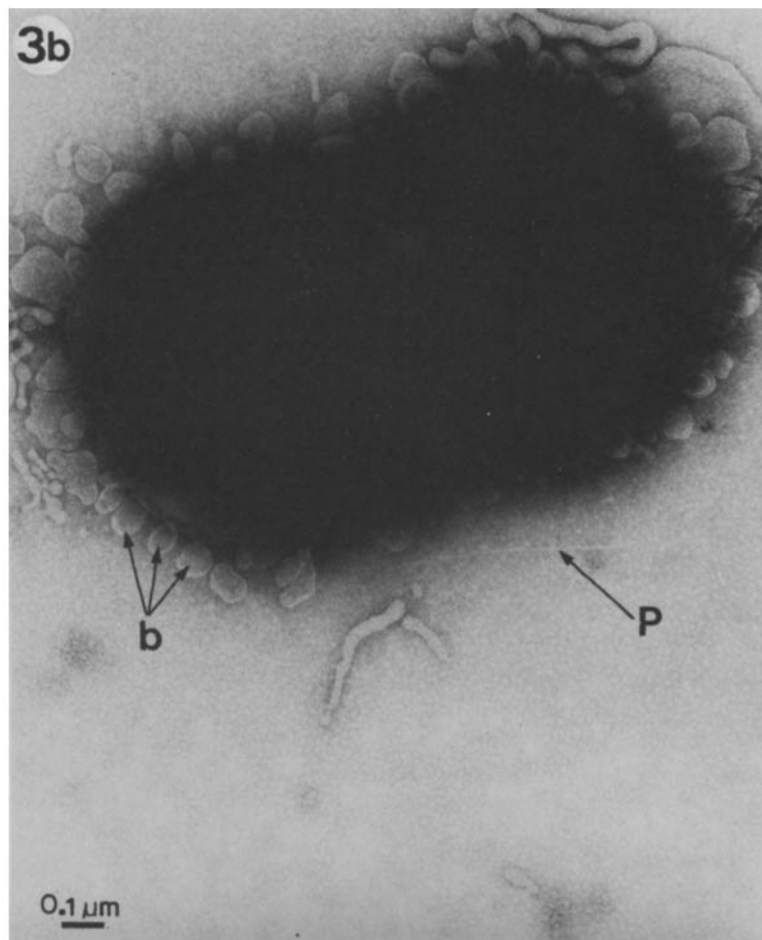
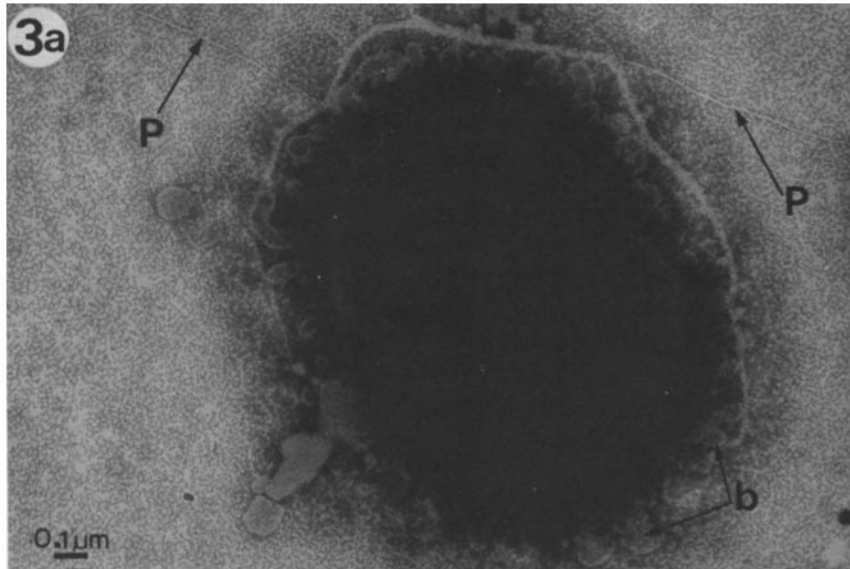


FIG. 3. Meningococcus from second serial culture from (a) nasopharynx of carrier ($\times 40,000$) and (b) cerebrospinal fluid ($\times 60,000$).

TABLE I
Characteristics of Strains from the Nasopharynx of Meningococcal Carriers‡*

Strain no.	Gram strain	Morphology	Oxidase	Glucose	Maltose	Other sugars	Serogroup
NS 39	-	Diplococcus	+	+	+	-	B
NS 2	-	Diplococcus	+	+	+	-	A
NS 36	-	Diplococcus	+	+	+	-	E
NS 184	-	Diplococcus	+	+	+	-	C
NS 165	-	Diplococcus	+	+	+	-	B
NS 38	-	Diplococcus	+	+	+	-	E
NS 195	-	Diplococcus	+	+	+	-	B
NS 109	-	Diplococcus	+	+	+	-	B
NS 49	-	Diplococcus	+	+	+	-	B
NS 19	-	Diplococcus	+	+	+	-	C

* All carrier strains were taken from the nasopharynx of Canadian Forces Recruits, Cornwallis, Nova Scotia.

‡ General characteristics of isolates from CSF were the same as above except all strains were serogroup A. All samples of CSF were taken from patients in Sao Paulo, Brazil.

Discussion

In a previous paper from this laboratory (2) we reported the presence of abundant pili on the surface of a serogroup B meningococcus (ATCC 13090) under a variety of growth conditions. In our experience, the presence of abundant pili on this laboratory strain is in marked contrast to the absence or near absence of pili in meningococcal populations of over 40 laboratory strains surveyed to date (DeVoe, unpublished). A small proportion (<5%) of cells in populations of three prototype strains were found generally to have one or two pili per cell but only rarely more than five. Such small proportions of piliated cells were not associated with a distinctive colony type, such as observed with the gonococcus (10), but to the contrary were representative of all colonies tested.

The work reported here provided some insight into the small numbers of pili found previously in laboratory strains of meningococci. The primary cultures of over 30 individual meningococcal carriers and of a similar number of patients with meningococcal disease were analyzed. Each colony tested from cultures of patients or carriers contained greater than 80% piliated cells. However, when randomly selected strains were serially subcultured, the pili were either lost or were present only in very low numbers similar to those previously reported for laboratory strains of meningococci.

That cells after serial subculture retain the ability to synthesize pili is indicated by the presence of small numbers of piliated cells in each colony. This suggests that commonly used laboratory media either contain a substance which represses the synthesis of pili or that some genetic inducer is present in the natural environment of the host. Another possible explanation which must be considered is that there may be a selective pressure in the host which permits only piliated cells to survive. Were this latter possibility valid, one might expect the loss of these pili in media in which such a selection pressure were absent. A

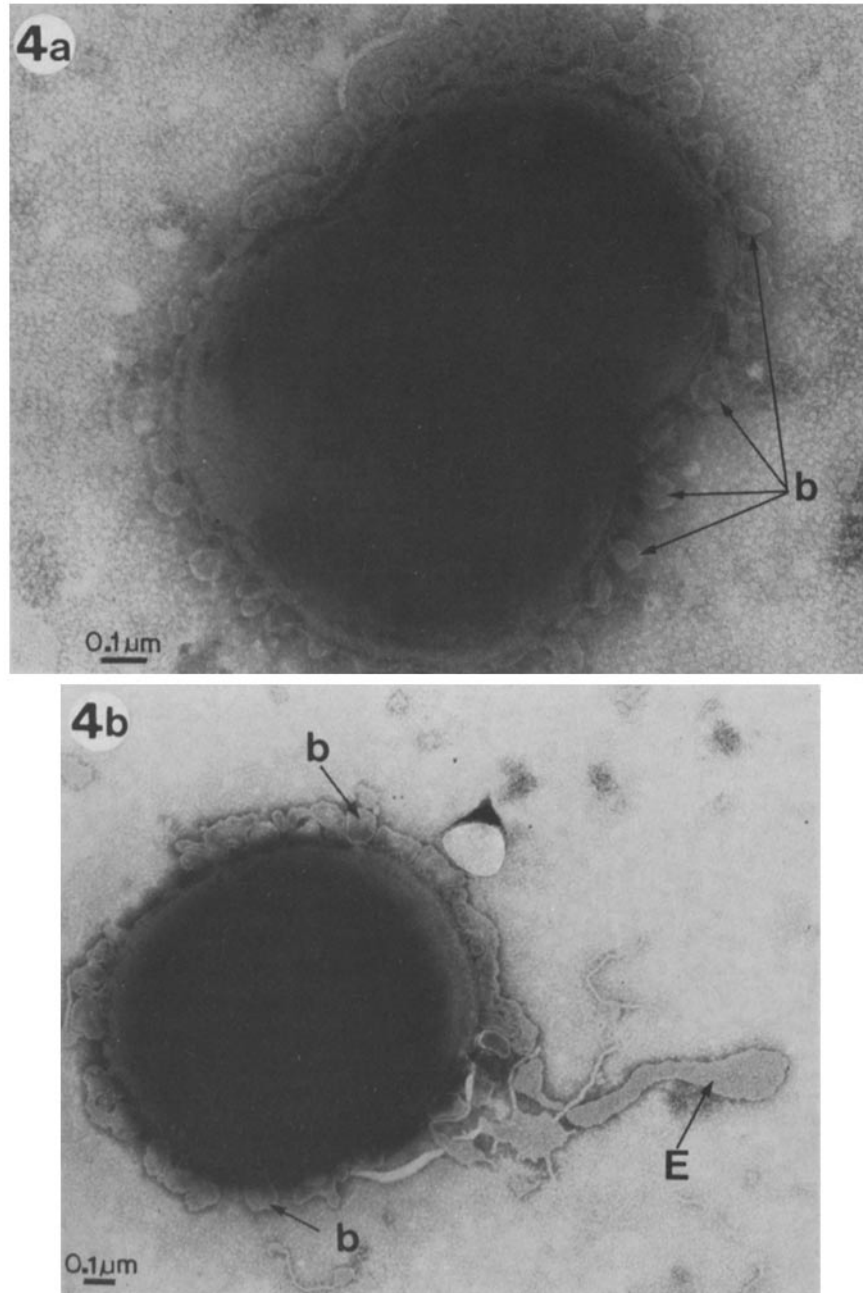


FIG. 4. Meningococcus from third serial culture of (a) nasopharynx ($\times 60,000$) and (b) cerebrospinal fluid ($\times 40,000$).

third possibility is that the meningococcus is not piliated in the host but synthesizes pili only on primary laboratory culture and loses them on subsequent subculture. For this possibility to be a valid one, one must rationalize a situation in which a medium both induces and represses pili synthesis, an unlikely

possibility. If the first suggestion is correct, that synthesis of pili is either repressed or not induced by the medium, then it is likely that the number of pili per cell in the host is probably greater than that observed in cells from primary cultures.

The function of pili in meningococcal disease or in the carrier state is not known. Evidence suggests that piliated gonococci are more resistant to phagocytosis (7) than nonpiliated forms and that pili aid these bacteria in adhering to tissue culture cell surfaces (9). One can readily visualize distinct selective advantages for meningococci in their natural environment if pili function in manners similar to those proposed for the gonococcus.

Summary

The nasopharynx of known meningococcal carriers without signs of acute meningococcal disease as well as cerebrospinal fluid from patients with acute meningococcal disease were cultured on Thayer-Martin agar. Pili were observed in negatively stained preparations of over 80% cells from all primary cultures of both nasopharynx and cerebrospinal fluids. Although pili were abundant on cells from all primary cultures, all pili were lost on serial subculture in the laboratory. This loss of pili from the cell surface on laboratory subculture was not accompanied by a concomitant loss of cell wall blebs.

We are grateful to the Faculty of Graduate Studies and Research of McGill University for support of the field trip to Sao Paulo, Brazil. We gratefully acknowledge the cooperation and aid of Warrant Officer R. J. Cusson (Canadian Forces Base Hospital, Cornwallis, Nova Scotia) and Dr. Gil Pessoa, Dr. Tuba Kuschnaroff, Dr. Carlos Bastos, and Dr. Augusto Taunay (Emilio Ribas Hospital, Sao Paulo, Brazil).

Received for publication 16 October 1974.

Bibliography

1. DeVoe, I. W., and J. E. Gilchrist. 1973. The release of endotoxin in the form of cell wall blebs during in vitro growth of *Neisseria meningitidis*. *J. Exp. Med.* **138**:1156.
2. DeVoe, I. W., and J. E. Gilchrist. 1974. Ultrastructure of pili and annular structures on the cell wall surface of *Neisseria meningitidis*. *Infect. Immun.* **10**:872.
3. James-Holmquest, A. N., J. Swanson, T. M. Buchanan, R. D. Wende, and R. P. Williams. 1974. Differential attachment by piliated and non-piliated *Neisseria gonorrhoeae* to human sperm. *Infect. Immun.* **9**:897.
4. 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. Microbiol. Immunol.* **79**:437.
5. Kellogg, D. S., W. L. Peacock, Jr., W. E. Deacon, L. Brown, and C. L. Pirkle. 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. *J. Bacteriol.* **85**:1274.
6. Kellogg, D. S., I. R. Cohen, L. C. Norris, A. L. Schroeter, and G. Reising. 1968. *Neisseria gonorrhoeae*. II. Clonal variation and pathogenicity during 35 month *in vitro*. *J. Bacteriol.* **96**:596.

7. Ofek, I., E. H. Beachey, and A. L. Bisno. 1974. Resistance of *Neisseria gonorrhoeae* to phagocytosis: relationship of colonial morphology and surface pili. *J. Infec. Dis.* **129**:310.
8. Swanson, John. 1972. Studies on gonococci infection. II. Freeze-fracture studies on gonococci. *J. Exp. Med.* **136**:1258.
9. Swanson, J. 1973. Studies on gonococcus infection. III. Pili: their role in attachment of gonococci to tissue culture cells. *J. Exp. Med.* **137**:571.
10. 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.
11. Vedros, N. *Neisseria* Repository Bulletin. U.S. Naval Research Unit No. 1, School of Public Health, University of California, Berkeley, Calif.