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Piliation and Colonial Morphology Among Laboratory Strains of Meningococci

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Colonial morphology and piliation were studied on twelve strains from various serogroups of *Neisseria meningitidis*. Six different colony types (M1 to M6) were identified. Most strains elaborated only an M1 colonial type, which is similar to gonococcus T_4. Several combinations of piliation and colonial morphology were observed: (i) colonial variation in which neither parent nor variant were piliated; (ii) colonial variation involving piliated and nonpiliated cells; (iii) dissociation of piliated from nonpiliated cells with no colonial change; and (iv) colonial variation in which both variants were piliated but with distinctly different pili. Results of this study demonstrate that correlations between piliation and colony morphology within *N. meningitidis* are exceptions rather than the rule.

Of the four major colonial types (T_1 to T_4) of the gonococcus, two (T_1 and T_2) consist of virulent (7, 8), piliated (6, 11) cells, whereas the two other colonial types (T_3 and T_4) are neither virulent nor piliated. These correlations suggest that pili are a virulence factor in the gonococcus, although recent results have cast some doubt on this hypothesis (9), and, of lesser importance, that pili could be a determining factor in colonial morphology, as well. The correlation between distinct colonial morphologies and the presence of pili on the surface of the gonococcus has provided an extremely useful laboratory tool for segregating and maintaining pure cultures of piliated gonococcal lines.

The finding of a group B meningococcus (M2092) that remained piliated under a variety of growth conditions (3) led us to survey both laboratory stock cultures of meningococci and primary cultures from the nasopharynx of 30 known asymptomatic carriers and from the cerebrospinal fluid of 30 patients with acute disease (4). Although stock cultures of laboratory strains generally produced populations in which 1 to 5% of the cells exhibited one to two pili per cell, no laboratory strain was found at that time in which a large proportion of cells in a colony were fully piliated, such as we had previously observed with strain M2092 (3). Our initial findings from primary cultures of carriers or the cerebrospinal fluid of patients led us to the tentative conclusion that the meningococci were piliated in nature. In this survey, cells from primary cultures from patients were analyzed by electron microscopy, and, without exception, >80% of cells from all colonies were piliated; however, subculture of these cells on the same medium resulted in a complete loss of detectable pili in some strains or a reduction of the proportion of piliated cells in a population to 1 to 5% in other strains. Unfortunately, this loss of pili from cells was not accompanied by a change in the colonial morphology, as one could expect with the gonococcus (6, 11).

Further studies on laboratory strains of meningococci have uncovered exceptions to our findings with fresh isolates. McGee et al. (10) reported two meningococcal strains that remained fully piliated after repeated subculturing. Using yet another piliated strain (M2092), D. Brener et al. (1a) found two colony types that were morphologically identical, except for colony size. The larger of the two colony types consisted of cells with long, thick (4.5-nm diameter) pili, whereas the cells from the smaller colony had short, thin (2.0-nm diameter) pili. In view of the apparent lack of correlation between meningococcal colony morphology and piliation, especially among laboratory strains, we have extended our studies in an attempt to define this area.

MATERIALS AND METHODS

Organisms. *Neisseria meningitidis* prototype strains A (791), B (SDIC), D(M-60), X (Slaterus), Y (Slaterus), Z (Slaterus), W-135, and 29E were obtained from the Neisseria Repository, NAMRU, University of California, Berkeley. *N. meningitidis* B (M2092) was obtained from the American Type Culture Collection (ATCC 13090). *N. meningitidis* group A strains SP3428, SP3424, and SP3453 were cultured from cerebrospinal fluid of patients in São Paulo, Brazil.

Cell growth and maintenance of cultures.

Stock cultures of all strains were lyophilized except for São Paulo (SP) strains, which were serially cultured three times and stored on Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) slants at −70°C. Working cultures of all strains were maintained on Mueller-Hinton agar (−70°C). Cultures of both parent and variant strains were checked routinely for purity (oxidase, Gram stain, sugar reactions, and serogroup) during these experiments by procedures described by N. Vedros (Neisseria Repository Bulletin, NAMRU, School of Public Health, University of California, Berkeley). Cells were grown both on plates of Mueller-Hinton agar and on GC medium (Difco) plus 2% IsoVitaleX (Baltimore Biological Laboratory, Cockeysville, Md.). Colonies were analyzed by means of a dissection light microscope at intervals ranging from 16 to 48 h of growth (37°C, candle jar, 100% humidity). Photographs of colonies were taken with a high-intensity side-arm illuminator focused to produce maximum intensity through a frosted glass stage.

Electron microscopy. Negatively stained preparations were prepared as previously described (2), except 0.05% phosphotungstate (pH 7.5) was used. Electron micrographs were taken on an AEI EM6B electron microscope.

RESULTS

The predominant colonial type (Table 1) among the meningococci tested was that designated M1 (Fig. 1 and 2). Moreover, the M1 colonial morphology, although previously not designated as such, is the one we had routinely observed in primary cultures of meningococci from either carriers or patients with acute disease (4).

One group B strain (SDIC), the prototype strain, dissociated into three colonial variants (M1, M3, and M5) (Fig. 1 and 2). Two of the colonial types (M1 and M3), although dramatically different in morphology after 36 h (Fig. 3C), were virtually indistinguishable after 18 h (not shown) and barely distinguishable, even with the aid of a dissection microscope, after 24 h of growth (Fig. 3A). Although these colonial variations among the meningococci are of interest in themselves, more important is the finding that >95% of cells from the rough colonial variant (M3) of SDIC were heavily piliated (Fig. 4B), whereas cells from the smooth colony (M1) did not have pili (Fig. 4A). The pili elaborated by the SDIC M3 colonial variant were the long, large-diameter (4.5 nm) pili described previously for other meningococci (1a, 3, 10) and not the short, small-diameter (2.0 nm) pili found to predominate (1a) on the M2 colonial variant of M2092 (Table 1). The cells of the third colonial variant (M5) of SDIC (Table 1), like the M1 variant, did not have pili.

The M1 and M3 variants of strain SDIC were repeatedly subcultured, and each time the results were similar. After numerous transfers, the smooth colony type (M1), made up of nonpiliated cells, consistently remained smooth, whereas subculture of the rough variant (M3) always yielded approximately 90% rough M3 colonies, consisting of piliated cells, and 10% smooth M1 type, consisting of cells without pili. The rough (M3) type dissociated into smooth (M1) as shown in Fig. 5. Such variants frequently appeared on colony edges, especially after 16 to 18 h of growth. A piliated group A organism, strain SP3428 (Table 1), elaborated only one colonial type (M4); however, a random check of thirty colonies revealed one in which >95% of cells were heavily piliated (4.5-nm diameter). Both piliated and nonpiliated variants of this

TABLE 1. *Colonial morphologies of various strains of N. meningitidis*[a]

Source	Serogroup	Strain	Colony type[b]
Neisseria Repository	A (prototype)	791	M1
Neisseria Repository	B (prototype)	SDIC	M1, (M3), M5
Neisseria Repository	D (prototype)	M-60	M1
Neisseria Repository	X (prototype)	X (Slaterus)	M1
Neisseria Repository	Y (prototype)	Y (Slaterus)	M1
Neisseria Repository	Z (prototype)	Z (Slaterus)	M1
Neisseria Repository	29-E (prototype)	29-E	M1
Neisseria Repository	W-135 (prototype)	W-135	M1
ATCC 13090	B	M2092	(M1), (M2)[c]
São Paulo, Brazil	A	SP3453	M1
São Paulo, Brazil	A	SP3428	M4, (M4)[d]
São Paulo, Brazil	A	SP3424	M6

[a] All cells were grown separately both on GC-IsoVitaleX and on Mueller-Hinton media. Colonies of any given strain were the same on both media.

[b] Parentheses indicate colonies consisting of piliated cells. In all variants, >95% of cells viewed in the electron microscope had more than 10 detectable pili per cell. A minimum of 50 cells was analyzed for each colony. Colony types were determined after 28 to 30 h of growth.

[c] Information extracted from Brener et al. (1a).

[d] SP3428 dissociated into one variant with pili and one without. Both variants had identical colonial morphologies (M4).

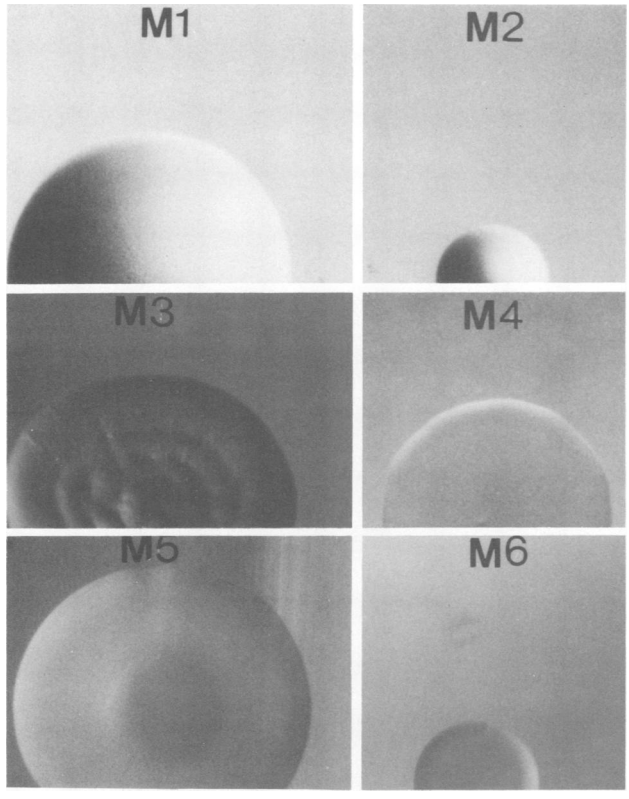

FIG. 1. *Various colonial morphologies of N. meningitidis after 30 h of growth. ×40.*

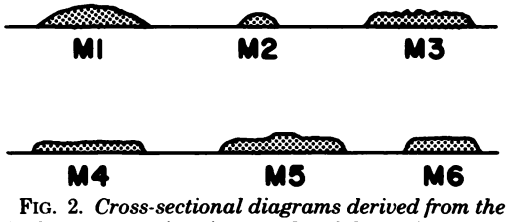

FIG. 2. *Cross-sectional diagrams derived from the shadow patterns in micrographs of the various colonial forms of N. meningitidis (after 30 h of growth). The relative sizes are in proportion to the colonies shown in Fig. 1.*

strain were subcultured on GC-IsoVitaleX and on Mueller-Hinton agar medium. The colonial morphologies of both strains were indistinguishable on either medium, i.e., both variants produced only an M4 colonial morphology. The three group A strains (SP) (Table 1) were taken on the same day from the cerebrospinal fluid of three patients (I. W. DeVoe and J. E. Gilchrist, unpublished data). We have cultured these strains in the laboratory three times since their isolation but have maintained them in the frozen state (−70°C) for over 3 years. Therefore, they must be considered laboratory strains, as are all other strains used in this study.

The two media upon which the cells were grown had no apparent effect either on colonial morphology or on the piliation of cells during this study. Colonial morphology was, however, affected by time of growth. A period of 28 to 30 h was sufficient, and required in some isolates, to distinguish between colonial variants.

In summary, we have presented examples here, as well as previously (1a), for the following combinations of pili and colonial morphology: (i) colonial variation involving no piliation, e.g., SDIC M1 and M5; (ii) colonial variation involving piliated and nonpiliated cells, e.g., SDIC M3 and M1; (iii) dissociation of piliated to nonpiliated cells without a change in colony type in strain SP3428; and (iv) colonial variation in which both variants were piliated, each elaborating a distinctly different pilus type in strain M2092 (1a).

DISCUSSION

Dissociation in bacterial species is a frequently observed phenomenon in which morphological or physiological variants appear spontaneously in pure cultures (1). We have presented evidence for dissociation manifested by changes in colonial morphology and/or piliation in meningococci. The characteristic of the gonococci to

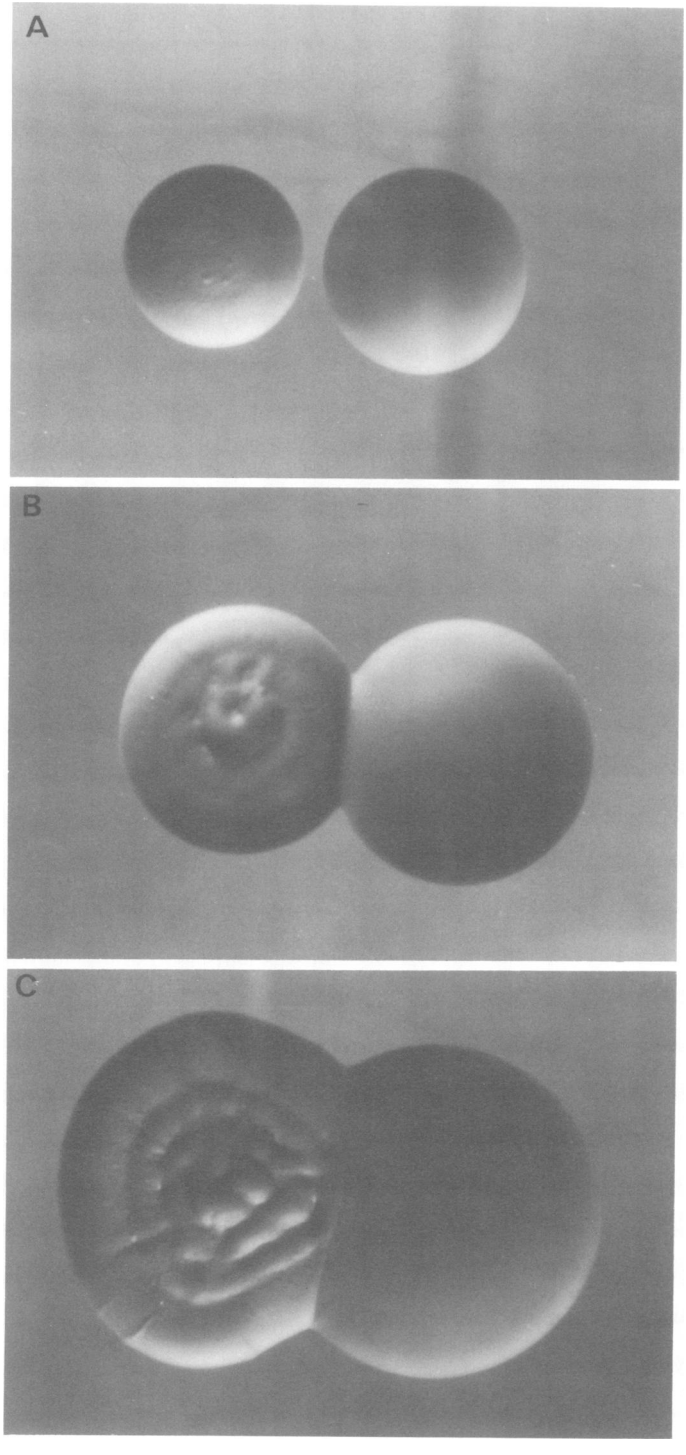

FIG. 3. *Development of two colonial variants, a smooth (M1) and a rough (M3) type, of group B N. meningitidis SDIC. (A) 24 h; (B) 30 h; (C) 36 h. ×56.*

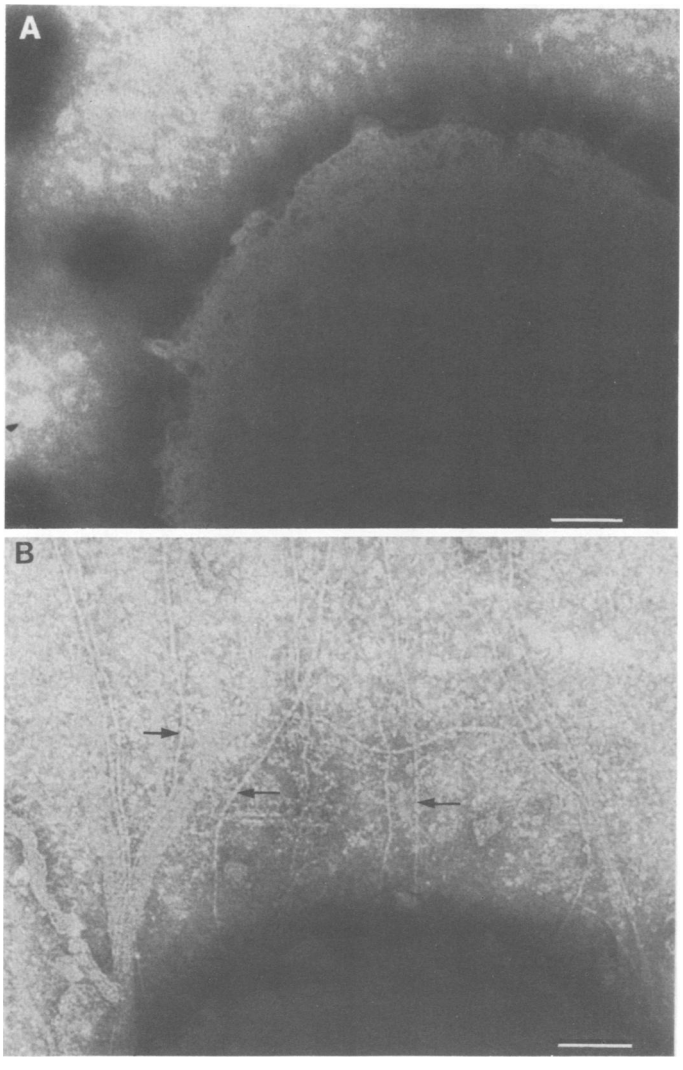

FIG. 4. *Negatively stained cells (30-h culture) from variant colonies of group B N. meningitidis strain SDIC. (A) Nonpiliated cell from the smooth M1 colonial type; (B) piliated cell from the rough M3 colonial type. Arrows point to pili. Bar = 0.1 μm.*

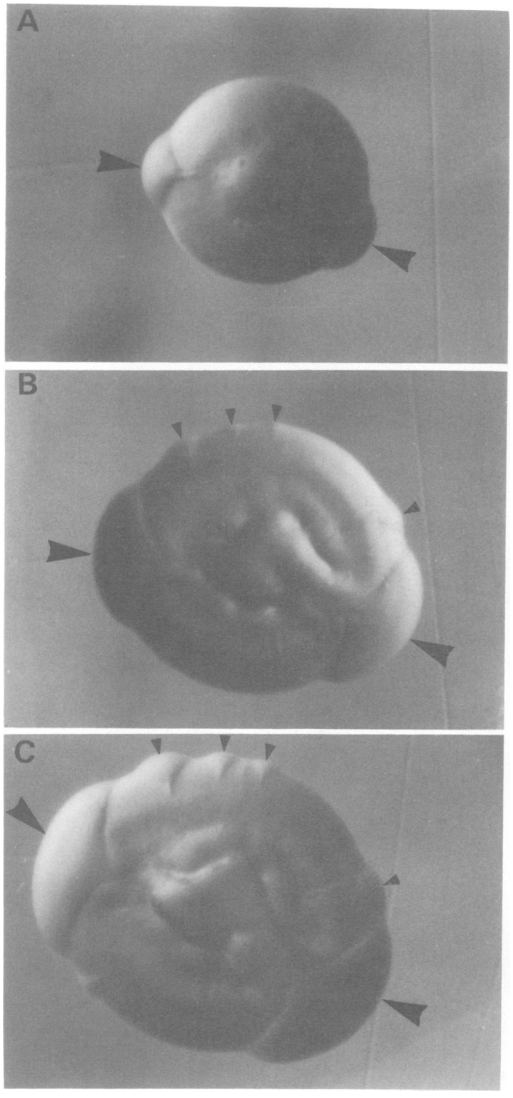

FIG. 5. *Multiple dissociations to smooth M1 variants within a single rough M3 colonial variant of N. meningitidis SDIC. Arrows point to areas where M1 variants are developing. (A) 24 h; (B) 28 h; (C) 32 h. ×56.*

dissociate into four colonial variants (7, 8) and the correlation of two of these variants with virulence in humans and piliation (6, 7, 10) has placed great importance on the colonial morphologies of this organism. Moreover, a fifth colonial variant of the gonococcus, with an extremely rough colonial morphology and containing piliated cells, has recently been reported (5).

McGee et al. (10) have compared the colonial variants of several nonpathogenic neisseriae and meningococci to the colonial forms of the gonococcus. They found some colonial forms roughly similar to, and some indistinguishable from, those of the gonococcus, but their findings among the meningococci failed to show similar correlations between colony morphologies and piliation. Of the four meningococci studied by McGee et al. (10), all had raised, convex colonies indistinguishable from the M1 type that we describe here or the T_4 type of the gonococcus (7). From the evidence presented here and elsewhere (1a, 3, 4, 10), it seems evident that the correlations between piliation and colonial morphology observed in the gonococcus do not hold for laboratory strains of meningococci. There are, no doubt, other combinations of colony morphology and piliation among meningococci that we have not observed.

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LITERATURE CITED

1. **Braun, W.** 1947. Bacterial dissociation. A critical review of a phenomenon of bacterial variation. Bacteriol. Rev. **11**:75–114.
1a. **Brener, D., J. E. Gilchrist, and I. W. DeVoe.** 1977. Relationship between colonial variation and pili morphology in a strain of *Neisseria meningitidis*. FEMS Microbiol. Lett. **2**:157–161.
2. **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**:1156–1167.
3. **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**:872–876.
4. **DeVoe, I. W., and J. E. Gilchrist.** 1975. Pili on meningococci from primary cultures of nasopharyngeal carriers and cerebrospinal fluids of patients with acute disease. J. Exp. Med. **141**:297–305.
5. **Jacobs, N. F., Jr., S. J. Kraus, C. Thornsberry, and J. Bullard.** 1977. Isolation and characterization of a rough colony type of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **5**:365–369.
6. **Jephcott, A. E., A. Reyn, and A. Birch-Andersen.** 1971. Brief report: *Neisseria gonorrhoeae*. III. Demonstration of presumed appendages to cells from different colony types. Acta Pathol. Microbiol. Scand. Sect. B **79**:437–439.
7. **Kellogg, D. S., Jr., I. R. Cohen, L. C. Norins, A. L. Schroeter, and G. Reising.** 1968. *Neisseria gonorrhoeae*. II. Colony variation and pathogenicity during 35 months in vitro. J. Bacteriol. **96**:596–605.
8. **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.
9. **Kraus, S. J., W. J. Brown, and R. J. Arko.** 1975. Acquired and natural immunity to gonococcal infection in chimpanzees. J. Clin. Invest. **55**:1349–1356.
10. **McGee, Z. A., R. R. Dourmashkin, J. G. Gross, J. B. Clark, and D. Taylor-Robinson.** 1977. Relationship of pili to colonial morphology among pathogenic and non-pathogenic species of *Neisseria*. Infect. Immun. **15**:594–600.
11. **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.