INFECTION AND IMMUNITY, Jan. 1981, p. 430-435
0019-9567/81/010430-06$02.00/0

Vol. 31, No. 1

Adherence of *Neisseria meningitidis* to Human Epithelial Cells

IRVING E. SALIT†* AND GLENN MORTON

Department of Medicine, Vancouver General Hospital and The University of British Columbia, Vancouver, British Columbia, Canada V5Z 1M9

Carrier strains of *Neisseria meningitidis* are recovered almost solely from the posterior pharynx and they are often nongroupable or rough. Invasive strains can be serogrouped (encapsulated). We studied adherence of both carrier- and patient-derived serogroupable and nongroupable meningococci to buccal epithelial and posterior pharyngeal cells. Fresh meningococcal isolates attached significantly better to pharyngeal than to buccal cells ($P = 0.01$). Strains that could be serogrouped adhered less than nongroupable strains ($P < 0.05$). Meningococci passed in vitro became hemagglutinin negative and nonpiliated. Hemagglutinin-negative meningococci always adhered less to both epithelial cell types than the hemagglutinin-positive variants of the same strain. These results indicate that meningococcal pili probably mediate attachment to oropharyngeal cells, but encapsulation may reduce adherence. Localization of meningococci in the posterior pharynx is in part explained by the receptivity of the epithelial cells in this area for meningococci.

Neisseria meningitidis colonizes the oropharynx and nasopharynx in 0.7 to 35% of the normal population in interepidemic periods (9). Many of these carrier strains are nongroupable by using the standard anticapsular antisera, whereas invasive strains possess capsular polysaccharides A, B, C, and Y (16). Disease is associated with absence of host bactericidal antibody; however, only 38% of the susceptible population develop disease when colonized by an encapsulated strain (10). Surface antigens such as capsules, pili, outer membrane proteins (7), and enzymes such as immunoglobulin A protease may influence the invasive and adhesive potential of meningococci and determine which susceptible people become infected.

Attachment of *Escherichia coli* and *Neisseria gonorrhoeae* to epithelial cells and erythrocytes is an attribute of virulent strains (14, 15, 17, 21, 24). The adhesive factors for these bacteria are pili (3, 18, 19, 22), and antibodies directed against pili diminish adherence (3, 19) and may prevent infection. Little is known about the mechanism of adherence of *N. meningitidis* even though a brief period of pharyngeal mucosal colonization is known to precede bacteremia and meningitis (10). Craven and Frasch (5) studied attachment of meningococci to buccal epithelial cells and concluded that encapsulation interferes with adherence and that invasive strains are less adhesive. Salit (Clin. Res. **28**:378A, 1980) found that freshly isolated, piliated meningococci attach to and agglutinate human erythrocytes. Rough and nongroupable strains had higher hemagglutination (HA) titers than groupable strains, and purified capsular polysaccharides inhibited HA. These studies imply that, for the meningococcus, cell attachment by itself is not a marker of virulence.

Carriers of meningococci harbor these bacteria almost solely in their nasopharynx and oropharynx and only rarely in the urethra, rectum, and oral mucosa. In vivo, gonococci, streptococci, and *E. coli* also specifically colonize some mucosal sites to the exclusion of others, and this colonization correlates well with in vitro attachment of the bacteria to isolated mucosal cells (1, 8, 19). Adhesins such as pili partly determine bacterial localization by binding preferentially to some human cells (4, 19).

In this study we examined the adherence of case and carrier strains of meningococci to human buccal and pharyngeal cells to determine whether meningococci possess such a specificity of attachment. HA has been used as a marker for piliation to determine the role of pili in adhesion to epithelial cells.

MATERIALS AND METHODS

Pharyngeal cells. Buccal epithelial cells were removed by scraping the buccal mucosa with a wooden applicator, from which cells were removed by swirling the applicator in 30 ml of phosphate-buffered saline.

Posterior pharyngeal cells were collected as follows: the pharyngeal wall was well visualized by depressing

† Present address: Division of Infectious Diseases, Toronto General Hospital, Toronto, Ontario, Canada M5G 1L7.

the tongue with an applicator stick, and a cotton-tipped sterile swab was then rubbed over the area. Cells were predominantly from the upper half of the posterior oropharynx. No attempt was made to collect material from the nasopharynx. The swabs were vigorously swirled in 30 ml of phosphate-buffered saline to remove adherent cells. Cells from three individuals were pooled and used on the day of collection.

Both cell types were then processed in an identical fashion: the cells were washed once and suspended in 15 ml of phosphate-buffered saline in plastic screw-cap tubes. They were then placed in an ultrasonic water bath for 5 min. Ultrasonic treatment removed most adherent bacteria and facilitated subsequent counting. This treatment did not affect experimental adherence, and 5 min was the minimal sonication period to remove most of the natural flora. The detached natural flora were separated from epithelial cells by washing three times in phosphate-buffered saline, using low-speed centrifugation in a clinical centrifuge (IEC clinical centrifuge). The cells were then suspended in PPS (proteose peptone no. 3, 10 g/liter; sodium chloride, 5 g/liter), and their concentration was measured in a hemacytometer. The suspensions were then diluted to 2×10^5 cells per ml in PPS for the experiments.

Bacteria. Meningococci were isolated from known throat carriers, or from patients with meningococcal disease, on gonococcal typing medium containing vancomycin, colistin, and nystatin (23) and were frozen in Greaves solution (13). Nonpiliated variants were produced by repeated daily subculture on the same medium. Piliation was monitored (18) with meningococcal HA of human erythrocytes and was confirmed by electron microscopy.

All strains were serogrouped by slide agglutination to detect capsular antigens and were serotyped to detect outer membrane protein antigens. Serotyping was performed by Carl Frasch, Bureau of Biologics, Food and Drug Administration, Bethesda, Md.

Attachment studies. Meningococci were removed from agar plates after overnight growth and suspended in PPS to an optical density of 0.3 at a wavelength of 540 nm in borosilicate tubes (using a Coleman Junior IIA linear absorbance spectrophotometer). This corresponds to 2.5×10^8 viable bacteria per ml. Samples of bacterial suspension (0.5 ml) were then mixed with 0.5 ml of pharyngeal cell suspension in 5-ml plastic snap-cap tubes. The mixture was rotated for 30 min at 23°C. Unattached bacteria ("background") were separated from human cells by repeated low-speed centrifugation. An acceptable background was achieved with four washes with 10 ml of phosphate-buffered saline each. Each experiment was done in triplicate.

Adherent bacteria and pharyngeal cells were placed on microscope slides, air-dried, and stained with Gram solution. Bacteria adherent to 50 unselected pharyngeal cells were counted, using a Zeiss photomicroscope and a final magnification of ×640. Nonadherent bacteria were counted over an equivalent area, and these were subtracted from counts of adherent bacteria ("corrected for background").

Results were expressed as meningococci per epithelial cell. Since the buccal and pharyngeal cells are of different mean sizes, the numbers of adherent bacteria were adjusted to the size of the buccal cells ("corrected for area") where noted. The cell areas were measured by photographing representative cells, cutting them out of the photographs, and calculating a ratio of weights which is equivalent to a ratio of areas. The ratio of areas could also be approximated by determining the diameters (D) of the cells by using an ocular micrometer, as follows: Ratio of areas = $\pi(D_x/2)^2 + \pi(D_y/2)^2 = 3.74$. The corrected numbers of bacteria adherent to pharyngeal cells were then equal to 3.74 × the absolute numbers of meningococci per pharyngeal cell.

RESULTS

The two epithelial cell types used for adherence studies had large numbers of attached bacteria (normal flora) when collected from subjects. Pharyngeal cells had 16 (±32) and buccal cells had 53 (±42) adherent bacteria per cell. Similar numbers of bacteria were present when corrected for size differences between the cell types (60 versus 53 bacteria per pharyngeal and buccal cell, respectively).

Buccal and pharyngeal cells were easily recognizable microscopically (Fig. 1): buccal cells were considerably larger and had a larger cytoplasm/nucleus ratio. Because the area of pharyngeal cells was 3.74 times less than that of buccal cells, the enumeration of adherent bacteria was appropriately adjusted to allow for meaningful comparison. Ten percent of buccal cells were viable by trypan blue exclusion, and 35% of pharyngeal cells were viable. Epithelial cells were pooled from three subjects, none of whom was a meningococcal carrier. A total of 4.6×10^5 pharyngeal cells and 1.8×10^7 buccal cells were collected from each subject.

Sonication of the cells for 5 min before adherence studies reduced the adherent normal flora by 90%; lengthening the period of sonication beyond 5 min did not result in any further loss of bacteria, and so this was selected as the optimal period. Before sonication, only 8% of cells had ≤5 adherent bacteria, compared to 94% of cells after sonication. Sonication facilitated counting of experimentally attached meningococci but did not affect the receptivity of the epithelial cells for the bacteria.

Comparison of media. Meningococci were passaged daily on three different media for 3 days, and their adherence was then determined. The three media used were gonococcal typing medium (22), typing medium with added iron dextran (20 μg/ml), and Thayer-Martin medium. These were previously shown to have different effects on HA of meningococci which were continuously passaged on each medium.

The mean adherence of four strains of buccal cells did not differ significantly on each medium:

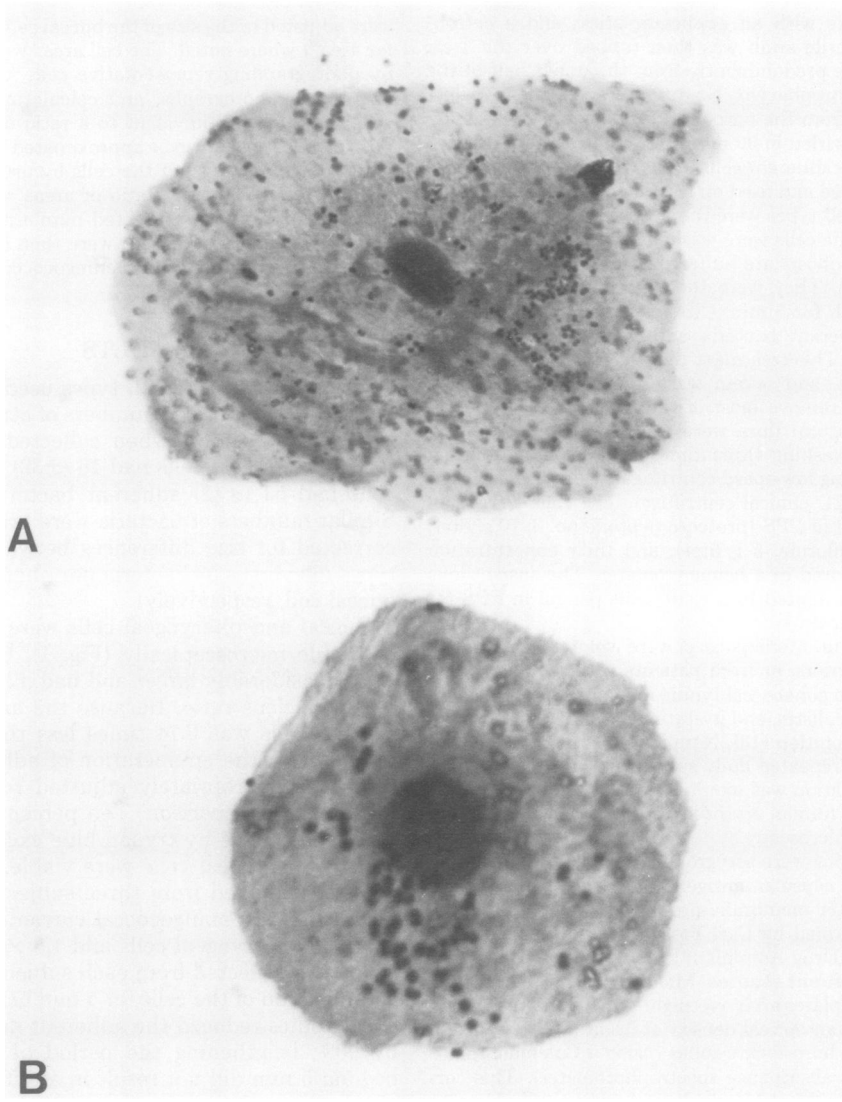

FIG. 1. *Adherence of hemagglutinin-positive meningococci to (A) buccal epithelial cells (×720) and (B) posterior pharyngeal cells (×1,800).*

48 (±42), 44 (±25), and 53 (±31) bacteria per cell, respectively, for the typing medium, typing medium with iron, and Thayer-Martin medium ($P > 0.1$, Student's t test). For all further studies, the typing medium was used without iron.

Comparison of epithelial cells. Freshly isolated meningococci, which had been passaged three times or less in vitro, were tested for their adherence to the two pharyngeal cell types. Newly isolated strains such as these are well piliated and have high HA titers. Ten of the 11 strains adhered to a greater extent to posterior pharyngeal cells than to buccal cells even if no correction was made for the smaller size of the pharyngeal cells ($P = 0.01$, Wilcoxon signed-rank test) (Table 1). If a correction was made for differences in epithelial cell size, then all strains adhered better to the pharyngeal cells.

Comparison of meningococcal isolates. Those strains that could be characterized as to capsular serogroup were then compared with nongroupable and rough meningococci with respect to adherence to buccal cells only (Table 2). Rough strains (which auto-agglutinate in saline) adhered best, and clumps of bacteria could be seen microscopically on the epithelial cells.

Serogroupable strains showed the least cell association, and nongroupable strains were intermediate.

Some strains had been collected from blood or cerebrospinal fluid (case strains), but most were from throat cultures (carrier strains). Carrier strains had a higher adhesive capacity than case strains (85 ± 37 versus 48 ± 42 bacteria per cell) ($P > 0.05$, Student's t test), but most case strains (3/4) were groupable and most carrier strains were nongroupable and rough (9/12).

There was no definite association between particular serotype antigens and level of adherence. Since there are many serotypes, a much larger number of isolates from each serotype would have to be examined to detect differences.

Correlation with HA. Five fresh isolates were used which had high HA titers. All strains eventually became HA negative and nonpiliated when passed daily on agar. The HA-positive parent strain (kept frozen at −80°C) and the HA-negative variant were then compared with respect to attachment to both epithelial cell types (Table 3). The HA-positive variant of each strain attached more avidly to both buccal and pharyngeal cells than did the HA-negative derivative. In addition, pharyngeal cells were more receptive than buccal cells for both HA-positive and HA-negative bacteria (Table 3). For the same unit area, pharyngeal cells had 1.6- to 13.8-fold more attached bacteria than buccal cells.

DISCUSSION

The specificity of attachment to human cells may be a critical ecological determinant affecting colonization. Gibbons and Van Houte (8, 25) studied bacterial adherence to teeth and oral epithelial surfaces. There was a correlation between the relative adherence of streptococci and their proportional distribution found naturally on oral surfaces. *E. coli*, which are not usually found in oral flora, did not adhere to cheek cells (8). *E. coli* do attach to cells of the urinary tract, and their relative ability to adhere to exfoliated uroepithelial cells may explain the propensity to cause different forms of urinary tract infections (21). Group A streptococci can cause pyoderma and pharyngitis; strains which cause the former adhere better to skin cells, whereas strains causing the latter adhere better to oral cells (1). *Staphylococcus aureus* may colonize the anterior nares, and they adhere more avidly to human nasal mucosal cells than viridans streptococci, which do not similarly colonize (2). Finally, the marked attachment of some bacteria to heart valve endothelial cells can explain why endocarditis is caused by these bacteria; nonadherent bacteria rarely cause endocarditis (12).

TABLE 1. *Adherence of N. meningitidis to buccal and posterior pharyngeal cells*

Bacteria			Adherence[a] (bacteria per cell) to cell type:	
Strain no.	Serogroup	Source	Buccal[d]	Pharyngeal[d]
96	Y	Blood	11 (±9)	13 (±9)
118	W135	Throat	25 (±47)	92 (±50)
123	NG[b]	Throat	72 (±52)	112 (±50)
124	W135	Throat	43 (±54)	126 (±35)
125	NG	Throat	73 (±48)	130 (±35)
126	Rough[c]	Throat	127 (±34)	>140
127	NG	Throat	60 (±47)	141 (±23)
128	NG	Throat	78 (±56)	123 (±38)
129	NG	Throat	124 (±42)	86 (±56)
130	NG	Throat	66 (±61)	87 (±55)
132	NG	Throat	88 (±53)	112 (±43)

[a] Arithmetic mean (±standard deviation), uncorrected for differences in cell size.
[b] NG, Nongroupable.
[c] Autoagglutinated in saline.
[d] $P = 0.01$, Wilcoxon signed rank test.

TABLE 2. *Influence of encapsulation on adherence to buccal cells*

Meningococci	No. of strains	Adherence[a] (no. of bacteria per cell)		
Serogroupable	6	73 (±29)	42 (±35)	[b]
Nongroupable	8		89 (±37)	
Rough	3	132 (±16)		

[a] Arithmetic mean (± standard deviation).
[b] $P < 0.05$, Student's t test.

TABLE 3. *Relationship of HA and adherence among meningococcal isolates*

Meningococci		Adherence (bacteria per cell) to epithelial cells:		Adherence ratio[c]
Strain	HA[a]	Buccal	Pharyngeal[b]	
96	+	11	49 (13)	4.4
	−	4	11 (3)	2.8
118	+	25	343 (92)	13.6
	−	4	51 (14)	13.8
129	+	124	322 (86)	2.6
	−	63	228 (61)	3.6
130	+	105	325 (87)	3.1
	−	66	109 (29)	1.6
131	+	88	418 (112)	4.8
	−	16	48 (13)	3.0

[a] Agglutination of human erythrocytes as detected by dilutions in microtiter plates. +, Hemagglutinating power ≥320; −, hemagglutinating power <80.
[b] Numbers of bacteria per pharyngeal cell corrected for buccal-pharyngeal cell area difference. Parentheses indicate absolute number of bacteria per pharyngeal cell, uncorrected for buccal-pharyngeal cell area difference.
[c] Adherence to pharyngeal cells/adherence to buccal cells.

N. meningitidis (the meningococcus) is a major cause of meningitis. Pharyngeal colonization probably precedes bacterial invasion in those who develop disease, but colonization exists much more commonly in asymptomatic carriers who never develop meningococcal disease. Meningococci colonize the throat almost exclusively; only rarely are they isolated from other mucosal sites (6). The nasopharynx (pharynx above the soft palate) is usually said to harbor the organisms but they are similarly present in the oropharynx (inferior to the soft palate). Meningococci do not usually colonize the mouth.

Adherence to human epithelial cells helps explain the localization of meningococci to the posterior pharynx. All strains tested, whether from cases or carriers, attached more avidly to pharyngeal than to buccal epithelial cells. We removed the normal flora by sonication so that observed differences were not due to interference from these attached bacteria. Pharyngeal cells likely have a greater intrinsic receptivity for meningococci than buccal cells. It is probable that this is a specific recognition reaction between the cell membranes of the epithelial cells and bacterial adhesins. A similar tropism for gonococci has been described and has been attributed to enhanced binding of pili to the cell membranes (4).

Meningococcal adhesins have not yet been defined. Pili are present on freshly isolated meningococci, and they are lost on serial transfer in vitro. Newly isolated meningococci agglutinate human erythrocytes, and this is also lost after passaging bacteria in vitro. HA is a useful marker for piliation, and purified meningococcal pili agglutinate human erythrocytes. In the present study, hemagglutinating bacteria adhered better to both epithelial cells than their nonhemagglutinating variants, and pili probably mediate adhesion to both cell types. A similar correlation between HA and adherence to epithelial cells exists for *E. coli* (14, 18, 19) and *N. gonorrhoeae* (17).

Invasive strains of *N. meningitidis* are encapsulated and can be classified by agglutination in anticapsular antisera. Noninvasive (carrier) strains are often nongroupable because they autoagglutinate or they do not react with standard antisera. Craven and Frasch (5) compared attachment of invasive and noninvasive serogroupable meningococci to buccal epitheal cells, and the former were less adherent. Potentially invasive strains may be less adhesive even when they exist initially on the mucous membranes, or they may develop this characteristic after growth in blood or cerebrospinal fluid (phenotypic variation). Isogenic pairs of meningococci from mucosal and extramucosal sites do differ phenotypically (J. T. Poolman and H. C. Zanen, FEMS Symp. Antimicrob. Envelopes, 26–30 May, 1980). The attachment capabilities of these bacterial variants are unknown.

Nongroupable meningococcal strains have higher HA titers, and they adhere more to human epithelial cells than serogroupable bacteria. Purified meningococcal capsular polysaccharides, which can inhibit bacterial HA, may modify attachment in vivo. Similarly, adherence of pneumococci to posterior pharyngeal cells is reduced when the capsule size is increased by passaging through mice (20). Adhesion of *E. coli* to human uroepithelial cells is also reduced when grown under conditions which enhance capsule formation (22). However, other factors change simultaneously, and piliation is reduced under similar conditions.

Differences in adhesion may help explain the greater prevalence of nongroupable strains in the throats of carriers, but other factors such as serotype 2 protein on bacteria and presence of serum bactericidal antibody are important. It is uncertain whether the greater adhesiveness among nongroupable strains actually contributes to diminishing their invasiveness.

ACKNOWLEDGMENTS

We thank Frances Hillier for typing the manuscript.

This study was supported by grant no. 68-0192 from the Medical Research Council of Canada.

LITERATURE CITED

1. **Alkan, M., I. Ofek, and E. H. Beachey.** 1977. Adherence of pharyngeal and skin strains of group A streptococci to human skin and oral epithelial cells. Infect. Immun. **18**:555–557.
2. **Aly, R., H. I. Shinefield, W. G. Strauss, and H. I. Maibach.** 1977. Bacterial adherence to nasal mucosal cells. Infect. Immun. **17**:546–549.
3. **Buchanan, T. M., and W. A. Pearce.** 1976. Pili as a mediator of the attachment of gonococci to human erythrocytes. Infect. Immun. **13**:1483–1489.
4. **Buchanan, T. M., W. A. Pearce, and K. C. S. Chen.** 1978. Attachment of *Neisseria gonorrhoeae* pili to human cells, and investigations of the chemical nature of the receptor for gonococcal pili, p. 242–249. *In* G. F. Brooks, Jr., E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D.C.
5. **Craven, D. E., and C. E. Frasch.** 1978. Pili-mediated and -nonmediated adherence of *Neisseria meningitidis* and its relationship to invasive disease, p. 250–252. *In* G. F. Brooks, Jr., E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D.C.
6. **Faur, Y. C., M. H. Weisburd, and M. E. Wilson.** 1975. Isolation of *Neisseria meningitidis* from the genitourinary tract and anal canal. J. Clin. Microbiol. **2**:178–182.
7. **Frasch, C. E., and S. S. Chapman.** 1973. Classification

of *Neisseria meningitidis* group B into distinct serotypes. III. Application of a new bactericidal-inhibition technique to distribution of serotypes among cases and carriers. J. Infect. Dis. **127**:149–154.

8. **Gibbons, R. J., and J. van Houte.** 1971. Selective bacterial adherence to oral epithelial surfaces and its role as an ecological determinant. Infect. Immun. **3**:567–573.
9. **Gold, R., I. Goldschneider, M. L. Lepow, T. F. Draper, and M. Randolph.** 1978. Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in infants and children. J. Infect. Dis. **137**:112–120.
10. **Goldschneider, I., E. C. Gotschlich, and M. S. Artenstein.** 1969. Human immunity to the meningococcus. I. The role of humoral antibodies. J. Exp. Med. **129**:1307–1326.
11. **Goldschneider, I., E. C. Gotschlich, and M. S. Artenstein.** 1969. Human immunity to the meningococcus. II. Development of natural immunity. J. Exp. Med. **129**: 1327–1348.
12. **Gould, K., C. H. Ramirez-Ronda, R. K. Holmes, and J. P. Sanford.** 1975. Adherence of bacteria to heart valves *in vitro*. J. Clin. Invest. **56**:1364–1370.
13. **Greaves, R. I. H.** 1960. Preserving living cells by freeze-drying. Ann. N.Y. Acad. Sci. **85**:723–727.
14. **Jones, G. W., and J. M. Rutter.** 1974. The association of K88 antigen with haemagglutinating activity in porcine strains of *Escherichia coli*. J. Gen. Microbiol. **84**: 135–144.
15. **Koransky, J. R., R. W. Scales, and S. J. Kraus.** 1975. Bacterial hemagglutination by *Neisseria gonorrhoeae*. Infect. Immun. **12**:495–498.
16. **The Meningococcal Disease Surveillance Group.** 1976. Analysis of endemic meningococcal disease by serogroup and evaluation of chemoprophylaxis. J. Infect. Dis. **134**:201–204.
17. **Punsalang, A. P., Jr., and W. D. Sawyer.** 1973. Role of pili in the virulence of *Neisseria gonorrhoeae*. Infect. Immun. **8**:255–263.
18. **Salit, I. E., and E. C. Gotschlich.** 1977. Hemagglutination by purified type I *Escherichia coli* pili. J. Exp. Med. **146**:1169–1181.
19. **Salit, I. E., and E. C. Gotschlich.** 1977. Type I *Escherichia coli* pili: characterization of binding to monkey kidney cells. J. Exp. Med. **146**:1182–1194.
20. **Selinger, D. S., and W. P. Reed.** 1979. Pneumococcal adherence to human epithelial cells. Infect. Immun. **23**: 545–548.
21. **Svanborg Eden, C., L. A. Hanson, U. Jodal, U. Lindberg, and A. Sohl-Akerlung.** 1976. Variable adherence to normal human urinary-tract epithelial cells of *Escherichia coli* strains associated with various forms of urinary-tract infection. Lancet **ii**:490–492.
22. **Svanborg Eden, 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.
23. **Swanson, J.** 1978. Studies on gonococcus infection. XII. Colony color and opacity variants of gonococci. Infect. Immun. **19**:320–331.
24. **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.
25. **van Houte, J., R. J. Gibbons, and S. B. Banghart.** 1970. Adherence as a determinant of the presence of *Streptococcus salivarius* and *Streptococcus sanguis* on the tooth surface. Arch. Oral Biol. **15**:1025–1035.