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Role of Adherence in the Pathogenesis of *Pseudomonas aeruginosa* Lung Infection in Cystic Fibrosis Patients

DONALD E. WOODS,[1] JOE A. BASS,[1] W. G. JOHANSON, JR.,[2] AND DAVID C. STRAUS[1]*

Departments of Microbiology[1] and Medicine,[2] The University of Texas Health Science Center at San Antonio, and the Veterans Administration Hospital, San Antonio, Texas 78284

A correlation has been demonstrated between the in vitro adherence of *Pseudomonas aeruginosa* to upper respiratory tract epithelium and colonization of the respiratory tract by this organism. Twenty patients with cystic fibrosis (CF) and 20 age-matched controls were examined in this study. All of the CF patients but none of the controls were colonized with *P. aeruginosa* at the time of study. *P. aeruginosa* adherence to isolated epithelial cells, as determined by an in vitro assay, was 19.1 ± 1.1 bacteria per buccal epithelial cell in the CF patients and 2.3 ± 0.3 bacteria per cell in the controls ($P < 0.01$). *P. aeruginosa* strains of the mucoid colony type adhered in significantly lower numbers to buccal epithelial cells than did strains of the rough colony type (1.8 + 0.1 versus 24.8 ± 0.9, $P < 0.001$). This difference might explain the common observation that the initial pseudomonas colonization of the respiratory tract of CF patients is due to organisms of the rough colony type. We have further demonstrated that increased *P. aeruginosa* adherence in vitro varies directly with the loss of a protease-sensitive glycoprotein, fibronectin, from the cell surface, as well as increased levels of salivary proteases in CF patients. When examined by a direct radioimmune binding assay, buccal cells from CF patients possessed only 17% of the total cell surface fibronectin present on similar cells obtained from controls. Salivary protease levels, as measured by ^{125}I release from an ^{125}I-labeled insoluble fibrin matrix, were increased about threefold in CF patients versus controls. Thus, colonization of the respiratory tract by *P. aeruginosa* in CF patients correlates well with buccal cell adherence of this organism; increased adherence is associated with decreased amounts of fibronectin on respiratory epithelial cell surfaces and increased levels of salivary proteases.

Cystic fibrosis (CF) is the most common lethal hereditary disease in Caucasians with an incidence of 1 in 2,000 live births (2, 25). More than 50% of patients with CF die before reaching the age of 21 (2). The biochemical or physiological lesions of the disease are not fully understood. Chronic obstructive pulmonary disease is the most serious aspect of CF. Among factors which contribute to chronic lung disease in CF patients are impaired ciliary movement, inadequate removal of bronchial mucous, and recurrent bacterial infections (2, 25).

Colonization of the upper respiratory tracts of CF patients with *Pseudomonas aeruginosa* is common and appears to be an important predisposing factor in the pathogenesis of pulmonary infection by this organism (12, 13). In most CF patients, the initial colonizing strains of *P. aeruginosa* are rough types similar to those isolated from other types of infections (1). With time, however, mucoid forms of *P. aeruginosa* predominate in the chronic pulmonary infections in CF patients, as first noted by Doggett (3).

The factors which regulate the bacterial flora of the upper respiratory tract have been intensively studied in the past few years (9). It has been found that the microbial composition of a region is proportional to the relative abilities of the individual species to attach to the epithelial cells of that region (8, 9, 17). Thus, selective bacterial adherence to mucosal surfaces of the respiratory tract seems to be a major determinant of the indigenous bacterial flora. Organisms which are unable to attach to surfaces are removed by secretions and thus fail to maintain colonization (18).

We have recently demonstrated that the ability of *P. aeruginosa* to persist in the respiratory tract is correlated with the organism's ability to adhere to upper respiratory epithelium (15). Normal individuals are infrequently colonized with *P. aeruginosa*, but when they are this organism adheres in low numbers to their buccal epithelial cells. Conversely, *P. aeruginosa* adheres in high numbers to the upper respiratory epithelial cells of seriously ill patients who are colonized with this organism. Although the exact

alteration of the epithelial cell surface which permits the adherence of *P. aeruginosa* has not been defined, we have demonstrated that *P. aeruginosa* adherence can be correlated with the loss of a protease-sensitive glycoprotein, fibronectin, from the epithelial cell surface (D. E. Woods, D. C. Straus, W. G. Johanson, Jr., and J. A. Bass, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, B27, p. 20).

The objectives of this study were to correlate the in vitro adherence of *P. aeruginosa* to CF buccal epithelial cells with respiratory tract colonization by this organism in CF patients. We further wished to determine the correlation, if any, between the in vitro adherence of *P. aeruginosa* and levels of buccal cell surface fibronectin and salivary proteases in CF patients. Additionally, we sought to determine the relative abilities of rough versus mucoid strains to adhere to buccal epithelial cells.

MATERIALS AND METHODS

Individuals seen in the Cystic Fibrosis/Chronic Pulmonary Clinic at Santa Rosa Children's Hospital, San Antonio, Tex., formed the group of 20 CF patients in this study. Diagnosis of CF was based on accepted criteria (26); the mean age of the group was 9.5 years; the mean Schwachman (21) score, clinical evaluation, was 63.8. Controls consisted of 20 age-matched noninfected pediatric patients with no history of CF. After obtaining the patient's and/or patient's parents' informed consent, swab specimens of the posterior pharynx and expectorated sputum were obtained. Specimens were cultured on appropriate media, and colonies of *P. aeruginosa* were identified by standard methods (16). Colonization was defined as the presence of any colonies of *P. aeruginosa* in either the swab or the sputum culture. All strains were classified serologically according to the procedure of Habs (10) and maintained in 1-ml portions of 5% skim milk at −70°C.

To prepare cultures for adherence testing, a 1-ml portion was thawed and inoculated into 200 ml of M-9 medium (20) containing 0.2% glucose. This was incubated without shaking for 12 h at 37°C. One milliliter of a 1:100 dilution of this 12-h culture (ca. 10^6 organisms) was transferred to 15 ml of fresh M-9 medium containing 0.2% glucose and 0.2 ml of uniformly labeled [^{14}C]lysine (New England Nuclear Corp.; specific activity, 50 μCi/ml) was added. This suspension was incubated at 37°C for 3 h in a shaking water bath at 500 rpm. At the end of the incubation period, the bacteria were washed twice with phosphate-buffered saline containing 0.001 M $MgCl_2$ at pH 7.2 (PBS) and resuspended in PBS at a concentration of 10^6 organisms per ml. The specific activity per milliliter of bacteria labeled was determined by pipetting 0.1-ml portions of the bacterial suspension into liquid scintillation vials. Scintillation cocktail was added, and the samples were counted for 1 min on a Searle Mark III scintillation counter equipped with computer conversion of counts per minute to disintegrations per minute.

Buccal epithelial cells were collected by vigorous swabbing of the buccal mucosa with a sterile cotton-tipped swab. The cells were dislodged by swirling the swab in 5 ml of PBS, washed three times by centrifugation (10 min at 150 × *g*) to remove unattached bacteria, and finally resuspended in PBS. The adherence of *P. aeruginosa* to epithelial cells in vitro was examined by mixing 1-ml samples of standardized suspensions of [^{14}C]lysine-labeled bacteria (5 × 10^6/ml) and epithelial cells (5 × 10^4/ml) together in a shaking water bath (1,000 rpm) at 37°C for 2 h. The epithelial cells were then washed free of unattached bacteria by continuous washing over 10-μm pore-size filters (Gelman) for 2 min. The filter was then placed in a scintillation vial and solubilized, and the scintillation cocktail was added and counted for 1 min to obtain the total disintegrations per minute per 10^4 epithelial cells. From the specific activity measurements of the bacterial suspension obtained previously, the results were expressed finally as the total adherent bacteria per epithelial cell.

In those experiments designed to assess the relative abilities of a large series of rough versus mucoid strains of *P. aeruginosa* to adhere to buccal epithelial cells, epithelial cells from normal healthy individuals were preincubated with 2.5 μg of trypsin per ml (Sigma Chemical Co., St. Louis, Mo.) for 10 min in PBS at 37°C. Trypsin was removed by washing in cold PBS, and the cells were resuspended in PBS. This procedure has been shown to alter the surfaces of normal cells in such a manner as to allow *P. aeruginosa* to adhere (15).

Plasma fibronectin was purified by affinity chromatography on gelatin-Sepharose (Pharmacia Fine Chemicals, Piscataway, N.J.) as described by Vuento and Vaheri (23) with 2 ml of plasma per ml of beads. Bound fibronectin was eluted with a linear gradient of L-arginine (0 to 2 M; gradient volume, 200 ml) in 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.5). Fractions eluted at between 50 and 75% of the gradient volume were pooled, dialyzed against 0.1 M ammonium bicarbonate, and lyophilized. The fibronectin preparation yielded a single component of 220,000 molecular weight on reduced polyacrylamide gel electrophoresis in sodium dodecyl sulfate. Anti-fibronectin antiserum was prepared by immunization of rabbits with 1-mg subcutaneous doses of purified fibronectin in complete Freund adjuvant for 6 weeks on a biweekly schedule. The anti-fibronectin was absorbed by passage through a gelatin-Sepharose column and a Sepharose column to which plasma depleted of fibronectin was coupled. Fibronectin antibodies were immunochemically purified on fibronectin-Sepharose (5 mg of fibronectin per ml of beads). After application of the antiserum and thorough washing, elution with 3.0 M potassium iodide in PBS yielded one major component of 160,000 molecular weight on nonreduced sodium dodecyl sulfate-polyacrylamide gel electrophoresis. For determination of relative levels of buccal cell surface fibronectin, the purified antibodies to fibronectin were radiolabeled with ^{125}I by the method of Fraker and Speck (6). One microgram of labeled antibody (specific activity, 10^4 cpm/μg) was then incubated with 10^4 epithelial cells in PBS at 37°C for 1 h. This level of antibody to fibronectin sufficiently saturated the epithelial cells.

The cells were then washed thoroughly with PBS and placed in a scintillation vial, the counts per minute of bound anti-fibronectin were determined with a gamma counter, and the results are expressed as the counts per minute of ^{125}I-anti-fibronectin bound per 10^4 epithelial cells.

Measurement of protease activity in secretions was performed utilizing the ^{125}I-labeled fibrin plate method of Unkeless et al. (22). Fibrin plates were prepared by distributing 0.1 ml of radioactive fibrinogen solution (Amersham, Arlington Heights, Ill., 100 mg/ml) over the surface of a 35-mm plastic petri dish with a spreader. The plates were dried a minimum of 1 day at 45°C and stored at this temperature until use. Fibrinogen was converted to fibrin by overlayering the plates with 2 ml of RPMI-1640 (Grand Island Biological Co., Grand Island, N.Y.) containing 10% fetal bovine serum and incubated at 37°C for 18 h. The medium was then removed, and the plates were thoroughly washed with incubation buffer to remove all traces of serum (three washes of 2 ml). One-milliliter portions of nonstimulated saliva, sterilized by filtration through 0.45-μm filters, from colonized patients and noncolonized controls, adjusted to 1 mg of protein per ml in 0.1 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 8.1), containing 10 μg of plasminogen, were deposited onto separate ^{125}I-fibrin plates. All determinations were performed in triplicate. After incubation at 37°C for 20 h, the entire reaction mixture from each plate was withdrawn and counted directly in a gamma counter. Results were expressed as the counts per minute of ^{125}I released per milliliter of secretions. This assay was chosen so as to allow the detection of proteolytic activity due to a wide variety of proteases, including plasminogen activators which might otherwise go undetected (22).

RESULTS

Each of the CF patients were colonized with both rough and mucoid variants of *P. aeruginosa*. In contrast, no gram-negative bacilli were recovered from any of the 20 controls. Results of the in vitro assay of *P. aeruginosa* adherence to buccal epithelial cells are shown in Table 1. Both a rough variant of *P. aeruginosa* (DG1-S) and a mucoid variant (DG1-M) adhered in low numbers to cells obtained from healthy controls. Similarly, low numbers of the mucoid variant but significantly greater numbers of the rough variant adhered to cells obtained from CF patients.

Adherence of rough and mucoid variants was studied further by measuring the adherence capabilities of a large series of 51 paired strains (Table 2). Adherence of organisms of the rough colony type was 24.8 ± 0.9 bacteria per epithelial cell, whereas that of the mucoid type was 1.8 ± 0.1 organisms per cell ($P < 0.001$). This difference in adherence between rough and mucoid types could not be explained by differences in serotype, as each rough and mucoid type constituting a pair was of the same serotype (Table 3).

Also noted in Table 3 is the preponderance of Habs type 6 among the CF strains examined, a finding reported by others (4, 21).

Table 4 summarizes the results of the cell surface fibronectin assays and salivary protease activity in CF patients and controls. Cell surface fibronectin was significantly decreased in CF patients ($P < 0.01$) and salivary protease activity was significantly increased ($P < 0.01$) over that of the controls. The amount of fibronectin present on CF buccal cells corresponded to ca. 17% of the total cell surface fibronectin present on similar cells from controls. Protease levels in the secretions from CF patients amounted to an approximate threefold increase over those of the controls.

DISCUSSION

There is increasing evidence that bacterial adherence to mucosal surfaces is an important etiological factor in infections. Recent evidence suggests that adherence is necessary for bacterial colonization and is the initial event in many bacterial diseases, including urinary tract infections (4, 5), gonorrhea (24), gastroenteritis (7, 11), and pulmonary infections due to gram-negative bacilli (15). *P. aeruginosa* is isolated infrequently from the respiratory tracts of healthy individuals (14, 19). In contrast, CF patients are frequently colonized by this organism. When studied in vitro, *P. aeruginosa* attached to buccal cells from CF patients in far greater numbers than to cells from controls, indicating that adherence of *P. aeruginosa* to upper respiratory epithelium of CF patients may be an important factor in respiratory tract colonization.

The adherence of *P. aeruginosa* to upper respiratory epithelium most certainly involves both host and bacterial factors. We have found that adherence of *P. aeruginosa* to mammalian cell surfaces requires the presence of pili (27). Decreased adherence of mucoid strains is not likely due to the absence of pili since we and

TABLE 1. *Adherence of P. aeruginosa to buccal cells obtained from patients with CF*

Source of buccal cell	*P. aeruginosa* adherence[a]	
	Rough colony type (DG1S)	Mucoid colony type (DG1M)
Controls (20)	2.3 ± 0.3[b]	3.4 ± 1.7
CF patients (20)	19.1 ± 1.1	6.1 ± 2.3[c]

[a] Number of *P. aeruginosa* per buccal cell; mean ± the standard error of the mean.

[b] Significantly different from CF patients ($P < 0.01$) by Student's t test.

[c] Significantly different from rough colony type ($P < 0.01$) by Student's t test.

TABLE 2. *Adherence of rough versus mucoid P. aeruginosa to mammalian epithelial cells*[a]

Rough strain	Adherence[b]	Mucoid strain	Adherence	Rough strain	Adherence[b]	Mucoid strain	Adherence
DNS	26.1	DNM	0.6	PM1S	17.8	PM1M	2.1
MGS	31.3	MGM	1.8	PM2S	19.5	PM2M	1.8
JWA1S	21.3	JWA1M	2.0	PM3S	16.2	PM3M	1.1
JWA2S	28.1	JWA2M	2.1	J6S	19.1	J6M	2.6
JWA3S	27.6	JWA3M	1.4	JC0S	24.4	JC0M	5.4
JWA4S	25.5	JWA4M	1.1	JW0S	31.7	JW0M	2.3
JWA5S	24.8	JWA5M	0.8	CW1S	19.8	CW1M	1.4
SLS	19.1	SLM	1.5	CW2S	23.6	CW2M	0.6
LWS	20.2	LWM	3.1	CW3S	20.4	CW3M	1.5
MRS	17.6	MRM	0.7	CW4S	27.6	CW4M	3.1
LSH	29.3	LHM	1.9	JCA1S	19.1	JCA1M	2.5
KC1S	27.8	KC1M	2.2	JCA2S	18.8	JCA2M	1.4
KC2S	26.4	KC2M	3.1	JCA2S	17.8	JCA3M	0.6
KC3S	29.1	KC3M	1.6	LSS	25.5	LSM	1.8
KC4S	27.7	KC4M	1.6	RBS	34.9	RBM	3.7
KC5S	28.3	KC4M	3.4	CNS	40.1	CNM	1.6
CG1S	16.4	CG1M	1.1	J1S	16.3	J1M	1.1
CG2S	15.9	CG2M	0.9	J2S	21.5	J2M	0.3
CG3S	14.3	CG3M	1.5	J3S	22.2	J3M	1.4
CG4S	17.1	CG4M	1.4	J4S	15.4	J4M	1.2
DG1S	34.8	DG1M	2.3	J5S	27.1	J5M	2.0
DG2S	36.9	DG2M	1.4	J6S	26.9	J6M	3.4
DG3S	33.4	DG3M	0.5	J7S	34.3	J7M	1.6
DG4S	35.3	DG4M	2.6	J8S	15.7	J8M	0.8
DG5S	39.9	DG5M	2.7	J9S	21.8	J9M	0.7
DG6S	34.7	DG6M	3.4				

[a] Buccal epithelial cells previously treated for 10 min at 37°C with 2.5 μg of trypsin per ml.
[b] Mean of three determinations, measured by radiolabeled adherence assay.
[c] Overall mean ± standard error of the mean.
[d] Significantly different from mucoid ($P < 0.001$) by Student's t test.

TABLE 3. *Agglutination types for Habs antisera of 51 P. aeruginosa rough and mucoid paired*[a] *isolates from 28 patients with CF*

Habs serotype	No. of strains	% of strains
1	1	1.9
2 5	2	3.9
3	5	9.8
4	4	7.8
6	22	43.1
7 8	10	19.6
9	1	1.9
10	1	1.9
11	2	3.9
12	1	1.9
13 14	2	3.9

[a] All rough and mucoid pairs were of the same serotype.

others (C. C. Brinton, University of Pittsburgh, personal communication) have found that most such strains are piliated. However, the mucoid material surrounding the organism may interfere with the interaction of pili with the mammalian cell surface. The observation that rough variants

TABLE 4. *Buccal cell surface fibronectin levels and salivary protease activity in CF patients*

Source of buccal cells	Cell surface fibronectin[a]	Protease activity[b]
Controls (20)	3,002 ± 17.8	20,084 ± 302.2
CF patients (20)	499.5 ± 63.8[c]	65,417 ± 3,179.1[c]

[a] Measured by radioimmunoassay. Values represent the mean counts per minute of ^{125}I-anti-fibronectin bound to 10^4 buccal cells ± the standard error of the mean.
[b] Values represent the mean counts per minute of ^{125}I released from insoluble ^{125}I-fibrin matrix exposed to 1.0-ml secretions for 20 h at 37°C ± the standard error of the mean.
[c] Significantly different from the value for the controls ($P < 0.01$) by Student's t test.

of *P. aeruginosa* adhere in increased numbers to buccal cells from CF patients compared with those of controls implies that there is some alteration in the surface of the CF cells. Trypsin treatment in vitro of normal buccal cells markedly increases *P. aeruginosa* adherence and causes release of fibronectin from the cell surface. We found that fibronectin was reduced on the surface of CF cells in vivo, suggesting that these cells had been exposed to protease activity

in the host. Since salivary protease levels in CF patients are significantly elevated over those of controls, it would be reasonable to assume that increased protease levels in these secretions which bathe the mucosal surfaces may be responsible for the decreased fibronectin levels. Although the loss of fibronectin may not be the only alteration which leads to increased *P. aeruginosa* adherence, the correlation of fibronectin loss with increased adherence and salivary protease levels makes this hypothesis an attractive one.

The finding that increased *P. aeruginosa* adherence to CF buccal cells was restricted to the rough colony type may explain the repeated observation that these organisms are the initial colonizers in CF patients. Huang and Doggett (13) reviewed a total of 5,055 respiratory tract cultures from 323 CF patients over a 10-year period; these authors demonstrated that while *P. aeruginosa* was acquired early in these patients, only 2 of 84 patients under 1 year of age were colonized with mucoid *P. aeruginosa*. The ability of the rough types to adhere to buccal cell surfaces may well provide a selective advantage over the mucoid types in the initial colonization of the CF respiratory tract.

The serotype distribution reported in this study agrees with the results reported by others (12, 28). The preponderance of one or two O serotypes isolated from CF patients indicates that there may be some association between O serotype and *P. aeruginosa* colonization in CF patients. It may be, however, that the investigation of the association between adherence capability and colonization would be a fruitful one. It is possible that the *P. aeruginosa* strains which colonize CF patients possess a common adherence factor which allows them a selective advantage in this situation.

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LITERATURE CITED

1. **Bergan, T., and N. Hoiby.** 1975. Epidemiological markers for *Pseudomonas aeruginosa*. 6. Relationship between concomitant non-mucoid and mucoid strains from the respiratory tract in cystic fibrosis. Acta Pathol. Microbiol. Scand. Sect. B **83**:553-560.
2. **diSant'Agnese, P. A., and P. B. Davis.** 1976. Research in cystic fibrosis. N. Engl. J. Med. **295**:597-602.
3. **Doggett, R. G.** 1969. Incidence of mucoid *Pseudomonas aeruginosa* from clinical sources. Appl. Microbiol. **18**: 936-937.
4. **Eden, C. S., U. Jodal, L. A. Hanson, U. Lindberg, and A. S. Akerlund.** 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.
5. **Fowler, J. E., Jr., and T. A. Stamey.** 1977. Studies on introital colonization in women with recurrent urinary tract infections. VII. The role of bacterial adherence. J. Urol. **117**:472-476.
6. **Fraker, P. J., J. C. Speck, Jr.** 1978. Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3, R, 6-tetrachloro-3A, 6A-diphenyglycoluril. Biochem. Biophys. Res. Commun. **80**:849-857.
7. **Freter, R.** 1972. Parameters affecting the association of vibrios with the intestinal surface in experimental cholera. Infect. Immun. **6**:134-141.
8. **Gibbons, R.J., D. M. Spinell, and Z. Skobe.** 1976. Selective adherence as a determinant of the host tropisms of certain indigenous and pathogenic bacteria. Infect. Immun. **13**:238-246.
9. **Gibbons, R. J., and J. vanHoute.** 1975. Bacterial adherence in oral microbial ecology. Annu. Rev. Microbiol. **29**:19-44.
10. **Habs, J.** 1957. Untersuchungen uber die O-antigens von *Pseudomonas aeruginosa*. Z. Hyg. Infektionskr. **144**: 218-228.
11. **Hohmann, A., and M. R. Wilson.** 1975. Adherence of enteropathogenic *Escherichia coli* to intestinal epithelium in vivo. Infect. Immun. **12**:866-880.
12. **Hoiby, N.** 1974. Epidemiological investigations of the respiratory tract bacteriology in patients with cystic fibrosis. Acta. Pathol. Microbiol. Scand. Sect. B **82**: 541-550.
13. **Huang, N. N., and R. G. Doggett.** 1979. Antibiotic therapy of *Pseudomonas aeruginosa*: clinical manifestations of infection and current therapy, p. 411. *In* R. G. Doggett (ed.), *Pseudomonas aeruginosa*: clinical manifestations of infection and current therapy. Academic Press, Inc., New York.
14. **Johanson, W. G., Jr., A. K. Pierce, and J. P. Sanford.** 1969. Changing pharyngeal bacterial flora of hospitalized patients. Emergence of gram-negative bacilli. N. Engl. J. Med. **281**:1137-1140.
15. **Johanson, W. G., Jr., D. E. Woods, and T. Chauduri.** 1979. Association of respiratory tract colonization with adherence of gram negative bacilli to epithelial cells. J. Infect. Dis. **139**:667-673.
16. **Lennette, E. H., E. H. Spaulding, and J. P. Truant (ed.).** 1974. Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
17. **Liljemark, W. F., and R. J. Gibbons.** 1972. Proportional distribution and relative adherence of *Streptococcus miteor* (*mitis*) on various surfaces in the human oral cavity. Infect. Immun. **6**:852-859.
18. **Mandel, I. D.** 1976. Oral secretions and fluid flow. J. Dent. Res. **55**(*Suppl.*):22-37.
19. **Rahal, J. J., Jr., R. H. Meade, C.M. Bump, and A. J. Reinauer.** 1970. Upper respiratory tract carriage of gram negative enteric bacilli by hospital personnel. J. Am. Med. Assoc. **214**:754-756.
20. **Roberts, R. B., P. Abelson, D. B. Cowie, E. T. Bolton, and R. J. Britten.** 1963. Studies of biosynthesis in *Escherichia coli*. Carnegie Inst. Washington Publ. **607**: 127-132.
21. **Schwachman, H., and L. L. Kulczycki.** 1958. Long-term study of one hundred five patients with cystic fibrosis: studies made over a five to fourteen year period. Am. J. Dis. Child. **96**:6-15.
22. **Unkeless, J. C., A. Tobia, L. Ossowski, J. P. Quigley, D. B. Rifkin, and E. Reich.** 1973. An enzymatic function associated with transformation of fibroblasts by onogenic viruses. J. Exp. Med. **137**:85-111.
23. **Vuento, M., and A. Vaheri.** 1978. Dissociation of fibronectin from gelatin-agarose by amino compounds. Bio-

chem. J. **175**:333-336.

24. **Ward, M. E., and P. J. Watt.** 1972. Adherence of *Neisseria gonorrhoeae* to urethral mucosal cells: an electron-microscopic study of human gonorrhea. J. Infect. Dis. **126**:601-605.
25. **Wood, R. E.** 1976. *Pseudomonas:* the compromised host. Hosp. Pract. **10**:91-100.
26. **Wood, R. E., T. F. Boat, and C. F. Doershuk.** 1976. Cystic fibrosis. Am. Rev. Respir. Dis. **113**:833-878.
27. **Woods, D. E., D. C. Straus, W. G. Johanson, Jr., V. K. Berry, and J. A. Bass.** 1980. Role of pili in adherence of *Pseudomonas aeruginosa* to mammalian buccal epithelial cells. Infect. Immun. **29**:1146-1151.
28. **Zierdt, C. H., and R. L. Williams.** 1975. Serotyping of *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis of the pancreas. J. Clin. Microbiol. **1**:521-526.