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Quantitation of Adherence of Mucoid and Nonmucoid *Pseudomonas aeruginosa* to Hamster Tracheal Epithelium

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Adherence of mucoid and nonmucoid isolates of *Pseudomonas aeruginosa* to tracheal epithelium was quantitated by using hamster tracheas mounted in a perfusion chamber. The strains of *P. aeruginosa* used were clinical isolates from cystic fibrosis patients and a series of laboratory strains. Aseptically excised hamster tracheas were mounted in perfusion chambers and embedded in minimal essential medium containing 1.5% agarose. The tracheas were infected with various numbers of bacteria for various periods, rinsed, homogenized, and plated on Trypticase soy agar. A 4-mm segment from each trachea was prepared for quantitation, and the other segment was prepared for examination by scanning electron microscopy. Adherence increased with time and with increasing concentrations of inoculum. Standard conditions of inoculation were set at an inoculum of 10^7 CFU/ml and a 2-h incubation. Under these conditions, the mucoid organisms adhered to the ciliated epithelium 10- to 100-fold better than did the nonmucoid organisms. Adherence of the mucoid isolates did not appear to be pilus mediated and did not involve hydrophobic interactions. The mucoid *P. aeruginosa* isolates could be seen adhering to the epithelium in the form of microcolonies embedded in an extracellular matrix which attaches the organisms to the cilia and to each other. The adherence may be involved in the establishment of infection of the lungs of these patients and in the inability to clear the organisms from the lungs. The model will be useful in determining the mechanism of adherence of the bacteria to the ciliated epithelium of the respiratory tract.

Pseudomonas aeruginosa is an opportunistic pathogen and is the major cause of serious bacterial infections of the respiratory tract in patients with cystic fibrosis (CF) (13, 14). The majority of the isolates from the lungs of chronically infected CF patients produce an alginate-like exopolysaccharide composed of an acetylated polymer of D-mannuronic and L-guluronic acids (6, 16). The factors responsible for the prevalence of these mucoid variants in CF patients are not clear. Current theories include increased resistance to attack by host phagocytes (5, 20), increased resistance to pulmonary surfactants (9) and antibiotics (10), and attachment to the ciliated epithelium (1, 4). Blackwood and Pennington reported that the clearance of mucoid and nonmucoid strains of *P. aeruginosa* from the lungs of normal animals was the same if the organisms were washed before instillation into the lungs (3). In contrast, Govan et al. have shown that the mucoid variants are cleared from the lungs less readily than are nonmucoid organisms if the organisms are not washed before infection (11). Thus, the loose exopolysaccharide layer apparently interferes with the normal clearance mechanisms of the host.

Previous work from our laboratory has shown that mucoid isolates adhere better than nonmucoid isolates to intact tracheal epithelium (1). In contrast, Woods et al. have shown that attachment of nonmucoid *P. aeruginosa* isolates to buccal cells from CF patients is pilus mediated and that mucoid isolates do not adhere as well as organisms of the rough colony type (23, 25). Ramphal et al. have also reported a pilus-mediated adherence of nonmucoid *P. aeruginosa* strains to acid-injured tracheal epithelium (17, 18). Interestingly, adherence of mucoid organisms was not inhibited by purified pili, which did inhibit the adherence of nonmucoid derivatives of the mucoid organisms, suggesting a different mechanism of adherence of the mucoid bacteria. Although some investigators have theorized that *P. aeruginosa* only colonizes respiratory tract tissue that has been damaged by a previous insult, available evidence indicates that the respiratory epithelium of CF patients may not be damaged before infection (21). Therefore, examination of the interaction of *P. aeruginosa* with the intact respiratory epithelium is necessary for studies relating to colonization of the respiratory tracts of CF patients.

Previous studies on the adherence of *P. aeruginosa* strains to the cells of the lower respiratory tract have relied on evaluation of scanning electron microscope images (17, 18). Such studies are tedious, time consuming, and somewhat subjective. Quantitation of adherent organisms required the counting of organisms in scanning electron microscope fields; we found this unsatisfactory for studies with intact ciliated epithelium, since many organisms could be hidden by the lawn of cilia. Therefore, we have developed a quantitative assay for adherence by modifying the perfused tracheal chamber described by Gabridge and Hoglund (8) to accommodate hamster tracheas. In addition, Govan has developed a series of laboratory strains which differ in the ability to make the alginate-like polysaccharide (10), permitting comparison of the adherence of closely related mucoid and nonmucoid strains of *P. aeruginosa* to the respiratory tract epithelium. The present study describes a quantitative method to analyze the adherence of *P. aeruginosa* strains to the tracheal epithelium and further emphasizes the importance of the bacterial exopolysaccharide in the adherence of *P. aeruginosa* strains to cells of the lower respiratory tract.

MATERIALS AND METHODS

Bacteria. *P. aeruginosa* isolates 105M, 117M, 151M, 251M, and 253M are mucoid clinical isolates from sputum samples of CF patients at Childrens Hospital, Columbus, Ohio. Isolates 32NM, 150NM, 201NM, 214NM, 250NM, and 255NM are clinical nonmucoid isolates from the same source. Spon-

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taneous nonmucoid variants, 253NM and 251NM, were obtained by transfer of 253M and 251M on Trypticase soy agar (BBL Microbiology Systems) and selection of nonmucoid colonies. Isolates 492a and 492a Rev are mucoid and nonmucoid clinical isolates, respectively, which were obtained from John Govan, University of Edinburgh, Edinburgh, Scotland. Strains PAO 581 and PAO 579 are mucoid variants isolated by carbenicillin selection from the nonmucoid parent PAO 381 by Govan (10). Strains PAO 552 and PAO 553 are spontaneous nonmucoid revertants from PAO 579. All isolates were tested for protease production by using an azocasein assay of overnight culture supernatants (12) and elastase by streaking the isolates on Trypticase soy agar plates containing 0.1% elastin and observing the plates for a zone of clearing around the growth after 24 to 48 h of incubation. Only strains 105M, 253M, and 150NM were negative for proteolytic enzymes, but these strains may produce low levels of proteases not detectable by these assays. The isolates were also tested for piliation by observing shadow-cast preparations of bacterial suspensions with a Zeiss transmission electron microscope.

Perfused tracheal explants. The tracheal perfusion model described by Gabridge for studies of mycoplasma (8) was modified for use with hamster tracheas. Adult male Syrian golden hamsters were sacrificed by intraperitoneal injection with 0.3 ml of 10% sodium brevitol. The trachea of each hamster was excised, cut to 11 mm in length, and placed in the groove in the perfusion chamber. The open ends of the trachea were blocked, and minimal essential medium (MEM) containing 1.5% agarose at 45°C was poured over the trachea. When the agar had solidified the blocks were removed, any agar trapped in the open ends of the trachea was removed carefully, and the trachea was perfused with MEM containing1.2%*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (MEM-HEPES) (pH 7.4).

Infection of the perfused tracheal explants. Overnight cultures (18 to 20 h) were obtained by inoculating 10 ml of the dialysate of Trypticase soy broth (TSBD) with an isolated colony from an agar plate and incubating the culture at 37°C in a shaking incubator, unless indicated otherwise. The concentration of the culture was estimated spectrophotometrically and adjusted to the appropriate concentration in MEM-HEPES for use as inoculum. The actual concentration of inoculum was determined by plate counts. To study the effect of inoculum size on adherence, it was necessary to wash the organisms by centrifugation and to resuspend the organisms in MEM-HEPES. The washing was required to remove the bacterial exoproducts which may alter the tracheal epithelium and to achieve high concentrations of bacteria. However, washing was undesirable since it removes much of the loose polysaccharide layer from the mucoid organisms. Therefore, in subsequent experiments the organisms were diluted to 10^7 CFU/ml in MEM-HEPES without washing. Previous results from our laboratory have shown that culture supernatants diluted similarly do not alter tracheal morphology even 48 h after exposure (2). In addition, the adherence of bacteria grown statically for 18 h in TSBD or M-9 containing 0.2% glucose, conditions which favor the development of pili (25), was compared with the adherence of organisms grown in TSBD with shaking.

A total volume of 4 ml of inoculum was added to each chamber, and the explants were incubated at 37°C in a 5% CO_2-air atmosphere for 2 h unless indicated otherwise. At the end of the incubation period, the bacterial suspension was removed and the explants were washed three times with MEM-HEPES to remove nonadherent organisms. The cut ends of the trachea were removed, and a 4-mm section of the trachea was homogenized in 1 ml of sterile double-distilled water in a Ten-Broeck tissue grinder. An additional 2 ml of double-distilled water was added to rinse each grinder, and the homogenate was diluted and plated on Trypticase soy agar plates for quantitation. The remaining tracheal section was processed for scanning electron microscopy (SEM).

Hydrophobicity assay. The hydrophobicity of the bacterial cell surface was tested by the method of Rosenberg et al. (19). Briefly, bacteria were grown as described previously and adjusted to an optical density of 1.2 to 1.4 at 540 nm in a spectrophotometer. A layer of hexadecane (0.2 to 0.5 ml) was added to each tube, and the mixture was vortexed for 2 min. The mixture was allowed to settle for 15 min, the hexadecane layer was removed, and the optical density of the aqueous phase was measured. Bacteria which have hydrophobic surface components separate into the hexadecane layer, resulting in a reduction in the optical density of the aqueous phase.

SEM. After the explants were removed from the chambers, they were rinsed three times with sterile MEM-HEPES and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 1 h at 4°C. The explants were then rinsed three times with 0.1 M phosphate buffer, post-fixed in 1% osmium tetroxide in phosphate buffer for 1 h at 4°C, washed three times in phosphate buffer, and dehydrated in a 30 to 100% ethanol series. The explants were transferred to amyl acetate, dried in a Bomar critical point drier, mounted, coated with gold, and observed with a Hitachi S-500 scanning electron microscope.

Analysis of data. The *t*-test was used to determine whether mean values were significantly different from each other.

RESULTS

Standardization of assay conditions. The standard conditions for assay of adherence of *P. aeruginosa* strains to the tracheal epithelium were established by varying the number of organisms in the inoculum and the time of exposure of the perfused explants to the inoculum. Adherence was clearly time dependent, as the number of adherent organisms increased logarithmically during the first 3 h of exposure to mucoid or nonmucoid organisms (Fig. 1). After a 30-min infection with 10^7 CFU/ml, approximately 10^3 CFU of the mucoid isolate and 2×10^2 CFU of the nonmucoid isolate were present on 4-mm sections of trachea. At subsequent times, the number of mucoid organisms was consistently 10- to 100-fold higher than the number of nonmucoid bacteria adhering at the same time point. Adherence of the mucoid organisms approached a maximum at 4 h after initiation of the infection, but the number of adherent nonmucoid organisms was still increasing logarithmically. The increase in the number of adherent organisms was not due to multiplication of bacteria in the medium, as the inoculum concentration remained constant up to 4 h after initiation of infection. The standard incubation time selected for further experiments was 2 h, since at that time there was a marked difference between the adherence of the mucoid and the nonmucoid organisms, the infecting organisms had not begun to multiply, and bacterial proteases were not detectable in the cell culture supernatants. The culture supernatants were screened for elastase and alkaline protease by a competitive enzyme-linked immunosorbent assay and were negative (<25 pg/ml) until after 6 h of culture.

Adherence was also dependent on the concentration of the inoculum (Fig. 2). The number of adhering mucoid *P. aeruginosa* organisms began to saturate after a 2-h infection

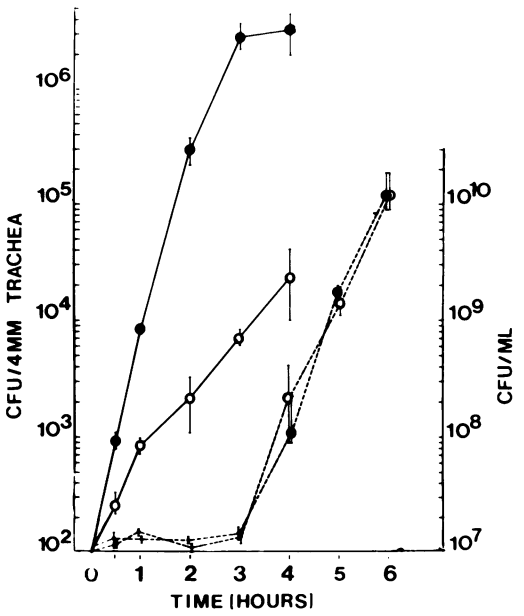

FIG. 1. Adherence of mucoid (●) and nonmucoid (○) *P. aeruginosa* strains to tracheal epithelium with time. Explants were perfused with MEM containing 10^7 CFU of bacteria per ml, rinsed at the appropriate time, and processed for counting of adherent bacteria. Dashed lines indicate the number of CFU of bacteria in the culture supernatants.

with 10^7 CFU/ml. To achieve the high concentrations of organisms used in this experiment, the cultures were centrifuged and suspended in MEM to the appropriate concentration. This washing resulted in a slight but insignificant reduction in adherence. Since washing removes some of the loose exopolysaccharide from the bacteria, washing was avoided in subsequent studies. The standard inoculum for further studies was 10^7 CFU/ml, prepared by diluting unwashed bacterial suspensions in MEM-HEPES, which does not transfer enough bacterial exoproducts to alter the tracheal morphology (2).

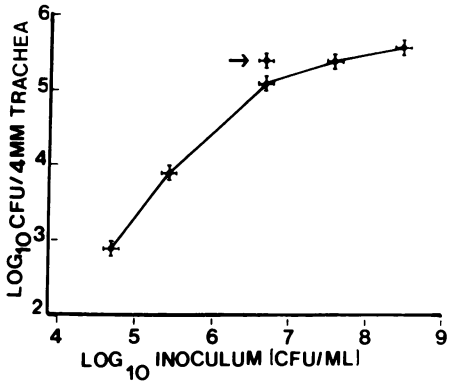

FIG. 2. Adherence of mucoid *P. aeruginosa* strains to tracheal epithelium as a function of inoculum size. An 18-h culture grown in TSBD with shaking was centrifuged and suspended in MEM. Tracheal explants were exposed to the appropriate inoculum in the perfusion chambers for 2 h and processed for quantitation of the adherent organisms. The arrow indicates the adherence of an inoculum diluted to 10^7 CFU/ml without washing.

TABLE 1. Adherence of mucoid and nonmucoid clinical isolates of *P. aeruginosa* to tracheal epithelium

Isolate[a]	No. (× 10^3) of CFU/4 mm of trachea (mean ± SD)[b,c]
105 M	120 ± 10
117 M	130 ± 20
492 M	130 ± 30
151 M	170 ± 70
251 M	200 ± 100
253 M	230 ± 100
32 NM	7.3 ± 1.2
150 NM	5.3 ± 1.6
201 NM	8.9 ± 5.7
214 NM	3.8 ± 0.4
250 NM	3.9 ± 0.4
255 NM	8.6 ± 0.2

[a] M, Mucoid; NM, nonmucoid.
[b] Average CFU adhering after a 2-h incubation. Each value is an average of three experiments.
[c] Average of mucoid isolates ([163 ± 40] × 10^3) is significantly different from average of nonmucoid isolates ([6.3 ± 2.2] × 10^3) ($P \leq 0.001$).

Adherence of clinical isolates of *P. aeruginosa*. Adherence of mucoid and nonmucoid clinical isolates of *P. aeruginosa* from sputum samples of CF patients was compared by using the adherence assay. The number of adherent mucoid organisms was consistently 10- to 100-fold higher than the number of adhering nonmucoid organisms (Table 1). Nonmucoid revertants of the mucoid isolates were 100-fold less adherent than were their mucoid parents (Table 2). The nonmucoid revertants were piliated and had the same serotype and exoproduct profile as the parents.

Adherence of mucoid and nonmucoid laboratory strains of *P. aeruginosa*. The adherence of mucoid and nonmucoid laboratory strains were compared in the perfused tracheal model (Table 3). Adherence of the mucoid strains PAO 579 and PAO 581 was similar to the adherence of the mucoid clinical isolates. The nonmucoid revertants of PAO 579, PAO 552 and PAO 553, were similar to other nonmucoid isolates in their adherence. However, the nonmucoid parent, PAO 381, adhered almost as well as the mucoid strains tested. The reason for this discrepancy is not clear, as there is no obvious morphological difference between PAO 381 and other nonmucoid isolates. It suggests, however, that these strains may differ in characteristics other than the mucoid phenotype.

Correlation of other bacterial properties with adherence. Since other bacterial properties could have been contribut-

TABLE 2. Adherence of mucoid clinical isolates and the respective nonmucoid variant of each isolate to tracheal epithelium

Isolate	No. (× 10^3) of CFU/4mm of trachea (mean ± SD)[a] for:	
	Mucoid isolates[b]	Nonmucoid isolates
251	600 ± 310	3.8 ± 0.7
253	270 ± 70	3.7 ± 0.4
492a	850 ± 210	3.8 ± 0.2

[a] See Table 1, footnote *b*.
[b] Significantly different from the value for the respective nonmucoid variant ($P \leq 0.05$).

TABLE 3. Adherence of mucoid and nonmucoid laboratory strains of *P. aeruginosa* to tracheal epithelium

Strain[a]	No. ($\times 10^3$) of CFU/4 mm of trachea (mean ± SD)[b]
PAO 579 M	200 ± 70[c]
PAO 581 M	380 ± 250[c]
PAO 381 NM	100 ± 100[c]
PAO 552 NM	3.5 ± 3.0[d]
PAO 553 NM	3.8 ± 2.1[d]

[a] M, Mucoid; NM, nonmucoid.
[b] Average of four experiments.
[c] Not significantly different from each other ($P > 0.05$) but significantly different from PAO 552 and PAO 553 ($P \leq 0.05$).
[d] Not significantly different from each other ($P > 0.05$).

ing to the adherence of the organisms, the bacteria were tested for piliation, cell surface hydrophobicity, and protease production. Observation of shadow-cast bacterial preparations by electron microscopy revealed the presence of pili on the mucoid and nonmucoid bacteria used in this study (Fig. 3). The extent of piliation was variable but did not correlate with adherence. Furthermore, growth of the organisms statically in M-9 medium with glucose, conditions reported to be optimal for pilus production, did not alter the adherence of either the mucoid or the nonmucoid bacteria to the tracheal epithelium (Table 4). Additionally, no hydrophobic properties were detectable by the hexadecane assay (data not shown), which is in agreement with a previous report (7). Adherence also did not correlate with the ability of the organisms to elaborate proteases, emphasizing the point that alteration of the epithelial surface by bacterial proteases was not involved in the adherence observed. Culture supernatants of the 2-h cultures contained less than 25 pg of bacterial proteases per ml.

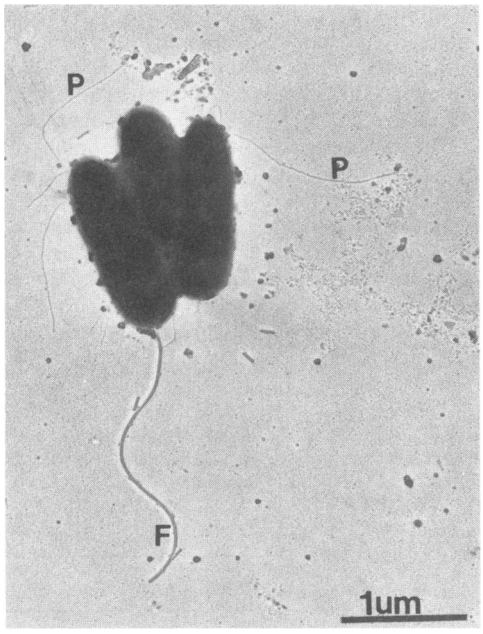

FIG. 3. Transmission electron micrograph of a shadow-cast preparation of a mucoid strain (581M) of *P. aeruginosa*, revealing the presence of pili (P). Nonmucoid organisms were similarly piliated, but none of the organisms were extensively piliated. Flagella (F) were also noted.

SEM. The epithelium of the infected perfused tracheal explants was observed by SEM. The infected explants were observed 4 to 6 h after initiation of infection, since most fields examined at this time contained adherent organisms if the organism was adherent. The mucoid *P. aeruginosa* isolates were observed to adhere predominantly to the ciliated cells of the tracheal epithelium in the form of microcolonies (Fig. 4). The mucoid organisms were embedded in mucus fibers condensed by the dehydration during processing, attached to each other, and attached to the ciliated epithelium. In contrast, no nonmucoid organisms could be detected to adhere to the epithelium, even after an 8-h infection period.

Adherence of the organisms to the anterior and posterior sections of the trachea was compared to determine whether different adherence patterns existed. Adherence to the anterior and posterior halves of the trachea was not significantly different, indicating that one trachea could be used for quantitation and SEM (data not shown).

The infected explants were washed extensively to remove nonadherent organisms, which also removed the mucus blanket of the epithelium. The data presented therefore represent organisms attached to the epithelium and not associated with the host mucus. The mucus blanket could be preserved for observation by electron microscopy by rinsing the infected explants gently. The mucoid organisms could be observed to be associated with the mucus blanket and attaching to the ciliated epithelium (Fig. 5).

DISCUSSION

In a previous study, we compared the adherence of mucoid and nonmucoid *P. aeruginosa* isolates to the ciliated epithelium of hamster tracheal explants by observing the adherent organisms in the scanning electron microscope (1). Although it was clear in these studies that the mucoid organisms have an advantage over the nonmucoid organisms in their ability to adhere, we needed a good quantitative assay to study the nature of the bacteria-host interaction in greater detail. Therefore, we modified the tracheal perfusion model developed by Gabridge and Hoglund (8) to quantitate the adherence of mycoplasma and studied the adherence of mucoid and nonmucoid *P. aeruginosa* isolates to the ciliated epithelium of hamster tracheas quantitatively and by scanning electron microscopy. Using the tracheal perfusion model, we have been able to detect adherent organisms as early as 30 min after addition of the bacteria to the explants

TABLE 4. The influence of bacterial growth conditions on adherence of *P. aeruginosa* to tracheal epithelium

Growth conditions[a]	No. ($\times 10^3$) of CFU/4 mm of trachea (mean ± SD)[b] for:	
	105 M[c]	214 NM[d]
TSBD shaker	130 ± 50	4.3 ± 0.4
TSBD static	130 ± 20	1.2 ± 0.2
M-9 static	90 ± 10	2.8 ± 0.4

[a] Organisms were grown in TSBD or M-9 with 0.2% glucose for 18 to 20 h at 37°C with or without shaking and diluted to 10^7 CFU/ml for inoculation.
[b] Average of two experiments.
[c] Not significantly different from each other ($P > 0.05$).
[d] Significantly different from each other and from 105 M ($P \leq 0.05$).

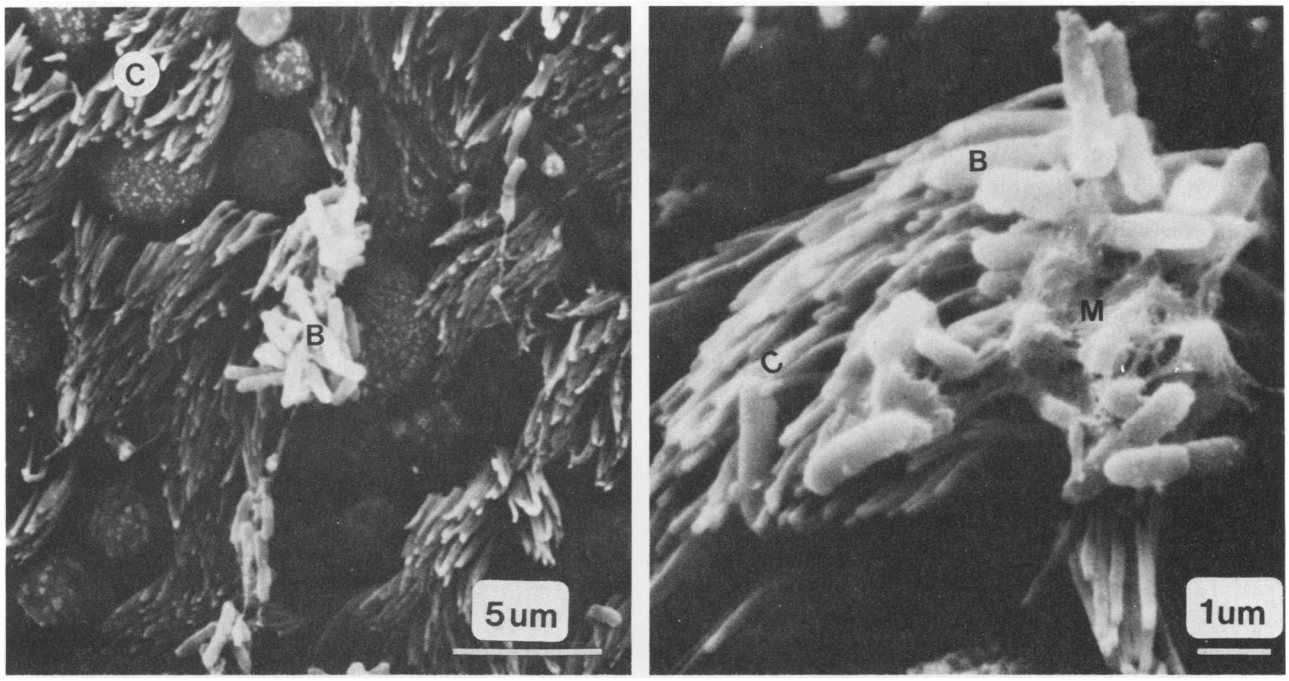

FIG. 4. Scanning electron micrograph (at two magnifications) of mucoid *P. aeruginosa* isolates attached to tracheal epithelium 5 h after initiation of infection. The mucus blanket is absent owing to extensive washing, and the bacteria (B) can be seen attached to the ciliated epithelium (C) in the form of microcolonies (M).

and to confirm the obvious advantage that mucoid organisms have in their ability to attach to the ciliated epithelium.

Ramphal and Pyle examined the adherence of *P. aeruginosa* strains to the cells of the lower respiratory tract and concluded that the organisms did not adhere unless the epithelium had been damaged before addition of the bacteria (17). In that study, adherence to normal tissue was examined after 1 h of incubation. Our results show that at 1 h, approximately 10^4 bacteria were present on the 4-mm section of trachea. On the average, therefore, only one organism would be present in each field scanned (1,000 μm^2), which could easily be missed. Even the approximate 10 organisms per field present at 2 h were not easy to find by SEM. We therefore relied on longer incubation times to observe the association of the bacteria with the epithelium. The number of adherent nonmucoid organisms was not sufficient for detection by electron microscopy in this study. Therefore, the perfused trachea model presented is a significant advancement toward a better understanding of the interaction of *P. aeruginosa* strains with the epithelium of the lower respiratory tract, and it will be useful in detailed studies of the initial interaction of mucoid *P. aeruginosa* strains with an intact ciliated epithelium.

Woods et al. showed that nonmucoid *P. aeruginosa* strains adhere to buccal cells from CF patients by a pilus-mediated mechanism and that mucoid organisms do not adhere to cells from the same patients (23, 25). Further analysis revealed the presence of a high level of proteases in the saliva of colonized patients and suggested that cleavage of fibronectin from the buccal cell surface exposed receptors for the *P. aeruginosa* pili (24). A recent report demonstrated the presence of mucoid *P. aeruginosa* strains on the tongues and in the saliva of CF patients (R. A. Lindeman, M. G. Newman, A. K. Kaufman, and E. R. Stehm, Int. Assoc. Dent. Res. abstr. no. 141, p. 186, 1984), supporting the concept that the oral cavity may be the reservoir for mucoid variants of *P. aeruginosa* which can be aspirated into the lungs. The mucoid forms may be selected by their ability to adhere and because of resistance of the microcolonies to ingestion by phagocytic cells and to antibiotics. Even when the sputum has apparently been cleared of *P. aeruginosa* strains after antibiotic therapy, the tenacious microcolonies may remain in the lungs and act as foci of infection when the antibiotic therapy is discontinued.

Whether or not the respiratory epithelium must be damaged before adherence by *P. aeruginosa* strains is debatable. The epithelium of the lower respiratory tracts of CF patients appears to be normal before infection (21). The ability of the mucoid forms of *P. aeruginosa* to adhere to the normal epithelium suggests that this may be an early event in the colonization of the lungs of these patients by strains of *P. aeruginosa*. However, nonmucoid *P. aeruginosa* strains adhere well to injured cells (17; unpublished data), and the possibility exists that a previous bacterial or viral infection may alter the epithelium and permit invasion of the lungs of CF patients by creating or exposing receptors for the organisms. Evidence for this will require close monitoring of patients to determine whether *P. aeruginosa* infection only follows other types of infection or can occur as a primary infection in CF patients. The quantitative-perfused-trachea-adherence assay should also be useful in detailed studies of the infection of damaged epithelium by *P. aeruginosa* or other pathogens.

The mechanism of adherence of the mucoid organisms to the ciliated epithelium is not clear but is currently under investigation. Adherence did not correlate with piliation, hydrophobicity, or the ability of the organisms to produce proteases. Results of inhibition studies indicate that interaction of the mucoid organisms with host mucin is an important early event in the colonization of the lower respiratory

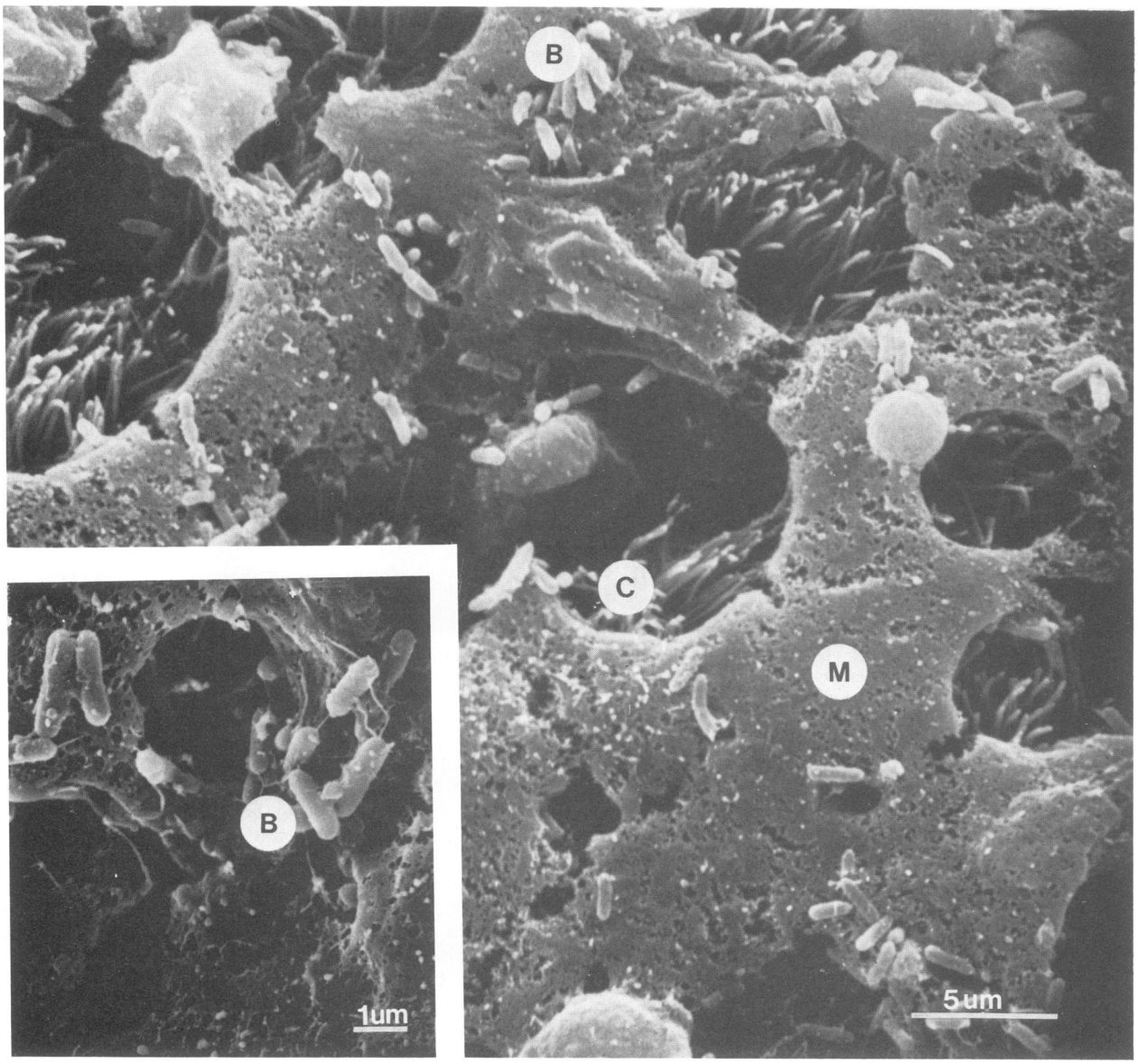

FIG. 5. Scanning electron micrograph of a mucoid *P. aeruginosa* isolate associated with the tracheal mucus blanket 6 h after initiation of infection. Bacteria (B) can easily be seen in association with the mucus blanket (M) and attached to the cilia (C). Inset, higher magnification, showing the bacteria in association with mucus and underlying cilia.

tract (22; H. Marcus and N. R. Baker, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, B149, p. 42). Recently, McArthur et al. reported the presence of a lectin in the lungs of rats (15). Such lectins or divalent cations may act to bridge the bacterial and host glycocalyxes, resulting in a firm bonding of the two components.

In summary, a quantitative assay has been developed which permits accurate measurement of the adherence of *P. aeruginosa* to the ciliated epithelium of the perfused trachea. The assay eliminates the earlier problems of attachment of organisms to cartilage and tissue that had been damaged during the preparation of explants. By using this assay, we could show that the mucoid forms of *P. aeruginosa* clearly adhere to the epithelium better than do nonmucoid forms. The adherent organisms could be observed to be associated with the host mucus layer and attached to the cilia of the epithelium. The ability of the mucoid forms of *P. aeruginosa* to adhere to the epithelium of the lower respiratory tract may help explain the prevalence and persistence of these organisms in CF patients.

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