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Tracheal Tube Biofilm as a Source of Bacterial Colonization of the Lung

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Biofilm formation in tracheal tubes, its bacterial content, and its interaction with ventilator gas flow were investigated. At least 50 mg (dry weight) of biofilm was found in 30 of 40 tracheal tubes used in intensive care patients for 2 h to 10 days. Electron microscopy showed bacteria in this layer, and quantitative studies showed that bacterial counts could reach up to 10^6/cm of tube length. Bacteria were cultured from the patient side of 18 of 78 heat and moisture exchanger-microbiological filter units removed from ventilator circuits. Particles were shown to detach from tracheal tube luminal biofilm and were projected up to 45 cm from the tracheal tube tip. Following contamination of the tracheal tube biofilm with a patient's own gastrointestinal flora, entrainment of bacteria in the inspiratory gas flow provides a mechanism for initial and repeated lung colonization.

Pneumonia is an important cause of morbidity and mortality in intensive care units (16). Nosocomial pneumonia is preceded by colonization of the lower respiratory tract. Although tracheal intubation is recognized as a risk factor for pneumonia (6), its relationship to colonization of the lung has yet to be fully defined. Because gastropulmonary spread of bacteria has been observed in adult patients with cuffed tracheal tubes (2, 8), an inflatable cuff does not appear to prevent access of bacteria to the lower trachea. Moreover, passage of secretions past the cuff has been documented by using a radio-opaque marker (14). We examined tracheal tube biofilm and tested whether the flow of gas during mechanical ventilation can produce a stream of contaminated particles.

MATERIALS AND METHODS

A series of experiments was conducted with tracheal tubes collected from intensive care patients to examine biofilm formation, bacterial content, and particle liberation. Different types of mechanical ventilators (including Erica [Engstrom AB, Bromma, Sweden] and Cape [Cape Engineering Co. Ltd., Warwick, United Kingdom]) and different types of humidification systems (including a cascade humidifier [Bennett Corp., Los Angeles, Calif.] and heat and moisture exchanger-microbiological filter units [Ultipor; Pall, Portsmouth, United Kingdom] and Edith heat and moisture exchanger units [Engstrom AB]) were used in these patients.

Amounts of biofilm in tracheal tubes. A consecutive series of adult tracheal tubes 7.5 to 9.5 mm in internal diameter was examined. The outsides of the tubes were cleaned, and the connectors and inflatable cuffs were removed. The tubes were marked at 1-cm intervals from the lower end to a level of 20 cm and cut into 20 1-cm lengths with minimum disturbance of the internal biofilm. The tube sections were freeze-dried overnight (Modulyo; Edwards High Vacuum, British Oxygen Company, Crawley, United Kingdom). Dried biofilm was then scraped off the tubing onto preweighed filter paper for weighing.

Electron microscopy of tracheal tube biofilm. Tubes with visible internal biofilms were collected for scanning electron microscope studies. Sections (1 cm) at the lower margin of the inflatable cuff were fixed in 2% glutaraldehyde. Smaller samples of these sections were subjected to critical-point drying (10) and sputter coated with gold. Specimens were examined in a scanning electron microscope (JEM 1200-EX; JEOL, Tokyo, Japan) equipped with a scanning image display device (EM-ASID10; JEOL).

Qualitative culture of tracheal tubes. Tracheal tubes were cultured immediately after removal from patients in an intensive care unit where heat and moisture exchanger-filter units (Ultipor) were in routine use. The inner surface 1 cm from each end, the outer surface at the lower cuff margin, and the tip of each tracheal tube were sampled with cotton swabs. Specimens were plated onto 5% horse blood agar (blood agar base; Oxoid, Basingstoke, Hampshire, United Kingdom), heated 5% horse blood agar, MacConkey agar (Oxoid CM 76), and Sabouraud agar (Oxoid CM41 with 0.5% chloramphenicol). Plates were incubated overnight at 37°C in 5% CO_2 in air. Identification of bacterial species was by standard laboratory practice (4).

Quantitative culture of tracheal tubes. A consecutive series of tubes was collected for quantitative studies of biofilm at the level of the cuff, where the inner and outer tracheal tube surfaces could be separated. A 1-cm ring of cuff and the corresponding tubing were cut from the tube. Biofilm was homogenized by vortex mixing 1-cm rings of tubing or cuff material in 2% *N*-acetylcysteine (in 0.15 M saline, pH 7). Samples (100 μl) of 10-fold dilutions of homogenized biofilm (in 0.15 M saline) were each spread on two 5% horse blood agar plates. Viable counts were made by counting colonies after overnight incubation in air at 37°C with an automated colony counter (Gallenkamp, Loughborough, United Kingdom).

The patient sides of heat and moisture exchanger-filter units were sampled with moist cotton swabs after 24 h of use. The swabs were inoculated onto 5% hemolyzed horse blood agar and incubated overnight at 37°C in 5% CO_2 in air. Identification of bacterial species was by standard laboratory methods, as described above.

Electron microscope studies of ventilator filters. Heat and

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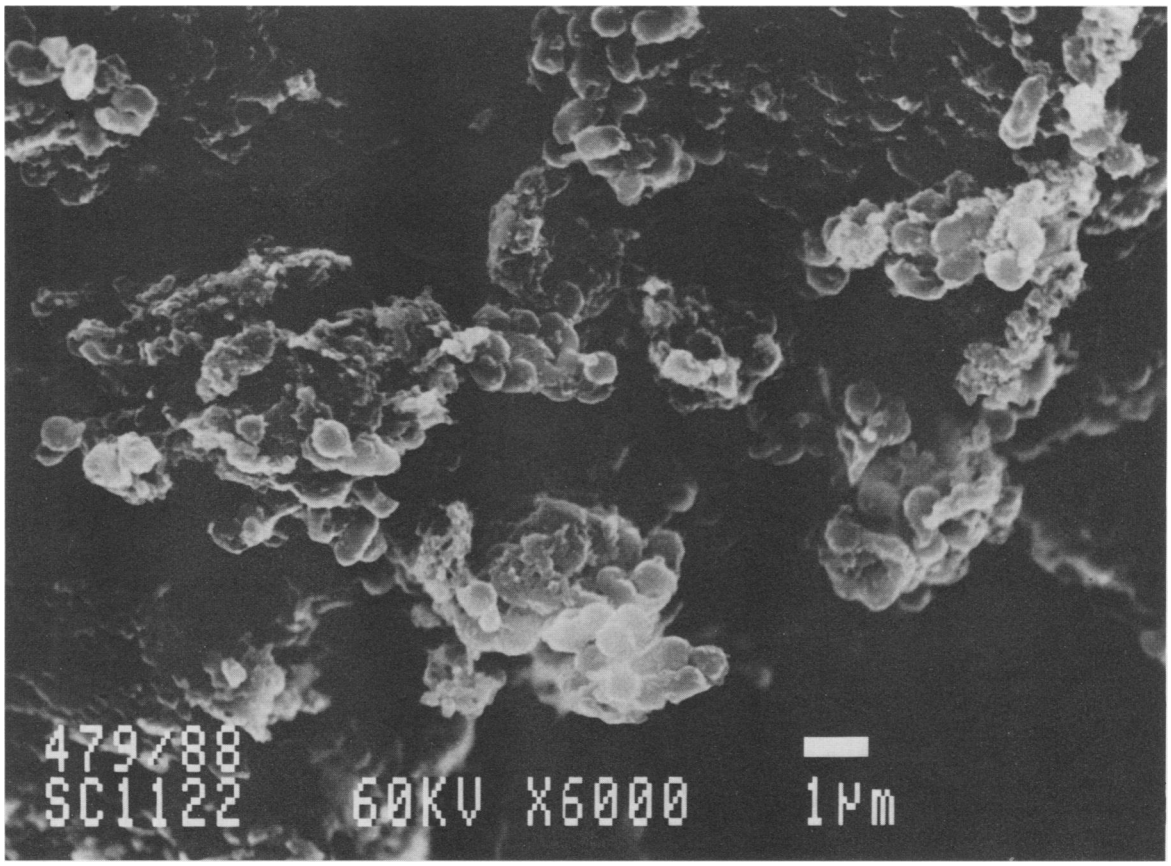

FIG. 1. Scanning electron micrograph of tracheal tube inner-surface biofilm. Coagulase-negative staphylococci were isolated from the adjacent luminal biofilm.

moisture exchanger-filter membranes (Ultipor) were released onto a sterile surface, and squares (6 by 6 cm) were cut from the strip. Two squares from each membrane were stored in sterile sealed polyethylene bags at −20°C. Two similar squares from adjacent parts of the membrane were each placed patient side down on a 5% horse blood agar plate and pressed firmly against its surface. Unused ventilator filter membranes were cultured by the same technique and used as controls. Plates were incubated at 37°C overnight in air. The stored membrane sections from heat and moisture exchanger-filter units that were used in two different patients and grew bacteria on the impression plates were prepared for scanning electron microscopy. Sections of each membrane were prepared by critical-point drying and sputter coated with gold.

Flow studies. Tracheal tubes 8.0 to 9.5 mm in internal diameter and containing 1 ml of blue ink were attached to a Manley ventilator. Tubes were pointed horizontally over a 1-m length of paper, and the ventilator was run for a single cycle, giving a maximum flow rate of 1 liter/s.

A similar procedure was repeated with tracheal tubes immediately after their removal from patients in the intensive care unit. The paper was replaced by two assay plates (23 by 23 cm) filled with nutrient agar and laid end to end. A heat and moisture exchanger-filter unit (Ultipor) was placed between the tracheal tube and the ventilator tubing. To simulate humidifier condensation, 1 ml of sterile saline was injected into the tube and run over the inner surface before the ventilator was switched on. The ventilator was switched off after one cycle, and the inner tube surface was sampled with a cotton swab at the lower margin of the inflatable cuff. Specimens were plated on 5% horse blood agar and MacConkey agar, and the plates were incubated in air at 37°C overnight. All flow studies were conducted in a self-contained laboratory, away from the intensive care unit.

Statistical methods. The Mann-Whitney U test was used to compare the means of two populations (13).

RESULTS

Amounts of biofilm in tracheal tubes. Substantial quantities of biofilm were found in a large proportion of tracheal tubes. In 30 of the 40 tubes examined (75%), the dry weight of the biofilm was greater than or equal to 50 mg. The 17 tracheal tubes used for 24 h or less contained 3 to 497 mg of biofilm ($\bar{x}$, 112 mg), and the 23 tubes used for more than 24 h contained 3 to 597 mg of biofilm ($\bar{x}$, 125 mg). While the mean biofilm weight was higher in the longer-use group, a Mann-Whitney U test showed that the difference was not significant.

Electron microscopy of tracheal tube biofilms. Scanning electron microscopy of luminal biofilms from six tracheal tubes did not reveal structures resembling bacteria on the surfaces. However, bacteria were visible in cracks in the biofilms produced by the critical-point drying procedure (Fig. 1).

Luminal biofilm had an appearance similar to that of material examined in other studies (15). The tracheal tube layer appeared to be composed of material other than the bacteria and bacterial glycocalyx found in other naturally

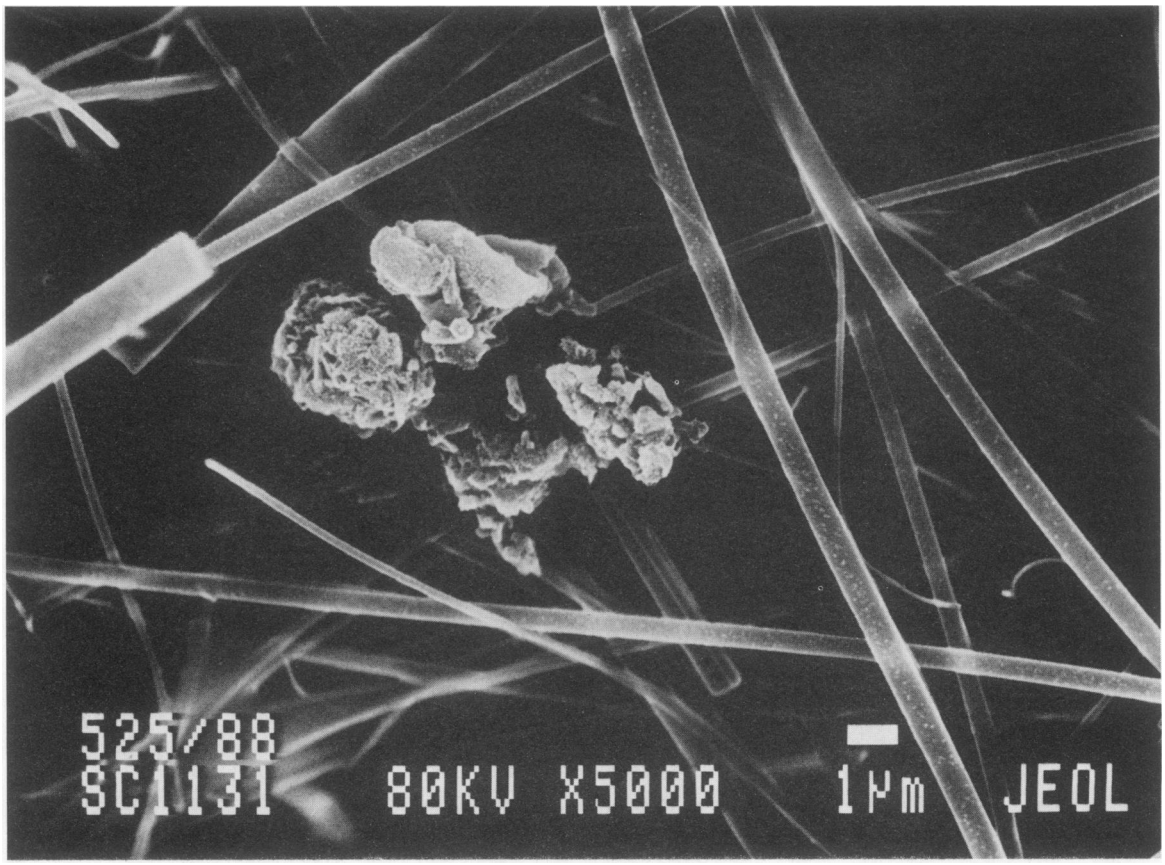

FIG. 2. Scanning electron micrograph of ventilator filter surface (patient side). Coagulase-negative staphylococci were isolated from the same filter.

occurring biofilms, consistent with the presence of respiratory secretions.

Culture of tracheal tube biofilms. Forty-five tubes were collected for culture. These had been used for 12 h to 26 days (mode, 2 to 3 days). In 33 (73%) of the 45 tubes, bacteria were isolated from the inner surface, and in 27 (60%), bacteria were isolated as far as the upper end of the inner surface. *Pseudomonas aeruginosa* and members of the family *Enterobacteriaceae* (including *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Providencia stuartii*) were isolated from 13 (29%) of 45 inner surfaces.

When bacteria had been isolated from the inner surface, the same species were isolated from the outer surface of the same tube in 31 (94%) of 33 tubes. In each of the remaining two tubes, at least one of the bacterial species was isolated from the inner and outer surfaces of the same tube. Quantitative culture of biofilm showed viable counts of up to 10^6 CFU/cm of tube length. *P. aeruginosa* was isolated from the tube with the highest viable count and was present as far as the upper end of the inner surface at levels in excess of 10^5 CFU/ml.

Bacterial culture of ventilator filters. Bacteria were isolated from the patient side of the filter in 18 (23%) of 78 filters used in 42 patients. In 15 (83%) of the 18 filters, these were either *P. aeruginosa* or members of the family *Enterobacteriaceae*.

Electron microscope studies of ventilator filters. Electron microscopy of a further two filters from which bacteria had been isolated by impression plate culture revealed a mesh-work of ceramic fibers and a random scatter of particles measuring 10 to 50 μm lodged among the fibers (Fig. 2). The depth of field of the electron microscope did not allow a search for particles lodged deeper inside the filter membrane. Impression plate cultures from the two filters prepared for electron microscopy grew coagulase-negative staphylococci and *Staphylococcus aureus*, respectively. In both cases, there was less than one colony per cm^2 scattered at random over the whole impression area, with no other growth on the plate.

Flow studies. Ink droplets were projected over an elliptical area up to 90 cm from the tip of a tracheal tube used for the preliminary flow studies (Fig. 3A). In subsequent experiments with used tracheal tubes, organisms were projected up to 45 cm from the tube tips and settled on nutrient agar in an elliptical distribution (Fig. 3B). A similar pattern of distribution was observed in all six tubes from which bacteria were isolated, although the maximum distance projected varied from a minimum of 15 cm. There was no detectable distribution of organisms from a further four tubes. In all four tubes, culture of the luminal biofilm yielded no growth.

DISCUSSION

We have shown that a layer of biofilm accumulates on the inner surfaces of tracheal tubes and that contaminated particles can be detached from this layer during mechanical ventilation.

Bacterial contamination of the insides of tracheal tubes may occur in over 50% of tubes and appears to increase with

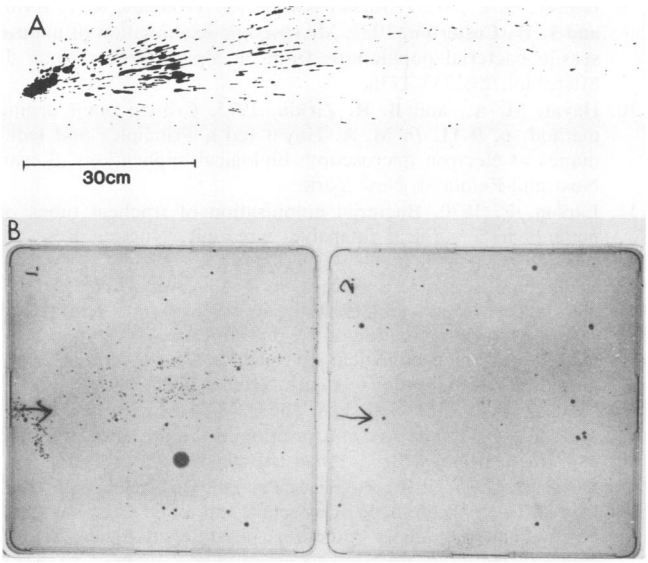

FIG. 3. Particle distribution in flow studies with tracheal tubes. (A) Ink spatter pattern. (B) Spread of organisms from tracheal tubes during one ventilator cycle. *C. albicans* and coagulase-negative staphylococci were isolated from the two nutrient agar plates shown and from the tracheal tube inner surfaces. In both cases, the tube tip was at the left edge, pointing toward the right.

duration of tube use (11). A biofilm has been shown to form on the inner surfaces of tracheal tubes during use, and it has been suggested that fragments of this become dislodged and are carried into the lower respiratory tract during tracheal suction procedures (15). Substantial amounts of biofilm were found on the inner surfaces of tracheal tubes removed from mechanically ventilated patients. The tendency of tubes used for longer periods to contain larger amounts of biofilm suggests that accumulation of this layer is a time-related process. However, lack of a simple correlation between biofilm dry weight and duration of use may mean that other factors determine the rate of accumulation. It is likely that the biofilm layer accumulates more rapidly in patients who produce larger volumes of respiratory secretions. Certain aspects of respiratory tract management, such as frequency of tracheal suction and possibly the type of humidification, may also influence the rate of accumulation; however, it is notable that biofilms were present in tubes from patients with a variety of respiratory support regimens.

The method used to determine viable counts may have underestimated the bacterial contents of biofilms because homogenization may have been incomplete. When a biofilm was spread over the entire luminal surface of a tube (>20 cm long), the maximum inner surface count was likely to be at least 10^7 CFU/cm. Bacteria inside tubes whose outer surfaces were free from contamination may have been protected from antibacterial substances by the tube walls. This protective effect may ensure the survival of a greater total number and a greater species variety of bacteria than elsewhere in a ventilated respiratory tract. Bacterial contamination and particulate debris on the patient side of the ventilator filter may arise from detachment of a luminal biofilm during the expiratory phase of ventilation and is evidence to support the occurrence of this phenomenon in vivo.

Flow studies with standard adult tracheal tubes and ink showed that low-viscosity fluid could be projected up to 90 cm in a straight line. Further flow experiments with tubes collected from patients showed that contaminated particles could be detached by gas flow during the inspiratory phase of ventilation and projected up to 45 cm. Although the maximum distance of projection was less than that with ink, the distribution of particles was in the same elliptical pattern.

Particles of 1 to 10 μm have traditionally been regarded as capable of reaching the alveoli; however, particle distribution experiments have usually used spontaneously ventilating volunteers. It is possible that the distribution of particles is different in an intubated respiratory tract because some of the anatomical defense systems are bypassed by the tracheal tube. Furthermore, recent work has shown that bacteria presented to the lungs of laboratory animals in bolus form or as larger particles require a smaller infective inoculum (1, 3). Detached particles of luminal biofilm may therefore have greater pathogenic potential than monodispersed bacteria.

Factors that raise gastric pH, such as histamine antagonists, promote pulmonary colonization and subsequent ventilator-associated pneumonia (5). Some agents used for stress ulcer prophylaxis, such as sucralfate, preserve a low gastric pH and may reduce the incidence of ventilator-associated pneumonia (7). Since contamination of the inner tube surface occurred with ventilator filters, bacteria must have gained access by an extension from an endogenous route. Respiratory secretions from below the tip of a tube might spread into the first few millimeters of the tube as a result of mucociliary transport, but it is more likely that spread further up the inner surface is due to regular passage of suction catheters through a pool of contaminated secretions.

The above-described flow studies presuppose an excess of water in the ventilator circuit. Large quantities of water accumulate as condensation in ventilator systems when added humidification is used. Condensation saturates the inner surface of the biofilm and flows over it, providing a source of low-viscosity fluid from which particles are more easily detached by gas flow. A tracheal tube biofilm may therefore be analogous to a biofilm that adheres to the bed of a stream and acts as a renewable source of bacteria for the water flowing over it (9).

Until now, the presence of biofilms in tracheal tubes has been seen mainly as a potential cause of tube obstruction. However, the presence of a biofilm inside a tube may alter gas flow, increase turbulence, and cause release of fluid droplets. Following contamination of a biofilm with a ventilated patient's own gastrointestinal flora, production of a cloud of contaminated particles from the biofilm surface is a mechanism for initial and repeated lung colonization.

The preventive strategies developed as a result of recognition of a gastrointestinal source of colonization do not prevent all cases of ventilator-associated pneumonia. Mechanically ventilated patients develop pneumonia despite selective decontamination of the digestive tract (12) or sucralfate ulcer prophylaxis; this suggests the presence of other major determinants of pulmonary colonization. If strategies to prevent ventilator-associated pneumonia are to be improved, it may be necessary to incorporate methods of reducing biofilm formation inside tracheal tubes.

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LITERATURE CITED

1. **Ansfield, M. J., D. E. Woods, and W. G. Johanson.** 1977. Lung bacterial clearance in murine pneumococcal pneumonia. Infect. Immun. **17**:195–204.
2. **Atherton, S. T., and D. J. White.** 1978. Stomach as a source of bacteria colonising respiratory tract during artificial ventilation. Lancet **ii**:968–969.
3. **Cash, H. A., D. E. Woods, B. McCullough, W. G. Johanson, Jr., and J. A. Bass.** 1979. A rat model of chronic respiratory infection with *Pseudomonas aeruginosa*. Am. Rev. Respir. Dis. **119**:453–459.
4. **Cowan, S. T.** 1974. Identification of medical bacteria. Cambridge University Press, Cambridge.
5. **Craven, D. E., L. M. Kunches, V. Kilinsky, D. A. Lichtenberg, B. J. Make, and W. R. McCabe.** 1986. Risk factors for pneumonia and fatality in patients receiving continuous mechanical ventilation. Am. Rev. Respir. Dis. **133**:792–796.
6. **Cross, A. S., and B. Roup.** 1981. Role of respiratory devices in endemic nosocomial pneumonia. Am. J. Med. **70**:681–685.
7. **Driks, M. R., D. E. Craven, B. R. Celli, M. Manning, R. A. Burke, G. M. Garvin, L. M. Kunches, H. W. Farber, S. A. Wedel, and W. R. McCabe.** 1987. Nosocomial pneumonia in intubated patients given sucralfate as compared with antacids or histamine type 2 blockers. N. Engl. J. Med. **317**:1376–1382.
8. **Du Moulin, G. C., J. Hedley-Whyte, D. G. Paterson, and A. Libson.** 1982. Aspiration of gastric bacteria in antacid-treated patients: a frequent cause of postoperative colonisation of the airway. Lancet **i**:242–245.
9. **Geesey, G. C., W. T. Richardson, H. G. Yeomans, R. T. Irvin, and J. W. Costerton.** 1977. Microscopic examination of natural sessile bacterial populations from an alpine stream. Can. J. Microbiol. **23**:1733–1736.
10. **Hayat, M. A., and B. R. Zirkin.** 1975. Critical point drying method, p. 9–11. *In* M. A. Hayat (ed.), Principles and techniques of electron microscopy: biological applications. 3. van Nostrand-Reinhold, New York.
11. **Larson, E.** 1970. Bacterial colonisation of tracheal tubes of patients in a surgical intensive care unit. Nursing Res. **19**: 122–128.
12. **Ledingham, I. M., S. R. Alcock, A. T. Eastaway, J. C. McDonald, I. C. McKay, and G. Ramsay.** 1988. Triple regimen of selective decontamination of the digestive tract, systemic cefotaxime, and microbiological surveillance for prevention of acquired infection in intensive care. Lancet **i**:785–790.
13. **Mattson, D. E.** 1981. Statistics. The C. V. Mosby Co., St. Louis.
14. **Mehta, S.** 1972. The risk of aspiration in the presence of cuffed endotracheal tubes. Br. J. Anaesth. **44**:601–605.
15. **Sottile, F. D., T. J. Marrie, D. S. Prough, C. D. Hobgood, D. J. Gower, L. X. Webb, J. W. Costerton, and A. G. Gristina.** 1986. Nosocomial pulmonary infection: possible etiological significance of bacterial adhesion to endotracheal tubes. Crit. Care Med. **14**:265–270.
16. **Stevens, R. M., D. Teres, J. J. Skillman, and D. S. Feingold.** 1974. Pneumonia in an ICU: a thirty month experience. Arch. Intern. Med. **134**:106–111.