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Binding of Staphylococci to Mucus In Vivo and In Vitro

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The association of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus* with tissues of the upper respiratory tract were compared by using an in vivo ferret model. Ferrets were challenged intranasally with a 1-ml volume of radiolabeled staphylococci (3 mg [dry weight]), were allowed to clear the bacteria in vivo for 90 min, and were sacrificed. Tissues from the right nasal fossa were harvested and processed for radioassay or histology. Of the recoverable staphylococci, ≥96% was associated with mucus gel overlaying mucosa of the turbinates. A quantitative radioassay was developed to study the binding of labeled staphylococci to immobilized crude ferret nasal mucin (FM) and bovine submaxillary gland mucin (BM). Binding showed saturation kinetics and was blocked specifically by BM but not by human Tamm-Horsfall glycoprotein nor orosomucoid. Binding to both FM and BM was significantly inhibited ($P \leq 0.01$) when cocci were pretreated with trypsin but not when treated with β-galactosidase or sodium metaperiodate (except for binding of *S. saprophyticus* to FM). These results suggest that mucin-binding receptors of the cocci may have protein components. The staphylococcus-binding receptors of both FM and BM appear to contain protein components, based on sensitivity to pretreatment with trypsin.

Staphylococci are ubiquitous gram-positive cocci that frequently colonize the skin and mucous membranes of the respiratory, gastrointestinal, and urinary tracts, usually without causing overt disease (34, 41). However, among the coagulase-negative staphylococci, *Staphylococcus epidermidis* has emerged as a significant cause of nosocomial bloodstream and biomaterial-centered infections (3, 8, 26, 45), while *Staphylococcus saprophyticus* is a frequent cause of urinary tract infection, particularly in young females (31, 39, 62). Coagulase-positive *Staphylococcus aureus* is a major human pathogen in superficial and deep-seated infections and can be a causal agent of tracheobronchitis or pneumonia, particularly in patients with influenza, in immunosuppressed patients, or in patients who are hospitalized (25, 38, 55, 64). *S. aureus* is also an initial pathogen colonizing the respiratory tract of patients with cystic fibrosis (9, 33, 35, 43), and, in a recent longitudinal study of sputa from 102 patients with cystic fibrosis, Bauernfeind et al. (5) reported the incidence of *S. aureus* and *S. epidermidis* isolation at ~36%. The mechanism by which these staphylococci colonize mucosa in vivo is unknown.

The hydrophilic mucus gel coating, or partial coating (16), of respiratory epithelium is generally considered to be a mechanical barrier to bacteria, facilitating their removal by mucociliary clearance (21, 40). However, trapping in mucus gel may be a necessary first step in the infectious process of certain potentially invasive microorganisms or may, in fact, be the only way some bacteria associate with the mucosa (23). The mechanism by which staphylococci colonize or infect mucosal surfaces is unknown. Presumably, cocci causing infection or chronic colonization of the lower respiratory tract are aspirates of microorganisms residing in mucus gel or on mucous membranes of the nasal cavity or nasopharynx.

In the present study, an in vivo ferret model was used to determine the association of *S. aureus*, *S. epidermidis*, and *S. saprophyticus* with mucosa of the nasal cavity. Also, an in vitro radioassay was developed to characterize the binding reaction between the three test strains of staphylococci and immobilized mucin.

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MATERIALS AND METHODS

Growth and labeling of staphylococci. *S. aureus* Ci-1 (54), *S. epidermidis* (ATCC 14990, American Type Culture Collection, Rockville, Md.), and *S. saprophyticus*, kindly supplied by Jack Horner, The University of Texas Health Science Center, San Antonio, were labeled by inoculating 500 ml of M199 medium (modified with Earle's salts and containing L-glutamine; GIBCO, Grand Island, N.Y.), which had been autoclaved and adjusted to pH 8.6 with $NaHCO_3$, with 1×10^8 to 2×10^8 CFU of staphylococci and incubating in stationary culture at 37°C for 18 h in the presence of 1 μCi of [methyl-^{3}H]thymidine (15 Ci/mmol; ICN Chemical and Radioisotope Div., Irvine, Calif.) per ml of medium. The ^{3}H-labeled staphylococci were harvested in stationary phase by centrifugation at 8,000 × *g* for 10 min at 5°C (model J21B; Beckman Instruments, Inc., Fullerton, Calif.); they were suspended in Hanks balanced salt solution (GIBCO) and stored at −70°C. Several samples were repeatedly washed with sterile deionized H_2O and freeze-dried for storage or for determining dry weight.

Ferrets. Twelve adult male ferrets, obtained from Triple-F (Fayre, Pa.), were anesthetized as previously described (52). Each animal was inoculated intranasally, on the right side only, with a 1-ml volume of washed (3×, Hanks balanced salt solution) ^{3}H-labeled staphylococci (3 mg [dry weight]; ~3×10^9 CFU) in Hanks balanced salt solution. The animals were allowed to clear bacteria for 90 min before sacrifice and harvesting of tissues from the nasal fossae, as previously described (53). Tissues were processed, without any wash steps, for radioassay (three animals per test strain), histology (one animal per test strain), or isolation of mucous gel.

In vivo radioassay. Each tissue specimen harvested from the right nasal fossa was transferred to a vial containing a 3-ml volume of 0.125% protease (type VI obtained from *Streptomyces griseus*; Sigma Chemical Co., St. Louis, Mo.) in sterile phosphate-buffered saline (PBS)–fluorescent trepo-

nemal antibody hemagglutination buffer, pH 7.2 (BBL Microbiology Systems, Cockeysville, Md.) and incubated at 37°C for 45 min with intermittent agitation. The tissue suspension was vortexed; the supernatant fluid was transferred to a test tube, and the tissue was rinsed with a 3-ml volume of sterile, deionized H_2O, which was added to the supernatant fluid. ^{3}H-labeled staphylococci were pelleted by centrifugation; the supernatant fluid was discarded, and the pellet was suspended in a 0.5-ml volume of H_2O and transferred to a vial. The test tube was rinsed with 0.5 ml of H_2O, which was transferred to the corresponding vial. A 10-ml volume of ScintiVerse I scintillation cocktail (Fisher Scientific Co., Fair Lawn, N.J.) was added to the 1-ml-volume cell suspension in each vial. Radioactivity was determined in a liquid scintillation spectrometer (Tracor Analytic model 6895; Tracor Instruments, Austin, Tex.); results, expressed as disintegrations per minute, were corrected for background and for quenching by using external standards and were converted to nanograms of staphylococci, as previously described (52).

Histology. Tissues were placed in a Uni-Cassette System (Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.), fixed in 10% neutral buffered Formalin (American Scientific Products, McGaw Park, Ill.), and processed in the Histopathology Laboratory, Department of Pathology, The University of Texas Health Science Center, San Antonio, where they were decalcified in TBD-2 (Shandon, Sewickley, Pa.), embedded in paraffin, and sectioned (thickness, 3 μm). Multiple sections were stained by the Brown and Hopps method for detection of gram-positive and gram-negative bacteria and by the periodic acid-Schiff method for visualization of mucopolysaccharides. Photomicrographs were made using Kodak Tmax 100 film.

Mucin. The anterior and posterior turbinates were harvested from the left nasal fossa of eight ferrets, placed in Hanks balanced salt solution containing 0.5% lactoalbumin hydrolysate (Difco Laboratories, Detroit, Mich.) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and gentamicin (50 μg/ml), and stored at −70°C. A crude mucin preparation was made by using a modification of the procedure described by Roberts (47). Briefly, tissue suspensions were thawed and vigorously vortexed for 60 s, and the supernatant fluids were pooled to give a total volume of 30 ml. A 20-ml volume of ethanol was added, which yielded a final concentration of 40% ethanol. The precipitate which formed was eliminated by centrifugation at 7,000 × *g* at 25°C for 30 min (model TJ-6; Beckman Instruments, Inc.). A 17.5-ml volume of ethanol was added to the supernatant fluid to give a final concentration of ethanol of 55%. The solution was allowed to incubate at 4°C for 18 h. After centrifugation, the supernatant fluid was discarded and the pellet was dissolved in sterile, deionized H_2O containing 0.1% NaN_3 and dialyzed (dialysis membrane tubing, 6,000 to 8,000 M_W cutoff; Spectrum Medical Industries, Inc., Los Angeles, Calif.) in repeated changes of deionized H_2O at 4°C for 18 h. The crude mucin preparation had a total protein of 140 μg/ml, as determined by the Bio-Rad protein assay performed according to the instructions of the manufacturer (Bio-Rad Laboratories, Richmond, Calif.), and 250 nmol of neutral hexoses per ml, as measured by the anthrone method (49) with glucose as the standard; the thiobarbituric acid assay for sialic acids (63) was negative. The crude ferret nasal mucin (FM) was kept stored at −70°C. FM and purified mucin type 1-S (obtained from bovine submaxillary glands; Sigma) were used as substrates in a solid-phase radioassay.

In vitro radioassay. ^{3}H-labeled staphylococci were assayed for capacity to bind to mucin immobilized in 96-well vinyl assay plates (Costar, Cambridge, Mass.). A 100-μl volume of mucin diluted in 0.1 M carbonate buffer, pH 9.6, was placed in each well, and uncovered plates were incubated at 37°C for 18 h. Control wells contained buffer only. Plates were covered and could be stored at 4°C if not used right away. Otherwise, each well was pretreated with 200 μl of PBS containing 0.05% Tween 20, 0.1% NaN_3, and 0.25% bovine serum albumin for 60 min at 25°C. This step removed any unbound mucin and also blocked sites not sensitized with mucin. At this point the method varied depending on which species of staphylococci was being tested. (i) For both *S. epidermidis* and *S. saprophyticus*, the following procedure was used. Buffer was removed, and plates were drained of excess fluid before addition of staphylococci. The test bacteria were thawed, washed 3× in PBS, and suspended in PBS. A 50-μl volume of labeled staphylococci was added to each well, and the wells were incubated at 37°C for 60 min. Unbound bacteria were removed by aspiration, and the wells were gently washed 3× with PBS; bacteria were removed by aspiration after each wash. Wells were examined by using an inverted microscope to determine whether all unbound staphylococci had been removed and whether wells required additional washes. Wells were drained, and each well was cut out and submerged in a 7-ml volume of Liquiscint scintillation cocktail (National Diagnostics, Manville, N.J.). Radioactivity was determined in a scintillation spectrometer (Tracor). The dpms were converted to nanograms of staphylococci bound as previously described (52). (ii) The method used for ^{3}H-labeled *S. aureus* was modified to optimize binding of the staphylococci to immobilized mucin and minimize background binding to control wells. After blocking buffer was removed, each well was gently washed 3× with PBS to remove any residual bovine serum albumin and wells were drained. Bacteria were thawed and washed 3× with Tris hydrochloride buffer, pH 7.2 (25°C). A 50-μl volume of the staphylococci, suspended in Tris hydrochloride buffer, was added to each well, and the wells were incubated at 25°C for 60 min. Unbound bacteria were removed, and wells were processed as described above. All assays were done in triplicate.

Inhibitors. Washed ^{3}H-labeled staphylococci were suspended in buffer, with or without inhibitors, and used in the in vitro assay. Inhibitors, obtained from Sigma, included α-L-(−)-fucose, D-(+)-galactose, D-(+)-mannose, D-(+)-galactosamine, D-(+)-glucosamine, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, *N*-acetylneuraminic acid type IV, bovine mucin, and α_1-acid glycoprotein (human orosomucoid purified from Cohn fraction VI). In addition, Tamm-Horsfall glycoprotein was purified from pooled human urine by repeated precipitation in 0.58 M NaCl as previously described (19, 20) and was used as an inhibitor.

Treatment of staphylococci. ^{3}H-labeled staphylococci were rinsed 2× in buffer and were suspended at a concentration of ~0.5 to 1.5 mg (dry weight) of staphylococci per ml of buffer alone or buffer containing trypsin type 1 (from bovine pancreas), β-galactosidase grade VIII (from *Escherichia coli*), and sodium metaperiodate, all of which were obtained from Sigma. The bacterial suspensions were incubated at 37°C for 60 min, except for bacteria suspended in the periodate solution, which were incubated in the dark at 25°C for 60 min. Treated bacteria and controls were washed 3×, suspended in buffer, and used in the in vitro radioassay. PBS buffer was used for both *S. epidermidis* and *S. saprophyticus*, and Tris hydrochloride buffer was used for *S. aureus* for each treatment and wash.

Treatment of immobilized mucin. After the blocking step, each well was treated with a 100-μl volume of trypsin type 1 in PBS, sodium metaperiodate in PBS, or receptor-destroying enzyme (from *Vibrio comma*; Whittaker M.A. Bioproducts, Walkersville, Md.) in calcium acetate saline (1), pH 6.2. Control wells were treated with the appropriate buffer solution only. After incubation at 37°C for 60 to 90 min, each well was aspirated, washed 3× with PBS, and used in the in vitro radioassay as described.

Statistical analysis. The arithmetic means, standard deviations, and standard errors of the means were determined for each set of data. Where appropriate, the coefficient of variation was determined and comparisons were made by a single-factor analysis of variance by using the StatView 512+ computer package (BrainPower, Calabasas, Calif.).

RESULTS

Growth conditions of staphylococci. Bacteria were labeled in a chemically defined tissue culture medium to avoid adsorption of protein components, conceivably present in a biologically complex medium, to the surface of bacteria. After growing for 18 h, *S. epidermidis* and *S. saprophyticus* were present as floccules that rapidly settled out of solution, while *S. aureus* formed floccules in early exponential growth phase that appeared to dissolve as the cocci reached the stationary phase of growth and the pH of the growth medium became acidic. The starting pH of the medium appeared to be critical for the formation of floccules and for the subsequent binding of all three test strains of staphylococci to mucin in vitro.

Distribution of staphylococci in vivo. Results of the in vivo radioassay are shown in Fig. 1. Of the total amount of labeled staphylococci introduced into the right nasal fossa, 8.1% of the *S. saprophyticus* inoculum was recovered from the four tissues harvested, versus 6.8% of the *S. epidermidis* and 5.4% of the *S. aureus* inocula. A total of 94.4% of recoverable *S. saprophyticus* was associated with the anterior turbinate. Similarly, 81.1% of recoverable *S. epidermidis* was associated with the anterior turbinate and 18.1% with the posterior turbinate. With *S. aureus*, the amount recovered from the anterior and posterior turbinates was 53.7 and 43%, respectively. The amount of cell-free label represented ~0.5% of the total inoculum with all three test strains. Storage conditions of the staphylococci made a significant difference in their capacity to associate with tissues of the upper respiratory tract. When freeze-dried bacteria were substituted for the fresh frozen staphylococci (as seen in Fig. 1), association with anterior turbinates was decreased 10-fold with *S. aureus* and ~200-fold with both *S. epidermidis* and *S. saprophyticus* (data not shown).

Association of staphylococci with mucus gel in vivo. The in vivo radioassay indicated the distribution and level of association of staphylococci with tissues of the nasal fossa; however, histological examination was able to define the nature of this association. Figure 2A shows the normal architecture of the anterior nasal turbinate, in which the air passages are lined with respiratory mucosa. The three test strains of staphylococci were seen only in association with mucus gel overlaying cilia of the columnar epithelial cells, as represented in Fig. 2B, or attached to mucus strands released into the air passage (Fig. 2A and B). Staphylococci were not observed interacting directly with host cells of the turbinate, even in areas where the epithelium appeared to be mucus free (Fig. 2A). The association of staphylococci with mucus was typical for all tissues examined except the vestibule, where cocci appeared to bind directly to keratinized tissue.

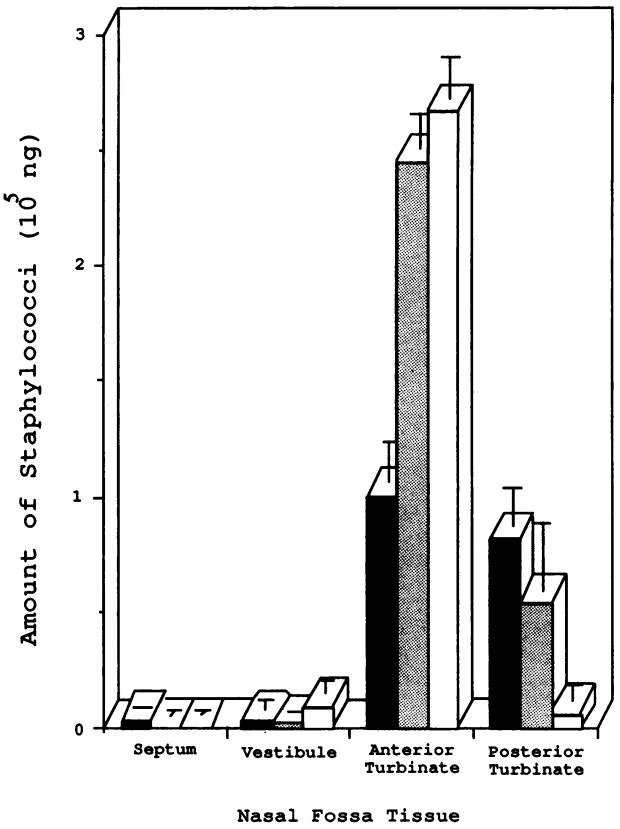

FIG. 1. Mean association of ^{3}H-labeled *S. aureus* (closed bar), *S. epidermidis* (stippled bar), and *S. saprophyticus* (open bar) with tissues from the right nasal fossa of adult ferrets. Thin bars represent the standard deviation of results. Specific activity: 5,828 dpm/μg (dry weight), *S. aureus*; 474 dpm/μg (dry weight), *S. epidermidis*; and 679 dpm/μg (dry weight), *S. saprophyticus*.

Binding of staphylococci to immobilized mucin in vitro. An in vitro radioassay was developed to study the interaction of ^{3}H-labeled staphylococci with immobilized crude FM and purified bovine submaxillary gland mucin (BM). Figure 3 demonstrates the binding of labeled staphylococci to various concentrations of FM and BM. Binding was saturable at a concentration of 2.25 μg of protein per ml (1:8 dilution) for FM and 0.28 mg (dry weight) of mucin per ml (1:32 dilution) for BM. *S. epidermidis* binding was 7.5-fold greater with FM and 60-fold greater with BM than with control wells treated with bovine serum albumin. Likewise, binding of *S. saprophyticus* was increased 3.8- and 7.5-fold with FM and BM, respectively. While the binding of *S. aureus* was also increased 3- to 4-fold with immobilized FM, subsequent testing against BM gave highly variable results in which binding to BM was often less than binding to control wells, and this data is not presented. Reproducibility of the radioassay was determined by replicate testing on different days over a 30-day period. The coefficient of variation for *S. epidermidis* was 10% (FM) and 8% (BM); for *S. saprophyticus* it was 11% (FM) and 10% (BM), and for *S. aureus* it was 19% (FM). For subsequent assays, 125 to 150 μg (dry weight) of *S. epidermidis* and *S. saprophyticus* and 60 μg (dry weight) of *S. aureus* were added to control wells or to wells of immobilized mucin sensitized at a concentration of 4.50 μg of protein per ml of FM and 0.56 mg (dry weight)/ml of BM.

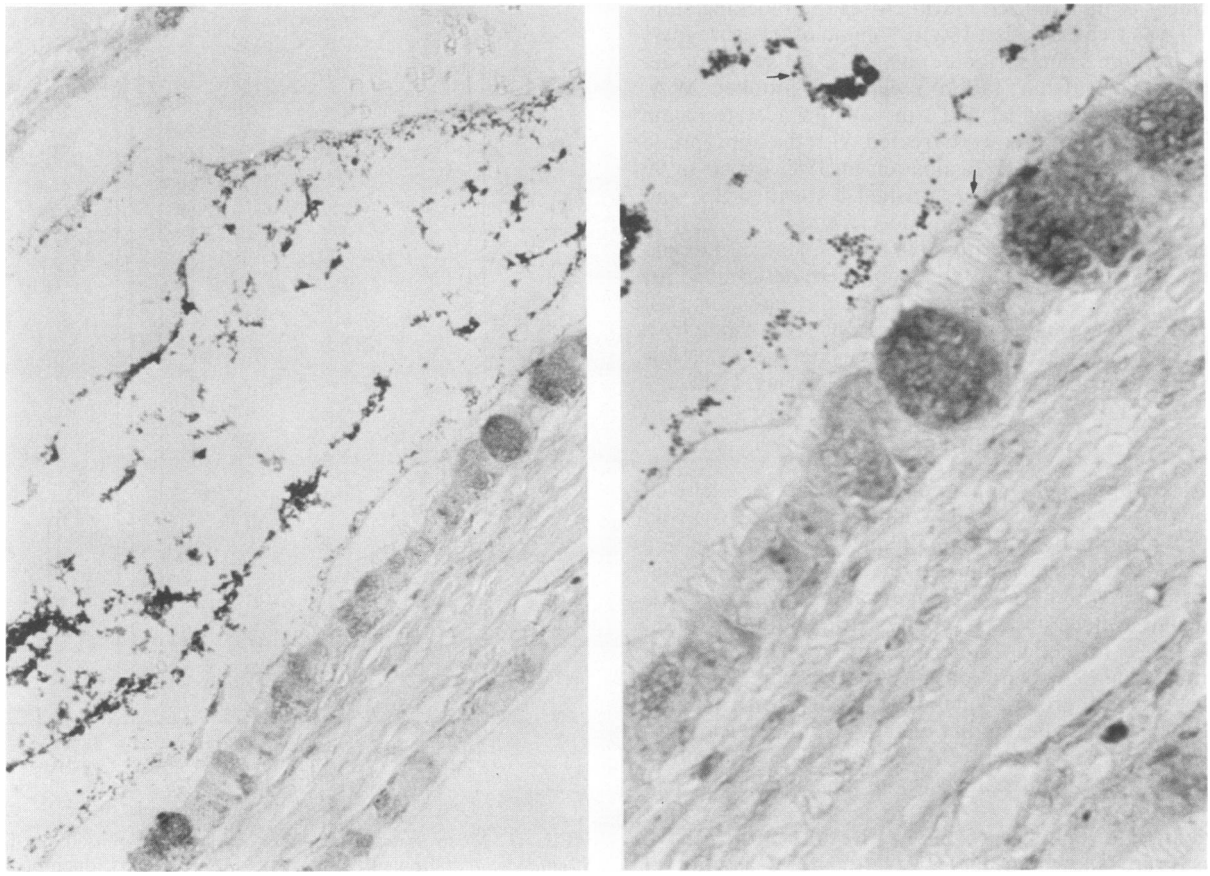

FIG. 2. Anterior turbinate from a normal ferret challenged with *S. saprophyticus*. The respiratory mucosa was covered by a characteristic ciliated columnar epithelium with interspersed goblet cells (A, left side); goblet cells, right center, were in the process of secreting mucus into a thin ribbon of gel overlaying the mucosa (Brown and Hopps; original magnification, ×200). In a higher-power view (B, right side), staphylococci (arrow) were only seen associated with the mucus gel overlaying cilia or present as strands in the air passage (Brown and Hopps; original magnification, ×500). No cocci were observed binding directly to mucus-free ciliated cells, lower right, panels A and B.

Inhibition of binding by mucin. Binding of staphylococci to immobilized FM and BM was significantly inhibited when bacteria suspended in BM were used in the in vitro radioassay (Table 1). A sufficient amount of FM was not available for use in the inhibition assay. Specificity of binding was indicated by the fact that human Tamm-Horsfall urinary mucin and orosomucoid did not significantly block binding of the staphylococci (Table 1).

Inhibition of binding by sugars. With one exception, the binding of staphylococci to immobilized mucin was not significantly inhibited when bacteria suspended in solutions of neutral and amino sugars were used in the in vitro assay (Table 2). The presence of *N*-acetylneuraminic acid did block binding of *S. epidermidis* to BM, but at a very low level. In the presence of amino sugars, *S. saprophyticus* exhibited a tendency to clump, which may account for the significant ($P = 0.01$) increase in binding to both FM and BM.

Characterization of mucin receptor(s). Treatment of immobilized mucin with trypsin significantly decreased subsequent binding of staphylococci (Table 3), which suggests that the mucin receptor has a protein component. In addition, oxidation of sugars in mucin by sodium metaperiodate decreased binding of *S. epidermidis* ($P = 0.002$) to BM, which suggests that a glycoprotein component may contribute to the binding of *S. epidermidis*.

Characterization of staphylococcal receptor(s). Table 4 summarizes the results obtained when ^{3}H-labeled staphylococci were subjected to various agents and treatments before being used in the in vitro radioassay. Compared with untreated controls, the binding of all three test strains of staphylococci to FM and BM was significantly reduced when the bacteria were treated with trypsin, which suggests that the surface receptors may have protein components. Binding to BM was significantly enhanced by treatment of *S. saprophyticus* with β-galactosidase ($P = 0.006$); one possibility is that this enzyme treatment actually unmasked receptors on the bacterial cell surface.

DISCUSSION

In the present investigation, localization of *S. aureus*, *S. epidermidis*, and *S. saprophyticus* in the upper respiratory tract (nasal fossae) was studied in a ferret model. All three strains appeared to bind to the keratinized lining of the anterior vestibule. These observations are similar to those previously reported for *S. aureus* in ferrets, uninfected and infected with influenza A virus (53), and with cell scrapings from the anterior nares of human subjects (7). Once past the nasal vestibule, however, the keratinized tissue is replaced by a thin layer of mucus that covers the epithelial-cell lining of respiratory mucosa. It was in this mucus that ≥96% of the

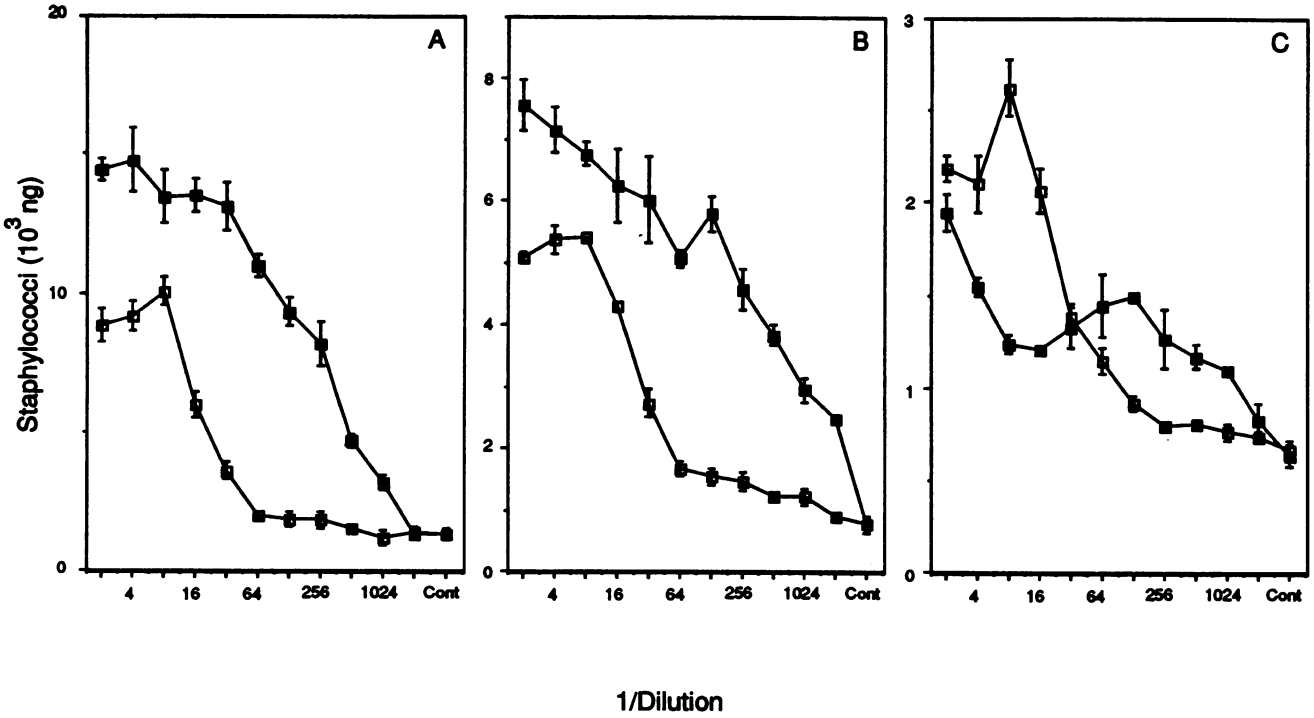

FIG. 3. Mean binding of ^{3}H-labeled *S. epidermidis* (A), *S. saprophyticus* (B), and *S. aureus* (C) to twofold dilutions of crude FM (open squares) and BM (closed squares). The starting concentration was 20 μg of protein per ml for FM and 9 mg (dry weight) of BM per ml. Labeled staphylococci (~100 μg [dry weight] of bacteria per 50 μl of buffer per well) were added to wells of immobilized mucin or to control wells without mucin. Bars represent standard error of the means of triplicate samples. Note that the scales are different for panels A, B, and C. Specific activity: 5,874 dpm/μg (dry weight), *S. epidermidis*; 3,325 dpm/μg (dry weight), *S. saprophyticus*; and, 12,276 dpm/μg (dry weight), *S. aureus*.

recoverable staphylococci localized (Fig. 1 and 2). Areas of the mucosa that appeared to be mucin free were also free of staphylococci (Fig. 2), which suggested that nasal mucus contained staphylococcus-binding components that were not present on respiratory epithelial cells. In fact, Tuomanen (59) has reported that *S. aureus* acquires the ability to adhere to cilia in vitro and in vivo after being coated with soluble protein adhesins of *Bordetella pertussis*, a bacterium known for its capacity to adhere specifically to the cilia of respiratory epithelial cells. In addition, we have observed *S. aureus* adherence to patches of ferret respiratory epithelial cells only when the anterior turbinates were in an advanced stage of desquamation due to a primary infection with influenza A virus (53). While mucus may act to trap and clear staphylococci from the respiratory tract, it is also possible that staphylococcal binding to mucus may be a mechanism for respiratory mucosal colonization similar to the mucus colonization of intestinal mucosa by *Campylobacter jejunum* (37).

TABLE 1. Effect of muco- and glycoproteins on the binding of staphylococci to immobilized mucin

Test substance	Concn/ well	Binding (mean % of control)[a]	
		FM	BM
Bovine mucin	400 μg		
Sa		40.1 (P = 0.02)	
Se		5.1 (P = 0.0001)	5.8 (P = 0.001)
Ss		6.0 (P = 0.003)	4.4 (P = 0.01)
Human Tamm-Horsfall glycoprotein	208 μg		
Sa		187.1	
Se		83.1	67.1
Ss		104.4	105.6
Human orosomucoid α_1-acid glycoprotein	208 μg		
Sa		102.8	
Se		97.3	81.8
Ss		113.0	88.9

[a] Binding of bacteria suspended in buffer only was used as the control valve and represented 100%.

Airway mucus, typically comprised of salts, plasma and nonplasma proteins, glycoproteins (mucin), lipids, and H_2O (14, 48), is rich in potential receptors for bacteria. Particular bacteria, including *S. aureus* (50), have been shown to have a high avidity for mucin in the upper respiratory tract (32), the intestinal tract (13), and saliva (65); the interaction may be highly specific for a particular mucin (17) and may involve multiple receptors (61). Vishwanath and Ramphal (60) reported that the human tracheobronchial mucin receptor for *Pseudomonas aeruginosa* contains *N*-acetylglucosamine and *N*-acetylneuraminic acid; treatment of immobilized mucin with pronase did not affect binding, which suggests that the receptor did not have a protein component. In the present study, we developed an in vitro radioassay to characterize the binding of ^{3}H-labeled staphylococci to immobilized crude FM and purified BM for comparison. Staphylococcal binding was dose dependent and saturable

TABLE 2. Effect of neutral and amino sugars on the binding of staphylococci to immobilized mucin

Test substance	Concn/ well	Binding (mean % of control)[a]	
		FM	BM
Fucose	100 mM		
Sa		97.8	
Se		80.6	77.0
Ss		107.8	96.8
Galactose	100 mM		
Sa		107.7	
Se		118.0	143.7
Ss		112.2	110.2
Mannose	100 mM		
Sa		107.8	
Se		87.6	112.9
Ss		95.9	88.2
Galactosamine	100 mM		
Sa		105.3	
Se		150.9	141.2
Ss		220.0	236.5
Glucosamine	100 mM		
Sa		89.0	
Se		128.0	108.4
Ss		192.1	219.7
N-acetylgalactosamine	100 mM		
Sa		127.5	
Se		100.7	113.8
Ss		124.1	119.5
N-acetylglucosamine	100 mM		
Sa		123.0	
Se		84.7	107.9
Ss		108.3	99.4
N-acetylneuraminic acid	100 mM		
Sa		78.0	
Se		90.7	72.3 ($P = 0.009$)
Ss		82.6	83.8

[a] Binding of bacteria suspended in buffer only was used as the control value and represented 100%.

(Fig. 3). Trypsin treatment of the mucin significantly reduced staphylococcal binding, by ≥42% (Table 3), which suggests that receptors are positioned on the segment of peptide core unprotected from enzyme attack by carbohydrate side chains. To a lesser extent, periodate treatment of BM, but not FM, inhibited the binding of *S. epidermidis*, which suggests that an α-glycol-containing carbohydrate which is rapidly oxidized by periodate or amino acids susceptible to oxidation by periodate (12, 56) might contribute to binding. Glycoprotein receptors on host cells have been described for both *S. epidermidis* (11) and *S. saprophyticus* (27); whether these host cell and mucin receptors are similar or distinct is unknown, but the subject merits further study.

TABLE 3. Binding of staphylococci to pretreated immobilized mucin

Pretreatment	Concn/ well	Binding (mean % of control)[a]	
		FM	BM
Trypsin	2 mg		
Sa		58.0 ($P = 0.05$)	
Se		35.0 ($P = 0.01$)	48.2 ($P = 0.01$)
Ss		29.7 ($P = 0.001$)	45.9 ($P = 0.01$)
Receptor destroying enzyme	12.8 U		
Sa		95.7	
Se		88.9	72.6 ($P = 0.02$)
Ss		90.9	79.0
Sodium metaperiodate	10 mM		
Sa		104.6	
Se		119.3	58.8 ($P = 0.002$)
Ss		129.0	70.1 ($P = 0.04$)

[a] Binding of bacteria to buffer-treated immobilized mucin was used as the control value and represented 100%.

TABLE 4. Binding of untreated or pretreated staphylococci to immobilized mucin

Pretreatment	Concn/ml	Binding (% of control)[a]	
		FM	BM
Trypsin	10 mg		
Sa		24.6 ($P = 0.001$)	
Se		7.5 ($P = 0.003$)	12.9 ($P = 0.005$)
Ss		11.7 ($P = 0.002$)	12.7 ($P = 0.01$)
β-galactosidase	100 U		
Sa		124.0	
Se		155.7	140.2
Ss		97.8	198.3
Sodium metaperiodate	10 mM		
Sa		149.2	
Se		132.5	149.0
Ss		62.6 ($P = 0.02$)	138.7

[a] Binding of bacteria pretreated with buffer only was used as the control value and represented 100%.

Specificity of the radioassay was tested in a series of blocking experiments. As expected, BM blocked the binding of staphylococci to immobilized BM; in addition, binding to FM was also blocked (Table 1). When human orosomucoid and Tamm-Horsfall urinary mucin were substituted for BM, binding was not significantly inhibited (Table 1). The in vitro radioassay was also used to characterize staphylococcal receptors responsible for mucin binding. Clearly, the binding of *S. aureus* to crude FM was considerately less than that obtained with *S. epidermidis* and *S. saprophyticus* (Fig. 3), which may reflect a quantitative or qualitative difference between surface receptors. In contrast to *S. epidermidis* and *S. saprophyticus*, the interaction of *S. aureus* with BM was not significantly and consistently above binding to control wells; increasing the concentration of *S. aureus* did increase binding to BM, but it also increased background binding. Possibly, test conditions were not optimal, because BM can coat *S. aureus* and significantly decrease binding to FM (Table 1). The FM-binding receptor of *S. aureus* was sensitive to trypsin treatment and resistant to treatment with β-galactosidase and sodium metaperiodate (Table 4), which suggests that the receptor has a protein component. At present it is unknown whether this mucin-binding receptor is similar to previously described surface proteins of *S. aureus* that mediate binding to fibrinogen (18), fibronectin (24), laminin (42), collagen (29), influenza A virus-infected and control cell monolayers (15, 51), the Fc piece of immunoglobulins (22), or silicone polymers (4). Surface proteins of other bacteria have been reported as receptors for mucin binding. For example, pili appear to play an important role in the binding of nonmucoid *P. aeruginosa* to respiratory mucin (46) and of *E. coli* to urinary (36, 44) and intestinal (61) mucin and saliva (28). For *S. aureus*, the mucin-binding receptor does appear to be distinct from the lipoteichoic acid adhesin (10) which mediates *S. aureus* adherence to human buccal cells (in vitro) and the teichoic acid component (2, 6)

reported to be one of the adhesins mediating attachment of *S. aureus* to human nasal mucosal cells (in vitro).

The mucin-binding receptors of both *S. epidermidis* and *S. saprophyticus* were sensitive to treatment with trypsin, which again suggested that the receptors were composed of protein components (Table 4). In a previous report (58), we have shown that the two strains of *S. aureus* and *S. epidermidis* used in the present study share four antigenically similar, if not identical, surface peptides; thus, it is not unreasonable to speculate that these staphylococci, as well as *S. saprophyticus*, might share similar mucin-binding receptors. Chugh et al. (11) recently reported that the *S. epidermidis* adhesin for human pharyngeal cells, prewashed to remove mucus, was sensitive to lipase but resistant to trypsin, which indicates that this adhesin is distinct from the mucin-binding receptor. From previous reports, it appears that lipoteichoic acid of *S. saprophyticus* is one of the adhesins mediating adhesion to uroepithelial cells (57) which is distinct from the trypsin-sensitive adhesin responsible for hemagglutinating sheep erythrocytes (30). Again the relationship between these adhesins and the mucin-binding receptor, if any, is yet to be established.

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LITERATURE CITED

1. **Ada, G. L., and J. D. Stone.** 1950. Electrophoretic studies of virus-red cell interaction: mobility gradient of cells treated with viruses of the influenza group and the receptor-destroying enzyme of *V. cholerae*. Br. J. Exp. Pathol. **31**:263–274.
2. **Aly, R., H. R. Shinefield, C. Litz, and H. I. Maibach.** 1980. Role of teichoic acid in the binding of *Staphylococcus aureus* to nasal epithelial cells. J. Infect. Dis. **141**:463–465.
3. **Archer, G. L.** 1984. *Staphylococcus epidermidis*: the organism, its diseases, and treatment. Curr. Clin. Topics Infect. Dis. **5**:25–48.
4. **Barrett, S. P.** 1985. Protein-mediated adhesion of *Staphylococcus aureus* to silicone implant polymer. J. Med. Microbiol. **20**:249–253.
5. **Bauernfeind, A., R. M. Bertele, K. Harms, G. Horl, R. Jungwirth, C. Petermuller, B. Przyklenk, and C. Weisslein-Pfister.** 1987. Qualitative and quantitative microbiological analysis of sputa of 102 patients with cystic fibrosis. Infection **15**:270–277.
6. **Bibel, D. J., R. Aly, H. R. Shinefield, and H. I. Maibach.** 1983. The *Staphylococcus aureus* receptor for fibronectin. J. Invest. Dermatol. **80**:494–496.
7. **Bibel, D. J., R. Aly, H. R. Shinefield, H. I. Maibach, and W. G. Strauss.** 1982. Importance of the keratinized epithelial cell in bacterial adherence. J. Invest. Dermatol. **79**:250–253.
8. **Blum, R. A., and K. A. Rodvold.** 1987. Recognition and importance of *Staphylococcus epidermidis* infections. Clin. Pharmacol. **6**:464–475.
9. **Burns, M. W., and J. R. May.** 1968. Bacterial precipitins in serum of patients with cystic fibrosis. Lancet **i**:270–272.
10. **Carruthers, M. M., and W. J. Kabat.** 1983. Mediation of staphylococcal adherence to mucosal cells by lipoteichoic acid. Infect. Immun. **40**:444–446.
11. **Chugh, T. D., G. J. Burns, H. J. Shuhaiber, and E. A. Bishbishi.** 1989. Adherence of *Staphylococcus epidermidis* to human pharyngeal epithelial cells: evidence of lipase-sensitive adhesin and glycoprotein receptor. Curr. Microbiol. **18**:109–112.
12. **Clamp, J. F., and L. Hough.** 1965. The periodate oxidation of amino acids with reference to studies on glycoproteins. Biochem. J. **94**:17–24.
13. **Cohen, P. S., J. C. Arruda, T. J. Williams, and D. C. Laux.** 1985. Adhesion of a human fecal *Escherichia coli* strain to mouse colonic mucus. Infect. Immun. **48**:139–145.
14. **Creeth, J. M.** 1978. Constituents of mucus and their separation. Br. Med. Bull. **34**:17–24.
15. **Davison, V. E., and B. A. Sanford.** 1981. Adherence of *Staphylococcus aureus* to influenza A virus-infected Madin-Darby canine kidney cell cultures. Infect. Immun. **32**:118–126.
16. **Dudley, J. P., and J. D. Cherry.** 1980. Scanning electron microscopic demonstration of goblet cell discharge and mucous layer on nasal ciliated respiratory epithelium. Otolaryngol. Head Neck Surg. **88**:439–441.
17. **Dulawa, J., K. Jann, M. Thomsen, M. Rambausek, and E. Ritz.** 1988. Tamm Horsfall glycoprotein interferes with bacterial adherence to human kidney cells. Eur. J. Clin. Invest. **18**:87–91.
18. **Duthie, E. S.** 1955. The action of fibrinogen on certain pathogenic cocci. J. Gen. Microbiol. **13**:383–393.
19. **Fletcher, A. P.** 1972. The Tamm and Horsfall glycoprotein, p. 892–908. *In* A. Gottschalk (ed.), Glycoproteins. Their composition, structure and function. Elsevier Science Publishing, Inc., New York.
20. **Fletcher, A. P., A. Neuberger, and W. A. Ratcliffe.** 1970. Tamm-Horsfall urinary glycoprotein. Biochem. J. **120**:417–424.
21. **Florey, H.** 1955. Mucin and the protection of the body. Proc. R. Soc. Lond. B Biol. Sci. **143**:147–159.
22. **Forsgren, A., and J. Sjoquist.** 1966. "Protein A" from *S. aureus*. I. Pseudo-immune reaction with human γ-globulin. J. Immunol. **97**:822–827.
23. **Freter, R.** 1980. Prospects for preventing the association of harmful bacteria with host mucosal surfaces, p. 441–458. *In* E. H. Beachey (ed.), Bacterial adherence: receptors and recognition, series B, vol. 6. Chapman & Hall, Ltd., London.
24. **Froman, G., L. M. Switalski, P. Speziale, and M. Hook.** 1987. Isolation and characterization of a fibronectin receptor from *Staphylococcus aureus*. J. Biol. Chem. **262**:6564–6571.
25. **Glasgow, L.** 1972. Interaction of viruses and bacteria in host-parasite relations. N. Engl. J. Med. **238**:42–43.
26. **Gristina, A. G.** 1987. Biomaterial-centered infection: microbial adhesion versus tissue integration. Science **237**:1588–1595.
27. **Gunnarsson, A., P.-A. Mardh, A. Lundblad, and S. Svensson.** 1984. Oligosaccharide structures mediating agglutination of sheep erythrocytes by *Staphylococcus saprophyticus*. Infect. Immun. **45**:41–46.
28. **Hasty, D. L., and W. A. Simpson.** 1987. Effects of fibronectin and other salivary macromolecules on the adherence of *Escherichia coli* to buccal epithelial cells. Infect. Immun. **55**:2103–2109.
29. **Holderbaum, D., G. S. Hall, and L. A. Ehrhart.** 1986. Collagen binding to *Staphylococcus aureus*. Infect. Immun. **54**:359–364.
30. **Hovelius, B., and P.-A. Mardh.** 1979. Hemagglutination by *Staphylococcus saprophyticus* and other staphylococcal species. Acta Pathol. Microbiol. Scand. Sect. B. **87**:45–50.
31. **Hovelius, B., P.-A. Mardh, and P. Bygren.** 1979. Urinary tract infections caused by *Staphylococcus saprophyticus*—recurrences and complications. J. Urol. **122**:645–647.
32. **Ishikawa, H., and Y. Isayama.** 1987. Evidence of sialyl glycoconjugates as receptors for *Bordetella bronchiseptica* on swine nasal mucosa. Infect. Immun. **55**:1607–1609.
33. **Kilbourn, J. P.** 1978. Bacterial content and ionic composition of sputum in cystic fibrosis. Lancet **i**:334.
34. **Kloos, W. E.** 1980. Natural populations of the genus *Staphylococcus*. Annu. Rev. Microbiol. **34**:559–592.
35. **Kulczycki, L. L., T. M. Murphy, and J. A. Bellanti.** 1978. Pseudomonas colonization in cystic fibrosis. A study of 160 patients. J. Am. Med. Assoc. **240**:30–34.
36. **Kuriyama, S. M., and F. J. Silverblatt.** 1986. Effect of Tamm-Horsfall urinary glycoprotein on phagocytosis and killing of type 1-fimbriated *Escherichia coli*. Infect. Immun. **51**:193–198.
37. **Lee, A., J. L. O'Rourke, P. J. Barrington, and R. J. Trust.** 1986. Mucus colonization as a determinant of pathogenicity in intestinal infection by *Campylobacter jejuni*: a mouse cecal model. Infect. Immun. **51**:536–546.
38. **Martin, C. M., C. M. Kunin, L. S. Gottlieb, and M. Finland.** 1959. Asian flu in Boston, 1957–1958. II. Severe staphylococcal

pneumonia complicating influenza. Arch. Intern. Med. **103:** 532–542.

39. **Maskell, R.** 1974. Importance of coagulase-negative staphylococci as pathogens in the urinary tract. Lancet **i:**1155–1158.
40. **McNabb, P. C.** 1981. Host defense mechanisms at mucosal surfaces. Annu. Rev. Microbiol. **35:**477–496.
41. **Morse, S. I., and M. E. Melish.** 1986. Staphylococci, p. 236–242. *In* A. I. Braude, C. E. Davis, and J. Fierer (ed.), Infectious diseases and medical microbiology, 2nd ed. The W. B. Saunders Co., Philadelphia.
42. **Mota, G. F. A., C. R. W. Carneiro, L. Gomes, and J. D. Lopes.** 1988. Monoclonal antibodies to *Staphylococcus aureus* laminin-binding proteins cross-react with mammalian cells. Infect. Immun. **56:**1580–1584.
43. **Myers, M. G., F. P. Koontz, and M. Weinberger.** 1983. Lower respiratory infections in patients with cystic fibrosis, p. 91–107. *In* J. D. Lloyd-Still (ed.), Textbook of cystic fibrosis. PSG Pub. Co., Littleton, Mass.
44. **Ørskov, I., F. Ørskov, and A. Birch-Andersen.** 1980. Comparison of *Escherichia coli* fimbrial antigen F7 with type I fimbriae. Infect. Immun. **27:**657–666.
45. **Pfaller, M. A., and L. A. Herwaldt.** 1988. Laboratory, clinical, and epidemiological aspects of coagulase-negative staphylococci. Clin. Microbiol. Rev. **63:**189–200.
46. **Ramphal, R., C. Guay, and G. B. Pier.** 1987. *Pseudomonas aeruginosa* adhesins for tracheobronchial mucin. Infect. Immun. **55:**600–603.
47. **Roberts, G. P.** 1974. Isolation and characterization of glycoproteins from sputum. Eur. J. Biochem. **50:**265–280.
48. **Roberts, G. P.** 1978. Chemical aspects of respiratory mucus. Br. Med. Bull. **34:**39–41.
49. **Roe, J. H.** 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. J. Biol. Chem. **212:**335–343.
50. **Saggers, B. A., and D. Lawson.** 1970. Affinity for glycoproteins of bacteria found in the respiratory tract in cystic fibrosis. J. Clin. Pathol. **23:**262–265.
51. **Sanford, B. A., V. E. Davison, and M. A. Ramsay.** 1986. *Staphylococcus aureus* adherence to influenza A virus-infected and control cell cultures: evidence for multiple adhesins. Proc. Soc. Exp. Biol. Med. **181:**104–111.
52. **Sanford, B. A., and M. A. Ramsay.** 1987. Bacterial adherence to the upper respiratory tract of ferrets infected with influenza A virus. Proc. Soc. Exp. Biol. Med. **185:**120–128.
53. **Sanford, B. A., and M. A. Ramsay.** 1989. *In vivo* localization of *Staphylococcus aureus* in nasal tissues of healthy and influenza A virus-infected ferrets. Proc. Soc. Exp. Biol. Med. **191:** 163–169.
54. **Sanford, B. A., V. L. Thomas, M. A. Ramsay, and T. O. Jones.** 1986. Characterization of clinical strains of *Staphylococcus aureus* associated with pneumonia. J. Clin. Microbiol. **24:** 131–136.
55. **Schwarzmann, S. W., J. L. Adler, R. J. Sullivan, Jr., and W. M. Marine.** 1971. Bacterial pneumonia during the Hong Kong influenza epidemic of 1968–1969. Arch. Intern. Med. **127:**1037–1041.
56. **Spiro, R. G.** 1966. Characterization of carbohydrate units of glycoproteins. Methods Enzymol. **8:**26–52.
57. **Teti, G., M. S. Chiofalo, F. Tomasello, C. Fava, and P. Mastroeni.** 1987. Mediation of *Staphylococcus saprophyticus* adherence to uroepithelial cells by lipoteichoic acid. Infect. Immun. **55:**839–842.
58. **Thomas, V. L., B. A. Sanford, B. S. Keogh, and R. G. Triplett.** 1989. Antibody response to *Staphylococcus aureus* surface proteins in rabbits with persistent osteomyelitis after treatment with demineralized bone implants. Infect. Immun. **57:**404–412.
59. **Tuomanen, E.** 1986. Piracy of adhesins: attachment of superinfecting pathogens to respiratory cilia by secreted adhesins of *Bordetella pertussis*. Infect. Immun. **54:**905–908.
60. **Vishwanath, S., and R. Ramphal.** 1985. Tracheobronchial mucin receptor for *Pseudomonas aeruginosa*: predominance of amino sugars in binding sites. Infect. Immun. **48:**331–335.
61. **Wadolkowski, E. A., D. C. Laux, and P. S. Cohen.** 1988. Colonization of the streptomycin-treated mouse large intestine by a human fecal *Escherichia coli* strain: role of adhesion to mucosal receptors. Infect. Immun. **56:**1036–1043.
62. **Wallmark, G., I. Arremark, and B. Telander.** 1978. *Staphylococcus saprophyticus*: a frequent cause of acute urinary tract infection among female outpatients. J. Infect. Dis. **138:**791–797.
63. **Warren, L.** 1959. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. **234:**1971–1975.
64. **Wheat, L. J., R. B. Kohler, and A. White.** 1983. Diagnosis and management of deep seated infection, p. 121–148. *In* C. S. F. Easmon and C. Adlam (ed.), Staphylococci and staphylococcal infections, 1st ed. Academic Press, Inc. (London), Ltd., London.
65. **Williams, R. C., and R. J. Gibbons.** 1975. Inhibition of streptococcal attachment to receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. Infect. Immun. **11:**711–718.