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Hemagglutination by *Bordetella bronchiseptica*

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A total of 53 isolates of *Bordetella bronchiseptica* from dogs and pigs were tested for their ability to agglutinate chicken, horse, sheep, dog, pig, and guinea pig erythrocytes. No differences in hemagglutinating activity were attributed to the animal origin of the bordetella isolates. Horse and dog erythrocytes consistently resulted in the strongest hemagglutination reactions, whereas only 4% of the *B. bronchiseptica* isolates produced weak agglutination of chicken erythrocytes. A total of 85% of the isolates agglutinated horse, sheep, dog, pig, and guinea pig erythrocytes. One canine isolate with hemagglutinating activity, strain 110H, was examined to determine the nature of the hemagglutinin(s) involved. Hemagglutination was always accompanied by hemadsorption, as determined by dark-field or phase-contrast microscopy. Treatment of cells and cell extracts with heat or protease K inhibited the hemagglutination reaction. Sonicated bacterial cells had a greater hemagglutinating ability than did unsonicated live bacteria. The hemagglutination reaction was not inhibited by any of 17 sugars nor by *N*-acetylglucosamine or ethylene glycol-bis-(β-aminoethyl ether)-*N,N*-tetraacetic acid. Hemagglutinins were not detected in sonic extracts nor in several bacterial subunit fractions, including isolated pili. Antigens in some of these preparations were, however, detectable by indirect hemagglutination with anti-*B. bronchiseptica* serum. Isolated pili could not be detected on the erythrocyte surface by electron microscopy; however, serial sections of erythrocytes agglutinated by the live *Bordetella* organisms showed that the bacterial outer membrane and the erythrocyte surface were separated by a space of approximately 20 nm. This study provided additional circumstantial evidence that *B. bronchiseptica* pili or at least heat-labile surface proteins which extend some distance from the bacterial surface are involved in hemagglutination. Multiple hemagglutinins are likely to exist within this species since one isolate lacking pili also agglutinated canine erythrocytes. The hemagglutinins of *B. bronchiseptica* need to be isolated and characterized before the hemagglutination reaction can be applied to studies of attachment.

The ability of *Bordetella bronchiseptica* to agglutinate erythrocytes has been recognized since the early reports of Keogh et al. (17). Hemagglutination has not, however, been a primary characteristic for the identification of *B. bronchiseptica*, and consequently only a few investigators since Keogh have reported on this activity in *B. bronchiseptica* isolates (11, 15, 16). It has previously been reported that the incidence of hemagglutination, piliation, and smooth colonial morphologies among 50 randomly chosen *B. bronchiseptica* isolates was 82, 82, and 92%, respectively, although the relationship between these three characteristics was uncertain (3).

Common bacterial pili are responsible for the initial colonization of several extracellular mucosal pathogens. Correlations between piliation and hemagglutination among some bacterial species have made the relatively simple and inexpensive hemagglutination test useful for studying mechanisms of attachment (5, 6, 18, 23, 24, 26) and identifying pathogenic isolates (2, 8–10).

The hemagglutinins of *B. pertussis* were recognized and purified long before the widespread recognition of pili and their hemagglutinating properties (17, 21). Pili have now been associated with the hemagglutinating activity of *B. pertussis*, and the so-called fimbrial hemagglutinin has been isolated and purified (1, 27).

Since piliation or other attachment factors or both are also important in the pathogenesis of *B. bronchiseptica* infections (4, 33), the hemagglutination test could be a useful adjunct to the study of such infections. Furthermore, it has been suggested that the hemagglutination reaction might provide a means of distinguishing *B. bronchiseptica* isolates from different animal species (16). The purpose of this study was to

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examine the hemagglutinating properties of *B. bronchiseptica* and to determine what relationship exists between hemagglutination and attachment to respiratory cells.

MATERIALS AND METHODS

Bacterial isolates. We studied 53 isolates of *B. bronchiseptica*, 42 of canine origin and 11 of swine origin. The sources of most of these isolates have been previously acknowledged (3). Twelve additional canine isolates were employed; their designations and sources are as follows: Lutton, 23153, Small Animal Clinic, Cornell University, Ithaca, N.Y.; Romark, N. McLain, Ithaca, N.Y.; Brook, J. Dodds, Albany, N.Y.; 35992, 36043, G. Marshall, North Rose, N.Y.; PR8053, PR78A, PR8125, J. Williams, Phillips Roxane Inc., St. Joseph's, Mo.; UT, D. Bemis, University of Tennessee, Knoxville; D54, JS34682, J. Rose, Jensen-Salsbery Laboratories, Kansas City, Kans.

Preparation of bacterial whole cell and subunit fractions. A hemagglutinating isolate, *B. bronchiseptica* 110H, was harvested from 24-h cultures on brucella agar (Difco Laboratories, Detroit, Mich.). Whole cell suspensions were made in phosphate-buffered saline (PBS) and adjusted to contain approximately 1×10^8 colony-forming units per ml. Bacterial suspensions were inactivated by Formalin (0.5%) treatment overnight at 37°C or by incubation for 1 h at 60°C. Bacteria were pretreated with trypsin or protease K (2.5% for 1 h at 37°C). Bacterial pretreatments were stopped by washing twice with PBS.

Bacterial pili were prepared by homogenizing a heavy bacterial suspension for 20 min in a Virtis homogenizer (Virtis Research Equipment, Gardiner, N.Y.). After removal of bacterial cells by two low-speed centrifugations, the supernatant was centrifuged for 6 h at 54,000 × *g*. The pellet was suspended in PBS and centrifuged twice more at 100,000 × *g* for 2 h. The final pellet was suspended in 20 ml of PBS and precipitated with 0.1 M magnesium chloride overnight at 4°C. After centrifugation the precipitate was suspended in 2 ml of PBS and dialyzed against water for 2 days.

Bacterial ribosomes were prepared by the method described by Kurland (19). Endotoxin was prepared by the phenol-water extraction method of Westphal et al. (32).

Bacterial suspensions were sonicated by three successive 60-s pulses at full power with a Sonifier cell disrupter (Ultrasonic, Inc., Plainview, N.Y.) equipped with a microtip. The sonicated material was centrifuged at 15,000 × *g* for 1 h; the supernatant was filter-sterilized (sonic extract), and the sonicated cells were suspended in PBS to the original volume.

Hemagglutination test. Whole blood was collected in sodium citrate (0.5% concentration [wt/vol]) from the following animal species: sheep, dog, pig, chicken, horse, and guinea pig. Blood cells were washed twice in PBS and suspended to a final concentration of 0.5%. Bacterial test suspensions (0.05 ml) were placed in round-bottom microtiter plates. Serial dilutions, where necessary, were made with 0.05-ml microdiluters. After adding an equal volume of erythrocyte suspension to each well and mixing, the plates were allowed to stand at room temperature for 2 to 4 h before reading. Hemagglutination titers were expressed as reciprocals of the highest dilutions of bacterial suspensions (before the addition of erythrocytes) having complete hemagglutination.

Evaluation of hemadsorption. Erythrocyte (RBC) suspensions containing live or killed bacterial cells were diluted with distilled water and examined by phase-contrast and dark-field microscopy to determine whether bacteria were attached to the RBC "ghosts." Adsorption of soluble bacteria subunits to RBCs was determined by passive hemagglutination with antiserum prepared against *B. bronchiseptica* 110H.

Preparation of RBC membranes. RBC membranes were prepared by washing packed sheep RBCs twice with distilled water and three times with PBS. Centrifugation was at 100,000 × *g* for 3 h. The final pellet was suspended in PBS to ⅓ of the original packed cell volume and sonicated as described above for the bacterial suspensions. The resultant clear fluid suspension was stored at −20°C.

Hemagglutination inhibition. Suspensions of *B. bronchiseptica* 110H containing 10^8 colony-forming units per ml were diluted with equal parts of the following sugars (2.0% in PBS) to test their ability to inhibit the hemagglutination reaction: cellobiose, galactose, dulcitol, trehalose, fructose, raffinose, xylose, salicin, rhamnose, sorbitol, inulin, mannitol, mannose, glycerol, dextrose, lactose, and L-fucose. All sugars except fucose were of the D configuration. *N*-acetylglucosamine (0.25 M), ethylene glycol-bis(β-aminoethyl ether)-*N,N*-tetraacetic acid (EGTA) (0.25 mM) and antiserum (adsorbed with sheep RBC before use) prepared in rabbits against Formalin-inactivated *B. bronchiseptica* 110H were also tested.

Electron microscopy. Bacterial preparations were suspended on Formvar-coated and carbon-stabilized grids (400 mesh) for 30 s to 1 min, grids were drained, a drop of 0.5 to 2.0% aqueous potassium phosphotungstic acid (pH 7.0) was added for 30 s to 1 min, and grids were drained until dry. Agglutinated RBCs were embedded in 1.5% agarose, fixed in 2% glutaraldehyde, postfixed in 1% osmium tetroxide, stained with 0.5% uranyl acetate, dehydrated through a graded series of alcohol-propylene oxide, and then embedded in Epon-Araldite (Ernest Fullam, Schenectady, N.Y.). Blocks were sectioned on an LKB Ultratome III ultramicrotome. Thin sections (60 to 70 nm) were mounted on uncoated 200-mesh copper grids and stained with 2.0% uranyl acetate and lead citrate (24). Specimens were observed with a Hitachi model H600 electron microscope (Hitachi Ltd., Tokyo, Japan).

RESULTS

Hemagglutinating patterns of *B. bronchiseptica* isolates from dogs and pigs. Forty-three canine isolates and eleven swine isolates were tested for their ability to agglutinate RBCs from six different animal species (Table 1). At least 81 and 91% of these isolates, respectively, agglutinated horse, dog, pig, guinea pig, and sheep RBCs. The magnitude of the hemagglutinating activity decreased in the same order from a geometric mean titer of 512 with horse RBCs to 35 with sheep RBCs. There was little or no hemagglutination of chicken RBCs; only 10% of the canine isolates agglutinated chicken RBCs

TABLE 1. Hemagglutination patterns of *B. bronchiseptica* isolates from dogs and pigs

Source of *B. bronchiseptica*	% Positive for hemagglutination[a] with RBC from:					
	Chicken	Horse	Sheep	Dog	Pig	Guinea pig
Dog (43 isolates)	10 (4)	81 (512)	86 (38)	91 (210)	88 (68)	81 (48)
Pig (11 isolates)	0	91 (512)	91 (28)	91 (194)	91 (137)	91 (42)

[a] Geometric mean hemagglutination titer is in parentheses.

(geometric mean titer = 4). Swine isolates did not differ significantly from canine isolates in their ability to agglutinate RBCs from various animal species.

Hemagglutinating and hemadsorbing activity of pretreated bacterial cells. Formalin-inactivated sonicated cells and live *B. bronchiseptica* 110H cells agglutinated sheep RBC (Table 2). Sonication resulted in a two- to fourfold increase in hemagglutinating activity of the bacterial cells. Pretreatment of the bacteria with trypsin did not prevent hemagglutination. Treatment with protease K or heat inactivation destroyed the bacterium's hemagglutinating activity.

When the agglutinated suspensions were observed by phase-contrast and dark-field microscopy, bacterial cells were seen attached to the surface of the RBC membranes and interconnecting the aggregates of RBCs (Fig. 1). All of the cells which caused hemagglutination also exhibited hemadsorption. Heat-killed bacteria did not hemadsorb.

Membrane suspensions prepared from sheep RBC caused agglutination of all of the bacterial suspensions except for the heat-killed or protease K-treated bacteria. Electron microscopic examination of the agglutinated bacteria revealed numerous small membrane vesicles surrounding and interconnecting the agglutinated bacteria (Fig. 2).

Effect of bacterial pretreatments on pili. Bacterial cell suspensions were examined by electron microscopy. Formalin- and trypsin-treated bacteria possessed pili which appeared identical to those of the live untreated organisms. Pili were not observed on the heated, sonicated, or protease K-treated bacteria.

Characterization of bacterial subunits. Pili preparations from *B. bronchiseptica* 110H (a heavily piliated and usually nonmotile isolate) appeared by negative contrast staining to contain primarily thin filamentous structures approximately 3 to 4 nm in diameter (Fig. 3A). Similar preparations from *B. bronchiseptica* 110NH (a heavily flagellated and usually nonpiliated isolate) contained many flagella, approximately 15 nm in diameter, but none of the thinner filaments observed in the preparation from *B. bronchiseptica* 110H (Fig. 3B). Total protein concentrations in these preparations were between 100 and 500 μg/ml, as determined by the Lowry method (20).

Ribosomal preparations from isolate 110H contained approximately 1.5 mg of RNA per ml (determined spectrophotometrically) and 1.0 mg of protein per ml.

Endotoxin preparations from isolate 110H produced dermal toxicity and pyrexia in rabbits, as previously described for other bacterial endotoxins (7, 30); pili and ribosomal preparations were unreactive in these assays. The preparations contained approximately 12 mg of endotoxin per ml, as determined by the *Limulus* assay (Pyrotell, Associates of Cape Cod, Inc., Woods Hole, Mass.). Protein concentrations were less than 0.1 mg/ml in the endotoxin preparations.

The extracts of sonicated cells contained approximately 1.5, 3.0, and 0.5 mg of protein, endotoxin, and RNA, respectively, per ml.

TABLE 2. Hemagglutintion, hemadsorption, and piliation of treated and untreated whole cells of *B. bronchiseptica* 110H

Treatment	Activity of cells in:			
	Hemagglutination[a]	Hemadsorption	Bacterial agglutination with RBC membranes	Pili observed by electron microscopy
Live	+ (32)[b]	+	+	+
Formalin	+ (16)	+	+	+
Heat	−	−	−	−
Sonicated	+ (128)	+	+	−
Trypsin	+ (38)	+	+	+
Protease K	−	−	−	−

[a] Sheep RBCs.
[b] Geometric mean titer.

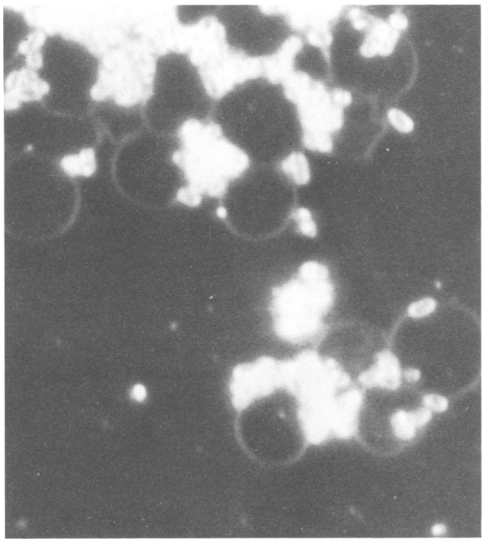

FIG. 1. Hemagglutination and hemadsorption by *B. bronchiseptica* strain 110H. RBC were lysed with water to improve visualization. Magnification, ×4200.

Hemagglutinating and hemadsorbing activity of bacterial subunits. None of the soluble bacterial subunits agglutinated sheep or dog RBCs (Table 3). However, when pili, sonic extract, or ribosomal preparations were incubated with the RBCs for 30 min and subsequently mixed with antiserum prepared against the whole bacterium, hemagglutination was observed, indicating that at least some reactive antigens were adsorbing to the RBCs. Endotoxin preparations did not react by passive hemagglutination.

Hemagglutination inhibition. None of the 17 sugars tested inhibited agglutination of sheep RBCs by *B. bronchiseptica*. Likewise, *N*-acetylglucosamine and EGTA were without effect. It was expected that *B. bronchiseptica* antiserum would inhibit the reaction between bacteria and RBCs, as had been reported by Thibault et al. (31); however, hemagglutination was also observed in the presence of *Bordetella* antiserum. The hemagglutination reaction occurred more rapidly in the presence of agglutinating or subbacterial agglutinating concentrations of *B. bronchiseptica* antiserum than in the absence of antiserum, and the RBC aggregates appeared to be larger and less densely packed. The antiserum did not agglutinate RBCs in the absence of *B. bronchiseptica*.

Electron microscopy. Examination of RBCs which had been agglutinated by *B. bronchiseptica* 110H by transmission electron microscopy revealed a close association between bacterium and RBCs. It initially appeared as if the bacterial outer membrane was in direct contact with the RBC membrane; however, examination of numerous serial sections at higher magnification revealed a consistent space of approximately 20 nm between the bacteria and the RBC (Fig. 4) at their point of closest contact.

DISCUSSION

An association of adhesion, piliation, hemagglutinating properties, and virulence is now well recognized among many bacterial species which colonize mucosal surfaces (2, 5, 6, 25). Most clinical isolates of *B. bronchiseptica* possess pili, agglutinate SRBCs, and attach to respirapili, agglutinate sheep RBCs, and attach to respiratory epithelial cells, presumably as a first step in establishing infection (3, 4a, 33). A causal relationship between these properties has not been established; should this correlation be made, the hemagglutination test would be useful in further studies of this organism's pathogenesis.

Hemagglutinins of *B. bronchiseptica* have not been isolated or characterized; however, their presence has long been suspected. Keogh et al. stated that "virulent, hemolytic strains of *H. bronchisepticus* are rich in hemagglutinins, which is not present in the avirulent, non-hemolytic variants" (17). The range of species whose RBCs are agglutinated by and the relative hemagglutinating ability of *B. bronchiseptica* isolates are not generally known. Keogh et al. (17) claimed that *B. bronchiseptica* (isolates unspecified) agglutinated RBCs of "man, mouse, fowl and other animals." Gallagher reported that a *B.*

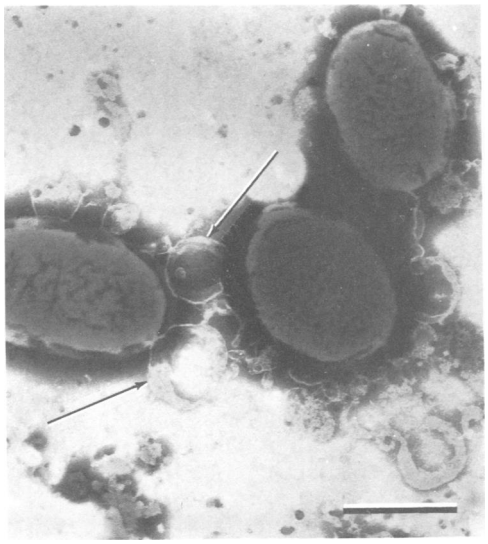

FIG. 2. *B. bronchiseptica* 110H agglutinated by membrane vesicles (arrows) prepared from SRBC. Magnification, ×50,000. Bar, 0.2 μm.

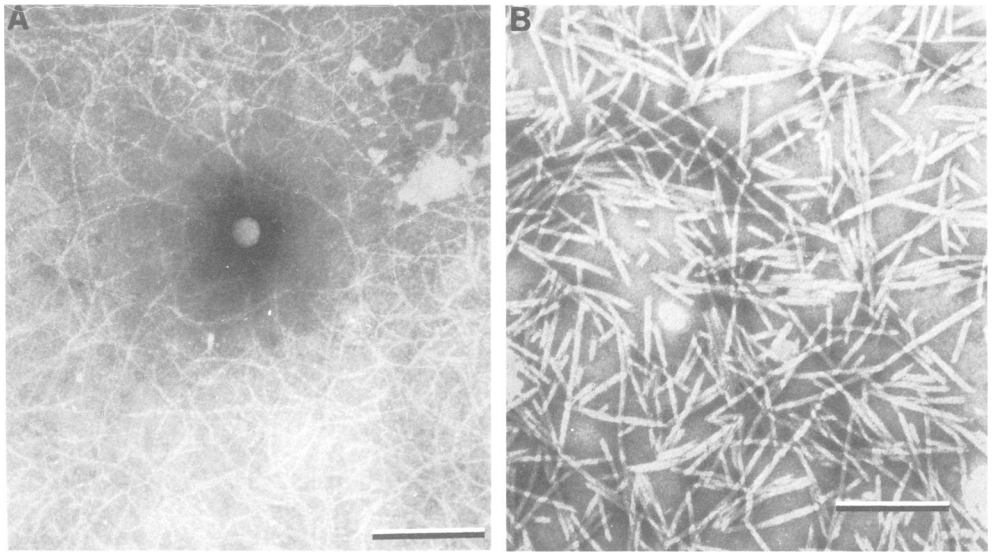

FIG. 3. Subunit fractions prepared from homogenates of *B. bronchiseptica*. (A) Preparation from strain 110H containing many aggregates of pili. The latex bead in the center of the photograph has a diameter of 0.08 μm. (B) Preparation from strain 110NH containing many flagella. No pili were observed in this preparation. Magnification for both, ×60,000. Bars, 300 nm.

bronchiseptica isolate of equine origin agglutinated human, horse, sheep, and budgerigar RBCs (11). In contrast, Joubert et al. (15) observed that an isolate of swine origin agglutinated human type O and sheep RBCs but not horse RBCs. Thibault et al. observed that at least 65% of 17 *B. bronchiseptica* isolates of human, dog, cat, pig, rabbit, guinea pig, or rat origin agglutinated human, monkey, guinea pig, pig, sheep, chicken, and horse RBCs (31). Kang et al. (16) found that 1 of 12, 1 of 4, 1 of 3, and 1 of 1 *B. bronchiseptica* isolates from pigs, guinea pigs, rabbits, and dogs, respectively, agglutinated horse RBCs. Furthermore, because the hemagglutination titer of the canine isolate was 15 times greater than those of the other isolates, they speculated that the differences in hemagglutination might be due to the species of origin of the *B. bronchiseptica* isolates. In the present study, most (>81%) of the *B. bronchiseptica* isolates agglutinated horse, dog, pig, guinea pig, and sheep RBCs. Only two isolates (<4%) were unable to agglutinate any of the RBCs tested. Chicken RBCs were generally unaffected by the isolates tested. Contrary to the speculation by Kang et al. (16), differences in the hemagglutinating activities of *B. bronchiseptica* isolates could not be attributed to their animal species of origin.

The relative agglutinability of the various RBC species decreased in the order listed above and seemed to correlate with the relative sedimentation rates of these RBCs in whole blood (28). Perhaps variables which affect RBC sedimentation, such as RBC size (28), also affect bacterial hemagglutination. RBC maturity and antigenic differences (due to breed, blood type, etc.) are additional factors which need to be examined with regard to bacterial hemagglutination.

The physiological state of the bacteria also undoubtedly affects hemagglutinin production. Such things as stage of growth, growth medium, and especially conditions of growth need to be further evaluated. We have observed that some, primarily rough and intermediate phase, *B. bronchiseptica* isolates do not hemagglutinate sheep RBCs during early stages of growth but have demonstrable hemagglutinating activity in later stages of growth (unpublished data).

Since most bacterial attachment and hemagglutination reflect the microorganism's ability to

TABLE 3. Hemagglutination and hemadsorption of bacterial subunits of *B. bronchiseptica* 110H

Bacterial subunit fraction	Activity of fraction in:	
	Hemagglutination[a]	Hemadsorption[b]
Sonic extract	−	+
Pili	−	+
Ribosomes	−	+
Endotoxin	−	−

[a] Sheep RBCs.

[b] Indirect hemagglutination with antiserum prepared against *B. bronchiseptica* 110H.

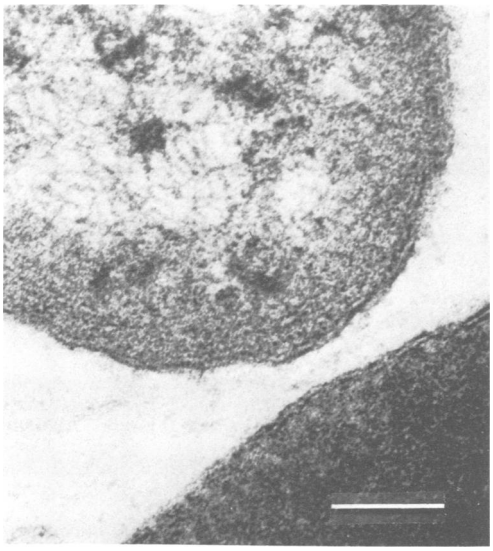

FIG. 4. Spatial relationship between the bacterial outer membrane and the cytoplasmic membrane of sheep RBC agglutinated by *B. bronchiseptica* strain 110H. High magnification of the point of closest contact between bacterium and RBC as determined from serial sections. A space of approximately 20 nm was consistently observed between these cells. Magnification, ×400,000. Bar, 60 nm.

overcome repulsive (negative) electrostatic surface changes (12), it is not surprising that Sedlock et al. (29) have found that bacterial hemagglutination assays can be made more sensitive by lowering the pH and ionic strength of the buffering medium used. Failure to recognize and control the above-mentioned variables may account for some of the reported discrepancies in *B. bronchiseptica*'s hemagglutinating ability.

In the present study bacterial hemagglutination was always accompanied by bacterial attachment to the RBCs. Furthermore, hemagglutination by *B. bronchiseptica* 110H appeared to be related to piliation in that treatments which did not affect filamentous appendages (Formalin, trypsin) did not alter the organism's hemagglutinating ability, but treatments (heat, protease K) which destroyed pili also destroyed the organism's hemagglutinating ability. One notable exception was the effect of sonication on *B. bronchiseptica*'s hemagglutinating ability. Sonicated cells had a significantly higher ($P < 0.05$) hemagglutinating capacity. Fragments were present; however, most of the sonicated cells appeared to be intact, of relatively normal size, and did not possess pili detectable by electron microscopy on their surfaces. The removal of pili by sonication may have resulted in greater exposure of another hemagglutinin, thus providing the cells with a larger total hemagglutinating surface area. Sonication of purified *E. coli* pili destroyed their ability to hemagglutinate (26). However, pretreatment of hamster lung fibroblasts with isolated pili from *B. bronchiseptica*, instead of inhibiting attachment as anticipated, resulted in greater attachment of *B. bronchiseptica* than that observed with untreated cells (unpublished data). Increased attachment by pili pretreatment and hemagglutination after sonication may reflect an increase in exposed pili surface available for binding as the result of redistribution of pili on the fibroblasts or bacteria (and not necessarily in their recognizable filamentous forms). This hypothesis is further supported by the observations that pili are sticky proteins that are capable not only of adhering to various surfaces but are also capable of sticking to themselves, causing autoaggregation (25). It will be interesting to determine whether pili-treated RBCs can be agglutinated by *B. bronchiseptica* more readily than untreated cells and whether sonicated *B. bronchiseptica* attaches better to hamster lung fibroblasts than untreated bacteria. It is alternatively possible that pili treatment or sonication enhances attachment and hemagglutination merely by altering surface charge.

The role of pili in hemagglutination is further suggested by the spatial relationship between bacterial and RBC membranes. The distances between eucaryotic cell membranes and attached bacterial cells at the point of attachment are not commonly measured; however, the distance of 20 nm between *B. bronchiseptica* and RBC membranes was approximately the same as the distance between RBCs which had been agglutinated by purified filamentous hemagglutinin (fimbriae) of *B. pertussis* (27) and is consistent with the distances observed in previously published electron micrographs of *B. bronchiseptica* attached to cilia (4, 33).

None of the soluble bacterial subunits tested, including isolated pili, agglutinated sheep or dog RBCs. This may have been due to low concentrations of the fractions involved or to the relative insensitivity of our assay procedure. Some antigens present in all but the endotoxin preparation were evidently bound to the RBCs since these cells were subsequently agglutinated by anti-*Bordetella* serum. Attempts to visualize pili adhered to the RBC surface, as has been reported for *B. pertussis* (27), were unsuccessful. Greater concentrations of pili or indirect methods of observation, such as by immunoelectron microscopy, or both may be needed to demonstrate the presence of pili on the cell surface.

Simple sugars are commonly used to block hemagglutination by piliated bacteria; however, the inability of simple sugars to inhibit hemagglutination does not rule out the possibility that

pili are mediators of hemagglutination by *B. bronchiseptica* since fimbrial hemagglutinins which are not affected by such sugars are known to occur among other bacteria (8, 10, 13, 22). Conversely, it should be pointed out that pili which have no known hemagglutinating ability have been isolated (14). Simple sugars also had no effect on the attachment of *B. bronchiseptica* to hamster lung fibroblasts; but the attachment of some strains (all heavily piliated) was both inhibited and reversed by the aminosugar *N*-acetylglucosamine, whereas attachment of the remaining strains (less heavily piliated) was inhibited and reversed by the organic acid EGTA (B. Plotkin and D. Bemis, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, B168, p. 42). Neither *N*-acetylglucosamine nor EGTA inhibited hemagglutination by *B. bronchiseptica*.

The role of hemagglutinins in the attachment of *B. bronchiseptica* to cells of the respiratory tract remains unclear. The observed patterns of hemagglutination in the present study were similar to the previously reported attachment patterns of *B. bronchiseptica* strains to ciliated canine tracheal outgrowth cells (4a). It was observed that not only did *N*-acetylglucosamine inhibit or reverse attachment of *B. bronchiseptica* 110H to hamster lung fibroblasts but that radiolabeled *N*-acetylglucosamine was capable of binding to *B. bronchiseptica* 110H (B. Plotkin, G. Merkel, and D. Bemis, Abstr. Annu. Meet Am. Soc. Microbiol. 1982, B55, p. 26). At least one hemagglutinin (multiple hemagglutinins are well known in other bacteria) (22) of *B. bronchiseptica* 110H was unaffected by *N*-acetylglucosamine since this compound did not inhibit hemagglutination or hemadsorption. This observation suggests that either the mechanisms of attachment of hamster lung fibroblasts and sheep RBC are different or that there is more than one hemagglutinin in this strain of *B. bronchiseptica*. The latter hypothesis is more likely since another strain of *B. bronchiseptica*, 110NH, which did not bind *N*-acetylglucosamine and whose attachment was not inhibited by *N*-acetylglucosamine, did agglutinate dog RBCs. The hemagglutinins of *B. bronchiseptica* need further characterization before accurate correlation can be made between hemagglutination and attachment to the respiratory epithelium.

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