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Morphology of Cells and Hemagglutinogens of *Bordetella* Species: Resolution of Substructural Units in Fimbriae of *Bordetella pertussis*

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The morphology of cells and the hemagglutinogens isolated from cultures of *Bordetella pertussis*, *Bordetella bronchiseptica*, and *Bordetella parapertussis* were studied by electron microscopy with the negative-staining technique. Cells of all three species had long, thin (3 nm thick), peritrichously arranged fimbriae on the cell surface. Similar structures were found in purified hemagglutinogen preparations together with shorter fimbrial structures 3 nm thick and from 40 to 100 nm long. In one experiment, long, thin fimbriae isolated from *B. pertussis* were found to be arranged in a crystalline structure on the specimen grid after negative staining. Optical diffraction analysis with a filtering technique performed on micrographs of these structures revealed 12.5-nm-long substructures within individual fimbriae. Further analysis resolved each of these structures into three globules, a central globule 3.5 nm in diameter and two diametrically opposed globules 2.5 nm in diameter. Based on this substructural composition, it is suggested that subunits of the individual fimbriae are connected by fragile regions. The presence of such regions would explain the size heterogeneity of the filamentous structures observed in preparations of hemagglutinogens isolated from cultures of *B. pertussis* and *B. bronchiseptica*. The concept that the short filamentous structures present in purified preparations of hemagglutinogens originate from the surface fimbriae present on the cells is supported.

Bordetella pertussis is known to be the pathogen that causes whooping cough (9). It seems well established that whooping cough in humans (14) is initiated when the bacteria become attached to the cilia of the epithelium in the upper respiratory tract, where they multiply during the acute catarrhal stage of the disease. Despite the absence of invasion of tissues, including the vascular system, infection with *B. pertussis* creates profound biological changes in the infected host. Some of these changes are suspected to be caused by two distinct hemagglutinogens, the filamentous hemagglutinogen (F-HA) and the leukocytosis-promoting factor hemagglutinogen (LPF-HA), which are known to be produced by *B. pertussis* (16, 17).

The morphology of the two hemagglutinogens isolated from *B. pertussis* is described in several papers (12, 13, 17). LPF-HA appears as spherical structures 6 nm in diameter, whereas F-HA is described as thin filaments about 2 to 3 nm in thickness and 40 to 100 nm in length. By cross protection tests in mice, Kendrick et al. (5) have shown antigenic relationship within the three *Bordetella* species, and Kloos et al. (6) recently concluded that *B. pertussis*, *Bordetella bronchiseptica*, and *Bordetella parapertussis* express sufficient DNA homology to be considered members of the same genus.

The present paper reports on the morphology of the cells of the three *Bordetella* species and of purified preparations of F-HA isolated from *B. pertussis* and *B. bronchiseptica*.

MATERIALS AND METHODS

Bacterial strains and growth. The *B. pertussis* strain used was 3803/57, one of the four pertussis strains in use for vaccine production at Statens Seruminstitut, Copenhagen, Denmark. *B. bronchiseptica* 4188/79 was received from K. B. Pedersen, State Veterinary Serumlaboratory, Copenhagen, Denmark. The strain was a primary isolate from a pig with atrophic rhinitis. *B. parapertussis* 5615/79 was received from K. Gaarslev, Diagnostic Department, Statens Seruminstitut. The strain was a primary human isolate.

The *Bordetella* strains were cultured on modified Bordet-Gengou (B-G) medium containing 30% defibrinated horse blood and 2 g of activated carbon per liter. The material from two to three Bordet-Gengou plates was transferred to a 10-liter laboratory fermentor containing 8 liters of modified Cohen-Wheeler

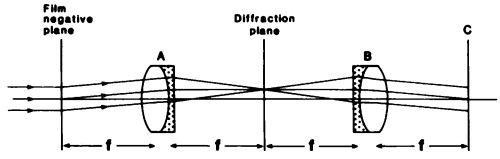

FIG. 1. Technique for optical diffraction and filtering (see text).

medium (8). The bacteria were cultivated at 35°C with an air flow rate of 1.7 liters/min.

Samples were withdrawn from the fermentor at different times during the cultivation period (1, 19, 23, and 47 h), and the growth rates, pH, and hemagglutinating activities (HA) were determined. Each of the samples was also examined under an electron microscope after negative staining.

Preparation of hemagglutinogens. Preparations of hemagglutinogens from *B. pertussis* and *B. bronchiseptica* were carried out as detailed below. The HA was determined by the method described by Sato and Arai (15). Differentiation between F-HA and LPF-HA activities was achieved by hemagglutination in tubes, as originally described by Irons and MacLennan (4), and the LPF activity was measured by the method of Morse and Morse (13). The protein content of the various fractions was determined by the method of Lowry et al. (10), with bovine serum albumin as the standard. Concentration of fractions obtained after gel filtration was performed by vacuum suction through semipermeable tubings (approximately 1 by 7 cm) or by means of a Millipore CX ultrafiltration unit. Chlorhexidine (0.002%) was added as a preservative to all buffers used for extraction or elution from the gel filtration columns.

Protocol for preparation of hemagglutinogens from *B. pertussis* and *B. bronchiseptica*.

Step 1. Cultivation of cells from a stock culture on two to three Bordet-Gengou plates at 35°C for 72 h.

Step 2. Cultivation in 8 liters of Cohen-Wheeler medium in a laboratory fermentor for 24 h.

Step 3. Cultivation in 80 liters of Cohen-Wheeler medium in a production fermentor. The cultivation was performed at 35°C, with an air flow rate of 25 liters/min and a stirring speed of 450 rpm. Samples were withdrawn during cultivation, and pH, growth rate, oxygen tension, and HA were measured. When maximum HA was reached, usually after 20 to 24 h at the end of the exponential growth phase, growth was terminated by cooling to 10°C and decreasing the pH to 3.5.

Step 4. Cells were allowed to sediment at 10°C at pH 3.5 for 72 h. The supernatant was siphoned off and discharged. The remaining sediment was centrifuged at 2,700 × *g* for 1 h.

Step 5. Three successive extractions were performed on the pellet with 500 ml of 0.1 M phosphate buffer (pH 8) containing 1.0 M NaCl. The extractions were performed on a magnetic stirrer at 4°C for 24 h, followed by centrifugation for 1 h at 2,700 × *g*.

Step 6. Precipitation of the combined extracts with 70% saturated ammonium sulfate. Precipitation was achieved at pH 6.4 after 24 h at 4°C. The precipitate was collected by centrifugation for 1 h at 2,700 × *g*.

Step 7. The precipitate was washed with 50 ml of distilled water by stirring for 24 h at 4°C with a magnetic stirrer and pelleted by centrifugation at 6,100 × *g* for 30 min.

Step 8. The washed precipitate was extracted with 3 volumes of 50 ml of 0.1 M Tris buffer (pH 10) containing 0.5 M NaCl to form three concentrated extracts. The three extractions were performed by stirring for 24 h at 4°C with a magnetic stirrer, followed centrifugation at 6,100 × *g* for 30 min.

Step 9. The concentrated extracts were purified on a Sephadex G-150 or a Sephacryl S-300 gel packed in a Pharmacia K 25/100, K 50/60, or K 50/100 column. The columns were eluted with 0.1 M Tris buffer (pH 10) containing 0.5 M NaCl. The fractions were screened for HA, and the active ones were pooled, concentrated, and chromatographed three times (in some experiments, four times).

The hemagglutinogens obtained from *B. pertussis*, batch no. 310878-0260, were made from one 80-liter culture. Those from *B. pertussis*, batch no. 220379-0260, were made from four 80-liter cultures, the extracts of which were pooled in preparation step 9. The material from *B. bronchiseptica*, batch no. 280679-0341, was obtained from three 80-liter cultures, and the concentrated extracts were pooled and reprecipitated according to step 6 above.

Preparation of specimens for electron microscopy. The cells of the different *Bordetella* strains and the

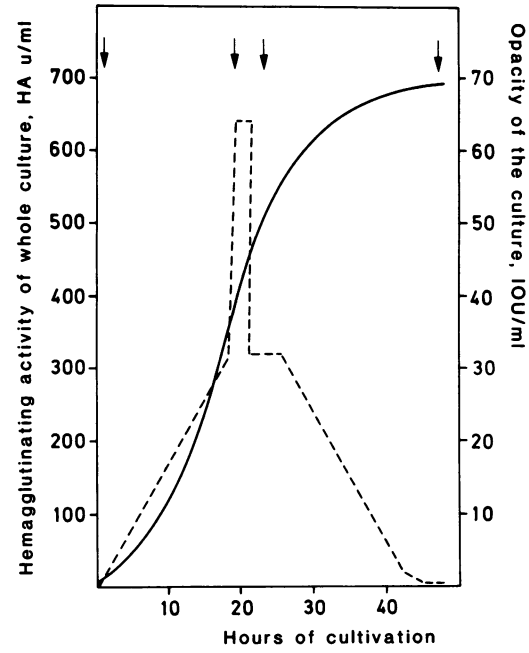

FIG. 2. HA (dotted line) and opacity (solid line) of a culture of *B. pertussis* during cell growth (8-liter culture). HA (units per milliliter) equals the reciprocal value of the smallest amount of the sample causing complete hemagglutination. IOU/ml, International opacity units per milliliter of culture. The arrows indicate times at which samples were taken for electron microscopy.

hemagglutinogens were examined under an electron microscope after negative staining by either of the two methods described below. The stain used was 1 or 2% (wt/vol) ammonium molybdate adjusted to pH 7.4 with NH_4OH. The samples obtained from cultures after 1, 19, 23, and 47 h of growth were diluted with distilled water or the staining solution to a suitable concentration for electron microscopy. Formvar-coated carbon-reinforced 200-mesh grids were irradiated for 10 min with UV light before use.

(i) Method A (suspension method). A grid was applied with the film side down on top of a drop of the culture placed on a strip of Parafilm. After 30 to 60 s, the grid was removed, and excess liquid was sucked off with filter paper. Finally, the grid was placed on a drop of the negative stain for another minute, after which it was removed, and the adhering stain was sucked off.

(ii) Method B (microdrop method). A microdrop of the culture was placed on the grid and allowed to dry partially for a few minutes at room temperature. Finally, the grid was stained by method A.

Electron microscopy was carried out at 60 kV on a Philips EM 300 electron microscope, and exposures were made on 35-mm film at a primary magnification of ×17,500. Internal calibration for determination of accurate magnification was performed with catalase crystals as described by Luftig (11). Negatives were obtained on Kodak fine grain release film type 5302, and suitable fields were photographically enlarged as desired.

Optical diffraction and filtering. The technique for optical diffraction and filtering on crystals of biological macromolecules is well described in the literature (7). In short, the procedure is as follows (Fig. 1). The electron micrograph showing the crystalline structure is illuminated by a plane-parallel beam of monochromatic light (e.g., from a helium-neon laser). The diffraction pattern is observed in the back focal plane of the lens (Fig. 1A) (focal length = f = 1,670 mm), and the diffraction pattern obtained is directly related to the structural detail contained in the electron micrograph. Another lens (Fig. 1B) is used to retransform the diffraction pattern within the plane (Fig. 1C), where it can be observed or photographed. To increase contrast and structural details, filtering masks are placed in the diffraction plane so that certain of the diffraction spots are filtered out and the background noise is thus suppressed.

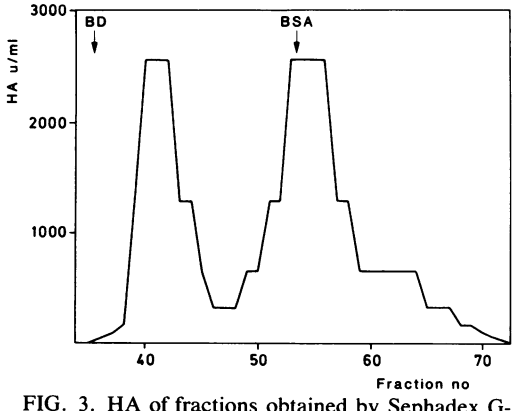

FIG. 3. HA of fractions obtained by Sephadex G-150 chromatography of a concentrated extract from a *B. pertussis* culture. Elution was done at 30 ml/h with 0.1 M Tris buffer (pH 10) containing 0.5 M NaCl. HA (units per milliliter) equals the reciprocal value of the smallest amount of the sample causing complete hemagglutination. BD, Pharmacia blue dextran; BSA, bovine serum albumin eluted in the fractions indicated with arrows.

TABLE 1. Results of analysis for HA and LPF activity of three preparations of hemagglutinogens from two batches of *B. pertussis* and one batch of *B. bronchiseptica*

Strain	Hemagglutinogen	HA activity[a]	LPF activity[b]
B. pertussis 310878-0260	F-HA	0.04	1.4
	LPF-HA	0.4	4.1
B. pertussis 220379-0260	F-HA	0.02	1.0
	LPF-HA	0.2	5.6
B. bronchiseptica 280679-0341	F-HA	0.02	1.0

[a] Minimal quantity of protein in micrograms (Lowry et al. [10]) which produced hemagglutination of chicken erythrocytes (15).

[b] Relative value of $WBC_{LPF\text{-}HA}/WBC_{saline}$ 3 days after intravenous injection of 1 μg of protein per mouse (13), where WBC is the leukocyte count per cubic millimeter of mouse blood.

RESULTS

Isolation of hemagglutinogens from *Bordetella* cultures. The HA of the cultivated bacteria of all *Bordetella* species examined was found to increase with the growth rate of the cells throughout the logarithmic phase of growth. When the stationary phase was reached (normally within 20 to 22 h), HA rapidly decreased to very low values (Fig. 2) within a few hours.

The material concentrated from *B. pertussis* cultures was, after chromatography, separated into two fractions which possessed HA (Fig. 3). The first fraction was eluted just after the void volume (indicated by blue dextran) and contained F-HA. The peak of the second fraction was eluted at the same volume as bovine serum albumin and expressed LPF-HA activity. By chromatography of the material obtained by concentration of extracts from *B. bronchiseptica* cultures, F-HA was eluted at the same volume as F-HA from *B. pertussis*, but there was no HA present in the fractions corresponding to those with the LPF-HA from *B. pertussis*.

Table 1 gives the results of analysis for HA and LPF activity of three preparations of hemagglutinogens arising from two different batches of *B. pertussis* and one batch of *B.*

bronchiseptica. It can be seen that *B. pertussis* 220379-0260 hemagglutinogens have been purified to a higher specific activity than *B. pertussis* 310878-0260. Table 1 also shows that the F-HA obtained from *B. pertussis* 220379-0260 and *B. bronchiseptica* 280679-0341 completely lack LPF activity, which is indicated by a relative value of $WBC_{LPF\text{-}HA}/WBC_{saline} = 1$ where WBC is the leukocyte count per cubic millimeter of mouse blood.

Electron microscopy of intact bacteria. Cells of *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* were removed after 1, 19, 23, and 47 h of growth in liquid cultures and examined after negative staining.

(i) *B. pertussis*. Small rod-shaped cells were found, often with a wrinkled cell surface (Fig. 4 and 5). After 1 day of culture (19 and 23 h), many of the cells presented two different types of surface appendages. One appeared as tentacular projections 15 to 20 nm wide, of varying length, and often with bulbous ends (Fig. 5). The other consisted of peritrichously arranged thin fimbriae about 3 nm thick and up to several micrometers long (Fig. 4). After 2 days of culture, the number of cells with fimbriae had decreased (not shown).

(ii) *B. parapertussis*. The morphology of *B. parapertussis* cells was similar to that of *B. pertussis*, except that very few cells presented the tentacular projections. The 1-day-old culture contained the highest number of fimbriated cells (Fig. 6).

(iii) *B. bronchiseptica*. *B. bronchiseptica* cells were rod shaped, and, in contrast to the other two species, they possessed flagella; again fimbriated cells were most numerous in 1-day-old cultures (Fig. 7).

Morphology of constituents of extracts obtained from *B. pertussis* and *B. bronchiseptica*. (i) Fractions containing F-HA. In fractions containing F-HA, isolated from *B. pertussis* 310878-0260, a mixture of filamentous and vesicular structures was seen (Fig. 8). However, the predominant structures were small, thin filaments about 3 nm wide and 40 to 100 nm long (Fig. 8). Together with these, long fimbriae about 3 nm wide and up to several micrometers long (Fig. 8 and 13) were found. These fimbriae showed a tendency to form bundles (Fig. 8 and 13), and their morphology was similar to that of the fimbriae present on the intact cells (Fig. 4 and 5). The vesicular structures measured from 30 to 200 nm in diameter (Fig. 8 and 13). Electron microscopy of a further purified fraction from *B. pertussis* 220379-0260, which contained increased HA activity (Table 1), showed a network of small filamentous structures, about 3 nm thick and 40 to 100 nm long (Fig. 9). In this case, however, very few of the long fimbriae were present.

Interesting fields of view were observed in a preparation of an F-HA fraction from one particular experiment originating from *B. pertussis* 310878-0260. The suspension was diluted with equal parts of 1% ammonium molybdate (pH 7.4) before preparation for negative staining by method A. In these fields, two-dimensional crystalline structures measuring up to 1 to 2 μm in width were seen. It was obvious (Fig. 13) that some of the long, thin fimbriae were in direct continuity with the crystalline regions, which appeared to be made up of individual layers of parallel fimbriae, deposited on top of each other at fixed angles. The angle between adjacent layers of the parallel fimbriae averaged 43° when measured on different parts of different crystals (Fig. 13). The center-to-center distance between the fimbriae in the crystal was about 9 nm, whereas the width of a single fimbria outside the crystals was about 3 nm. Optical diffraction with filtering techniques was used on the photographic negatives of these crystalline structures to achieve further resolution of substructural details (see below).

When the fractions containing F-HA from *B. bronchiseptica* 280679-0341 were examined after staining by method A, long, thin fimbriae dominated the picture, but vesicular structures were also present, together with an occasional piece of a flagellum (Fig. 12). When the same material was prepared by method B, the distribution of the structural elements had changed radically. Now small filamentous structures, about 3 nm wide and 40 to 100 nm long, were the predominant structures (Fig. 10).

(ii) Fraction containing LPF-HA. A fraction containing LPF-HA was isolated from the cultures of *B. pertussis* 310878-0260. This fraction contained a homogeneous suspension of small round structures with a diameter of about 6 nm (Fig. 11). Fimbriae-like elements were never seen.

Optical diffraction and filtering. A characteristic diffraction pattern (Fig. 14) was obtained when the original film negative for Fig. 13 was analyzed with a diffractometer. The diffraction pattern corresponding to the framed area in Fig. 13 showed a series of bright spots arranged symmetrically around the central spot (Fig. 14). This diffraction pattern was further analyzed by application of a filtering mask which reduced the background noise and the intensity of the central spot by as much as 70%. The retransformed picture obtained from the pattern after the filtering is shown in Fig. 15.

A regular substructure consisting of three globules was now revealed as the structural constituents of the individual fimbriae. A large globule A with a diameter of about 3.5 nm and two diametrically opposed globules B about 2.5 nm in diameter were resolved within a repeating unit of 12.5 nm.

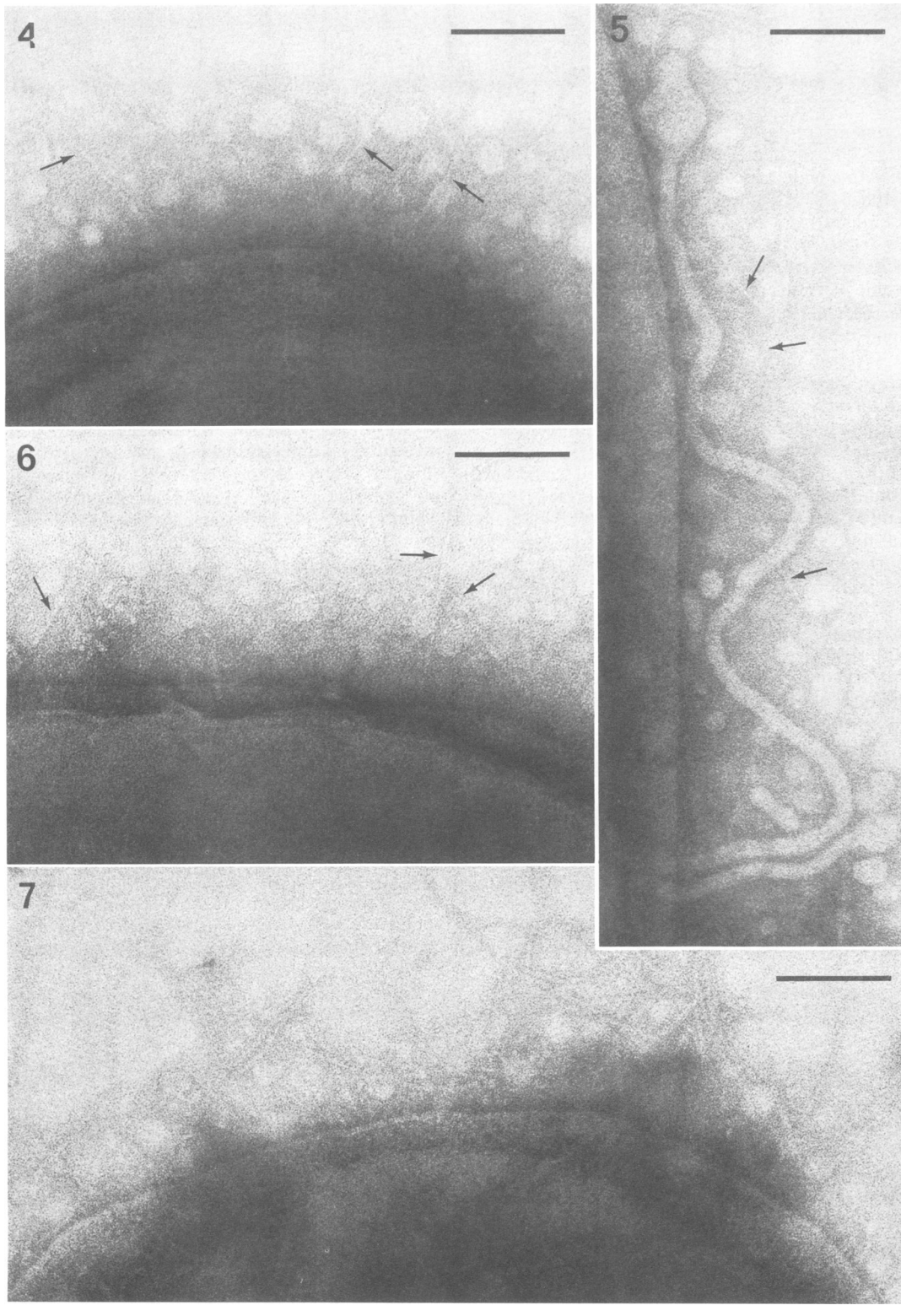

FIG. 4. *B. pertussis*. Cell from a 23-h-old liquid culture (170579-0250) negatively stained with 1% ammonium molybdate by staining method A. Part of the polar region of a rod-shaped cell. The cell surface is wrinkled. Thin fimbriae (arrows) about 3 nm wide are seen radiating from the cell surface. Bar, 100 nm. ×175,000.

FIG. 5. *B. pertussis*. Cell from the same culture and stained as for Fig. 4. Part of the cell surface of a rod-shaped cell. Two tentacular projections with bulbous ends protrude from the cell. Round vesicular structures 30 to 50 nm in diameter and some thin fimbriae (arrows) are also present. Bar, 100 nm. ×175,000.

FIG. 6. *B. parapertussis*. Cell from a 19-h-old liquid culture (220580-1351) negatively stained with 1% ammonium molybdate by staining method A. Part of the cell surface of a small rod-shaped cell. A few thin fimbriae 3 nm thick (arrows) are present. Bar, 100 nm. ×175,000.

FIG. 7. *B. bronchiseptica*. Cell from a 19-h-old liquid culture (300879-0341) negatively stained with 1% ammonium molybdate by staining method A. Part of the polar region of a rod-shaped cell. Several fimbriae 3 nm thick are seen radiating from the cell surface. Bar, 100 nm. ×175,000.

The two small globules B appeared to be connected with the bigger central globule A by small, thin rods oriented at a fixed angle α (Fig. 16) with respect to the long axis of the fimbriae. The fimbriae arranged in parallel within the crystalline layers seemed to be attached to each other through the subunits B (Fig. 15).

A schematic drawing based on measurements and calculations made on different fields with crystalline structures which were analyzed and reconstituted is given in Fig. 16. Our measurements have shown that the angle α between the subunits and the axis through the centers of subunit A is $21.3 \pm 0.3°$, and the angle β between adjacent layers of parallel fimbriae is $42.8 \pm 0.5°$. The center-to-center distance between subunits A in the same fimbria was found to be about 12.5 nm, and that between subunits A in parallel fimbriae was found to be about 9 nm. The total substructure consisting of the elements B-A-B measured about 12.5 nm.

DISCUSSION

Recent studies (12, 13, 16, 17) on *B. pertussis* have firmly established that two morphologically distinct antigens can be isolated from these cells. One is the F-HA with a high HA; the other is a substance which appears as small spherical entities under the electron microscope. This latter substance possesses the ability to produce marked leukocytosis when injected into experimental animals and is consequently called the LPF. It also possesses the ability to agglutinate erythrocytes from various species and has been found only in cultures of *B. pertussis* and not in cultures of other *Bordetella* species (14).

In our study the HA of fermentor cultures of the three *Bordetella* species increased during the logarithmic phase of growth (19 to 23 h). This finding correlated with the number of fimbriated cells, which was greatest in 1-day-old cultures. The fimbriae on the cells were long and thin and similar to those first described on *B. pertussis* by Morse and Morse (12). These authors also mentioned that fimbriae were present on cells of the other *Bordetella* species, but no illustrations were given. However, in a paper by Bemis et al. (2), a cell of *B. bronchiseptica* with long, thin fimbriae is shown.

Electron microscopy in the present study on purified preparations from *B. pertussis* and *B. bronchiseptica* with high HA showed that these contained practically only filamentous structures about 3 nm wide, but varying in length from 40 nm to several micrometers. The long filaments were morphologically identical to the thin fimbriae found on the surface of cells obtained from cultures in the logarithmic phase of growth. The marked variation in length of the filamentous structures observed contradicts the uniform filamentous elements about 2 by 40 nm demonstrated by Sato et al. (17). However, the preparations from *B. pertussis* with the highest HA (Table 1) also showed an increased number of short units and a lower number of long, thin fimbriae, and the discrepancy may be explained by the use of different isolation and purification procedures.

Similarly, different procedures for preparation of the negatively stained material may also be of importance for the results obtained. In the present study only the suspension method revealed long, thin fimbriae, frequently mixed with a small amount of shorter filamentous structures, whereas the microdrop method always showed the short elements as the dominating structure (compare Fig. 10 and 12). At this point it should be added that staining methods similar to the microdrop method have been used in previous studies (12, 15).

Sato et al. (17) have proposed that the F-HA of *B. pertussis* is derived from the fimbriae on the bacterial surface. If so, this must indicate that during the purification process the long fimbriae can be divided into shorter subunits of more or less equal size. The chemical composition of F-HA is at present unknown, but a highly purified preparation contains only protein (18). However, polyacrylamide and sodium dodecyl sulfate gel electrophoresis on such a preparation have disclosed that F-HA contains components with different mobility (3).

The reason why the long fimbriae crystallized in the fractions with F-HA activity from one of our *B. pertussis* preparations and not in others is at present unknown to us. Based on the dimensions of individual fimbriae in purified F-HA material, we are convinced that this material originates from the surface of the intact cells. Our optical diffraction analysis revealed a repeating unit consisting of three globules so connected that altogether they measure about 12.5 nm along the axis of the fimbriae of *B. pertussis*. If the observation by Sato et al. (17) is taken into consideration, it is tempting to postulate that fragile regions are located between subunits of the individual fimbriae. The existence of such "weak" regions would explain the heterogeneity in length (from 40 nm [equal to three subunits] to more than 100 nm) found in different preparations of F-HA, depending on the purification procedure.

In a recent paper Ashworth et al. (1) concluded that the "Sato concept" (17) of filamentous F-HA originating from the fimbriae of *B. pertussis* should be questioned. These authors (1) described immune electron microscopy experiments in which fimbriae from *B. pertussis* did not label specifically with antibody to F-HA, but did so with antibody to serotype-specific aggluti-

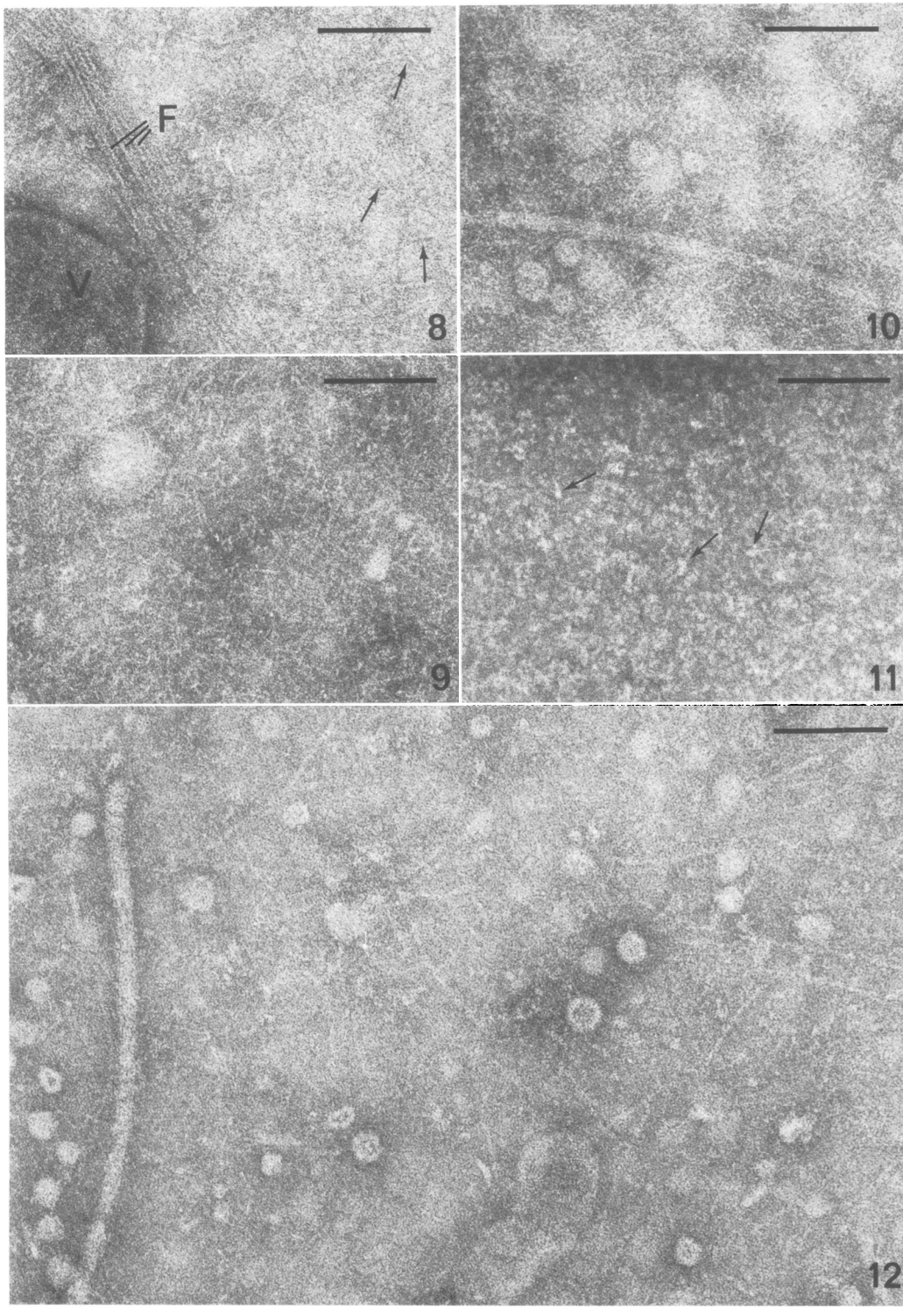

FIG. 8. Material from a fraction with F-HA activity obtained from a culture of *B. pertussis* (310878-0260). The material was diluted with equal parts of 1% ammonium molybdate before negative staining by method A. Part of a large vesicular structure (v) and a small bundle of fimbriae (F) are illustrated. Numerous filamentous elements (arrows) about 3 nm thick and from 40 to 100 nm long are also present. Bar, 100 nm. ×175,000.

FIG. 9. Material from a purified fraction with F-HA activity obtained from cultures of *B. pertussis* (220379-0260). Preparation method B was used for negative staining with 2% ammonium molybdate. A network of filamentous elements about 3 nm thick and 40 to 100 nm long is seen. Bar, 100 nm. ×175,000.

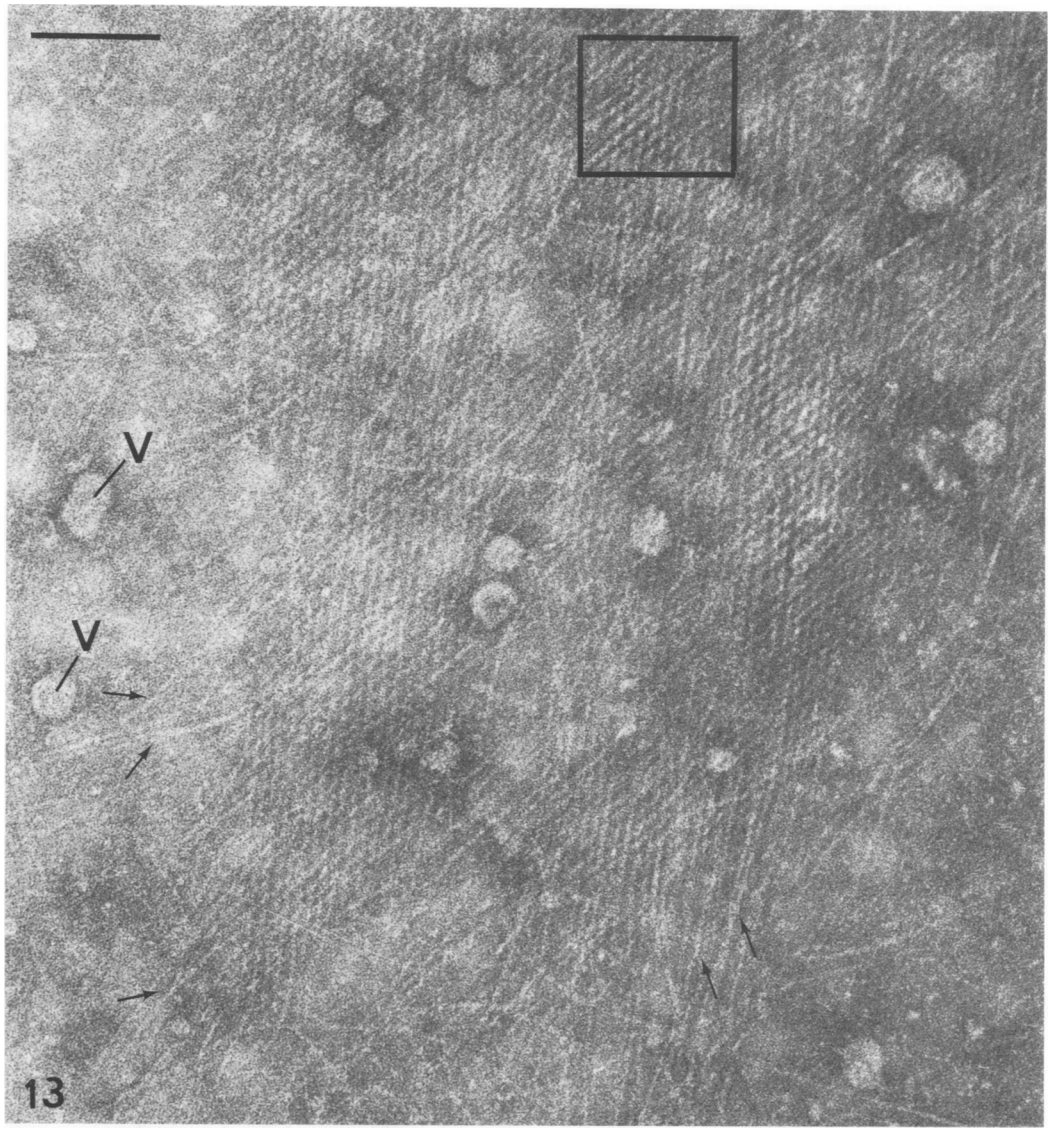

FIG. 13. Fraction with F-HA activity from a culture of *B. pertussis* (310878-0260). Representative field from the same grid as the field shown in Fig. 8. Some of the fimbriae (arrows) within the network of long fimbriae about 3 nm thick and some more than 1 μm long appear directly to continue into a two-dimensional crystalline structure. Some vesicular structures (v) are also present. Bar, 100 nm. ×175,000.

FIG. 10. Material from a fraction with F-HA activity obtained from cultures of *B. bronchiseptica* (280679-0341). The specimen was diluted with equal parts of 2% ammonium molybdate before negative staining by method B with 2% ammonium molybdate. Most of the field is occupied by a network of filamentous elements about 3 nm thick and 40 to 100 nm long. A piece of a flagellum is also present together with some small vesicular structures. Bar, 100 nm. ×175,000.

FIG. 11. Material from a fraction with LPF-HA activity from a culture of *B. pertussis* (310878-0260). The specimen was negatively stained with 1% ammonium molybdate by method B. A homogeneous picture is presented in which the basic element is a round structure with a diameter of about 6 nm (arrows). Note the absence of fimbriae and other filamentous elements. Bar, 100 nm. ×175,000.

FIG. 12. Material from the same fraction with F-HA activity as in Fig. 10, but the specimen was prepared by method A after dilution with 2% ammonium molybdate and was negatively stained with 2% ammonium molybdate. Long fimbriae about 3 nm thick and up to 1 μm long are now the major structural elements. Note also the presence of vesicular structures and part of a flagellum. Bar, 100 nm. ×175,000.

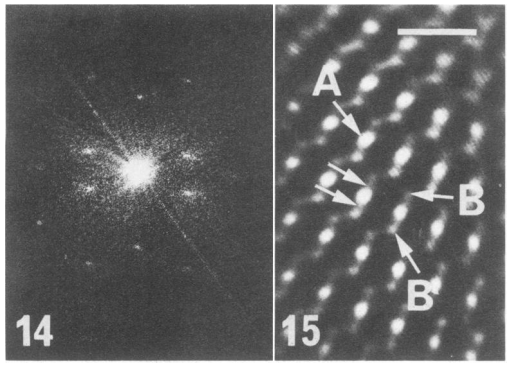

FIG. 14. The diffraction pattern obtained from the crystalline structure of fimbriae present within the framed area of Fig. 13. This is the unfiltered pattern as observed directly in the diffraction plane (see text).

FIG. 15. The retransformed picture obtained after having filtered the diffraction pattern by a copper foil with small holes punched at the position of the diffraction spots of Fig. 14. The central spot is further weakened about 70% in amplitude by a fine mesh grating grid. The effect is a suppression of superimposed structures and background noise. A substructure consisting of three little globules (B, A, B) connected by small rods (arrows), is revealed within individual fimbriae of the crystalline structure. Bar, 20 nm.

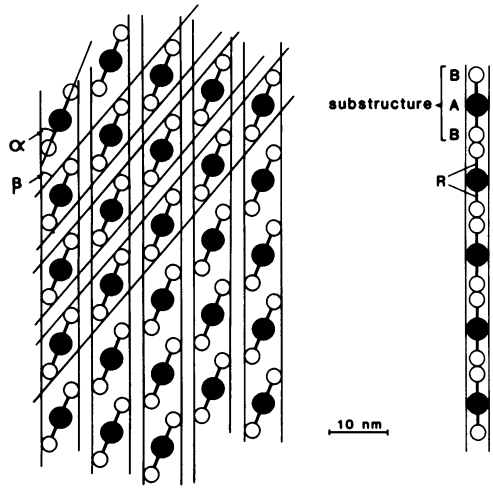

FIG. 16. Schematic drawing of the arrangement of individual fimbriae within the crystalline structure formed in a concentrated fraction of F-HA from *B. pertussis*. The substructural units resolved by optical diffraction and filtering within the fimbriae are also shown. The central globule A (diameter, about 3.5 nm) is connected to two smaller globules B (diameter, 2.5 nm) by two somewhat thinner rods R. The weak regions between the subunits are proposed to be localized between some adjacent B globules. α, Angle between the subunits and the axis through the centers of subunit A; β, angle between adjacent layers of parallel fimbriae.

nogens. No alternative suggestion as to the specific location of F-HA in or on the cells was given.

The present report is, to the best of our knowledge, the first to demonstrate a substructure in the fimbriae of *B. pertussis*. The elementary subunits appear to be arranged in a manner favoring the concept that the F-HA hemagglutinogens from *B. pertussis* and *B. bronchiseptica* are derived from the surface fimbriae of the cells.

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