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Histamine-sensitizing Factor, Mouse-protective Antigens, and Other Antigens of Some Members of the Genus *Bordetella*

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The three species of the genus *Bordetella*—*B. pertussis*, *B. parapertussis*, and *B. bronchiseptica*—have many antigens in common. Studies on representative strains of these species have shown that there are only a few specific antigens in each species. Whole-cell vaccines and extracts from *B. pertussis* contained specific mouse-protective antigen and a histamine-sensitizing factor. In addition, whole-cell vaccines and some saline extracts protected mice against intracranial challenge with *B. bronchiseptica*. Cells and a saline extract of *B. parapertussis* also protected against *B. bronchiseptica* but not against *B. pertussis*. Whole cells of *B. bronchiseptica* protected against *B. bronchiseptica*, but only one of three saline extracts protected against this challenge. Neither whole cells nor saline extracts from *B. bronchiseptica* protected against *B. pertussis*. The antigen in *B. pertussis* responsible for cross-protection against *B. bronchiseptica* was less resistant to heat than the protective antigen in *B. bronchiseptica*. Since histamine-sensitizing factor was not detected in *B. bronchiseptica* or *B. parapertussis* cells or extracts, this factor is not required to protect mice against *B. bronchiseptica* challenge. Whether *B. pertussis* vaccines protected against *B. bronchiseptica* by a nonspecific mechanism was not established, but it is clear that the specific antigen responsible for protection against *B. pertussis* was found only in *B. pertussis* and not in *B. bronchiseptica* or *B. parapertussis*.

The antigenic relationships of *Bordetella pertussis*, *B. parapertussis*, and *B. bronchiseptica* have been studied with various serological tests (1, 2, 5, 6, 8). Andersen (1) described strain- and species-specific heat-labile and heat-stable agglutinogens which Eldering et al. (5, 6) then employed to develop a useful scheme for typing the three species. The mouse-protective antigen of *B. pertussis*, thought to be distinct from the agglutinogens, has received considerable attention but has not been fully characterized. This antigen is known to be associated with the cell wall, to be inactivated by heating at 80 C for 0.5 hr, and to be sensitive to certain proteolytic enzymes and resistant to other enzymes (11). The mouse-protective antigen in *B. pertussis* is similar in many respects to the histamine-sensitizing factor (HSF), and some investigators believe that the two substances are identical (13).

Evidence that mouse-protective antigens are present in *B. parapertussis* and *B. bronchiseptica* was presented by Kendrick et al. (8), who showed that *B. pertussis* and *B. bronchiseptica* shared antigens which were immunogenic in cross-protection tests, and that *B. parapertussis* was a potent immunogen against *B. bronchiseptica* but not against *B. pertussis*. Histamine-sensitizing activity has not been detected in *B. bronchiseptica* or *B. parapertussis* (13).

Presence of cross-protective antigens in the three species of *Bordetella* and lack of HSF activity in *B. bronchiseptica* and *B. parapertussis* prompted us to investigate further the activities of these antigens. A better knowledge of the antigenic relationship of these related organisms should help in understanding the relationship between protective and HSF activities in *B. pertussis*.

MATERIALS AND METHODS

Origin of cultures. *B. pertussis* strains 353/Z (serotype 1), T122 (serotype 1, 2, 4), and J20 (serotype 1, 2) were obtained from Noel Preston, University of

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Manchester, Manchester, England; strain 2753 (serotype 1, 2, 3, 4, 5), originally derived from P. Kendrick's mouse virulent strain 18-323, was obtained from Merck Sharp and Dohme Research Laboratories, West Point, Pa.; and strain 04965 (serotype 1) was obtained from Parke, Davis & Co., Detroit, Mich. *B. parapertussis* 17903 (serotype 7, 8, 9, 10, 14) and *B. bronchiseptica* 22067 (serotype 7, 8, 9, 12, 13) and 214 (serotype 7, 9, 12, 13) were obtained from Grace Eldering, Michigan Department of Public Health, Grand Rapids. *B. bronchiseptica* 2320 (serotype not known) was isolated from swine nasal secretions. All cultures were stored in lyophilized form at −20 C, reconstituted on Bordet-Gengou (Difco) agar containing 15% horse blood, and then transferred twice before being used for cell production or for preparation of live challenge suspensions.

Media and culture procedures. Bacteria used in fractionation experiments were grown under constant agitation in the modified Casamino Acids medium of Verwey et al. (19). The cells were harvested with a Sorvall RC 2 centrifuge equipped with a continuous-flow collection attachment. Packed cells were stored as a paste at −20 C. Cells used for challenge of mice were grown on Bordet-Gengou agar. Cultivation was carried out at 35 to 37 C.

Preparation and fractionation of cells. Dried whole cells were prepared by dialyzing fresh liquid-grown cells against four changes (approximately 10 volumes per change) of cold distilled or deionized water; these cells were lyophilized and stored at 4 C.

The procedure for preparation of acetone-dried disrupted cells was as described previously (14). Frozen cultures were thawed, suspended in cold saline at a concentration of approximately 300 billion cells per ml, and ruptured in a Sorvall-Ribi cell fractionator at 40,000 psi. The ruptured cells were extracted twice with three volumes of acetone and were dried in vacuo.

Alkaline saline extract (SE) was prepared by homogenizing the acetone-dried cells in cold 0.85% NaCl (1 g per 100 ml). The *p*H was adjusted to 8.5 with NaOH, and the mixture was stirred overnight at 2 to 5 C. The *p*H was readjusted to 7.5, and the mixture was centrifuged at 27,000 × *g* for 90 min. The clear amber supernatant fluid was then dialyzed against four changes (approximately 10 volumes each) of cold distilled water and lyophilized.

The HSF was further purified by adding, to a dialyzed aqueous solution of SE at *p*H 5.6, enough 2 M $MgSO_4$ to give a final concentration of 0.03 M. The precipitate was collected by centrifugation at 35,000 × *g* for 40 min. The clear supernatant fluid and the sediment were separately dialyzed at 2 to 5 C for 3 days against daily changes of 20 liters of distilled water. The slight precipitate formed was collected by centrifugation and pooled with the dialyzed precipitate from the first centrifugation; the pool was lyophilized. The resulting white-gray powder thus obtained contained most of the HSF in the starting material.

An alternative method for preparation of a soluble antigen from the *Bordetella* cells was extraction with sodium deoxycholate (DOC) (3, 18). A 4-g amount of whole cells (wet paste) was washed once with 0.85% NaCl and suspended in 100 ml of 1% DOC in 0.06 M phosphate buffer (*p*H 8.5). The mixture stood overnight at room temperature, the *p*H was adjusted to 7.5 with HCl, and the solution was treated for 2 hr at room temperature with 10 μg of deoxyribonuclease and 5 μg of ribonuclease per ml. The preparation was centrifuged at 27,000 × *g* for 60 min, and the supernatant fluid was dialyzed against many changes of cold distilled water and lyophilized.

Boivin-type endotoxin was extracted [as described by Kabat (7)] with trichloroacetic acid, precipitated with ethyl alcohol, and lyophilized. Pyrogenicity and chick embryo lethality of endotoxin and SE were tested by the methods of Milner and Finkelstein (10).

Immunodiffusion and immunoelectrophoresis. Double diffusion in agar was performed in 0.5% Ionagar in 0.85% NaCl. Immunoelectrophoresis was done on standard microscope slides in 1.5% Ionagar in barbituric acid buffer (μ = 0.01, *p*H 7.5) at 7 v per cm for 75 min. Immunoelectrophoresis was also performed on 10 × 8.25 cm slides under similar conditions, except that 5 v per cm was applied for 2 hr.

Starch-block electrophoresis. Starch-block electrophoresis was performed as described previously (14). The starch block (50 × 9.5 × 1.2 cm) was made in phosphate buffer (μ = 0.02, *p*H 6.2). SE (100 mg) was applied at the center of the block, and electrophoresis was performed at 6 to 10 ma and 200 v at 4 C for 40 hr. The block was cut into twenty-five 2-cm strips starting at the negative pole, and each strip was extracted with phosphate buffer. The eluates were dialyzed against distilled water and lyophilized.

Mouse-protection test. Protective activity of the various preparations was determined in female albino mice of the Rocky Mountain Laboratory strain by a procedure reported previously (15). To prevent toxic effects from heat-labile toxins, whole cells were heated at 56 C for 20 min before administration to mice. The PD_{50} of the various preparations was calculated by the method of Reed and Muench (17).

Histamine-sensitization test. Histamine-sensitizing activity was determined in male and female Swiss Webster mice of the CFW strain raised at Rocky Mountain Laboratory or purchased from Carworth Farms, New City, N.Y. Sensitization was induced by one intravenously (iv) administered dose of the substance to be tested, and was followed 24 hr later by an intraperitoneal challenge dose of 0.5 mg of histamine base, given as histamine diphosphate (12). The SD_{50} of the substances was calculated by the method of Reed and Muench (17).

Preparation of antisera. Rabbits were immunized subcutaneously with DOC extracts in complete Freund's adjuvant or with whole-cell suspensions given intravenously. Various schedules of immunization were used, and sera producing the greatest number of lines by immunodiffusion were selected and in some cases pooled.

RESULTS

Different antigenic components of Bordetella species. Immunoelectrophoretic analysis of SE or

DOC extract from *B. pertussis* 04965 revealed many antigens, most of which were present in both extracts. However, there were at least two antigens in DOC extracts that were not detectable in SE. One of these formed a band close to the antigen well (well 2, Fig. 1) and another migrated toward the anode. SE (well 1, Fig. 1) seemed to have a few antigens close to the anode which were not clearly seen in DOC extracts. These minor discrepancies could have been due to differences in antigen concentration. Protective and histamine-sensitizing activities were comparable for the two extracts. A diagrammatic sketch of the immunoelectrophoretically separated antigens of SE and DOC extracts developed with various antisera to *B. pertussis* is shown in Fig. 2. A minimum of 15 different antigens were detected. Tests of the antigens eluted from the agar after electrophoresis on large slides showed that the mouse-protective and histamine-sensitizing activities were always in fractions close to the point of application.

Many of the antigens in *B. pertussis* 04965 were shared by *B. bronchiseptica* and *B. parapertussis*, as is shown by the results of immunodiffusion tests illustrated in Fig. 3. Few bands of precipitate appeared to be unique for *B. pertussis*.

The close antigenic similarity became more apparent when anti-*B. pertussis* serum was absorbed with heterologous SE. Anti-*B. pertussis* serum absorbed with 20 mg of *B. pertussis* 04965 SE per ml removed all antibodies, as judged by complete disappearance of the bands. A 20-mg amount of SE from *B. parapertussis* 17903 removed all precipitins reacting with this extract, but left antibodies for at least four antigens in *B. pertussis* 04965 SE and three for *B. bronchiseptica* 22067 SE (Fig. 4). A 20-mg amount of *B. bronchiseptica* 22067 SE absorbed all precipitins which reacted with this SE and with SE from *B. parapertussis* 17903 (except for a weak minor band in each case), but left at least three antibodies specific for *B. pertussis* SE (Fig. 4). *B. bronchiseptica* SE contained all the antigens present in *B. parapertussis* SE but had at least three extra antigens (Fig. 4). In these tests, *B. parapertussis* SE did not appear to contain antigens different from those of *B. pertussis* SE or *B. bronchiseptica* SE, but, when antiserum prepared against *B. parapertussis* SE was absorbed with *B. bronchiseptica* SE or *B. pertussis* SE, two specific antigens for *B. parapertussis* were demonstrated by immunodiffusion.

Trichloroacetic acid extracts (endotoxins) from *B. pertussis* were serologically distinct from *B.*

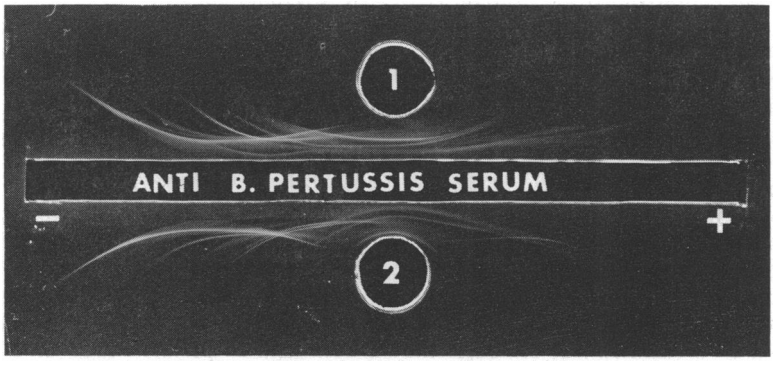

FIG. 1. *Immunoelectrophoretic patterns showing the similarity of SE (well 1) and DOC extract (well 2) from B. pertussis 04965.*

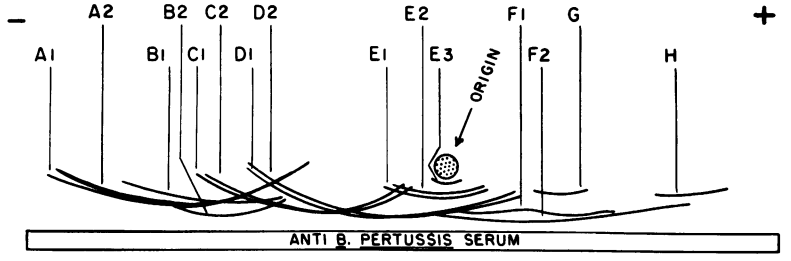

FIG. 2. *Diagrammatic illustration of all antigens detected in B. pertussis 04965 SE and DOC extract by immunoelectrophoresis. The letters were used to group the antigens by their relative electrophoretic mobility: "A" antigens were the most positively charged, and "H" the most negatively charged.*

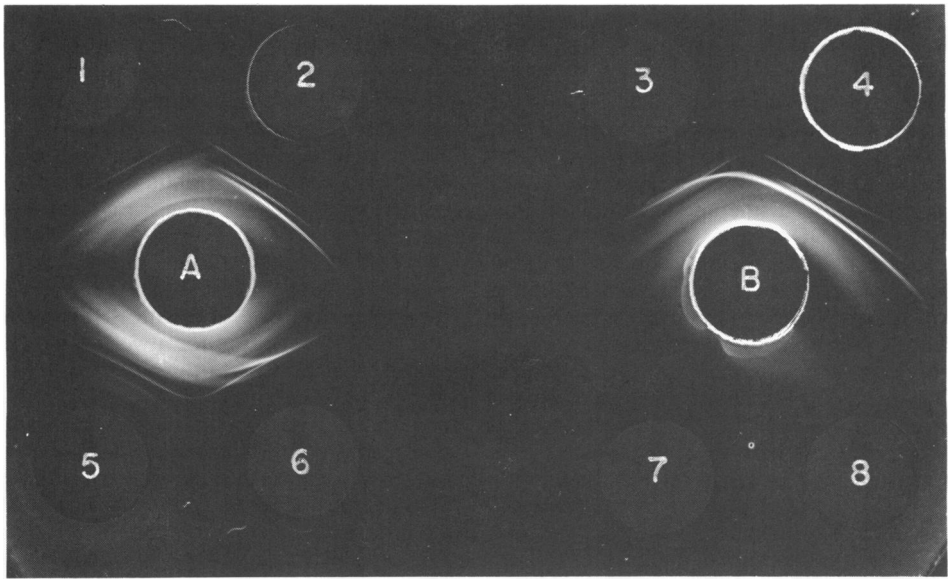

FIG. 3. *Comparison of antigens of B. pertussis 04965, B. parapertussis 17903, and B. bronchiseptica 22067 by gel diffusion. Wells A and B contained a pool of antiserum to B. pertussis 04965. Wells 1 and 5 contained SE from B. pertussis 04965; wells 2 and 4, SE from B. parapertussis 17903; wells 3 and 6, SE from B. bronchiseptica 22067; and well 7, the medium used to grow the cells; well 8 was not filled.*

bronchiseptica and *B. parapertussis* trichloroacetic acid extracts, but endotoxins made from different *B. pertussis* strains were serologically identical (Fig. 5).

Cross-protection among Bordetella species. The close antigenic relationship of these species led us to test whether cross-protection could be demonstrated in mice immunized with whole-cell vaccines or extracts from these cells. The results (Table 1) showed that *B. pertussis* cells or extracts protected against *B. pertussis*; whole cells also protected against *B. bronchiseptica* challenge. Curiously, except for strain 04965, SE from most *B. pertussis* strains showed at best only weak protection against *B. bronchiseptica*, although they gave good protection against *B. pertussis*. Protection against *B. pertussis* was not observed with whole cells or extracts from *B. bronchiseptica* and *B. parapertussis*, although protection against *B. bronchiseptica* was obtained with whole cells of *B. bronchiseptica* or *B. parapertussis*. SE prepared from these cells gave little protection against *B. bronchiseptica* at the doses tested (Table 1). Since a mouse-virulent strain of *B. parapertussis* was not available, protection tests against this organism could not be performed.

These results suggested that at least two substances may be involved in the protection against *B. pertussis* and *B. bronchiseptica*: one specific for *B. pertussis* and one specific for *B. bronchiseptica*. *B. pertussis* cells must contain both substances, unless protection with these cells is nonspecific. If two different substances are involved, it should be possible to separate them or at least to demonstrate that they have different properties.

Effect of heat on protective antigens from B. pertussis and B. bronchiseptica. To determine the heat stability of the protective antigens of *B. pertussis* 04965, *B. bronchiseptica* 22067, and *B. parapertussis* 17903, cell suspensions (2.5 mg of dry cells/ml of *p*H 7.3 buffered saline) were heated in a water bath at 56, 60, or 80 C for 0.5 hr. Mice in groups of 15 were injected intraperitoneally with 5, 25, or 125 μg of these preparations. The animals were challenged intracranially 14 days later with *B. pertussis* 2753 or *B. bronchiseptica* 22067. This experiment showed (Table 2) that the antigens of *B. pertussis* cells that immunize against *B. pertussis* and *B. bronchiseptica* are heat-labile, whereas the antigens in *B. bronchiseptica* and *B. parapertussis* that protect against *B. bronchiseptica* are rather resistant to 80 C for 0.5 hr. In another experiment, autoclaved *B. parapertussis* cells or *B. bronchiseptica* cells no longer protected, and endotoxins (trichloroacetic acid extracts) from any of the three species also failed to protect against *B. bronchiseptica*. These experiments indicated that the protection induced by *B. pertussis* was due to a substance other than the protective substance found in *B. bronchiseptica* or *B. parapertussis*, since the substance in *B. pertussis* cells protecting against *B. bronchiseptica* was more

susceptible to heat than that in *B. bronchiseptica* or *B. parapertussis*.

B. pertussis 04965 SE, which gave good protection against both *B. pertussis* and *B. bronchiseptica*, was submitted to electrophoresis in starch. Each of twenty-five 2-cm fractions cut from the block was extracted with phosphate-buffered saline (*p*H 7.3), and the extracts were pooled as indicated in Fig. 6. These pools were dialyzed against distilled water, lyophilized, and then tested for their ability to protect against *B. pertussis* or *B. bronchiseptica* challenge. The optical density at 280 nm of eluted fractions and the qualitative results of the protection tests against *B. pertussis* and *B. bronchiseptica* are given in Fig. 6. Both protective antigens were found in the same fractions of the block (Fig. 6, pools 4 and 5).

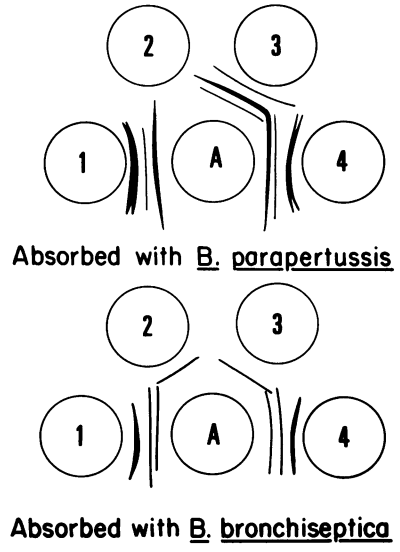

FIG. 4. *Diagrammatic tracings of gel diffusion tests showing the effect of absorption of anti-B. pertussis serum with B. parapertussis SE (upper well A) and B. bronchiseptica SE (lower well A). Well A contained absorbed antiserum; upper and lower wells 1 and 4, SE from B. pertussis 04965; upper well 2 and lower well 3, SE from B. parapertussis 17903; upper well 3 and lower well 2, SE from B. bronchiseptica 22067.*

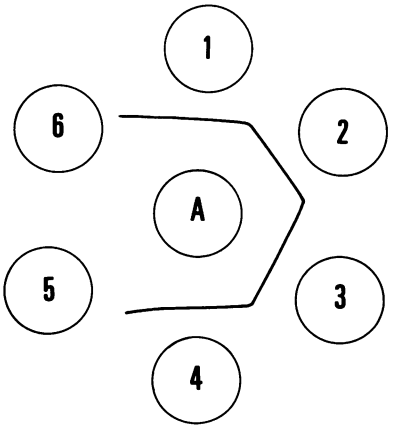

FIG. 5. *Diagrammatic tracing of gel diffusion test showing the serological relationship among trichloroacetic acid extracts (endotoxins) from B. pertussis strains and B. bronchiseptica and B. parapertussis. Well A contained pool of anti-B. pertussis serum. Peripheral wells contained saline solutions of endotoxin (625 μg/ml) prepared from: well 1, B. pertussis 353/Z; well 2, 04965; well 3, 3779; well 4, J20; well 5, B. parapertussis 1703; and well 6, B. bronchiseptica 22067.*

Histamine-sensitizing activity of Bordetella species. The histamine-sensitizing property of cells or extracts from *B. pertussis*, *B. bronchiseptica*, and *B. parapertussis* was also investigated. Mice were inoculated intravenously with 1, 5, or 25 μg of whole cells, acetone-dried cells, SE, $MgSO_4$-precipitated HSF, or trichloroacetic acid extracts, and were challenged with histamine 24 hr later. The results (Tables 3–4) showed that only whole cells, acetone-dried cells, SE, and $MgSO_4$-precipitated HSF from *B. pertussis* induced histamine sensitization. Cells or extracts from *B. bronchiseptica* and *B. parapertussis* did not induce sensitization at the doses given. Endotoxins extracted from *B. pertussis* by the trichloroacetic acid method, in doses that were already somewhat toxic for mice, failed to sensitize mice to histamine (Table 4). For comparison, Table 4 also gives the histamine-sensitizing ability of two highly active extracts from *B. pertussis*.

DISCUSSION

The close antigenic relationship among the three species of the genus *Bordetella* has been noted by various workers (1, 2, 5, 6, 8). *B. bronchiseptica* is considerably less fastidious in its growth requirements than *B. pertussis* and *B. parapertussis*, but the antigenic similarities of the three species are striking. The absorption experiments reported in this paper clearly showed that only a few antigens of *B. pertussis*, *B. bronchiseptica*, and *B. parapertussis* were different. A few specific antigens were detected in each of the three species. The heat-labile toxin is antigenically similar in all three species (1, 4). In this work, endotoxin (trichloroacetic acid extract) was similar in various strains of *B. pertussis* but was serologically different from the endotoxins from *B. bronchiseptica* and *B. parapertussis*. In the doses employed, HSF was not detected in *B. bronchiseptica* or *B. parapertussis*. Other workers have claimed that endotoxins from these organisms, as well as from *B. pertussis*, sensitize mice to

histamine, but the amounts employed were large and were lethal to some of the mice (9, 16). Our work demonstrated that *B. bronchiseptica* and *B. parapertussis* cells, extracts, or endotoxins do not have histamine-sensitizing ability comparable to that of *B. pertussis* cells, SE, or $MgSO_4$-precipitated HSF of *B. pertussis*. The *B. pertussis* endotoxin also failed to sensitize mice to histamine.

TABLE 1. *Mouse-protective dose (PD_{50}, μg per mouse) of either whole cells or alkaline saline extract from various strains of Bordetella species*[a]

Vaccine made from	Challenge strain			
	B. pertussis 2753		*B. bronchiseptica* 22067	
	WC[b]	SE[c]	WC	SE
B. pertussis 353/Z	5.69	27	20.9	>125
B. pertussis 2753	35.1	100	67.8	>125
B. pertussis T122	13.3	31	13.3	75.9
B. pertussis J20	28.1	37	17.0	>125
B. pertussis 04965	10.3	ND[d]	12.7	20.1
B. parapertussis 17903	>125	>625	13.8	62.6
B. bronchiseptica 22067	>125	>625	16.5	67.8
B. bronchiseptica 214	>125	>625	45.3	>125
B. bronchiseptica 2320	>125	>625	32.9	>125
Brucella abortus strain 19	>125	>625	>125	>625

[a] To determine the PD_{50} of each sample, three groups of 15 mice each were immunized with graded doses of each sample. The mice were challenged intracranially 14 days later with 40,000 cells of either *B. pertussis* 2753 or *B. bronchiseptica* 22067. Deaths were recorded for 14 days. In all cases, 90 to 100% of normal control mice similarly challenged died.

[b] WC = whole cells.

[c] SE = alkaline saline extract.

[d] Not done.

TABLE 2. *Effect of heat on the protective antigens of Bordetella species*

Immunizing antigen	Antigen treated for 0.5 hr at	Challenge culture	PD_{50}[a] (μg/mouse)	
			Expt 1	Expt 2
	C			
B. pertussis 04965	56	*B. pertussis* 2753	18.6	—[b]
	60	*B. pertussis* 2753	—	16.1
	80	*B. pertussis* 2753	>125[c]	90.1
	60	*B. bronchiseptica* 22067	—	13.5
	80	*B. bronchiseptica* 22067	—	>125
B. bronchiseptica 22067	56	*B. pertussis* 2753	>125	—
	80	*B. pertussis* 2753	>125	—
	56	*B. bronchiseptica* 22067	21	10.2
	80	*B. bronchiseptica* 22067	16.6	7.6
B. parapertussis 17903	56	*B. pertussis* 2753	>125	—
	80	*B. pertussis* 2753	>125	—
	56	*B. bronchiseptica* 22067	10.9	—
	80	*B. bronchiseptica* 22067	37.8	—

[a] For calculation of PD_{50}, three doses of antigen (5, 25, and 125 μg) were used and 15 mice per dose were immunized intraperitoneally. The animals were challenged intracranially 14 days after immunization with approximately 40,000 live cells. Deaths were recorded for 14 days after challenge. In all cases, 90 to 100% of the control mice died after challenge.

[b] Not done.

[c] Slight protection was obtained at the 125-μg dose level.

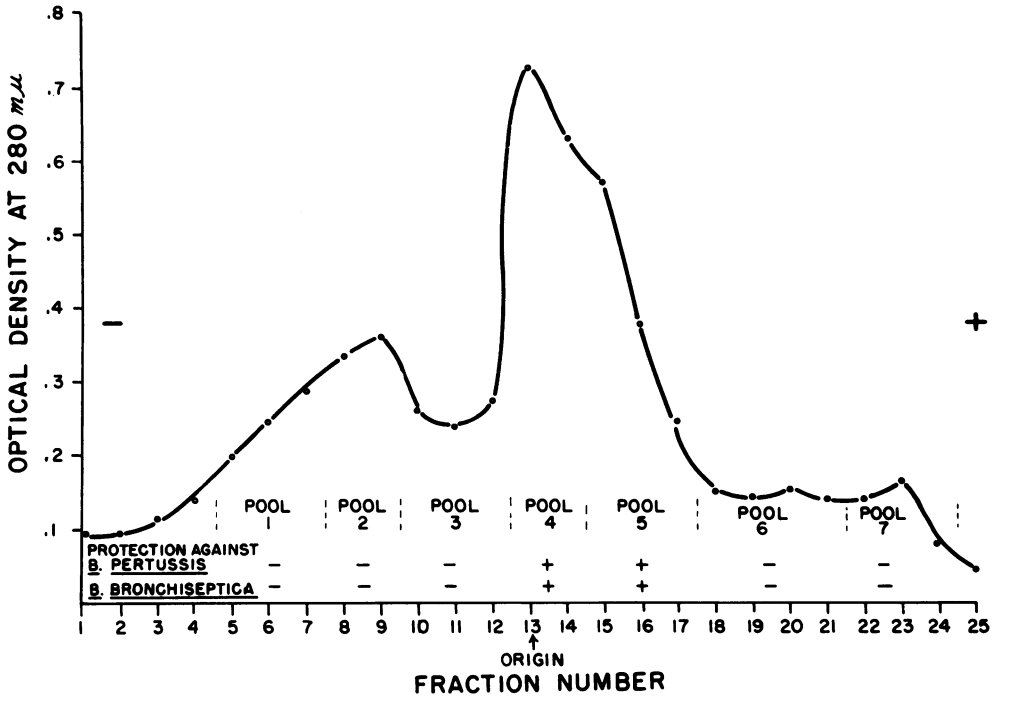

FIG. 6. *Relationship of optical density and mouseprotective activity against B. pertussis and B. bronchiseptica of starch-block electrophoresis fractions of B. pertussis 04965 SE. The fractions were pooled as follows: pool 1, fractions 5–7; pool 2, fractions 8–9; pool 3, fractions 10–12; pool 4, fractions 13–14; pool 5, fractions 15–17; pool 6, fractions 18–21; and pool 7, fractions 22–24.*

TABLE 3. *Histamine-sensitizing dose (SD_{50}, μg per mouse) of whole cells, acetone-dried cells, and alkaline saline extract made from various strains of Bordetella species*[a]

Cells used to prepare sensitizing materials	SD_{50} (μg per mouse)		
	Whole cells	Acetone-dried cells	Saline extract
B. pertussis 353/Z	10.2	2.76	5
B. pertussis 2753	12.9	>25	12.9
B. pertussis T122	21.6	7.36	3.45
B. pertussis J20	5.87	4.19	1.5
B. bronchiseptica 22067	>25	>25	>25
B. bronchiseptica 214	>25	>25	>25
B. bronchiseptica 2320	>25	>25	>25
B. parapertussis 17903	>25	>25	>25

[a] To determine the SD_{50} of each sample, three groups of 10 CFW female mice were sensitized intravenously with 1, 5, or 25 μg of sample, and challenged intraperitoneally 24 hr later with 0.5 mg of histamine base (given as histamine diphosphate).

TABLE 4. *Histamine-sensitizing dose (SD_{50}) of three semipurified extracts from B. pertussis strains*[a]

B. pertussis strain	SD_{50} (μg per mouse)		
	$MgSO_4$ ppt[b]	SE[c]	Endo[d]
353/Z	1	5	>25
5374	<1	<1	>25
J20	1	1.5	>25

[a] The SD_{50} was determiled as in Table 3.
[b] $MgSO_4$-precipitated HSF.
[c] SE = alkaline saline extract.
[d] Endo = trichloroacetic acid extract of whole cells. These preparations were pyrogenic for rabbits and lethal for chick embryos, whereas 100 times more SE or $MgSO_4$ precipitated HSF was required to elicit a similar pyrogenic response in rabbits or to kill 11-day-old chick embryos.

The cross-protection afforded by *B. pertussis* and *B. parapertussis* cells to mice challenged with *B. bronchiseptica* is due to a different antigen than that responsible for *B. pertussis* protection. The protective antigen of *B. pertussis* is a heat-labile substance very closely associated with, if not

identical to, HSF. It is not known with certainty whether this antigen is involved in protection against *B. bronchiseptica*, since the protective effect of *B. pertussis* cells against *B. bronchiseptica* was also sensitive to heating at 80 C for 0.5 hr. On the other hand, extracts (SE and $MgSO_4$-precipitated HSF) which are active in protecting mice against *B. pertussis* challenge and in sensitizing them to histamine failed or were very poor in protecting against *B. bronchiseptica* challenge. The antigen in *B. pertussis* that protects mice against *B. bronchiseptica* is more susceptible to heat than that in *B. bronchiseptica*; most likely, the two antigens are different. These antigens are not related to endotoxin.

ACKNOWLEDGMENTS

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