ANTIGENIC RELATIONSHIPS OF HAEMOPHILUS PERTUSSIS, THE PARAPERTUSSIS BACILLUS, AND BRUCELLA BRONCHISEPTICA AS SHOWN BY CROSS PROTECTION TESTS IN MICE[1]

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Received for publication January 26, 1953

Antigenic relationships among the three organisms, *Haemophilus pertussis*, the parapertussis bacillus, and *Brucella bronchiseptica*, have been demonstrated by various workers, using different methods. Ferry and Noble (1918), by means of agglutination tests, and Ferry and Klix (1918), using complement fixation procedures, showed cross reactions between *H. pertussis* and *B. bronchiseptica*. In connection with the description of the parapertussis bacillus Eldering and Kendrick (1938) outlined the differential characteristics and demonstrated relationships with *H. pertussis* and *B. bronchiseptica* using agglutination and agglutinin absorption techniques. Evans (1940) studied the toxins of the three organisms and showed that they were all neutralized by pertussis antitoxic serum. Further evidence of these relationships was presented in the work of Eldering (1942) with protection tests in mice challenged by the intraperitoneal route. The results indicated cross protection among the three species. In fact, *B. bronchiseptica* antigens, either the whole culture or a carbohydrate fraction, protected against *H. pertussis* infection fully as well as did *H. pertussis* antigen itself.

It is of interest that both the parapertussis bacillus and *B. bronchiseptica* occasionally are found associated with clinical whooping cough in children. A recent paper by Eldering and Kendrick (1952) reports that approximately 2 per cent of the cultures isolated in the Grand Rapids area during a 16 year period from children with whooping cough symptoms were parapertussis. The organism has been found also in other parts of the United States and in other countries. *B. bronchiseptica* has been isolated from patients with whooping cough-like disease in a few instances (Brown, 1926; Medical Research Council Investigation, 1951; Chang Shih Man, 1950; Alexander, 1950).

With the development of the mouse protection test, using the intracerebral route for challenge as a measure of potency of pertussis vaccine, another method was available for studying the antigenic relationships within the group. Such a study seemed particularly important because of its bearing on the question raised by several workers (Miller *et al.*, 1941; Rambar *et al.*, 1947) concerning the inclusion of parapertussis cultures in pertussis vaccine. It was planned therefore to inject groups of mice with killed suspensions of each of the cultures. Following a suitable rest period the mice injected with each vaccine were to be divided into three groups for intracerebral challenge with *H. pertussis*, the parapertussis bacillus, and *B. bronchiseptica*, respectively. The study could not be completed as planned because of the failure to find a parapertussis culture which would kill mice inoculated by the intracerebral route. In the search for a virulent strain, 102 parapertussis cultures were tested in mice. These included cultures isolated in Grand Rapids and others obtained from Mexico, Denmark, Australia, and England. Mouse passage to increase virulence was unsuccessful. Finally it was decided to proceed with the study omitting the challenge with parapertussis.

MATERIALS AND METHODS

Antigens. The cultures used for preparation of antigens for immunization of mice included two strains each of *H. pertussis* and parapertussis, all isolated in the Western Michigan Section Labora-

[1] This study was sponsored by the Committee on Research and Standards of the American Public Health Association, with the aid of funds granted by laboratories in the Biologic Section of the American Drug Manufacturers Association.

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tory of the Michigan Department of Health from children with symptoms of whooping cough, and three strains of *B. bronchiseptica*, one isolated from a rabbit, and two[3] isolated from children. The cultures were revived from the dried state not more than two months prior to their use and were checked for characteristics of smooth strains. The antigens were prepared according to the procedure used for pertussis vaccine. The 48 hour growth on Bordet-Gengou agar was harvested in saline, centrifuged, and resuspended in saline containing merthiolate 1:5,000. The turbidity was adjusted by means of a Cenco photelometer using as standard the pyrex glass suspension distributed by the National Institutes of Health.

Challenge cultures. H. pertussis, strain 18-323, and *B. bronchiseptica*, strain 22-067, were used for challenge in the protection tests. The pertussis culture is the one widely used in potency tests of *H. pertussis* vaccine and has an LD/50 of approximately 500 organisms. The *B. bronchiseptica* strain has an LD/50 of about 300 organisms. The same range of virulence was observed for several other strains. Symptoms in nonimmunized mice injected intracerebrally with *B. bronchiseptica* are the same as in mice infected by the same route with *H. pertussis*, as described in a previous publication (Kendrick *et al.*, 1947).

Mice. The mice were white Swiss, obtained from the "disease-free colony" of the Michigan Department of Health Laboratories, Lansing.

Procedure. The procedure for the mouse protection test was essentially as described in a previous publication (Kendrick *et al.*, 1947) and in line with the method used by the National Institutes of Health (1948). Three groups of 14 mice each were given graded doses of vaccine in one intraperitoneal injection. After a rest period of 14 days, an infecting dose of 100,000 organisms contained in 0.03 ml of a one per cent solution of casamino acids was injected intracerebrally. In one experiment an infecting dose of 50,000 organisms was used (experiment 329).

Following an observation period of 14 days the results of the experiments were analyzed by the regression curve method described by Litchfield and Fertig (1941).

[3] These cultures were sent to us by Dr. Hattie Alexander, Children's Hospital, New York, and Communicable Disease Center, Atlanta. The culture from the Communicable Disease Center was isolated in Boston City Hospital.

RESULTS

Seven experiments were carried out in which 41 groups of immunized mice were challenged, 20 groups with *H. pertussis* and 21 with *B. bronchiseptica*. In each experiment the LD/50 of each of the challenge cultures was determined, and eight nonimmunized mice were injected intracerebrally with the challenge dose. A sample protocol is shown in table 1.

TABLE 1

Sample protocol: Experiment no. 355 Brucella bronchiseptica infection of 3 groups of mice immunized with graded doses of Haemophilus pertussis, parapertussis, and B. bronchiseptica antigens, respectively

IMMUNIZATION		RESPONSE	
Antigen	Dose in billions	S/T*	ED/50 in billions
Haemophilus pertussis, strain 10-536	0.06	1/14	
	0.3	3/13	1.2
	1.5	8/14	
Parapertussis, strain 22-656	0.06	7/13	
	0.3	9/14	0.08
	1.5	13/14	
Brucella bronchiseptica, strain 22-067	0.06	11/14	
	0.3	13/14	Not
	1.5	11/13	graded
	no. of organisms		LD/50
Titration of infecting culture *Brucella bronchiseptica*, strain 22-067	80	5/9	
	400	4/10	130 organisms
	2,000	2/9	

*S/T indicates number of survivors over total.

In experiment 355, groups of mice were immunized with graded doses of the three antigens, and all were challenged with *B. bronchiseptica*. The ED/50 of 1.2 billion with pertussis vaccine indicates protection but not of the same order as that shown with the other two antigens.

The protection afforded by the *B. bronchiseptica* antigen was greater than could be calculated from the response to the dosage scheme used. Therefore in another test, smaller doses of *B.*

bronchiseptica antigen were used, and the end point in this experiment was 0.03 billion. The end point of 0.08 billion obtained with the parapertussis antigen was lower than in other experiments and was considered to be an extreme result. The LD/50 was 130 organisms in comparison with a range of from 74 to 430 in other experiments.

To simplify presentation of data, the results obtained in the different experiments are summarized in separate tables according to the two challenge cultures. In table 2 are shown the

TABLE 2

Haemophilus pertussis infection of mice immunized with Haemophilus pertussis, parapertussis, and Brucella bronchiseptica antigens

ANTIGEN	ED/50 RESULTS IN SEVERAL EXPERIMENTS			
	Experiment 329	Experiment 343	Experiment 355	Experiment 364
Haemophilus pertussis				
strain 18-323	0.21	0.38	0.51	0.16
strain 10-536	0.48	0.52	0.10	—
Parapertussis				
strain 21-353	NG*	NG	—	NG
strain 22-656	NG	NG	NG	NG
Brucella bronchiseptica				
strain 899	—	1.0	—	1.1
strain R370	6.5	—	—	—
strain 22-067	—	NG	0.52	NG

*NG = response not graded; very little, if any, protection.

results of four experiments in which the mice were immunized with pertussis, parapertussis, and bronchiseptica vaccines, respectively, and challenged with *H. pertussis*.

The pertussis injected mice challenged with *H. pertussis* showed definite protection with both the vaccine suspensions, with end points ranging from 0.10 to 0.52 billion. These end points are in line with those obtained in potency tests of production lots of pertussis vaccine.

So few of the mice survived in any of the seven tests in which parapertussis vaccinated mice were challenged with *H. pertussis* that ED/50 values could not be calculated. Only 36, or 12 per cent, of the 290 mice tested survived the infection, indicating very slight protection. In the same experiments none of the 35 nonimmunized mice survived the challenge dose. Bronchiseptica vaccinated mice challenged with *H. pertussis* gave variable results, with end points from 0.52 to 6.5. In 2 of 6 tests the results were not graded and end points could not be calculated.

Table 3 shows the results of challenge with *B. bronchiseptica*.

TABLE 3

Brucella bronchiseptica infection of mice immunized with Haemophilus pertussis, parapertussis, and Brucella bronchiseptica antigens

ANTIGEN	ED/50 IN BILLIONS OF ORGANISMS			
	Experiment 337	Experiment 345	Experiment 355	Experiment 363
Haemophilus pertussis				
strain 10-536	1.6	0.49	1.2	4.0
strain 18-323	3.6	0.72	NG*	4.1
Parapertussis				
strain 21-353	1.3	NG*	—	1.5
strain 22-656	0.66	0.13	0.08	2.9
Brucella bronchiseptica				
strain 899	—	0.48	—	NG*
strain R370	0.23	—	—	—
strain 22-067	—	0.04	0.03†	0.03

*NG = response not graded; very slight protection.

†ED/50 outside of dosage range used.

The ED/50 values for the *H. pertussis* vaccines varied from 0.49 to 4.1 billion organisms. The results with mice injected with parapertussis vaccine indicated definite protection, the end points ranging from 0.08 to 2.9. The two lowest end points, 0.13 and 0.08, should be pointed out; in potency tests of pertussis vaccines these values would be interpreted as indicating a high degree of protection. The results with mice immunized with *B. bronchiseptica* vaccines showed good protection. There was some indication that *B. bronchiseptica*, strain 22-067, vaccine, which was prepared with the culture used for challenge, gave slightly better protection than the other two *B. bronchiseptica* vaccines, but further work would have to be done before any conclusion could be made as to differences in strains.

ACKNOWLEDGMENT

The authors acknowledge the assistance of Dr. Elaine Updyke during the early part of the work.

SUMMARY

The results of the cross protection tests are summarized in the following chart.

ANTIGEN	EVIDENCE OF PROTECTION WITH CHALLENGE CULTURE	
	Haemophilus pertussis	*Brucella bronchiseptica*
Haemophilus pertussis....	Definite	Slight to good
Parapertussis..	Very slight	Fair to good
Brucella bronchiseptica....	Slight to good	Definite

Haemophilus pertussis and *Brucella bronchiseptica* antigens afforded definite protection against infection with the homologous culture. Cross protection tests between *H. pertussis* and *B. bronchiseptica* gave evidence of a fair degree of protection, with results varying within a wide range from experiment to experiment.

When mice injected with parapertussis vaccine were infected with *H. pertussis*, there was very little protection. The survival rate was so low that end points could not be calculated. Parapertussis-vaccinated mice challenged with *B. bronchiseptica* showed protection, which in some tests approached that obtained with *B. bronchiseptica* vaccine.

Although the cross protection tests are incomplete because of the lack of a parapertussis culture suitable for challenge, the results obtained are additional evidence of antigenic relationships among the three species. The data suggest that from the standpoint of mouse protection both *H. pertussis* and the parapertussis bacillus are related more closely to *B. bronchiseptica* than they are to each other. The poorest protection was with parapertussis injected mice challenged with *H. pertussis*, suggesting that addition of parapertussis organisms to pertussis vaccine could not be expected to add to its effectiveness against *H. pertussis* infection.

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